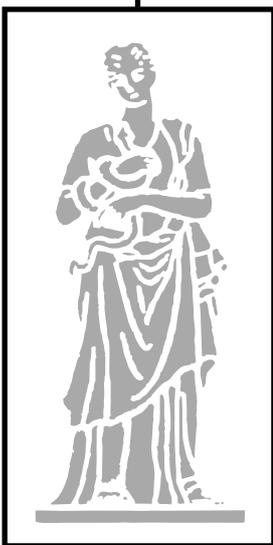


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The role of vitamin D in selected autoimmune diseases. <i>Aleksandra Wiśniewska, Alicja Szypowska</i>	111
Link between methyl nutrients and the DNA methylation process in the course of selected diseases in adults. <i>Paulina Łoboś, Bożena Regulska-Iłow</i>	123
Associations between diet and acne lesions. <i>Magdalena Daszkiewicz</i>	137
The relationship between seeds consumption, lipid profile and body mass index among patients with cardiovascular diseases. <i>Jana Kopčėková, Petra Lenártová, Jana Mrázová, Martina Gažarová, Marta Habánová, Kristína Jančichová</i>	145
Long-term effect of one-time nutritional education in school on nutritional knowledge of early school-aged children. <i>Aleksandra Chojnacka, Magdalena Górnicka, Kacper Szewczyk</i>	155
Nutrition habits and frequency of consumption of selected food products by the residents of urban and rural area from the Subcarpathian voivodeship. <i>Mateusz Szalajko, Wiktoria Stachowicz, Monika Dobosz, Martyna Szalankiewicz, Aneta Sokal, Edyta Łuszczki</i> ,	165
An evaluation of the nutritional status of elderly with the use of the MNA questionnaire and determination of factors contributing to malnutrition. A pilot study. <i>Małgorzata Kostecka, Monika Bojanowska</i>	175
Attitude and acceptance of Covid-19 vaccine amongst medical and dental fraternity - a questionnaire survey. <i>Avineet Kaur, Gagandeep Kaur, Akanksha Kashyap, Gaganpreet Singh, Harnoor Singh Sandhu, Iqra Khilji, Ramandeep Singh Gambhir</i>	185
Appraisal of awareness on medical emergencies and its management among dentists in Bhubaneswar, India. <i>Ramesh Nagarajappa, Ipsita Mahapatra, Dharmashree Satyarup, Sharmistha Mohanty</i>	193
<i>In vitro</i> evaluation of antimicrobial efficacy of silver zeolite against common oral pathogens. <i>Sangram Panda, Shakti Rath, Mirna Garhanayak, Sourav Chandra Bidyasagar Bal, Ramesh Nagarajappa</i>	203
Dietary recommendations during the Covid-19 pandemic. Statement of the Committee of Human Nutrition Science of the Polish Academy of Sciences. <i>Lidia Wądołowska, Małgorzata Drywień, Jadwiga Hamulka, Piotr Socha, Maria Borawska, Mariola Friedrich, Ewa Lange and other Members of the Human Nutrition Science Committee of the Polish Academy of Sciences</i>	209
Instruction for Authors	221

THE ROLE OF VITAMIN D IN SELECTED AUTOIMMUNE DISEASES

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ABSTRACT

The authors of recently published scientific papers are focusing increasingly often on the effect of vitamin D on immune processes. In the case of deficiencies of this vitamin, an imbalance in the immune system is observed, which is associated with the intensification of the inflammatory reaction in the body and the increased possibility of an autoimmune reaction. Therefore, due to the growing interest of scientists in the relationship between the effects of vitamin D and the development of autoimmune diseases, this paper considers the use of Vitamin D in autoimmune therapies. However, the mechanism of vitamin D on individual autoimmune diseases has not been elucidated so far, therefore there is a need for further research. The importance of maintaining normal plasma vitamin D levels to reduce the risk of developing autoimmune diseases has been demonstrated by the authors of other studies. They showed that vitamin D levels influenced the course, severity of symptoms and frequency of relapses of autoimmune thyroid disease, inflammatory bowel disease, and rheumatoid arthritis.

Key words: *vitamin D, autoimmunity, rheumatoid arthritis, inflammatory bowel disease, autoimmune Hashimoto's thyroiditis, autoimmune thyroid disorder*

STRESZCZENIE

Autorzy prac naukowych, opublikowanych w ostatnim czasie, coraz częściej skupiają się na wpływie witaminy D na procesy immunologiczne. W przypadku wystąpienia niedoborów tej witaminy obserwuje się upośledzenie równowagi działania układu immunologicznego, co wiąże się z nasileniem reakcji zapalnej w organizmie i zwiększeniem możliwości wystąpienia reakcji autoimmunologicznej. Ze względu na rosnące zainteresowanie naukowców tematem zależności występujących pomiędzy działaniem witaminy D a rozwojem chorób autoimmunologicznych rozważa się wykorzystanie tej witaminy w ich terapii. Mechanizm wpływu witaminy D na poszczególne choroby autoimmunologiczne nie został dotychczas wyjaśniony, dlatego istnieje potrzeba przeprowadzenia dalszych badań. Istotne jest utrzymanie prawidłowego stężenia witaminy D w osoczu w celu zmniejszenia ryzyka rozwoju chorób autoimmunologicznych. Powyższy wniosek został wykazany przez autorów badań w odniesieniu do autoimmunologicznych chorób tarczycy, nieswoistych chorób jelit oraz reumatoidalnego zapalenia stawów. Stężenie witaminy D wpływało na przebieg tych chorób, nasilenie objawów oraz częstotliwość występowania nawrotów.

Słowa kluczowe: *witamina D, autoimmunizacja, reumatoidalne zapalenie stawów, nieswoiste zapalenia jelit, choroba Hashimoto, choroby autoimmunologiczne tarczycy*

INTRODUCTION

Vitamin D is an essential vitamin that is necessary in the proper functioning of the body. It performs many important functions by influencing the course of basic metabolic processes, as a result ensuring the proper functioning of the body. The range of vitamin D's activity in the body depends on its serum concentration. Vitamin D deficiencies are often an indirect cause in the multistage pathogenesis of diseases, and its supplementation is crucial for

preventing these diseases [1, 2, 5, 13, 20, 21, 39, 47, 49, 53].

The most widely described function of vitamin D is its effect on calcium-phosphate metabolism and its role in the prevention of rickets and osteoporosis [49]. However, the authors of recently published scientific papers are increasingly focusing on the effect of vitamin D on immune processes, including autoimmune diseases. In the event of vitamin D deficiencies, an impairment of the immune system balance is observed, which is associated with

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intensification of the inflammatory reaction in the body and the increased possibility of an autoimmune reaction. The correct vitamin D level in the blood determines the secretion of anti-inflammatory cytokines and regulatory elements of the immune system. The serum vitamin D levels also have an indirect effect on, among other things, the microbiome and the integrity of the intestinal epithelial barrier. In the case of a deficiency of vitamin D in the plasma, this relationship can have an effect on the development of autoimmune reactions. Due to the growing interest of scientists in the relationship between the effects of vitamin D and the development of autoimmune diseases, this paper considers the use of Vitamin D in autoimmune therapies. The mechanism of vitamin D on individual autoimmune diseases has so far not been elucidated, therefore there is a need for further research [1, 8, 9, 14, 31, 51, 55, 59].

The aim of this study is to review the scientific literature with regard to the effects of plasma vitamin D levels on the immune system, pathogenesis, and development of autoimmune diseases, such as autoimmune thyroid disease, inflammatory bowel disease (IBDs), and rheumatoid arthritis (RA).

VITAMIN D – METABOLISM AND BIOLOGICAL FUNCTIONS

Vitamin D is part of a specific group of compounds known as secosteroid compounds. The term “vitamin D” refers to two main compounds, ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Both molecules have an analogous structure, consisting of A, B, C, D rings and a side chain. Vitamin D is considered to be a secosteroid compound because of the presence of a characteristic, open B chain. The ergocalciferol and cholecalciferol are synthesized from provitamins – ergosterol and 7-dehydrocholesterol, respectively – under the influence of ultraviolet B radiation of a specific wavelength. The central point of the process of converting provitamins into vitamins is a modification within the molecule B-ring by thermal isomerization [17,25,33,46]. Vitamin D supplied to the body undergoes numerous metabolic processes, thanks to which it reaches its active form [25].

Ergocalciferol, a derivative of exogenous ergosterol, differs from cholecalciferol in the structure of its side chain, but both forms undergo the same metabolic process pathways [17,25,46]. The vitamin D₃ precursor, which is 7-dehydrocholesterol, is mainly found in keratinocytes and fibroblasts. The reaction of 7-dehydrocholesterol conversion into its active form of cholecalciferol (calciol) takes place in the skin under the influence of UVB radiation with an appropriate wavelength of 290-315 nm. The resulting calciol molecule has a lasting and stable structure, and is

then released into the intercellular space to be further transported in the blood [25].

The next stage of metabolic transformations is the double enzymatic hydroxylation reaction which takes place in the liver and kidneys. The first hydroxylation reaction takes place in the liver, catalyzed by the vitamin D 25-hydroxylase enzyme. The product of this reaction is 25-hydroxy-cholecalciferol (calciol), with a chemical formula of 25(OH)D₃. Calciol is the main form of vitamin D found in the blood serum. The second hydroxylation reaction takes place in the kidneys with the participation of 1 α -hydroxylase. During this reaction, the A-ring of the molecule is hydroxylated, and a compound called calcitriol, otherwise known as 1 α ,25-dihydroxycholecalciferol, is formed with the chemical formula 1 α ,25(OH)₂D₃. The reaction is controlled by parathyroid hormone (PTH), the metabolism of which depends on the concentration of 1 α ,25(OH)₂D₃, calcium, and phosphate content in the blood serum [25].

The vitamin D receptor (VDR receptor) is part of a group of steroid hormone receptors that includes vitamin D. Through this receptor, vitamin D acts on target cells of the body [25]. The VDR receptors have been detected in a large number of tissues, including adipose tissue, skin, cells of the immune system, and the placenta. The largest number of receptors are found within the endocrine and digestive systems, especially in the small intestine, which fact proves the specific effect of vitamin D in these areas [10, 56].

The effect of vitamin D at the genomic level is related to the effect of the VDR/RXR complex. It is a combination of the vitamin D receptor (VDR) and the retinoid X receptor (RXR), which forms a heterodimer complex. The complex is activated by 1 α ,25(OH)₂D₃, which shows an analogous activity to the transcription factor. An example of an action of the 1 α ,25(OH)₂D₃/VDR complex is its effect on bone remodeling [3]. The particular effect of VDR/RXR complex has also been observed in the case of so-called transcriptional pathways, for example, cytokine-activated pathways. Additionally, vitamin D can modulate such immunological processes as autophagy and apoptosis [7].

Vitamin D can control its own level through the VDR receptor using negative feedback. In the process, 1,25(OH)₂D inhibits expression and synthesis of the parathyroid hormone. As a consequence 1 α -hydroxylase synthesis in the kidneys is decreased. 1 α -hydroxylase is responsible for the major stage of the vitamin D synthesis process. The vitamin D 25-hydroxylase enzyme is responsible for the degradation of calciferol, which causes catabolic reactions. As a result, a water-soluble inactive metabolite is formed. The produced metabolite is excreted from the body along with bile [10].

Calcitriol is a substance with hormone properties, including the ability to bind to the VDR receptor, which acts as a transcription factor and is necessary for vitamin D to induce biological effects [3,48].

THE EFFECT OF VITAMIN D ON THE IMMUNE SYSTEM

The immune system works by controlling processes in all body systems. The enzyme 1- α -hydroxylase and the VDR receptor that activate vitamin D have been detected in many cells of the immune system. The presence of VDR receptors has been detected in monocytes, antigen-presenting cells, as well as T and B lymphocytes. The highest content of VDRs in the immune system is observed in monocytes and antigen-presenting cells [51,56].

The mechanism of vitamin D on cells of the non-specific immune response

Innate immunity, otherwise known as non-specific immunity, is the body's first line of defense. The mechanisms of non-specific immunity act immediately, but are not very precise. The main line of defense in the case of this type of immune mechanism are phagocytic cells (macrophages, granulocytes), the complement system, lysozyme, interferon and cells capable of cytotoxicity [31].

Macrophages

Vitamin D stimulates the maturation of phagocytic cells and their transition to the macrophages' active form. A deficiency of vitamin D is associated with inhibition of the secretion of lysosomal enzymes and H_2O_2 , which are essential factors in the phagocytosis of pathogens by macrophages [45]. Because of their functions in the immune system, macrophages are referred to as phagocytic and antigen-presenting cells. In the event of an infection, the macrophages' action has two steps: production of pro-inflammatory cytokines, and mobilization of other cells of the immune system. With regards to the functional profile of the cytokines they produce, we distinguish two groups of macrophages. The first are M1 macrophages that produce pro-inflammatory cytokines, such as interleukin 12 (IL-12), interleukin 1 (IL-1), tumor necrosis factor α (TNF α) and interleukin 23 (IL-23). The cytokines take an active part in the elimination of pathogens and also have an effect on T-cell differentiation. The other, M2 subgroup macrophages, are mainly responsible for the anti-inflammatory activity by producing interleukin 10 (IL-10) [44].

In the case of autoimmune diseases, changes in the distribution and number of macrophages of a specific subtype are observed. In the area of inflammation, there is an increase in the number of macrophages

and macrophage-dependent lymphocytic cells. The loss of balance between M1 and M2 macrophages is also observed. The appropriate concentration of $1,25(OH)_2D_3$ in the body restores the balance between the activities of the M1 and M2 macrophages. This restoration occurs through the reduced production of pro-inflammatory factors by M1 macrophages and increased synthesis of anti-inflammatory cytokines produced by M2 macrophages. This macrophage regulation results in anti-inflammatory effects which prevent the excessive reactivity of M2 macrophages, while also maintaining the correct immune response mechanisms of the body [14].

Calcitriol also has an effect on the synthesis of cathelicidin and defensin antimicrobial peptides. The process of synthesizing an antimicrobial peptide controlled by vitamin D concentration begins with identification of the pathogen. Using toll-like receptors (TRL), macrophages and monocytes capture the pathogen. The TRL receptor is assisted by cytokines produced by T lymphocytes, and causes transition of the vitamin D precursor to its active form and its binding to the VDR receptor. Among the cytokines, interferon γ (IFN- γ) has an active effect on vitamin D metabolism, while interleukin 4 (IL-4) has an inhibitory effect. Vitamin D bound to the VDR receptor binds to the retinoid X receptor, stimulating the synthesis of the antimicrobial peptide. The resulting cathelicidin has an antibacterial effect and influences the immune system's neutralization of pathogenic microorganisms [16, 22, 51]. Wang et al. [57] showed that vitamin D also influences the synthesis of β_2 defensin by stimulating receptors for the molecular patterns nucleotide-binding oligomerization domain containing 2 (NOD $_2$), responsible for the activation of β_2 defensin synthesis.

Dendritic cells

Dendritic cells stimulate specific immunity cells multidirectionally, however, the most important factor is immunostimulation, which is dendritic cells' ability to transform into a tolerogenic state. However, calcitriol can weaken their ability to immunostimulate – under calcitriol's influence, the expression of surface molecules that are necessary for the presentation of the antigen is weakened. In addition, the synthesis of pro-inflammatory cytokines (such as IL-6, -12, -23), is inhibited with a simultaneous increase in the synthesis of anti-inflammatory factors (IL-10, prostaglandin E_2 ; PGE $_2$, chemokine CCL22). Tolerogenic dendritic cells regulate the production of regulatory T cells (Treg), which are responsible for regulation of immune processes and control autoimmune reactions. These elements of the immune system influence the maintenance of the immune balance and support the regulation of autoimmune processes in the human body [1, 9, 23, 24].

Zhongxiang et al. [62] demonstrated the effect of vitamin D-induced tolerogenic dendritic cells on immunological processes in multiple sclerosis (MS). Their study was performed on animal models using experimental autoimmune encephalomyelitis (EAE) material. Researchers collected blood from EAE-infected dead mice and grew populations of dendritic cells from bone marrow cells. The cells were made tolerogenic under the influence of vitamin D. The researchers then created three groups: a control group, a group with dendritic cells administered, and a group with dendritic cells administered in the presence of $1,25(\text{OH})_2$. The scientists reported the effect of tolerogenic dendritic cells in the presence of $1,25(\text{OH})_2$ in mice in the form of an increased number of IL-10 secreting T helper 2 (Th2) cells and regulatory B lymphocytes produced in the spleen. Less penetration and activity of T helper 1 (Th1) and T helper 17 (Th17) cells in the area of the spinal cord were also observed. The authors speculate that vitamin D-induced tolerogenic dendritic cells alleviate EAE. In addition, the authors believe that in the future, use of this type of therapy could be introduced into autoimmune diseases therapy. However, the form of therapy presented above requires further research.

As a result of the action of vitamin D, anti-inflammatory and regulatory effects are stimulated, which are key in the autoimmune process. Therefore, the tolerogenic dendritic cells formed under the influence of vitamin D are being considered for use in autoimmune disease therapy [14,26,30].

The effect of vitamin D on selected elements of specific immune responses

Specific immunity is not an immediate reaction. Specific immune mechanisms are targeted precisely against a specific antigen. This type of immune reaction uses antibodies produced by B lymphocytes and T lymphocytes. In the case of specific immunity, there is also a secondary specific response. This response is the immediate response of the immune system to repeated contact with a given antigen [31].

B lymphocytes

The effect of $1,25(\text{OH})_2\text{D}_3$ on B lymphocytes is dependent on whether the cell is activated or resting. Calcitriol inhibits the proliferation of activated B lymphocytes, resulting in reduced production of immunoglobulins. In addition, calcitriol also significantly influences the activation of the apoptosis process of B lymphocytes, and inhibits the differentiation of plasma cells present in the maturation and proliferation stage. Vitamin D reduces the amount and production of antibodies, but does not affect the existing antibodies. The effect of vitamin D on B lymphocytes via VDRs depends on the duration

of the action and its concentration, as well as on the developmental stage of B lymphocytes [12, 45].

In autoimmune diseases, B lymphocytes are responsible for the production of antibodies against the body's own antigens. The mechanism of vitamin D is based on its effect on the main stage of B lymphocytes' formation, by which it prevents the differentiation of plasma cells. For this reason, it inhibits the production of antibodies in the autoimmune process [14].

T lymphocytes

The effect of $1,25(\text{OH})_2\text{D}_3$ on T lymphocytic cells can cause changes in the synthesis of specific cytokine profiles mediated by T helper cells (Th), and also affect VDR expression. Naive CD4+ T cells are differentiated functionally by cytokines, the secretion of which is controlled by vitamin D. Vitamin D inhibits IFN- γ synthesis and activates IL-4, as a result of which increased Th2 lymphocyte synthesis and decreased Th1 lymphocyte synthesis can be observed. Vitamin D also inhibits Th17 synthesis and activates increased Treg synthesis [40].

Th1s are lymphocytes that synthesize interleukin 2 (IL-2), IFN γ , interferon α (IFN- α). While Th2 lymphocytes synthesize interleukin 3 (IL-3), IL-4, interleukin 5 (IL-5) and IL-10. T cells that secrete interleukin 17 (IL-17) are Th17 lymphocytes. Vitamin D affects the suppression of IL-17 transcription during gene expression, thus reducing the production of IL-17. The inhibition of IL-17 synthesis results in the reduction of autoimmune processes in the body [36]. The correct vitamin D level regulates the physiologically correct balance of cytokine production [28].

Treg lymphocytes are responsible for stimulating the correct immune response. Their mechanism is part of the control of inflammatory processes, reactivity and sensitivity to the body's own antigens. Vitamin D can affect the synthesis of Treg lymphocytes by antigen-presenting cells or by expression of genetic factors conditioning the synthesis of regulatory T lymphocytes. Antigen-presenting cells increase the synthesis of IL-10, which stimulates the synthesis of Treg. Vitamin D can influence the transcription factor Forkhead box protein E3 (FOXE3, which determines the secretion of Treg [12,45].

It has been suggested that $1,25(\text{OH})_2\text{D}_3$ has an immunomodulatory effect. Prietl et al. [50] assessed whether high-dose supplementation with vitamin D_3 has an effect on autoimmune processes by influencing the mechanism of regulatory lymphocytes CD4+ FoxP3+ (Treg). 60 healthy people were qualified for the study and were randomly divided into two groups. The study group (n=30) received vitamin D_3 (140,000 IU/month), while the control group (n=30) received a placebo. Prietl et al. [50] also assessed the safety of this supplementation and its effect on the metabolism

of other immune cells (including monocytes, dendritic cells). After 3 months, a 116% increase in the vitamin D₃ level in serum was observed in the study group. Additionally, in these patients, an increase in regulatory T cells was assessed, which was not interfering with the function of other immune cells. The appropriate serum 25(OH)D concentration was associated with an increase in Treg cells, which are crucial in the treatment of autoimmune diseases.

THE RELATIONSHIP BETWEEN THE IMMUNE RESPONSE AND GUT MICROBIOTA

Vitamin D has an effect on the condition of connections of the intestinal epithelial cells. The action of vitamin D increases the synthesis of the zonulin protein. Zonulin connects intestinal epithelial cells and determines the connections integrity with the intestinal epithelial tissue. Increasing the connections integrity between the epithelial cells reduces the penetration of pathogens through the intestinal barrier, which significantly reduces the possibility of inflammation. The described mechanism exerts a significant effect on immunological processes and is considered a predisposing factor in the occurrence of autoimmune diseases. Changes in serum vitamin D levels affect the barrier in different, often opposite ways. Vitamin D deficiency may be associated with the increased permeability of the intestinal wall to pathogenic substances, resulting in a decreased anti-inflammatory effect, which may then contribute to the intensification of autoimmune processes [59, 61].

Genetic predispositions can influence the metabolism and concentration of vitamin D, the connection integrity of intestinal epithelial cells, and the immune activity level in the body. Low plasma vitamin D levels increase the penetration of microorganisms through the intestinal epithelium walls, and also stimulates immune activity. The stimulated immune activity increases the likelihood of an autoimmune reaction. On the other hand, the increased penetration of pathogenic substances through the intestinal walls indirectly increases the body's immune activity, which can lead to the development of autoimmune diseases. A deficiency of vitamin D also influences changes in the microbiome composition, which increases the microbes' ability to penetrate the intestinal barrier. As a result of the increased diffusion of microorganisms, the immune system is activated, and can trigger an autoimmune reaction. All of these consequences of low vitamin D levels in plasma contribute directly or indirectly to the intensification of autoimmune processes, and the occurrence of vitamin D-related diseases [59].

AUTOIMMUNE DISEASES

A meta-analysis by *Skaaba et al.* [55] reviewed studies on patients with selected autoimmune diseases, including: psoriasis, autoimmune thyroid disorders (AITD), type 1 diabetes, multiple sclerosis, iritis, IBD, and RA. The meta-analysis assessed the plasma vitamin D levels in patients with these diseases. In each of these disease entities, the exact relationship with the vitamin D levels was different and resulted from the vitamin effect on the pathophysiological processes characteristic of a given disease entity. In the case of autoimmune diseases of the thyroid gland, a relationship was observed between the vitamin D levels and the development of diseases. However, in the case of IBDs and RA, the conclusions are ambiguous. The authors of this meta-analysis confirmed a significantly lower incidence of autoimmune diseases in the case of higher vitamin D levels in the blood of the tested subjects, while vitamin D deficiencies were associated with an increased risk of developing these diseases in the future.

Autoimmune diseases of the thyroid gland

Autoimmune diseases of the thyroid gland include, among others, chronic lymphocytic thyroiditis (for example, Hashimoto's thyroiditis (HT)), and Graves' disease. The prevalence of these diseases is 5% of the overall population, and the observed vitamin D deficiency in 70% of patients those autoimmune diseases. The autoimmune process in the thyroid gland usually takes the form of lymphocytic infiltrates with the presence of antibodies against thyroid cells, for example antibodies against thyroglobulin (anti-Tg), thyroid stimulating hormone (TSH) receptor (anti-Tr) and thyroid peroxidase (anti-TPO) [6]. There are also disturbances in the production of thyroid hormones. In the case of autoimmune thyroid diseases, increased serum TSH levels and decreased levels of triiodothyronine (T3) and tyrosine (T4) are observed. TSH is secreted by the pituitary gland, and coordinates secretion of hormones by the thyroid gland [34].

Vitamin D plays a significant role in the pathogenesis of these diseases through anti-inflammatory activity. For example, in HT, increased levels of anti-TPO and anti-Tg have been observed in the blood. In addition, there is a characteristic presence of thyroid lymphocytic infiltrates caused by cytokines synthesized by Th1 lymphocytes (IL-2, IFN- γ , TNF- α). In the case of Graves' disease, cytokines are synthesized by Th2 lymphocytes (IL-1, IL-5, IL-10), which stimulate the secretion of anti-Tr [6].

The mechanism of vitamin D reduces the proliferation of B lymphocytes in plasma cells, which reduces the amount of secreted antibodies affecting the development of autoimmune reactions within the

thyroid gland. In addition, the mechanism of vitamin D is associated with a reduction in the secretion of pro-inflammatory cytokines by Th1 lymphocytes, and an increase in the secretion of specific anti-inflammatory cytokines by Th2 [6,41].

Hashimoto's disease

A relationship between the concentrations of vitamin D, TSH and anti-TPO was observed in the course of Hashimoto's disease. In patients with a vitamin D deficiency there is a much higher concentration of TSH and anti-TPO in the blood [8]. Due to limited data on the impact of vitamin D supplementation on the development of AITD, Chaudhary et al. [11] assessed the effectiveness of vitamin D supplementation in patients with newly diagnosed AITD. The subjects were divided into a control group (n=51), and a study group (n=51). The participants in the study group received cholecalciferol at a dose of 60,000 IU per week for a period of 8 weeks. The authors of this study observed a reduction in anti-TPO values compared to the baseline concentration in the study group. In an unhealthy group with a baseline serum TSH \leq 10 mIU, a much greater decrease in anti-TPO values was observed. According to Chaudhary et al. [11], the above dependence was caused by the severity of the disease. In the case of patients in the early stages of AITD, whose serum TSH concentration does not exceed 10 mUI, vitamin D3 supplementation will be the most beneficial.

El Rawi et al. [18] assessed the relationship between abnormal vitamin D levels in the plasma and the incidence of hypothyroidism in the course of Hashimoto's disease. As part of their study, a clinical picture of the thyroid gland and the concentration of triiodothyronine, thyroxine, TSH, anti-TPO, and anti-Tg were assessed. The participants were divided into a study group (n=35), consisting of patients with hypothyroidism, and a control group (n=35), composed of healthy people. The scientists found a relationship between vitamin D levels and the occurrence of hypothyroidism. The authors of this study estimated that higher plasma vitamin D levels were associated with lower anti-TPO and anti-Tg secretion. However, no correlation was found between the blood vitamin D and TSH levels. Low vitamin D levels were associated with a lower thyroid weight and a larger area of nodularity. T3 and T4 hormones levels were higher with the increase of vitamin D levels in the blood.

Kim et al. [38] also assessed the relationship of vitamin D levels in the blood and AITD. The study group involved the Korean population (n=4181) and was based on a comparative analysis of patient study results. The authors found no correlation between vitamin D levels and the incidence of autoimmune hypothyroidism.

Simsek et al. [54] assessed whether vitamin D supplementation reduced the severity of autoimmune diseases of the thyroid gland. The group that qualified for the study consisted of people with diagnosed AITD, who were additionally diagnosed with a vitamin D deficiency (<20 ng/mL). The subjects were randomly divided into a study group (n=46), which received a daily dose of 1,000 IU of vitamin D for a period of 1 month, and a control group (n=36), which did not receive supplementation. In the patients receiving vitamin D, lower levels of anti-TPO and anti-Tg were observed as compared to the control group. The authors did not observe any significant changes in TSH, T3, T4 concentrations during the study. It has been suggested that vitamin D deficiency is associated with the pathogenesis process of autoimmune thyroid disease.

Inflammatory bowel disease

Inflammatory bowel diseases (IBDs) are autoimmune diseases caused by a genetic predisposition that affects the immune mechanisms of the intestinal barrier and the microbiome. Inflammatory bowel diseases include Crohn's disease (CD) and ulcerative colitis (UC). The diseases are differentiated according to the area of the gastrointestinal tract in which the inflammatory changes are observed. In the case of CD, the inflammatory foci occur in all parts of the digestive system – the mouth, throat, esophagus, stomach, small intestine and large intestine. Most often, though, CD occurs in the final section of the small intestine. In CD, inflammation in all layers of the intestinal wall is observed. In contrast, UC affects only the mucosa of the large intestine. The course and severity of the diseases are associated with gastrointestinal symptoms. The most common symptoms of UC are diarrhea, end-digestive bleeding, weight loss, weakness, and fever. In the case of CD, symptoms include fever, weakness, weight loss, diarrhea, vomiting and ulceration. The symptoms are related to the part of the digestive system that is affected by the inflammation, and depend on the intensity of the inflammation [4, 43].

Mechie et al. [43] assessed whether vitamin D levels in the plasma had an effect on the activity of IBD (UC, CD). The study group consisted of 233 patients with IBD. Vitamin D deficiency was diagnosed in 54% of patients. Lower vitamin D levels (<20 ng/mL) were observed more frequently in patients with UC compared to patients with CD. The authors of the study showed a relationship between higher plasma vitamin D levels and remission in those IBDs. The scientists presented a disease activity scale depending on vitamin D concentration. In CD, the value > 19 ng/mL 25(OH)D was assessed by the authors as a clinical remission, and > 26 ng/mL as deep remission. In UC, concentration of 25(OH)D >32 ng/mL was defined as a state of clinical remission. The authors of this study

speculated that when disease exacerbated, plasma vitamin D levels fell, reaching the levels seen with severe vitamin D deficiencies. In case of remission, an increase in vitamin D levels in the blood is observed.

Janssen et al. [32] assessed the effect of vitamin D levels on IBD activity and plasma vitamin D levels depending on the season of the year. Their study included 384 patients enrolled in the first blood vitamin D measurement, and 208 enrolled in the second measurement after 24 months. The scientists found vitamin D deficiencies in 63% of CD patients, and 7% in patients with UC. Additionally, differences in plasma vitamin D levels depending on the season were determined, with a deficiency associated with the spring and winter seasons. The reduced activity of IBD was associated with an increase in plasma vitamin D levels in CD. The scientists observed lower plasma vitamin D levels when the disease was active, compared to when it was in its remission state.

Martin et al. [42] assessed the effectiveness of high-dose supplementation of vitamin D in children diagnosed with IBD. The study group consisted of children ($n=23$) aged 3-16 who were administered vitamin D in a single dose of 100,000-800,000 IU. The appropriate dose was selected depending on the patient's body weight. *Martin et al.* [42] observed an increase in blood vitamin D levels in all subjects within a period of 1-2 months after its administration. The scientists noted decreased value in the inflammatory markers: the number of platelets, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), 3 months after the vitamin D's administration and a decrease in disease activity.

Jørgensen et al. [35] conducted a randomized, double-blind study on the effect of vitamin D supplementation on the course and severity of symptoms in patients with CD. The subjects were randomized into 2 groups. The study group ($n=46$) took a daily dose of 1,200 IU of vitamin D for a period of 12 months, and the control group ($n=48$) took a placebo. The scientists observed a significant increase in the blood vitamin D levels in the study group, and a reduction in relapses from 29% to 13%. The authors of the study suggest introducing a safe supplementation dose to supplement the IBD treatment.

In the studies conducted so far, the adverse effect of vitamin D deficiency on the course and intensity of symptoms in IBD was observed. There is a need to conduct clinical trials on the impact of supplementation with specific doses of vitamin D on the condition of IBD patients, and to develop specific guidelines on supplementation [35,51,58,59].

Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune disease that affects the cells of the joints. It is associated with chronic

inflammation in the joints. RA occurs in 0.3-1% of the overall population, more often in women. The exact pathogenesis of the disease is not known, however, environmental factors and genetic predisposition are believed to be the causes. The clinical picture of the disease is the presence of synovial infiltration caused by the immune system cells, such as macrophages, T cells, B cells, dendritic cells and neutrophils. The infiltrations are associated with damage to joint tissue, and pain. The main symptoms include severe pain in the symmetrical joints, most often in the arms and legs, and joint stiffness. Additionally, symptoms of low-grade fever, or fever, muscle pain, and fatigue are observed. Inflammatory changes also occur in the tendons, ligaments and synovial bursae. The occurrence of inflammation within the above-mentioned elements causes the damage and disappearance of the function of the locomotor apparatus. The inflammation caused by RA is, among other things, caused by IL-17 and interleukin 23 (IL-23). Rheumatoid arthritis markers are anti-citrullinated peptide antibodies (ACPA) in plasma, also known as anti-CPP, and the presence of rheumatoid factor (RF) in the serum. Antibodies are not present in all patients, but their occurrence is associated with a more severe course of the disease. In the case of RA, increased erythrocyte sedimentation rate (ESR) and CRP are also observed [19,29].

Hong et al. [29] assessed the relationship between serum 25(OH)D concentration and pro-inflammatory cytokine activity in the course of RA. A total of 130 patients with RA and 80 people constituting a control group were enrolled in the study. A vitamin D deficiency was found in 98.5% of RA subjects. *Hong et al.* [29] found that the disease was more active, with more severe pain symptoms, in RA patients with lower plasma vitamin D levels. The researchers also observed an inverse relationship between vitamin D levels and the synthesis of IL-17 and IL-23. The increase in interleukin levels was observed with exacerbation of the disease. The authors speculate that vitamin D deficiency can influence the pathogenesis of RA.

Yang et al. [60] analyzed the relationship between the vitamin D levels in the blood and the risk of RA's recurrence. The study included 377 participants who were divided into two groups according to their plasma vitamin D concentration. The group with normal vitamin D levels ($n=168$) was separated from the blood vitamin D deficiency group. The group with inadequate concentration of vitamin D was randomly divided into two groups. In the first group ($n=84$), vitamin D was administered in the form of alfa-calcidol in a twice-daily dose of 0.25 μg for 24 months. The second group ($n=88$) did not receive vitamin D supplementation. *Yang et al.* [60] found a relationship, and concluded that as vitamin D levels in the body decreased, the risk of the disease's recurrence increased. The relapse rate

was 16.7% in the patients with normal serum vitamin D levels, 19% in the patients with hypovitaminosis receiving supplementation, and 29.5% in the patients not receiving supplementation.

Di Franco et al. [15] assessed the effect of serum vitamin D levels on the diagnosis and progression of the disease in patients with RA. Observation of the study group ($n=37$) lasted 12 months. The pharmacological treatment of RA included corticosteroids (prednisone of up to 10 mg/day), and methotrexate (MTX) at a dose of 7.5-15 mg/week. The authors showed that RA patients with hypovitaminosis occurring from the early stages of the disease are more prone to acute disease, and less prone to treatment and remissions.

On the basis of observations of 1,191 RA patients, *Rossini et al.* [52] estimated that the occurrence of deficiencies in patients is common and reaches 40-50%. In addition, the authors of this study showed that as plasma vitamin D levels increased, disease activity and physical disability decreased.

Khajoei et al. [37] assessed the effect of the concentration of vitamin D, adiponectin, copper and zinc in the plasma on RA activity. The study group consisted of people diagnosed with RA ($n=90$), and a control group ($n=30$) composed of healthy people characterized by a lack of elevated levels of IgM rheumatoid factor and plasma anti-CCPs. The condition for participation in the study was the patients' declaration that they had not supplemented vitamin D and calcium during the last 2 months. Disease activity was assessed using the Disease Activity Score 28-joint count (DAS28). The study group continued pharmacological therapy of RA with the following drugs: MTX 7.5–25 mg weekly; sulfasalazine 500-3000 mg daily, and hydroxychloroquine 200 mg daily. Participants from the study group were divided according to the disease's clinical status: remission ($n=22$); mild disease activity ($n=20$); moderate disease activity ($n=32$), and severe disease ($n=16$). When assessing the concentration of copper and zinc in the serum, the authors of this study did not notice any significant differences. The adiponectin levels increased with increasing disease activity. Vitamin D concentration averaged 35 ng/mL in the control group. In the study group, in disease remission, the vitamin D levels were 32.5 ng/mL; in mild disease the level was 19 ng/mL; in moderate disease the level was 20 ng/mL, and 14.5 ng/mL in severe disease. The authors of this study showed a decrease in plasma vitamin D levels in patients with mild, moderate and severe disease. The differences between the groups were slight.

Herly et al. [27] assessed the effect of the concentration of active vitamin D metabolites in plasma on the activity of RA. The study group ($n=160$) consisted of people with RA who had never been treated and had not received vitamin D and calcium

supplementation. The concentration of vitamin D metabolites (25OHD₂, 25OHD₃ and 1.25(OH)₂D) was assessed and the total vitamin D levels were calculated as the sum of 25OHD₂ and 25OHD₃. Each study participant underwent tests in which disease activity was assessed on the basis of, among other things, DAS28-CRP and CRP protein concentration, anti-CCP, and RF. *Herly et al.* [27] showed low total vitamin D levels in 42% of the subjects (<50 nmol/L). Low 1.25(OH)₂ concentration was associated with low total vitamin D levels. However, the authors of the study observed a low percentage of people with low 1.25(OH)₂ levels, and a relationship between the blood 1.25(OH)₂ levels with disease activity (DAS28-CRP), and the CRP levels. Both parameters showed a downward trend with an increase in the concentration of 1.25(OH)₂. *Herly et al.* also showed a significant relationship between the 1.25(OH)₂D and the concentration of anti-CCP. As the concentration of 1.25(OH)₂D increased, the concentration of anti-CCP decreased.

CONCLUSIONS

Vitamin D deficiencies in the body exacerbate inflammation-promoting responses and disturb the balance of anti-inflammatory activity in the immune system. This increases the chances of inflammation, and therefore, autoimmune diseases. The analyzed literature shows that it is important to maintain the proper concentration of vitamin D in the plasma to reduce the risk of developing autoimmune diseases. This conclusion was demonstrated by the authors of the studies analyzed herein, in relation to autoimmune thyroid disease, IBD and RA. Vitamin D levels influenced the course of these diseases, the severity of the symptoms, and the frequency of relapses. In the case of AITD and RA, the authors of the studies demonstrated the effect of low plasma vitamin D levels on the increase in the amount of secreted antibodies, which in turn determined disease activity. Low vitamin D levels were also associated with a higher incidence of relapses in IBD and RA. The relationships between lower vitamin D levels and a greater degree of damage and nodular changes in the thyroid gland in AITD, as well as a greater degree of motor disability in RA, were all shown. The studies' authors also observed an increase in the secretion of IL-17 and IL-23, anti-CCP, and an increased risk of relapse in the presence of lower blood vitamin D levels in RA. The studies showed the positive effect of vitamin D supplementation in IBD patients. This beneficial effect was associated with the reduction of inflammatory markers such as: platelet count, CRP, and ESR. The timely correction of vitamin D deficiencies was associated with a better response to pharmacological treatment and a reduction in symptoms

in RA patients. The authors of the analyzed studies emphasize the importance of maintaining correct blood vitamin D levels and preventing deficiencies that may worsen the symptoms of the diseases, or cause development of an autoimmune disease. In the case of insufficient amounts of vitamin D supplied to the body, supplementation is recommended. The supplementation should be consulted with a physician and selected on an individual basis.

Conflict of interest

The authors declare no conflict of interest.

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LINK BETWEEN METHYL NUTRIENTS AND THE DNA METHYLATION PROCESS IN THE COURSE OF SELECTED DISEASES IN ADULTS

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ABSTRACT

DNA methylation is a reversible epigenetic modification that plays a crucial role in transcriptional gene silencing. Both excessive (hypermethylation) and reduced DNA methylation (hypomethylation) can contribute to the disturbance of the proper course of many important processes in the human body. The aim of the study was to discuss the relationship between methyl nutrients and the DNA methylation process in the course of selected diseases in adults. Methyl nutrients include folates (vitamin B9), riboflavin (vitamin B2), cobalamin (vitamin B12), pyridoxine (vitamin B6) and choline (vitamin B4), as well as methionine and betaine. These substances play the role of both substrates and cofactors in transformations related to one-carbon metabolism. The deficiency of methyl nutrients in the body can lead to disturbances in SAM synthesis, which is the primary donor of methyl groups in the DNA methylation process. However, the mechanism explaining the discussed relationship has not been fully explained so far. Both the concentration in the body and the intake of folate and vitamin B12 in the diet can, to some extent, have an effect on the level of DNA methylation in healthy people. In comparison, data on the effect of excessive intake of vitamin B12 in the diet on the risk of cancer development are inconsistent. An adequate betaine and choline intake in the diet might not only affect the overall improvement of the DNA methylation profile, but, to some extent, also reduce the risk of cancer, the effect of which can depend on the content of folic acid in the body. Research results on the effect of supplementation of methyl nutrients on the DNA methylation process are inconclusive. It is therefore necessary to conduct further research in this area to draw clear conclusions.

Key words: *DNA methylation, diet, methyl nutrients, one-carbon metabolism, epigenetics*

STRESZCZENIE

Metylacja DNA jest odwracalną modyfikacją epigenetyczną, która odgrywa kluczową rolę w transkrypcyjnym wyciszaniu genów. Zarówno nadmierna (hipermetylacja), jak i zmniejszona metylacja DNA (hipometylacja) mogą przyczyniać się do zaburzenia prawidłowego przebiegu wielu istotnych procesów zachodzących w organizmie człowieka. Celem pracy było omówienie związku między metylowymi składnikami diety a procesem metylacji DNA w przebiegu wybranych chorób u osób dorosłych. Do metylowych składników pokarmowych zaliczane są: foliany (witamina B9), ryboflawina (witamina B2), kobalamina (witamina B12), pirydoksyna (witamina B6) i cholina (witamina B4) oraz metionina i betaina. Substancje te pełnią zarówno rolę substratów, jak i kofaktorów w przemianach związanych z metabolizmem jednego atomu węgla. Niedobór metylowych składników pokarmowych w organizmie może prowadzić do zaburzeń w syntezie SAM, będącej podstawowym donorem grup metylowych w procesie metylacji DNA. Mechanizm wyjaśniający omawianą zależność nie został jednak dotychczas w pełni wyjaśniony. Zarówno stężenie w organizmie, jak i podaż w diecie folianów oraz witaminy B12 mogą w pewnym stopniu oddziaływać na poziom metylacji DNA u osób zdrowych, przy czym dane dotyczące wpływu nadmiernej zawartości witaminy B12 w diecie na ryzyko rozwoju nowotworów są niespójne. Odpowiednia podaż betainy i choliny w diecie może wpływać korzystanie nie tylko na ogólną poprawę profilu metylacji DNA, ale w pewnym stopniu również na zmniejszenie ryzyka wystąpienia nowotworów, przy czym efekt ten może być zależny od zawartości kwasu foliowego w organizmie. Wyniki badań odnoszące się do wpływu suplementacji metylowymi składnikami pokarmowymi na przebieg procesu metylacji DNA są niejednoznaczne. Konieczne jest prowadzenie dalszych badań w tej dziedzinie w celu sformułowania jednoznacznych wniosków.

Słowa kluczowe: *metylacja DNA, dieta, metylowe składniki pokarmowe, metabolizm jednego atomu węgla, epigenetyka*

ABBREVIATIONS

ADCY3 – adenylate cyclase type 3
BHMT – betaine homocysteine methyltransferase
BMI – body mass index
BRCA1 – breast cancer gene 1
C – cytosine
CBS – β-cystathionine synthase
CG – cytosine-guanine
CIN – cervical intraepithelial neoplasia
CpG – cytosine-phosphate group-guanine
DMG – dimethylglycine
DNA – deoxyribonucleic acid
DNMTs – DNA methyltransferases
EC-SOD – extracellular superoxide dismutase
ESR1 – estrogen receptor 1
GST – glutathione S-transferase
HDACs – histone deacetylases
HATs – histone acetyltransferases
HPV – human papilloma virus
LINE-1 – long interspersed nucleotide element-1
MBDs – methyl-CpG binding domains
MBPs – methyl-CpG binding proteins
MLH1 – human MutL homolog 1
MTHFR – methylenetetrahydrofolate reductase
PBMCs – peripheral blood mononuclear cells
RAPGEF4 – rap guanine nucleotide exchange factor 4
RARB – retinoic acid receptor beta
SAH – S-adenosyl-L-homocysteine

INTRODUCTION

Every human body contains a unique genome, which consists of two main components. The first is a double-stranded deoxyribonucleic acid (DNA) packed in condensed chromatin form that contains specific nucleotide sequences (genetic information record), and the second is methylation. Methylation is a complex molecular mechanism that regulates the course of numerous biochemical processes responsible for the correct reading of the genetic information stored in DNA, maintains genome stability, and controls the course of gene transitions [26, 42]. Methylation is one of the epigenetic processes that is not related to modifications of nucleotide sequences in DNA [17, 19, 33, 42]. While epigenetic changes occur at every stage of life and can be inherited, they do undergo modification under the influence of external factors, such as: lifestyle (including, in particular, diet, weight disorders and physical activity), as well as drugs and toxic substances [19, 22, 42].

In recent years, there has been a significant progress in studies on nutriepigenetics, including the analysis of nutrients influence on epigenetic processes, especially on their effect on modifying susceptibility to the development of many chronic diseases. It has been indicated that some nutrients may influence the regulation of gene expression. More and more attention is also paid to the analysis of the influence of selected

nutrients on the course of the DNA methylation process [3, 13, 22, 26]. DNA methylation plays a key role not only in controlling processes in determining the gene expression level in cells, but also regulates the course of important biological processes taking place in the human body, such as genomic imprinting and X-chromosome inactivation [14, 26, 32, 33, 42]. DNA methylation may also condition the silencing of genes related to the aging process [20, 44]. Both excessive (hypermethylation) and reduced DNA methylation (hypomethylation) may contribute to the abnormalities of the proper course of many key body processes [3, 10, 13, 44]. Abnormalities in the DNA methylation profile are very often associated with the development of genetic diseases (including *Prader-Willi*, *Beckwith-Wiedemann*, and *Silver-Russell* syndromes) [14]. Numerous scientific research has also shown that abnormal DNA methylation can contribute to the initiation of the carcinogenesis process [19, 31, 40, 44, 48]. The research indicates that abnormalities in the DNA methylation profile may also be associated with the occurrence of cardiovascular diseases [17, 28, 36, 73], metabolic disorders (including obesity and type 2 diabetes) [10, 35, 50, 61] and schizophrenia [47, 55, 62] or depression [5, 9, 34]. It has also been assumed that the abnormal course of DNA methylation may play a significant role in the development of *Alzheimer's* disease pathogenesis [59, 64, 71] and liver diseases [12, 43, 67]. However, due to the inconsistent results of scientific analyzes carried out in this field, it is not possible to formulate clear conclusions on this subject. Understanding the relationship between the selected nutrients and the course of the DNA methylation process may be significant both in the context of controlling the rate of body aging, and in the prevention and treatment of diseases which pathomechanism is associated with DNA hypo- and/or hypermethylation [3, 10, 17, 19, 26].

The aim of the present study is to discuss the relationship between methyl nutrients and the DNA methylation process in the course of selected diseases in adults, based on a review of the current literature.

DNA METHYLATION

DNA methylation is a reversible, post-replication, enzymatic modification of DNA that plays a crucial role in transcriptional gene silencing. It occurs primarily during the S phase of the cell cycle. Methylation is a process of covalently attaching methyl groups (-CH₃) onto nitrogen bases of nucleotides – in particular to cytosine, but less often to adenine – and forming products of which the most frequent are C⁵-methylcytosine (m⁵C), and sometimes also N⁴-methylcytosine (m⁴C), and N⁶-methyladenosine (m⁶A) [16, 33, 42].

The DNA methylation process requires DNA methyltransferases (DNMTs) family enzymes such as adenine-specific and cytosine-specific DNA methyltransferases, which have an ability to transfer methyl groups between individual structures in the body [17, 19, 26, 33, 36, 40, 42]. Among DNMTs, DNA methyltransferase 1 (DNMT1) plays the most crucial role in regulating the DNA methylation process. This enzyme is responsible for copying the DNA methylation pattern to the newly synthesized DNA strands during the replication process. It also has the ability to repair errors that appear in the DNA methylation pattern. On the other hand, the DNA-methyltransferase 3-like (DNMT3L) participates indirectly in the DNA methylation process, and despite lacking catalytic properties, has the ability to stimulate activity of DNMT3a and DNMT3b methyltransferases, which are responsible for *de novo* methylation [22, 33, 36, 40, 42]. The primary role of DNMTs is to catalyze the additional reactions of methyl groups derived from the donor S-adenosyl-L-methionine (SAM), to the C⁵ carbon of the cytosine pyrimidine ring, or to the amine group of adenine (N⁶) or cytosine (N⁴) [22, 26, 33]. This process leads to the formation of methylated DNA and S-adenosyl-L-homocysteine (SAH) [17, 26, 33, 39, 42]. The conversion reaction of SAM to SAH is a complex biochemical process known as one-carbon metabolism [16].

Methylation is a targeted process that mainly occurs on cytosines contained in CpG islands. CpG islands are short stretches of DNA (about 1000 bp) characterized by a high content of specific sequences of 5'-CpG-3' dinucleotides (cytosine-phosphate group-guanine) [17, 26, 33, 36, 39, 42]. The islands occur mainly within or in close proximity to the promoter regions that are the gene origin and are essential for cell functioning. About 70% of CpG dinucleotides in the human body have a methyl group attached to cytosine (CG – cytosine-guanine), and as a result the genes encoded by this sequence are not accessible for cellular transcription systems. There is an inverse relationship between the transcriptional activity of certain genes and the degree of methylation in the promoter regions – the higher the methylation level, the weaker the gene expression. On both DNA strands, the methylation at CpG sites occurs in a mostly symmetrical way and only a small number of CpG sequences undergo asymmetrical methylation [17, 26, 33, 39, 42]. The structure that results from the interaction of transcription factors with promoter genes is an open chromatin – a highly acetylated chromatin containing CpG islands [26, 33, 42]. About half of all human genes contain CpG islands, and these are called housekeeping genes, as well as tissue-specific genes. It has been considered that transcriptionally inactive sequences are most

often methylated, while transcriptionally active genes are hypomethylated [17, 26, 33, 42].

Increasing the frequency of CpG islands methylation in the promoter regions leads to silencing of a certain gene expression due to chromatin condensation resulting from a decrease in histone acetylation. Consequently, this phenomenon leads to blocking or disturbing synthesis of the encoded products (mainly proteins) by a certain gene. The exact mechanism that determines this relationship is not yet fully understood. One of the proposed models assumes that this process involves methyl-CpG binding proteins (MBPs) that bind to the methylated DNA, with the participation of methyl-CpG binding domains (MBDs). The MBPs interact with chromatin remodeling complexes and with a histone deacetylase. These modifications result in histone H3 lysine 9 methylation, followed by the deacetylation of histones, causing a change in the chromatin structure from the active, relaxed form (euchromatin) to its concentrated form (heterochromatin), which is inaccessible to transcription factors. On the other hand, histone protein acetylation of lysine, which occurs at histone H4, leads to partial decondensation of chromatin, making it more available to transcription factors, and causing an increase in the gene expression level. This is due to the fact that the condensation and decondensation of chromatin take place in a dynamic way, because both forms can intertwine with each other to either allow or block gene transcription. Therefore, it has been indicated that gene expression can be modified both by DNA methylation and by acetylation or deacetylation of histones. Histones are transformed with the participation of histone deacetylases (HDACs) and histone acetyltransferases (HATs). However, it is important to note that due to the possibility of 5-methylcytosine deamination to thymine, epigenetic changes can be transformed into permanent mutations [16, 19, 22, 40, 42].

Methylation is a reversible process that is independent of DNA, because when necessary genes can be reactivated. However, the exact mechanism determining the course of this process has not yet been fully understood. It has been recognized that DNA demethylation can take place through enzymatic processes, and in a passive way. The enzymatic process occurs independently of the cell division cycle and under the influence of demethylases, stimulating the detachment of methyl groups from DNA, including the ten-eleven translocation proteins (TETs). The passive way occurs due to a lack of methylation of the newly synthesized DNA chain by DNMTs during replication [26, 33, 40, 42].

The DNA methylation pattern changes during the body's development, growth, and aging. Nevertheless, all properly functioning cells have

a stable DNA methylation pattern, which is specific to each cell type [3, 13, 26, 33, 36, 42]. Some of the changes in the DNA methylation pattern tend to be a physiological adaptation to changing environmental conditions, however, some changes may be related to a developing disease or intensification of the cell aging processes in the human body [20, 33, 44]. Regulatory mechanisms, such as DNA demethylation, chromatin structure modification, and active transcription, should prevent those changes in the DNA methylation pattern. If the functioning of these mechanisms is disturbed, an uncontrolled DNA methylation process can occur. This consists of a lowering of the genome methylation level, or the attachment of *de novo* methyl groups, the main consequence of which can be the disturbance of DNA integrity and stability. Changes in the DNA methylation pattern include, but are not limited to, hypo- and hypermethylation as well as m⁵C transition to thymine. 5-methylcytosine formed as a result of cytosine (C) methylation in the DNA strand can undergo spontaneous deamination to thymine (T), resulting in the C → T transition. Due to the fact that DNA thymine glycosylase exhibits low repair activity that is additionally inhibited by DNMTs, this mutation is often not repaired, which can lead to its fixation. It has been shown that the DNA methylation pattern in the course of selected chronic diseases can differ from that which occurs under physiological conditions [26, 32].

MECHANISMS CONDITIONING THE INFLUENCE OF METHYL NUTRIENTS ON THE COURSE OF DNA METHYLATION

It is believed that diet can influence the course of DNA methylation as a result of the interaction of at least three different mechanisms [19, 26]. It should be emphasized that exact explanation of the biochemical mechanisms that determine the dependencies described below is not fully possible because in most of the scientific analyzes, the authors relied mainly on hypotheses and general assumptions. Therefore, it is necessary to conduct further research in this area in order to draw clear conclusions [26].

The first and most often-described mechanism is the optimal supply of methyl nutrients in one-carbon metabolism, which enables the proper course of the DNA methylation process. An adequate supply of methyl nutrients determines the production of SAM, which is the primary donor of methyl groups in the DNA methylation process. Methyl nutrients include vitamins, the most important of which are: folates (vitamin B₉), riboflavin (vitamin B₂), cobalamin (vitamin B₁₂), pyridoxine (vitamin B₆), choline (vitamin B₄), as well as amino acids such as

methionine and betaine. These substances play the role of both substrates and cofactors in transformations related to one-carbon metabolism [16, 17, 26, 39, 40]. Methyl nutrient deficiencies in the body can lead to disturbances in SAM synthesis, and thus to the formation of abnormalities in various biochemical pathways in the DNA methylation process [16, 26, 40]. The analysis conducted by *Inoue-Choi et al.* [24] showed that the concentration of choline, methionine and SAH in the subjects' blood serum was positively correlated with the concentration of SAM in their blood. The authors emphasized the significant role of controlling the concentration of methyl nutrients in the blood serum in the context of preventing the occurrence of DNA methylation disorders resulting from insufficient SAM content in the body.

Another mechanism that can determine the course of the DNA methylation process is the influence of nutrients on the change in the activity of enzymes related to the regulation of the DNA methylation process, especially with regard to one-carbon metabolism [16, 19, 26, 40]. It has been indicated that a deficiency of methyl nutrients can indirectly interfere with the activity of DNA methyltransferases as a result of the insufficient supply of SAM. Moreover, it is presumed that some nutrients can play the role of DNMTs inhibitors in the human body [19, 26].

It has been assumed that another mechanism explaining the discussed dependence can also be the indirect influence of nutrients on the process of DNA demethylation [26]. This is because individual publications describe vitamin C as potentially affecting the process of DNA demethylation by supporting the reactions associated with TETs proteins [41, 69]. It has also been indicated that disturbances in the regulation of transformations related to TETs may also result from insufficient supply of methyl nutrients [66]. However, it should be emphasized that the hypotheses explaining the influence of diet on the process of DNA demethylation are still inconsistent [26, 41, 66, 69].

The DNA methylation process includes the interconnected cycles of folate and methionine, in which individual nutrients play both the role of substrates and cofactors of biochemical reactions (Fig. 1) [39]. Folates are found in many food products. Their rich sources include green leafy and cruciferous vegetables, legume seeds, avocados, liver, beets and eggs [39, 40]. Dietary folates are metabolized to tetrahydrofolate (THF), which through numerous changes is converted to 5-methyl-THF under the influence of, among other things, serine hydroxymethyltransferase (SHMT) and vitamin B₆, as well as methylenetetrahydrofolate reductase (MTHFR) and vitamin B₂ [16, 17, 39, 40]. Vitamin B₆ is mainly found in fish, meat, offal, as well as in legume seeds, nuts and seeds. Wholegrain cereal products are also valuable sources of this vitamin.

Whereas the basic nutritional sources of vitamin B₂ are milk and its products, eggs and offal [40]. 5-methyl-THF transfers the methyl group to the methionine cycle as a result of homocysteine remethylation to methionine [16, 17, 39, 40]. This reaction requires the participation of vitamin B₁₂, which is present only in animal products – meat, fish, eggs, dairy products and offal [39, 40]. The richest sources of methionine in food are primarily protein-rich products, e.g. meat, fish, eggs, dairy products, soybeans and Brazil nuts [39]. 5-methyl-THF can be converted back to THF and reused in the above DNA methylation process. Moreover, homocysteine can also receive a methyl group, which is necessary for the production of methionine, as a result of the conversion of betaine to dimethylglycine (DMG) taking place under the influence of betaine homocysteine methyltransferase (BHMT) [16, 26, 39, 40]. Betaine can originate from both food and the process of endogenous choline oxidation. Foods such as quinoa, wheat germ, bran, beetroot, spinach and seafood are a rich source of betaine in the diet [39]. The main food sources of choline are egg yolk, offal, wheat germ, soybeans, meat and fish [39, 40]. The methionine formed from homocysteine is then converted to SAM, from which, with the participation of DNMTs, methylated DNA (in the form of 5-methylcytosine) and SAH are formed. Then, under the influence of SAH hydrolase,

SAH is hydrolyzed to adenosine and homocysteine. Subsequently, homocysteine can be reused in the methionine cycle or be broken down to cystathionine and cysteine by trans-sulfuration, which is dependent on β-cystathionine synthase (CBS) and vitamin B₆ [16, 17, 39, 40]. Cysteine can then be used for the synthesis of body proteins or be transformed, for example, into taurine and glutathione. Glutathione shows strong antioxidant properties [16, 40].

Upon the transfer of methyl groups, SAM is converted to SAH, which competes with SAM for influence over the DNA methyltransferases. SAH acts as a DNMT inhibitor, therefore its excess in the body can disturb the course of reactions catalyzed by this group of enzymes [16, 26]. It has been shown that changes in SAM and SAH levels resulting from a methyl-deficient diet for a period of over 18 weeks led to irreversible changes in DNA methylation in the liver cells of F344 male rats [54]. Moreover, it has also been found that a moderate increase of homocysteine levels in the blood serum is associated with an increase in SAH levels, with no changes in SAM levels, while the increased SAH levels are associated with the occurrence of DNA hypomethylation [70]. Maintaining the balance between SAM and SAH, which relies on stimulating SAM synthesis and supporting SAH removal, seems to be crucial in ensuring the proper DNMT activity. Nevertheless, due

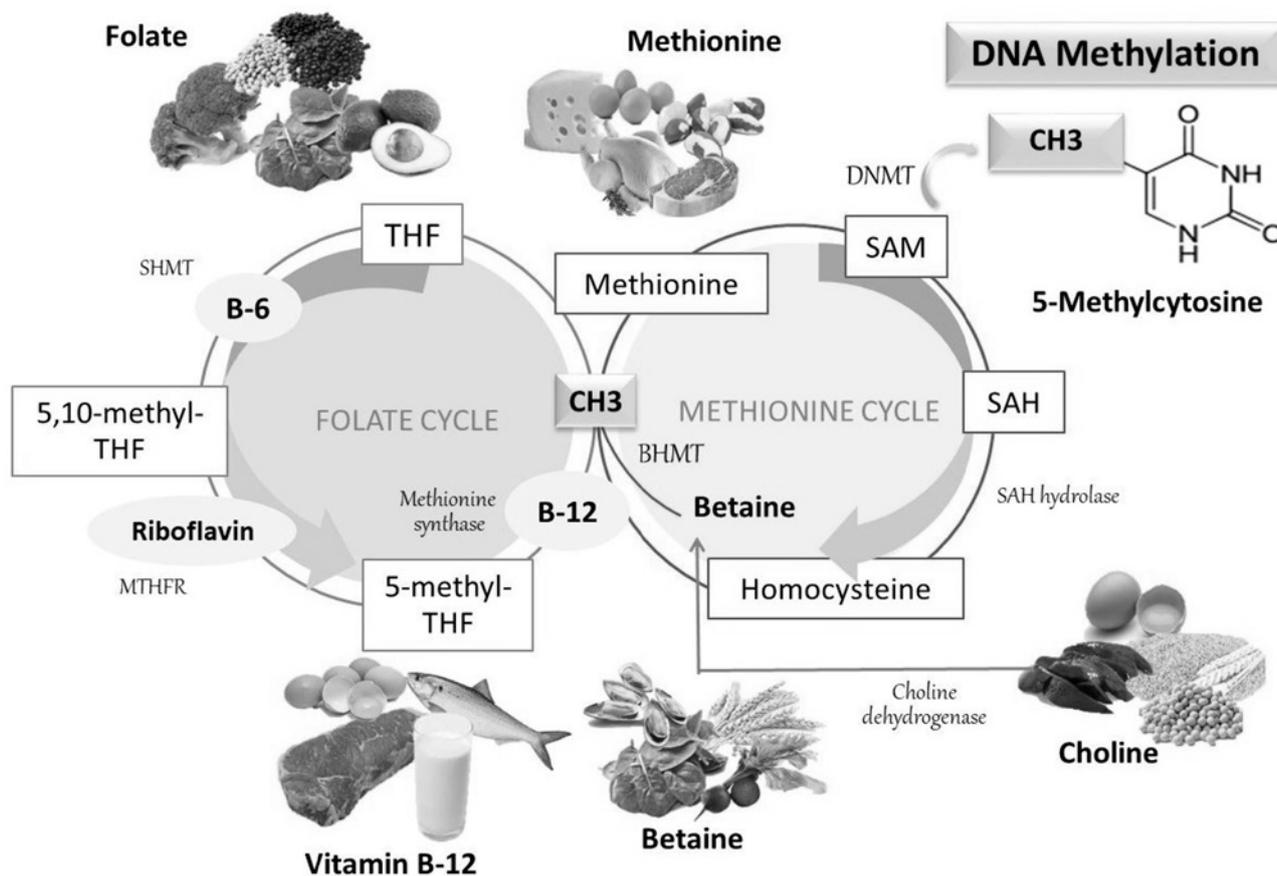


Figure 1. Nutrients involved in one-carbon metabolism [39]

to the complexity of SAM and SAH transformations in the human body, it is not possible to formulate unambiguous conclusions indicating that the SAM to SAH ratio could be a common indicator determining the risk of DNA hypo- or hypermethylation in the human body [26].

EFFECT OF METHYL NUTRIENTS ON THE PROCESS OF DNA METHYLATION IN THE COURSE OF SELECTED DISEASES IN ADULTS

The relationship between methyl nutrients and the course of the DNA methylation process has been described in several animal studies [11, 27, 54]. *Pogribny et al.* [54] found that in F344 male rats, which were fed a methyl-low diet for 9 weeks (low in methionine, choline and folates), SAH did not change but SAM content decreased by 70% compared to rats on a control diet. Thus, a significant imbalance between SAM and SAH was demonstrated in the organisms of the tested animals. In addition, it was also observed that the number of unmethylated CpG sites in liver cells increased by 60% as a result of the methyl-deficient diet. Interestingly, the reintroduction of a diet with the correct methyl nutrient content made it possible to reverse previously induced DNA hypomethylation only in the rats that were fed a deficient diet for 9 weeks. For animals that were exposed to methyl nutrient deficiencies for 18, 24, or 36 weeks, reintroducing these nutrients did not influence the normalization of the DNA methylation levels. It is worth noting, however, that the presence of glutathione S-transferase (GST), which is associated with the process of hepatocarcinogenesis, was confirmed in the liver tissue of the studied rats even after 9 weeks of using a methyl-low diet. In a study by *Kalani et al.* [27] for 6 weeks C57BL/6J mice were fed a diet containing increased methionine content (1.2%) and a reduced amount of folate (0.08 mg/kg body weight), vitamin B₆ (0.01 mg/kg body weight), and vitamin B₁₂ (10.4 mg/kg body weight). Based on analysis, it was shown that the C⁵-methylcytosine levels in the brain of the tested mice had increased as a result of the applied diet. Moreover, in mice on a methionine-rich diet and deficient in folates and vitamins B₆ and B₁₂, a decrease in the expression of the netrin-1 protein was observed as a result of the increase in the methylation level in the promoter region of the netrin-1 gene, which resulted in the occurrence of long-term memory disorders in the tested animals. Interestingly, the mice's long-term memory improved when netrin was readministered. The authors indicated that, based on the results obtained in the study, it should be assumed that a methyl-deficient diet can contribute to the development of learning and memory disorders.

The authors also stated that the decrease in netrin-1 expression due to hypermethylation of its gene can, presumably, be associated with impairment or loss of long-term memory. In a study by *Cordero et al.* [11], *Wistar* rats were fed one of three diets: a Western-type diet (defined as increasing the risk of obesity), a Western-type diet additionally supplemented with methyl nutrients (choline, betaine, vitamin B₁₂ and folic acid), and a control group. It was shown that after 8 weeks, the rats in both of the intervention groups gained body weight and increased their body fat. Interestingly, the intensification of fatty liver was observed only in the animals on the Western-type diet. In rats that were additionally supplemented with methyl nutrients in addition to the Western-type diet, the fatty liver was reduced. The authors indicated that methyl nutrients can have a potentially protective effect against the accumulation of excess fat tissue caused by a high-fat diet, however, the effect is probably limited to only the liver. *Cordero et al.* also suggested that the potential mechanism determining this link might, to some extent, be related to the influence of methyl nutrients on the course of the DNA methylation process in liver cells. However, it was emphasized that in the rats from both intervention groups there were some changes in the DNA methylation profile.

Analysis of the overall relationship between methyl nutrients and the course of the DNA methylation process has also been described in human studies [3, 8, 38, 51]. A systematic review conducted by *Amenyah et al.* [3] demonstrated a relationship between methyl nutrients that are specific to one-carbon metabolism, and the DNA methylation process. In a study by *Li et al.* [38] involving the 6-year observation of 5,687 women aged 65-79, it was found that a diet that is rich in vitamin B – which is a methyl donor in the DNA methylation process (along with folate, vitamin B₂, B₆ and B₁₂) – can modify long-term changes in body weight and body fat. This happens through the effects of these nutrients on DNA methylation determined by the rs752579 variant of the sterol regulatory element binding factor 1 (SREBF1) gene. However, not all scientific analyses in this area have obtained similar conclusions. In a study by *Perng et al.* [51], conducted on 987 healthy people aged 45-84, no correlation was found between the supply of methyl nutrients in the diet and the DNA methylation level in the tested subjects. However, it was found that a higher body mass index (BMI) in the subjects was associated with a higher degree of methylation of the long interspersed nucleotide element-1 (LINE-1). In another analysis by *Chamberlain et al.* [8] conducted on 5,168 healthy adults, no correlation was found between the methyl nutrients content in the diet and the CpG island methylation level in the tested subjects.

Nevertheless, most scientific studies have focused on the assessment of the effect of individual methyl nutrients on the course of DNA methylation [7, 18, 45, 58]. The compounds most often reported in this context were folate and vitamin B₁₂. Foliates are the primary methyl-group donors in the DNA methylation process. In comparison, appropriate amounts of vitamin B₁₂ are required to remethylate homocysteine to methionine because vitamin B₁₂ acts as a cofactor in this reaction. Therefore, it has been assumed that a deficiency of both of these components can lead to disturbances in the course of changes related to SAM and SAH in the body [16]. In an analysis by *Hirsch et al.* [18], conducted on a group of healthy people, it was found that higher folic acid levels in the blood serum (>45 nmol/L) was associated with an increased content of SAM and SAH in the erythrocytes of the tested subjects. This is not, however, related to the SAM-to-SAH ratio, and the CpG island methylation level in the promoter region of the extracellular superoxide dismutase (EC-SOD) gene. Neither was a correlation found between the vitamin B₁₂ levels in the blood serum and the SAM or SAH content in the erythrocytes of the tested subjects. Alternatively, *Pufulete et al.* [58] showed that the folic acid levels both in blood serum and in erythrocytes were inversely correlated with the plasma homocysteine levels ($r = -0.573$, $p < 0.001$ and $r = -0.307$, $p = 0.01$, respectively), as well as with the presence of DNA hypomethylation in the colon mucosa ($r = -0.311$, $p = 0.01$ and $r = -0.356$, $p = 0.03$). When gender, age, BMI, smoking, and genotype were taken into account, however, it was found that this relationship was much weaker ($p = 0.07$ and $p = 0.08$). On the other hand, in an analysis by *Morris et al.* [45] conducted on 1,858 people ≥ 60 , it was found that the concentration of 5-methyl-THF in the body was associated with better results of tests assessing cognitive functions in tested subjects with no vitamin B₁₂ deficiency in the blood serum. A study by *Bednarska-Makaruk et al.* [7] carried out on a similar age group, showed that patients with dementia had lower folic acid levels ($p = 0.002$) and 5-methyl-THF ($p = 0.005$) in the blood than those without memory impairment. Moreover, a positive correlation was also observed between the concentration of folic acid in the blood serum and the DNA methylation level in the tested subjects ($p = 0.013$).

A deficiency of folic acid and vitamin B₁₂ in the body can also be a risk factor for the development of certain types of cancer [4, 53, 57]. An analysis by *Piyathilake et al.* [53] of 315 women who tested positive for human papilloma virus (HPV) type 16, who were diagnosed with cervical intraepithelial neoplasia (CIN), stage CIN2+ or \leq CIN1, showed that in women with a higher degree of HPV 16 DNA methylation and a higher concentration of both folic acid and vitamin B₁₂ in the

plasma, the probability of CIN2+ was lower by 75% ($p < 0.01$) and 60% ($p = 0.02$), respectively. The authors suggested that maintaining the appropriate methyl donors content in the body, including in particular folic acid and vitamin B₁₂, can play a critical role in ensuring a high degree of CpG islands methylation, among other things, within the promoters of the HPV E6 gene, which are associated with a higher probability of being diagnosed with CIN2+. On the other hand, a study by *Badigi et al.* [4] found that women with higher blood homocysteine levels were more likely to be diagnosed with CIN2+ (OR = 1.86, $p = 0.005$). In contrast, the higher folic acid levels in the plasma were a significant determinant of lower blood homocysteine levels in the tested subjects (OR = 0.4, $p = 0.0002$). In women with homocysteinemia, a lower degree of methylation of LINE-1 of peripheral blood mononuclear cells (PBMCs) (OR = 2.3, $p = 0.0007$) was observed significantly more frequently (PBMCs are a potential biomarker of CIN2+ development). The authors emphasized that maintaining the appropriate content of folic acid in the body can have a potentially beneficial effect on reducing homocysteine blood levels and increasing the degree of PBMC LINE-1 methylation, which could, consequently contribute to the reduction of the risk of developing CIN2+. In another analysis by *Pufulete et al.* [57] it was showed that patients diagnosed with colorectal cancer had a 26% lower folic acid content in the body (95% CI: 6-44%, $p = 0.01$) and a 21% lower vitamin B₁₂ level in the blood (95% CI: -38-1%, $p = 0.06$), compared to a control group of people without colorectal disease. Moreover, it was also found that high folic acid levels in the blood were associated with a reduced risk of developing colorectal cancer ($p = 0.01$). While the DNA hypomethylation of colon cells and leukocytes was associated with an increased risk of colorectal adenoma ($p = 0.02$ and $p = 0.01$, respectively).

Apart from the assessment of the relationship between the total content of folic acid and vitamin B₁₂ in the body and DNA methylation, scientific research also analyzed the impact of the supply of these components in the diet on the course of the discussed process of DNA methylation [1, 29, 52, 63]. In a study by *Agodi et al.* [1] carried out on 177 healthy, non-pregnant women, it was shown that subjects whose fruit consumption was lower than the median value for the whole group (<201 g/day) were 3.7 times more likely to develop LINE-1 hypomethylation, compared to women with fruit consumption higher than the median (OR = 3.7, 95% CI: 1.4-9.5). Similarly, the female subjects whose dietary folate intake was 3.6 times lower than the demand, were more likely to develop LINE-1 hypomethylation compared to women with normal dietary content of folates in their diet (OR = 3.6, 95% CI: 1.1-12.1). The authors have indicated

that low fruit consumption and too low dietary folate content may possibly increase the risk of cancer development because of LINE-1 hypomethylation. Notwithstanding, in the study by *Pirouzpanah* et al. [52], an inverse relationship was found between the folate supply in the diet and the intensification of the hypermethylation process in the promoter regions of retinoic acid receptor beta (RARβ) gene, such as in female patients <48 years of age and breast cancer gene 1 (BRCA1), and in tested subjects >48 years old age. It should be emphasized that with regard to the vitamin B₁₂ content in their diet, this relationship was significant only with the RARβ gene, and only in female patients aged <48 years old. The authors indicated that an insufficient supply of folate and vitamin B₁₂ in the diet can contribute to the increased likelihood of breast cancer development as a result of hypermethylation of the promoter regions of the discussed genes. However, this effect is probably dependent on the age of the female patients. In a study by *Kawakita* et al. [29], covering over 12.5 years of observation, it was shown that higher intake of folate in the diet and higher consumption of folic acid-fortified products were associated with a reduced risk of head and neck cancer. However, this effect was dose-dependent (for folate intake in the diet: quartile 1 vs. 4: HR = 0.35, 95% CI: 0.18-0.67, and for the consumption of folic acid-fortified products: quartile 1 vs. 4: HR = 0.49, 95% CI: 0.3-0.82). The authors suggest that the above results are presumably related to the role of folates in regulating the DNA methylation process, but this relationship was not analyzed in the study. Interestingly, in a study by *Steluti* et al. [63], no correlation was found between dietary folate supply and the level of DNA methylation, even in people who consumed folic acid-fortified flours.

One of the best-studied issues in the context of the discussed topic is the analysis of the effect of supplementation with selected methyl nutrients, including folic acid and vitamin B₁₂, on the DNA methylation level and the risk of developing diseases associated with disturbances in this process [3, 6, 21, 25, 30]. In a randomized, double-blind study by *Kok* et al. [30] conducted on healthy people aged 65-75 with moderately elevated levels of homocysteine in the blood serum, it was shown that 2-year supplementation with folic acid at a dose of 400 µg/day and vitamin B₁₂ at a dose of 500 µg/day contributed to the occurrence of changes in the DNA methylation profile of several genes, including genes related to neurological functions and the process of carcinogenesis. Nonetheless, in a study by *Bea* et al. [6] conducted on 408 healthy, postmenopausal women, a dependence between periods of folic acid supplementation and its concentration in erythrocytes was observed, compared to the DNA methylation level. It was shown

that in women with a higher concentration of folic acid in erythrocytes (compared to the lower concentration of this parameter), the average DNA methylation level was higher in the period before supplementation (5.12 vs. 4.99%, $p = 0.05$), but decreased in the post-supplementation period (4.95 vs. 5.16%, $p = 0.03$). The authors emphasized that the increased concentration of folic acid in erythrocytes as a result of supplementation can affect the DNA methylation levels in leukocytes in postmenopausal women. However, not all scientific analyses have obtained similar results on the effect of supplementation with selected methyl nutrients on the course of the DNA methylation process. In a randomized, double-blind study conducted by *Jung* et al. [25] it was found that a 3-year supplementation with folic acid at a dose of 800 µg/day did not affect the DNA methylation level in a population of healthy women and men aged 50-70 with moderate hyperhomocysteinemia. Moreover, in the study by *Hübner* et al. [21] no relationship was found between the 1-year supplementation of B vitamins (500 µg of folic acid, 500 µg of vitamin B₁₂ and 50 mg of vitamin B₆ per day), vitamin D (1,200 IU/day) and calcium (456 mg/day) on the SAM and SAH levels in the blood serum and the LINE-1 methylation level, in a group of people aged 46-88. Nevertheless, in a meta-analysis conducted by *Amenyah* et al. [3] it was found that despite many methodological inaccuracies, it could be concluded that supplementation with folic acid, both alone and in combination with vitamin B₁₂, can increase the DNA methylation level. However, a need for further analyses on this topic was emphasized, to obtain data enabling the formulation of unambiguous conclusions in this area.

A study by *Li* et al. [37] conducted on obese C57BL/6J mice showed that a 10-week, fat-rich diet, combined with folic acid supplementation, contributed to the reduction of body fat and blood glucose levels, and improved insulin sensitivity in the tested mice. The animals that were fed only with a high-fat diet showed increased weight gain compared to mice in the control group. In addition, it was shown that animals on a high-fat diet without additional folic acid supplementation developed type 2 diabetes and insulin resistance. The authors also indicated that the observed differences between the intervention groups resulted from the fact that folic acid supplementation contributed to the reduction of DNA methylation within adipose tissue of adenylyl cyclase type 3 (ADCY3) gene, and rap guanine nucleotide exchange factor 4 (RAPGEF4) gene, which have the ability to influence adipose tissue metabolism and regulate insulin sensitivity in the body. Conversely, *Park* et al. [49] found in their analysis that the changes in DNA methylation patterns as a result of folic acid supplementation were different in women aged 18-35, and depended on their body

weight. As a result of folic acid supplementation at a dose of 800 µg/day for 8 weeks, serum folic acid levels increased both in women with normal body weight (from 38.36 ± 2.5 to 71.41 ± 3.03 nmol/L), and those with obesity (from 27.12 ± 3.09 to 56.85 ± 3.9 nmol/L). However, in the obese women, this level was significantly lower than in the women with normal body weight, before and after the introduction of folic acid supplementation in both groups. Nevertheless, the change in serum folic acid levels as a result of the introduced supplementation was greater in the obese women than those with normal body weight (109.6% vs. 86.2%). Moreover, it was indicated that the number of changes occurring in DNA methylation as a result of folic acid supplementation was higher in the obese women, compared to those with normal body weight (99 vs. 56 CpG stretches). The authors suggested that the need to modify the recommendations for folic acid supplementation doses depending on women's body weight should be considered.

There have also been studies that analyzed the effect of folic acid supplementation on the risk of developing cancer, and the course of cancer, in relation to disturbances in the DNA methylation process [2, 15, 29, 46, 56, 60]. In a randomized study by O'Reilly et al. [46], which included post-polypectomy patients using folic acid supplementation at a dose of 600 µg/day for 6 months, an increase in folic acid levels was observed that was determined directly in colonocytes, as well as a decrease in the DNA methylation level compared to a placebo group. The authors suggested that folic acid supplementation in patients at risk of developing colorectal cancer can affect the folic acid supplementation deficiency directly in the colonocytes, which presumably reduces the risk of initiating the process of carcinogenesis due to DNA hypomethylation. A randomized study by Pufulete et al. [56] found that DNA hypomethylation caused by insufficient folate intake in the diet can be normalized as a result of the implementation of folic acid supplementation. The same study showed that folic acid supplementation for 10 weeks at a dose of 400 µg/day in patients with colorectal adenoma had the following effects: 81% increased concentration of folic acid in blood serum (95% CI: 57-104%, $p < 0.001$ vs. placebo), 57% increased concentration of folic acid in erythrocytes (95% CI: 40-74%, $p < 0.001$ vs. placebo), 12% decreased serum homocysteine levels (95% CI: 4-20%, $p < 0.001$ vs. placebo), 31% increased methylation in leukocytes (95% CI: 16-47%, $p = 0.05$ vs. placebo), and 25% increased methylation in the colon mucosa (95% CI: 11-39%, $p = 0.09$ vs. placebo). Interestingly, not all studies have formulated similar conclusions about these dependencies. In the previously mentioned study by Kawakita et al. [29], no evidence of the relationship between folic acid supplementation

and the risk of developing head and neck cancer was found. Moreover, a randomized, double-blind study [2] of a 10-week folic acid supplementation at a dose of 400 µg/day in patients with colorectal adenoma, with no deficiency of folic acid and vitamin B₁₂ in the body, found no effect on the improvement of methylation of promotor regions of the estrogen receptor 1 (ESR1) and human *MutL* homolog 1 (MLH1) genes. ESR1 and MLH1 genes can undergo hypermethylation in the course of colon tumors. Surprising conclusions were formulated in the study by Farias et al. [15], carried out using human colonosphere formation in a colon cancer cell line grown in vitro. It was shown that long-term increased folic acid intake from both folic acid supplements and folic acid-fortified food may lead to abnormalities in DNA methylation patterns, especially those related to DNMT activity. It was suggested that this relationship can cause initiation of carcinogenesis, or the progression of pre-existing neoplastic tumors. The authors have emphasized the need for further analyses on the impact of excessive supplementation with methyl nutrients on the risk of development or progression of cancerous tumors. A meta-analysis conducted by Qiang et al. [60] found that each increase in dietary folate content by 100 µg/day was associated with a 12% reduction in the risk of development of esophageal cancer. Moreover, each increase of B₆ vitamin intake in a diet by 1 mg/day contributed to the reduction of the risk of esophageal cancer development by 16%. Interestingly, it was shown, however, that each increase of B₁₂ vitamin intake in a diet by 1 µg/day was associated with a 2% increase in esophageal cancer risk, particularly in the United States and Europe, which could indicate both geographic and histological differences in this regard. The authors concluded that the increased intake of dietary one-carbon metabolism-related B vitamins could be a protective factor in the risk of developing esophageal cancer, with the exception of vitamin B₁₂, the supply of which in the diet should be controlled.

There are not many studies analyzing the effects of choline and betaine on the course of DNA methylation in adults [23, 63]. Both of these components play a crucial role in one-carbon metabolism. Betaine is involved in the process of converting homocysteine to methionine, and can be obtained from diet or as a result of choline oxidation in endogenous processes [16]. In the study by Steluti et al. [63], it was found that of all methyl nutrients, only the supply of betaine in the diet had an effect on modifying the DNA methylation level. Imbard et al. [23] conducted a study on 109 healthy volunteers, analyzing the relationship between the concentration of choline and betaine in the blood serum, folate, SAM, SAH and homocysteine levels in the blood, and DNA methylation levels. Based on the obtained data, a strong positive correlation

was found between the choline levels and folic acid levels in the blood. A strong positive correlation was also found between choline and SAM and SAH levels in blood serum, although in the multivariate linear regression model no correlation was found between blood choline levels and the SAM-to-SAH ratio. Moreover, it was pointed out that the serum betaine levels were positively correlated with folic acid levels and negatively with the blood homocysteine levels. Interestingly, no statistically significant relationship between the analyzed indicators' levels in the blood serum and DNA methylation levels was confirmed. Nevertheless, the authors emphasized that there is a need to extend the scope of the scientific research evaluating the effect of methyl nutrients on the course of the DNA methylation process by analyzing the role of choline and betaine in this regard. Several studies have also assessed the effect of dietary choline and betaine intake on the risk of developing various types of cancer [65, 68, 72]. One such analysis was a two-stage case-control study by *Zhang et al.* [72], with the participation of 807 women diagnosed with breast cancer, and 807 healthy women qualified for the control group. Based on the obtained data, an inverse relationship was found between the choline and betaine content in the diet and the risk of breast cancer development. The adjusted odds ratio (OR) for the highest quartile intake of the analyzed nutrients, in comparison with their lowest amount in the diet, was, respectively: 0.4 for choline (95% CI: 0.28-0.57, $p < 0.001$), 0.58 for betaine (95% CI: 0.42-0.8, $p < 0.001$), and 0.38 for choline and betaine combined (95% CI: 0.27-0.53, $p < 0.001$). Interestingly, it has also been found that the described relationship was statistically significant only in subjects with low dietary folate intake ($<242 \mu\text{g/day}$). *Zhang et al.* [72] emphasized the importance of the role of appropriate choline and betaine content in the diet, with regards to reducing the risk of breast cancer development in women. However, the potentially beneficial effects can also depend on the possible occurrence of folic acid deficiency in the body. Another study, by *Ying et al.* [68], analyzed the effect of choline and betaine intake in the diet on the risk of developing lung cancer, depending on smoking. Higher betaine content in the diet was statistically significantly associated with a reduced risk of lung cancer in smokers, with the protective effect being more pronounced in current smokers than in ex-smokers. Similar relationships were also observed with regard to choline content in the diet, however, the effect was less pronounced. The authors indicated that based on their results, it can be assumed that increased betaine intake in the smokers' diet, as well as choline (but to a lesser extent), can be a protective element in the risk of lung cancer development, through some kind of mitigation of the

adverse effects of smoking. Meta-analysis conducted by *Sun et al.* [65] found that choline and betaine intake in a diet may reduce the risk of developing neoplastic diseases, however, the authors emphasized the need for further analyses in this area due to the small number of studies conducted so far. It was shown that the relative risk (RR) of cancer occurrence for the lowest and the highest ranges was, respectively: 0.82 (95% CI: 0.7-0.97) for the choline intake, 0.86 (95% CI: 0.76-0.97) for betaine in the diet, and 0.6 (95% CI: 0.4-0.9) for the total choline and betaine in the diet. It was determined that increasing the choline and betaine content by 100 mg/day contributed to the reduction of risk of developing cancer by 11% (0.89, 95% CI: 0.87-0.92).

CONCLUSIONS

Despite significant scientific progress in the field of nutriepigenetics, it is not possible to draw unambiguous conclusions about the relationship between methyl nutrients and the DNA methylation process in the course of selected diseases in adults. There are few randomized studies of high methodological quality that have analyzed this relationship in humans. Due to the fact that some of studies conducted so far were performed on animals and others on human cell lines, the comparison of these results is difficult. It is necessary to conduct further analyses on this topic, in order to obtain data enabling the formulation of unambiguous conclusions in this area.

Based on the research results examined above, it should be assumed, however, that both folic acid and vitamin B₁₂ levels in the body, as well as folate and vitamin B₁₂ intake in the diet of healthy people, can affect their DNA methylation level to some extent. In contrast, the data on the effect of excessive vitamin B₁₂ content on the risk of developing cancer is inconsistent. Moreover, it has also been indicated that the control of the folic acid content, and to a lesser extent also of vitamin B₁₂ in the body, can be particularly significant in patients at risk of developing cancer.

Research results on the effect of the supplementation of methyl nutrients on the DNA methylation process is inconclusive. Some studies have shown that folic acid supplementation, both alone and in combination with vitamin B₁₂, can increase the DNA methylation level. Nevertheless, many analyses have not confirmed this effect, or even an increase in the risk of developing cancer as a result of such diets.

There are few studies assessing the effect of choline and betaine on the course of DNA methylation in adults. On the basis of the cited studies, however, it should be concluded that the appropriate intake of both betaine and choline in the diet can have a beneficial effect not only on the overall improvement of the DNA methylation profile, but also to some

extent, on reducing the risk of developing cancer, and this effect may depend on the folic acid content in the body. Nevertheless, there is a need to extend the scope of scientific research evaluating the effect of methyl nutrients on the course of DNA methylation based on analyses of betaine and choline effect in this context, due to the small number of valuable studies carried out on this topic so far.

It should be emphasized, however, that the exact explanation of the biochemical mechanisms that determine the dependencies described above is not fully possible because in most of the scientific analyses, the authors relied mainly on hypotheses and general assumptions. More research is needed in this area in order to draw clear conclusions and fully understand these relationships. This could contribute to obtaining data that could be used to formulate guidelines for recommended intakes of methyl nutrients for dietary amounts, and supplementation. This recommendation could be valuable both in the context of prevention and treatment of selected diseases in adults, including cancer diseases in particular.

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ASSOCIATIONS BETWEEN DIET AND ACNE LESIONS

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ABSTRACT

Acne is one of the most common dermatological conditions affecting millions of people worldwide. It is assumed that the main etiological factors acne are considered to be the excessive production of sebum and hormones, and the colonisation of *P. acnes* bacteria. The relationship between diet and acne lesions is still the subject of debate and scientific criticism. On the one hand, studies suggest that an elimination diet is of no benefit in the treatment of acne, while other studies indicate that there is a close relationship between diet and the occurrence of acne lesions. Already in the 1970s, some scientists claimed that the course of acne could be modified by specific dietary components. Several studies have confirmed, among others, the role of a diet with a high glycemic index diet in acne exacerbation and suggested that a low glycemic index diet for several weeks may lead to a reduction in the number of acne lesions. The effect of milk consumption was also investigated in terms for its potential role in acne pathogenesis, including its ability to increase insulin levels. In the last decade, studies have also emerged to determine the effect of dark chocolate on the exacerbation of acne symptoms. To date, it has not been conclusively established which component of chocolate may have a potentially adverse effects on the course of acne. The article summaries the current knowledge on the relationship between diet and acne lesions, and discusses the results of recent studies on selected dietary components that may exacerbate acne.

Key words: *acne vulgaris, diet, chocolate, risk factors*

STRESZCZENIE

Trądzik jest jednym z najczęstszych schorzeń dermatologicznych dotyczących miliony ludzi na całym świecie. Przyjmuje się, że głównymi czynnikami etiologicznym trądziku jest nadmierna produkcja łoju i hormonów oraz kolonizacja bakterii *Propionibacterium acnes* (*P. acnes*). Związek pomiędzy dietą a zmianami trądzikowymi nadal stanowi przedmiot dyskusji i krytyki naukowej. Z jednej strony wyniki badań sugerują, że dieta eliminacyjna nie przynosi korzyści w leczeniu trądziku, inne badania wskazują, na istnienie ścisłego związku między dietą a występowaniem zmian trądzikowych. Już w latach 70. naukowcy twierdzili, że przebieg trądziku może być modyfikowany przez określone składniki diety. Wyniki kilku badań potwierdziły między innymi rolę diety o wysokim indeksie glikemicznym w zaostrzeniu trądziku i sugerowały, że stosowanie przez kilka tygodni diety o niskim indeksie glikemicznym może prowadzić do zmniejszenia liczby zmian trądzikowych. Zbadano także wpływ spożycia mleka pod kątem jego potencjalnej roli w patogenezie trądziku, w tym zdolności do zwiększania poziomu insuliny. W ostatnim dziesięcioleciu pojawiły się również badania mające na celu określenie wpływu ciemnej czekolady na zaostrzenie objawów trądzików. Dotychczas nie ustalono jednoznacznie, który ze składników czekolady może mieć potencjalnie niekorzystny wpływ na przebieg trądziku. W artykule podsumowano dotychczasową wiedzę na temat zależności pomiędzy dietą i zmianami trądzikowymi oraz omówiono wyniki najnowszych badań dotyczących wybranych składników diety, które mogą wpływać na nasilenie trądziku.

Słowa kluczowe: *trądzik pospolity, dieta, czekolada, czynniki ryzyka*

INTRODUCTION

Acne is a skin disease commonly encountered throughout the world. During adolescence, most adolescents struggle with varying degrees of acne lesions. Data on the prevalence of acne in the general population range from 50-95% [12]. The

main factors contributing to the pathophysiology of acne are the inflammatory response, colonisation by *Propionibacterium acnes* (*P. acnes*) and increased sebum production [22]. Dietary factors, especially sweets (including chocolate), milk and fat, are considered by many researchers as a cause or exacerbating factors of acne [11]. The aim of this study

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is to present the relationship between diet and acne vulgaris based on a review of recent world literature.

Pathophysiology of acne vulgaris

During puberty there is an increase in adrenal androgen production, resulting in the increased sebaceous glands production of sebum, a substance consisting of sterol esters, triglycerides, cholesterol and free fatty acids that cause inflammation. The increased production of sebum and the accumulating epithelial and keratin cells block the hair follicle outlet causing swelling and the formation of a keratinous plug, which lead to the formation of an acne lesion called microcomedone (early acne). As the keratinous plug enlarges, there is an increased risk of colonisation by *Propionibacterium acnes* bacteria, which are the skin microbiome, causing the progression of microcomedones into comedones or vesicles. Acne lesions most commonly occur in areas of the skin where there is a high number of sebaceous glands, namely the face, neck, chest, back and upper limbs [4].

Glycaemic index and glycaemic load in acne vulgaris

The authors of many scientific studies have focused on assessing the relationship between dietary glycaemic index and insulin resistance and the risk of acne lesions or their severity. Glycaemic Index (GI) and glycaemic load (GL) may be related to the development of acne because they are closely linked to the insulin response that occurs when blood glucose levels rise after a meal. The glycaemic index is a method to assess the quality of carbohydrates.

Glycaemic Index is defined as the area under the blood glucose curve that develops within 2 hours of consuming a product containing 50 g of assimilable carbohydrate, and is expressed in relation to the area of the glycaemic curve that develops after consuming 50 g of pure glucose. For glucose, the GI is 100, so the smaller the increase in glycemia, the lower the GI value. Products with a low glycaemic index have a GI value of 50 or lower, with a medium GI value of 56-69 and a high GI value of 70 or higher [18].

The glycaemic load, on the other hand, takes into account both the quality of carbohydrates consumed with the product and their quantity. The glycaemic load is the product of the GI of a product and the quantity of carbohydrates it contains. The result is then divided by 100. The value of products with a low GL is less than 10, with a medium GL 10-20, and high GL includes products with a GL value higher than 20 [18].

Kaymak et al. [16] studied the relationship between acne and glycaemic load and index, insulin sensitivity and plasma somatomedin C (IGF-1) levels. It was shown that serum levels of IGF-1 and significantly lower levels of insulin-like growth factor binding protein

(IGFBP-3) were higher in acne subjects compared to healthy subjects. Furthermore, as assessed by an intake questionnaire, the diet of acne subjects had a significantly higher glycaemic index than the diet of subjects without acne lesions [16].

Cerman et al. [8] conducted a study involving a 50-person study group and a 36-person control group. Respondents with acne were divided into three subgroups: with "mild" lesions (a few to several blackheads, papules and pustules; no nodules); with "moderate" lesions (several blackheads, papules and pustules; from several to more than ten nodules); with "severe" lesions (numerous blackheads, papules and pustules; many nodules). Patients who had previously received topical or systemic acne treatment were excluded from the study. The reference product was milk. Participants provided the researchers with notes every seven days listing the products and foods present in their 24-hour diets. The researchers then calculated the energy value of the diet, its glycaemic load and the proportion of energy from protein, carbohydrate and fat in the 24-hour diet using a nutrition software program (Foodworks Xyris Software). Venous blood samples were collected for examination at different times and analysed for glycaemic factors. Glycaemic index and glycaemic load values were significantly higher (respectively $p = 0.022$ and $p = 0.001$) and serum adiponectin levels were significantly lower ($p = 0.015$) in patients with acne than in control group. There was also an inverse correlation between serum adiponectin levels and glycaemic index ($p = 0.049$, $r = -0.212$). The study concluded that diets with a high glycaemic load were positively associated with acne vulgaris. Adiponectin may be a pathogenic cofactor contributing to disease development [8].

In contrast, Burris et al. [6] conducted a study involving 66 participants divided into two subgroups: 34 people on a low glycaemic index and glycaemic load diet and 32 control subjects. The aim of the study was to determine changes in biochemical markers associated with moderate to severe acne after a low GI and GL diet or an usual diet plan for 2 weeks. Insulin-like growth factor IGF-1 concentrations decreased significantly among respondents following a low-GI and GL diet between pre- and post-intervention time points (pre-intervention = 267.3 ± 85.6 mg / ml, post-intervention = 244.5 ± 78.7 ng / ml) ($P = 0.049$). The study showed no differences in changes in glucose, insulin, IGFBP-3 protein or insulin resistance concentrations between the study groups after 2 weeks. However, the proportion of energy derived from carbohydrates ($P < 0.001$), GI ($P < 0.001$) and GL ($P < 0.001$) were significantly reduced among participants following a low-GI/GL diet between pre- and post-intervention time points [6].

A diet rich in dairy products and acne vulgaris

Researchers are also investigating possible links between dairy product consumption and the pathogenesis of acne vulgaris. The probable reason for the possible comedogenic effect of milk and its products are the hormones it contains, which are produced by cows during pregnancy. However, the component of milk which stimulates the hair-sebum duct is insulin-like growth factor (IGF-1), the blood concentration of which changes depending on the severity of acne [27]. IGF-1 levels increase during puberty under the influence of growth hormone and positively correlate with the clinical course of acne. The highest IGF-1 concentrations are observed in women with acne, and the number of acne lesions positively correlates with IGF-1 plasma concentrations [23]. High plasma IGF-1 concentrations caused by milk consumption stimulate sebaceous cell proliferation, which leads to the development and progression of acne lesions [20]. A study by *Ulvestad et al.* [28] found that high consumption (≥ 2 glasses per day) of full-fat dairy products was associated with moderate to severe acne development. In boys consuming large amounts of full-fat dairy products, the odds ratio for developing acne was 4.81 (1.59-14.56). On the other hand, high total dairy consumption was associated with the development of acne lesions in girls (1.80, 1.02-3.16). In contrast, no significant associations were found between acne and consumption of semi-skimmed or skimmed dairy products or with moderate consumption of any fatty dairy products [28].

A study by *Adebamowo et al.* [1] investigated the alleged association between milk and dairy consumption and acne. Nearly 50,000 women participated in the study. The authors found that acne positively correlated with the reported amount of milk consumed, especially skimmed milk. The researchers hypothesised that the bioavailability of comedogenic components may increase when skimmed milk is produced. Skimmed milk contains less estrogen than full-fat milk. Estrogen is a hormone that can reduce acne lesions. Furthermore, the authors showed a positive correlation between acne and the consumption of instant products and cottage cheese. These results were explained by the cow's milk content in both products [1].

LaRosa et al. [19] investigated a possible association between the consumption of dairy products and the occurrence of acne in adolescents. Conducting a case-control study among 225 respondents aged 14-19 years, the authors hypothesised that adolescents with facial acne (120) would consume more dairy than those in the control group (105). Participants taking oral contraceptive pills or isotretinoin in the past 6 months were excluded from the study. The data on milk and dairy products intake were then compared

with the nutritional values in both groups. The three study variables included total daily intake of dairy products, as well as total daily intake of full-fat, reduced-fat and low-fat dairy products. The variables included in the study were also data on total daily energy intake from carbohydrates, fats and protein. The mean body mass index (BMI) in the acne group was 22.8, compared to 23.8 in the control group. The total dairy content of the diet in the acne group was found to be significantly higher ($p = 0.02$) than in the control group. In this study, there were no statistically significant differences in the total intake of full-fat ($p = 0.95$) and low-fat ($p = 0.36$) dairy products between the groups. It was also found that total energy was not statistically different between the two groups ($p = 0.12$). Total fat intake ($p = 0.34$), carbohydrate ($p = 0.14$) and protein ($p = 0.08$) were found to be similar in both the group of people with acne as well as in the control group [19].

Chocolate and acne vulgaris

The effect of chocolate ingredients on the exacerbation of acne lesions has been a subject of debate for several decades. Although it has been hypothesised that several factors, including body mass index, hormone levels, glucose levels, stress and glycaemic index may influence acne, the role of diet in the formation and/or worsening of acne lesions remains controversial.

To date, several hypotheses have been proposed to explain the possible influence of dairy and chocolate consumption on the exacerbation of acne symptoms. One hypothesis suggests that the sugars in dairy products and chocolate trigger insulin secretion, activating signaling pathways that ultimately lead to increased keratinocyte proliferation, which may lead to acne lesions development [26]. This may explain why some studies have found an effect of sweets and chocolate on acne symptoms [32]. Other researchers suggest that chocolate consumption increases the secretion of inflammatory cytokines by cells and thus may influence the formation of acne lesions [26].

In Western societies, acne vulgaris is a common skin disease that affects 79-95% of the adolescent population. Epidemiological data show that acne incidence rates are much lower in non-Western societies. A study by *Cordain et al.* [10] found that among Westerners a higher proportion of the population developed acne vulgaris than two non-Western populations, including Kitavan islanders from Papua New Guinea and Aché hunters from Paraguay. Both non-western populations followed low glycaemic load diets devoid of western refined foods such as cereals, chips, biscuits and bread. The diet of the Kitavan population consisted of vegetables, fruits, fish and coconuts. Consumption of dairy, coffee, alcohol, cereals, sugar, oils and salt was

minimal. The diet of the Aché community included local foods (69%, including sweet cassava, peanuts, maize and rice), game (17%), western foods (8%, containing mainly pasta, flour, sugar, yerba tea and bread), home-grown meat (3%) and harvested forest products (3%). Researchers have suggested that a low fat and low glycaemic load diet may account for the lack of acne in both populations [10]. The differences in acne prevalence between non-Western and fully modernised societies cannot be explained solely by different genetic factors between populations, as they are likely due to different environmental factors. Identification of these factors may help with developing a treatment regimen for acne in Western populations.

In contrast, the crossover study found no effect of chocolate on acne or on sebum production or composition. The study involved 65 people with moderate acne symptoms who were asked to consume two chocolate bars per day for a month of time. The placebo group consumed identical-looking bars that did not contain chocolate in their composition, but had a similar fat and sugar composition. Both bars weighed between 112 g and 114 g, but the bar consumed by the control group contained more calories. Excessive consumption of chocolate and fat did not alter the composition or amount of sebum. Diets rich in carbohydrates or fat have not been confirmed to stimulate sebum secretion and adversely affect the development of acne vulgaris [14]. *Fulton's* study [14] was criticised by *Rasmussen* [24], who believed that an incorrect method of lesion quantification was used because all lesion types (blackheads, papules and pustules) were included to the same extent, thus making an accurate assessment of acne severity impossible.

In the 1970s, another important study was carried out with the primary aim of assessing the effects of chocolate on acne. Twenty-seven medical students participated in the study. They consumed six servings of chocolate bars (39 g each) over seven days. Facial acne lesions were counted at the beginning of the study and then daily for the seven days of the study. Although the assessment methods used were not properly described, the study did not observe a causal relationship between chocolate consumption and acne proliferation [3].

In 2011, *Block et al.* [5] investigated whether 100% chocolate could directly exacerbate acne in men with a history of acne vulgaris. The study involved 10 men aged 18 to 35 years with 1 to 4 acne lesions (limited to blackheads and papules) present on the face. Participants consumed up to 340 g of chocolate bars on the first day of the study and followed their regular diets for a further week. The total number of blackheads, papules and pustules on the face was counted on days 4 and 7 of the study. There was

a statistically significant increase in the mean number of acne outbreaks (blackheads, papules and pustules) on both day 4 ($p = 0.031$) and day 7 ($p = 0.050$) compared to baseline. No serious adverse events occurred in the male subjects. Rare transient adverse events included gastric complaints, nausea, vomiting and headache. The results of this study indicate that participants experienced significant changes in the severity of acne after a single ingestion of chocolate [5]. However, the authors did not provide information on the type of chocolate consumed by the subjects and the cocoa content of the samples consumed, which may have influenced the results. Dark chocolate contains more antioxidants than milk chocolate, leading to the conclusion that it may have significantly less comedogenic effects. However, this issue still remains unclear.

A study conducted by *Netea et al.* [21] aimed to determine the mechanisms through which chocolate may influence the development of acne. They investigated the effect of chocolate on the growth of *P. acnes* and on cytokine production stimulated by *P. acnes* or *S. aureus*. Blood samples were taken from 7 participants, before and after consumption of 50 grams of chocolate (30% cocoa contents) for four consecutive days. Respondents had not consumed chocolate for at least one week prior to the study. No direct effect of chocolate on *P. acnes* growth was observed. On the other hand, chocolate had a stimulatory effect on pro-inflammatory cytokines, such as TNF and IL-1b, induced by *P. acnes*. Chocolate consumption increased the production of the anti-inflammatory cytokine IL-10 induced by *S. aureus*, while decreasing the release of IL-22 [21].

The aim of the study carried out by *Caperton et al.* [7] was to evaluate the effect of chocolate on acne exacerbation in men aged 18-35 years. Women were deliberately excluded from the study to avoid the likelihood of a hormonal effect of menstruation on acne development. In addition, subjects with a history of diabetes or hypertriglyceridemia were excluded from participation in the study, as well as those who were diagnosed with obesity based on BMI. The 14 study participants were divided into three groups. The first group received capsules filled with unsweetened 100% cocoa, the second with hydrolysed gelatin powder and the third with a mixture of cocoa and hydrolysed gelatin. A statistically significant increase in the mean number of total acne lesions (blackheads, papules, pustules, nodules) was detected on both day 4 ($p = 0.006$) and day 7 of the study ($p = 0.043$), compared to baseline. No serious adverse events occurred during the study. Minor transient events included gastric discomfort, nausea and vomiting. In acne-prone men, chocolate consumption has been found to correlate with an increase in acne lesions [7].

The findings suggest that chocolate ingredients stimulate blood cells to release more of the pro-inflammatory cytokines, interleukin-1 β and TNF α . *Delost et al.* [13] conducted a study involving 54 students with acne vulgaris who were divided into two groups. The first group received for consumption a bar of *Hershey's* milk chocolate (43 g), while the second group received 15 Jelly Belly jellybeans, which provided the same glycemic load. The number of acne lesions was counted from photographs by a dermatologist after 48 hours. There was a statistically significant ($p = 0.0001$) increase in acne lesions (14.8 lesions) in the chocolate eating group compared to the jelly bean eating group (0.7 lesions). Linear regression analysis (SPSS) was performed to assess the effect of associated variables on the number of acne lesions. None of the associated variables (age, $P = 0.424$; stress, $P = 0.901$; gender, $P = 0.843$) showed statistical significance for the number of acne lesions [13]. Since dark chocolate contains more antioxidants than milk chocolate, it shows a weaker comedogenic effect [17].

On the other hand, *Vongraviopap and Asawanonda* [29] conducted a study involving 25 men, aged between 18 and 30 years, with a tendency to acne. Exclusion criteria included systemic conditions that could cause acne-like lesions and local or systemic acne treatments performed in the past 4-12 weeks, including light- and laser therapy. Participants were then asked to consume 25 g of dark chocolate (99%) per day for 4 weeks. Before the start of the study, a 4-week pre-study period was conducted during which the subjects did not consume cocoa-containing products. For 8 weeks (pre-study and study phase), subjects did not use any new sunscreen or moisturiser, and washed their face with a soap-free cleanser provided by the study authors. The number of acne lesions was assessed once a week. Increased numbers of blackheads and inflammatory papules were detected as early as 2 weeks after starting the study. After 4 weeks, the changes remained statistically significant compared to baseline values [29].

The effect of dark chocolate on the development of acne lesions was also confirmed by the study of *Chylak et al.* [9] conducted on a group of 33 men who were divided into a "young" group (20-30 years) and a middle-aged group (45-75 years). Only non-smokers and non-consumers of large amounts of alcohol (i.e., more than 35 UK units per week) participated in the study. A further inclusion criterion was the absence of visible acne symptoms. Body mass index was determined for all study participants. Values below 25 kg/m² were taken as body weight within the normal range, those between 25 kg/m² and 30 kg/m² as overweight and values above 30 kg/m² - as obesity. The subjects were then asked to consume 10 g of Green & Black's dark chocolate (70% cocoa contents)

every day immediately after lunch for four weeks. Participants were also instructed not to consume any other cocoa-containing products during the study period. Prior to the dietary intervention and at the end of the study, RSSC samples were taken from participants' faces to measure lipid droplet size, the number of exfoliated corneocytes and to assess the presence of microorganisms. The results indicate that chocolate consumption increased the number of exfoliated corneocytes in both groups of men. However, this increase was statistically significant only in young men ($p < 0.001$). The result of the t-test indicated a highly significant ($p < 0.001$) increase in corneocyte exfoliation in normal weight subjects, whereas in overweight or obese subjects was non-significant. In addition, the consumption of dark chocolate resulted in a significant increase in microbial presence in both young and middle-aged men. These changes could potentially contribute to the development of acne [9].

Currently, scientists have not established whether chocolate exacerbates acne lesions, and if so, what factors determine this process. Cocoa contains a large amount of flavonoids, which should have anti-inflammatory effects and beneficial effects on the functioning of many organs. However, dark chocolate also contains cocoa butter, which among other things contains 33% monounsaturated oleic acid and 33% stearic acid. When used in animal models, oleic acid has been shown to alter epithelial keratinisation, leading to the formation of blackheads [15].

Fat intake and acne vulgaris

High fat intake may also be a potential dietary risk factor for acne development. Despite the inconsistent results of various studies, several studies have shown that high fat intake increases the likelihood of acne. Although researchers have suggested potential mechanisms to explain the relationship, there is insufficient evidence to support this [31]. More research needs to be conducted to establish the relationship between fat intake and acne and clarify the mechanisms involved. Nevertheless, several dietary factors may have a protective effect on the development of acne lesions. The relationship between high fish intake and reduced acne risk has been debated [2]. However, *Wang et al.* [30] observed that seafood consumption was associated with increased acne risk, suggesting that consumption of other types of seafood may be associated with increased rather than decreased acne risk. The results of some studies suggest that a high intake of fruit and vegetables may reduce the risk of acne. The protective effect may be due to the omega-3 fatty acids in fish and fiber in fruit and vegetables, which reduce insulin-like growth factor 1 (IGF-1) levels, thus reducing the risk of acne [25].

CONCLUSIONS

Further research is needed to fully elucidate the role that diet may play in both development and treatment of acne. As the typical diet in developed countries is a high-glycaemic diet often causing insulin resistance, it may therefore exacerbate sebum production and thus inflammation and acne. Contemporary research is being conducted to determine whether a diet low glycemic index diet can effectively treat or even prevent the development of acne. The question of why not every person with insulin resistance develops acne, if this is one of their risk factors, is still being sought. On the other hand, studies on the exacerbation of acne as a result of chocolate consumption remain controversial due to the additives found in it, such as fat and sugar. The role of antioxidants, omega-3 fatty acids, zinc, vitamin A, dietary fiber and iodine in acne remains unclear. It can be assumed that if a patient notices an association between a particular dietary factor and acne severity, then they should exclude it from their diet or limit their intake.

Conflict of interest

The Author declares no conflict of interest.

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THE RELATIONSHIP BETWEEN SEEDS CONSUMPTION, LIPID PROFILE AND BODY MASS INDEX AMONG PATIENTS WITH CARDIOVASCULAR DISEASES

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ABSTRACT

Background. Cardiovascular disease (CVD) is the leading cause of death worldwide and largely preventable through improving diet quality and other lifestyle factors. In recent years, dietary recommendations have shifted towards diets high in plant-based foods and low in animal-based foods for the prevention of chronic diseases.

Objective. This study aims to evaluate the relationship between seeds consumption, lipid profile and body mass index (BMI) in a group of 889 (313 female and 576 male) randomly selected patients hospitalized in the Nitra Cardio Center, Slovakia.

Material and Methods. The data necessary for the evaluation the relationship between seeds consumption, lipid profiles and BMI were obtained by a questionnaire method in closed-ended format. Data collection was carried out simultaneously with the anthropometric and biochemical examinations of the respondents ensured by the Nitra Cardio Center. The following parameters were assessed in the blood: total cholesterol (T-C), low-density cholesterol (LDL-C), high-density cholesterol (HDL-C), triglycerides (TG), and glucose (GLU). Comparisons between groups were made utilizing one-way analysis of variance (ANOVA) followed by *Tukey's post hoc* test.

Results. Respondents indicated pumpkin seeds as the most commonly consumed seeds (45.19% of women and 53.06% of men) and their preference did not cause changes in lipid profile. Women who consume seeds 1-2 times/month had significantly lower levels of T-C and LDL-C compared to women non-consuming seeds ($P < 0.05$). In men, we did not observed any changes in lipid profile. The lowest BMI in women was associated with flaxseed preference and in men with sunflower preference. Increasing frequency of seeds consumption of women and men did not cause changes in BMI.

Conclusions. Based on beneficial effects of seeds on cardiovascular risk factors, it seems that seeds consumption can be considered as a useful therapeutic approach for reducing lipid profile and body mass index.

Key words: cardiovascular disease, lipid profile, seeds, flaxseed, pumpkin, sunflower, sesame, body mass index

STRESZCZENIE

Wprowadzenie. Choroby ukłdu krżenia (CVD) s głwn przyczyn zgonw na całm świcie i mořna im w duřej mierze zapobiec poprzez poprawę jakości diety i innych czynnikw zwizanych ze stylem Źycia. W ostatnich latach zalecenia Źywieniowe przesunęły się w kierunku diety bogatej w Źywność pochodzenia roślinnego i ubogiej w Źywność pochodzenia zwierzęcego w celu zapobiegania chorobom przewlekłm.

Cel. Niniejsze badanie ma na celu ocenę zwizku międy spořyciem nasion, profilem lipidowym i BMI w grupie 889 (313 kobiet i 576 męzczyzn) losowo wybranych pacjentw hospitalizowanych w Nitra Cardio Center na Słowacji.

Material i metody. Dane niezbędne do oceny zaleřności międy spořyciem nasion, profilami lipidowymi i BMI uzyskano metod ankietow stosujc pytania zamknięte. Zbieranie danych odbywało się równolegle z badaniami antropometrycznymi i biochemicznymi respondentw zapewnianymi przez Centrum Nitra Cardio. Oceniano we krwi stężeńia następujących parametrw: cholesterol całkowity (T-C), cholesterol o niskiej gęstości (LDL-C), cholesterol o duřej gęstości (HDL-C), trjglicerydy (TG) i glukozę (GLU) we krwi. Porwnania międy grupami dokonano wykorzystujc jednokierunkow analizę wariancji (ANOVA), a następnie test *post hoc Tukeya*.

Wyniki. Respondenci wskazali pestki dyni jako najczęściej spořywane nasiona (45,19% kobiet i 53,06% męzczyzn), a ich preferencje nie powodowały zmian w profilu lipidowym. Kobiety, które spořywały nasiona 1-2 razy w miesicu, miały statystycznie znacznie niřszy poziom T-C i LDL-C w porwnaniu z kobietami nie spořywajcymi nasion ($P < 0,05$). U męzczyzn nie zaobserwowaliśmy Źadnych zmian w profilu lipidowym. Najniřsze BMI u kobiet wizało się

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z preferowaniem nasion lnu, a u mężczyzn z preferencją słonecznika. Rosnąca częstotliwość spożycia nasion przez kobiety i mężczyzn nie powodowała zmian wskaźnika BMI.

Wnioski. Opierając się na korzystnym wpływie nasion na czynniki ryzyka chorób układu sercowo-naczyniowego, wydaje się, że spożycie nasion można uznać za przydatne podejście terapeutyczne do obniżania profilu lipidowego i wskaźnika masy ciała.

Słowa kluczowe: choroby układu krążenia, profil lipidowy, nasiona, siemię lniane, dynia, słonecznik, sezam, wskaźnik masy ciała

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide and largely preventable through improving diet quality and other lifestyle factors [36, 50, 55]. Multiple cardiovascular risk factors, including hyperlipidemia, hypertension, diabetes and smoking are associated with excessive production of reactive oxygen species and increased oxidative stress and inflammation, and promote vascular cell damage. Consequently, increased inflammation and oxidative stress is considered the major mechanism of the pathogenesis of endothelial dysfunction [4, 23]. Diet can affect CVD directly by modulating the composition of vascular plaques, and indirectly by affecting the rate of aging [36].

In recent years, dietary recommendations have shifted towards diets high in plant-based foods and low in animal-based foods for the prevention of chronic diseases [25, 27, 37, 47]. This dietary pattern has been associated with lower risk of CVD [11, 16, 20, 28] and is widely recommended for heart health [32].

Most of these plant-based dietary patterns highlight the intake of nuts as a key component [49, 55]. The integral role of seeds in pre-agricultural diets is understandable given their high energy and nutrient density [52]. In general, they are dense in a variety of nutrients and provide protein, fat (mostly unsaturated fatty acids), dietary fiber and many bioactive constituents such as vitamins (e.g. folic acid, niacin, vitamin E, vitamin B₆), minerals (e.g. copper, magnesium, potassium, zinc), antioxidants, phytoestrogens and other phytochemicals [19, 50, 51, 54, 55].

Nuts and edible seeds are recognized for their nutritional value and potential contribution to health promotion [38, 48]. Nuts and seeds may be considered an important component of a healthy diet and are regularly consumed, either as snacks or part of a meal [19]. In the last decade a large body of scientific evidence has been built on the beneficial effects of increasing consumption of plant seeds and derived products on various health outcomes, chiefly CVD, type 2 diabetes mellitus (T2DM) and intermediate markers [51, 52]. There is substantial evidence that increased consumption of seeds is associated with lower risk of CVD and T2DM or a significant

reduction in CVD risk factors such as serum cholesterol or blood pressure [51]. In addition, nuts and edible seeds provide antioxidant, anti-microbial, anti-inflammatory, anti-mutagenic, anti-cancer, anti-diabetic and glucoregulatory properties [30, 41].

Functional foods with positive effects on health are good choices for reducing the risk of chronic diseases [8, 43]. Flaxseed is the richest source of plant α -linolenic acid (ALA) and lignans [44]. Due to its high content of lignans, fiber, phytosterols and ALA, flaxseed was recently recognized as a functional food that may reduce cardiovascular disease risk factors such as hypertension and hyperlipidemia [8, 13, 14, 17, 29, 46, 62]. Results from studies have reported that some bioactive compounds found in the pumpkin seed oils are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) act as a natural anti-oxidant [3, 7, 12]. The pumpkin seed oil has also demonstrated various other beneficial properties like anti-microbial, anti-hypertensive, anti-arthritis, anti-inflammatory, and anti-depression activities [2, 33, 61, 63] and pumpkin seed oil is also well recognized for the treatment of urinary tract infection and other infectious diseases [39]. Sunflower is an excellent source of phenolics and showed the highest antioxidant capacity even though its lignan content is limited. Sesame provide a good source of phenolics, but their peculiar contribution is in term of lignans [21, 60]. This study aims to evaluate the relationship between seeds consumption, lipid profile and body mass index in a group of randomly selected patients hospitalized in the Nitra Cardio Center, Slovakia.

MATERIALS AND METHODS

Study design and participants

The study was conducted using the direct PAPI (*Paper And Pen Personal Interview*) method. A total of 1255 subjects (455 female and 800 male) were recruited, of which 366 were eliminated because they did not answer all of the questions in the questionnaire. Finally, 889 participants (313 females and 576 males) aged 20-101 years were included for further analysis, translating to a response rate of 70.84%. The average age of women was 64.30 ± 10.03 years and the average age of men was 60.79 ± 9.94 years. Respondents

included in the study had either overcome myocardial infarction or were diagnosed with angina pectoris and hospitalized after undergoing catheterization. Data were collected during the years 2015–2020.

Ethical aspects

The research was conducted according to the guidelines of the Declaration of Helsinki and approved by Nitra Cardio Center and by the Ethics Committee at the Specialized Hospital St. Zoerardus Zobor, n. o. Nitra, Slovak Republic (protocol number 10.6.2014).

Dietary data

The data necessary for the evaluation the relationship between seeds consumption, lipid profiles and BMI were obtained by a questionnaire method in closed-ended format. The questionnaire was compiled by the Department of Human Nutrition (Slovak University of Agriculture in Nitra) and approved by the Nitra Cardio Center. The questionnaire was applied individually by a single interviewer. The questionnaire contained two parts. The first part included questions concerning the socio-demographic situation of the subjects, physical activity, use of tobacco, and medical history. The second part of the questionnaire concerned the analysis of selected dietary habits, including the number of the consumed meals, their regularity, snacking between meals, types of snacks consumed, and the eating frequency of selected groups of food products. In the dietary questionnaires, we asked participants how often they had consumed a seeds during the preceding year: never, 1-2 times/month or 1-2 times/week. The questionnaire was anonymous; its completion was voluntary, with only one response to be circled for each question.

Biochemical parameters

Data collection was carried out simultaneously with the somatometric and biochemical examinations of the respondents ensured by the Nitra Cardio Center. All biochemical parameters were measured in certified

hospital laboratory using the Automatic Biochemical Analyzer BioMajesty® JCA-BM6010/C (DiaSys Diagnostic System GmbH, Holzheim, Germany). The following parameters were evaluated: total cholesterol (T-C), low-density cholesterol (LDL-C), high-density cholesterol (HDL-C), triglycerides (TG), and glucose (GLU).

Anthropometric parameters

Anthropometric measurements were performed in the morning with a standard manner (on an empty stomach, after urination and defecation). Anthropometric parameters - body weight (kg) and height (cm) were measured on outpatient electronic medical scales (Tanita WB-3000, Tanita Co., Tokyo, Japan). The body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters.

Statistical analysis

Statistical analysis was carried out using the Statistica version 10 and MS Excel 2007 (Microsoft Corporation, Redmond, Washington, USA). Data were expressed in figures and tables as average \pm standard deviation (SD). We also presented minimum and maximum, categorical variables are reported as percent values. Comparisons between groups were made utilizing one-way analysis of variance (ANOVA) followed by *Tukey's post hoc* test. The Chi-square test was used for categorical variables. The statistical significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Characteristics of study participants

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

Of the 889 participants, 481 (54.10%) were retiree, 559 (62.88%) were married and 331 (37.23%) participants had attained a graduation. The demographic characteristics of study participants is shown in Table 2.

Table 1. Basic characteristics of study participants

n=889 Parameters	Women (n=313)		Men (n=576)	
	average \pm SD	min. – max.	average \pm SD	min. – max.
Age (yrs)	64.30 \pm 10.03	35 – 90	60.79 \pm 9.94	20 – 101
BMI (kg.m ⁻²)	29.31 \pm 4.02	19.00 – 43.11	29.70 \pm 4.51	17.90 – 52.01
T-C (mmol.L ⁻¹)	4.83 \pm 1.24	1.95 – 8.69	4.62 \pm 1.17	1.99 – 9.18
LDL-C (mmol.L ⁻¹)	2.79 \pm 1.04	0.69 – 5.89	2.88 \pm 0.98	0.61 – 7.19
HDL-C (mmol.L ⁻¹)	1.47 \pm 0.51	0.57 – 4.55	1.16 \pm 0.39	0.45 – 4.01
TG (mmol.L ⁻¹)	1.62 \pm 1.14	0.35 – 8.27	1.74 \pm 0.93	0.43 – 7.39
GLU (mmol.L ⁻¹)	6.92 \pm 3.49	2.23 – 27.07	6.67 \pm 2.31	3.98 – 24.12

Data are expressed as average \pm standard deviation (SD), min. – max.; BMI, body mass index; T-C, total cholesterol; LDL-C, low density cholesterol; HDL-C, high density cholesterol; TG, triglycerides; GLU, glucose

Table 2. Demographic characteristics of study participants

Parameters	Women (n=313) n (%)	Men (n=576) n (%)	All participants (n=889) n (%)
<i>Social status</i>			
employed	78 (24.92)	207 (35.94)	285 (32.06)
unemployed	25 (7.99)	98 (17.01)	123 (13.84)
retiree	210 (67.09)	271 (47.05)	481 (54.10)
<i>Family status</i>			
married	158 (50.48)	401 (69.62)	559 (62.88)
divorced	52 (16.61)	92 (15.97)	144 (16.20)
widower	103 (32.91)	83 (14.41)	186 (20.92)
<i>Education</i>			
basic	66 (21.09)	67 (11.63)	133 (14.96)
apprenticeship	77 (24.60)	176 (30.56)	253 (28.46)
graduation	130 (41.53)	201 (34.90)	331 (37.23)
higher	40 (12.78)	132 (22.92)	172 (19.35)

Basic characteristics of participants based on frequency of seeds consumption

The results revealed significant differences ($P < 0.001$) of basic characteristics among three different seeds consumption categories (Table 3). Consumption of seeds (1-2 times/week) was most common among

younger women, lower BMI, fewer smokers, more physically active, those with positive family history of dyslipidemia, lower alcohol drinking, more consumption of red meat, fruits, vegetables and lower consumption of whole dairy products. We observed different results in men, seed consumption (1-2

Table 3. Basic characteristics of participants based on frequency of seeds consumption

Characteristics	Frequency of consumption seeds			P
	1-2 times/week	1-2 times/month	never	
Women (n=313)				
Age (years)	63.43 ± 7.68	64.75 ± 8.92	63.50 ± 10.97	-
BMI (kg.m ²)	28.31 ± 3.52	29.49 ± 4.3	29.74 ± 4.52	-
Current Smoking (%)	16.00	17.54	16.13	P < 0.001
Physical activity <30 min/day (%)	18.67	30.70	28.23	P < 0.001
Family history of dyslipidemia (%)	45.33	28.95	29.84	P < 0.001
Alcohol >5 drinks/week (%)	10.67	14.03	14.52	P < 0.001
Red meat >3 times/week (%)	9.33	8.77	8.87	P < 0.05
Fruits - no daily (%)	4.0	7.02	18.55	P < 0.001
Vegetables - no daily (%)	13.33	24.56	30.65	P < 0.001
Whole dairy product (%)	49.33	61.40	57.26	P < 0.001
Men (n=576)				
Age (years)	61.55 ± 9.69	59.09 ± 9.12	61.84 ± 9.60	-
BMI (kg.m ²)	29.57 ± 3.55	29.60 ± 4.51	29.67 ± 5.28	-
Current Smoking (%)	12.62	23.96	26.17	P < 0.001
Physical activity <30 min/day (%)	22.33	14.75	21.88	P < 0.001
Family history of dyslipidemia (%)	30.10	33.64	44.53	P < 0.001
Alcohol >5 drinks/week (%)	7.76	9.68	16.40	P < 0.001
Red meat >3 times/week (%)	8.74	5.99	11.71	P < 0.001
Fruits - no daily (%)	18.45	18.89	25.39	P < 0.001
Vegetables - no daily (%)	20.39	24.42	30.86	P < 0.001
Whole dairy product (%)	69.97	62.67	63.28	P < 0.001

times/week) was most common in older men, higher BMI, fewer smokers, less physically active, lower positive family history of dyslipidemia, lower alcohol consumption, higher consumption of fruits, vegetables and whole dairy products.

Effect of the species and frequency of consumption of seeds on lipid profile

Respondents indicated pumpkin seeds as the most commonly consumed seeds (45.19% of women and 53.06% of men). The second most frequently consumed was sunflower seed which consume 16.74% of women and 25.55% of men ($P < 0.001$) (Figure 1).

Thus, recent studies on pumpkin seed oil indicate that they have gained much attention not only as an edible oil but also as a potential nutraceutical [3, 7,

12, 35]. Pumpkin seed is being used traditionally for curing and protecting various ailments [4].

The preference of pumpkin seeds in women and men did not cause changes in lipid profile. The mean serum lipids of participants based on species of seeds consumption is shown in Table 4. Some human clinical trials found that consumption of 30-50 g of flaxseed per day for four to 12 weeks resulted in an 8-14% decrease in LDL-C levels [5, 15, 42, 56], while other trials did not observe significant reductions in LDL-C after adding 30-40 g flaxseed per day to the diet [31, 34, 57]. Daily consumption of flaxseed (40 g) decreased LDL-C, T-C and TG lipoprotein and increased HDL-C concentrations in healthy menopausal women after 12 months [18]. Significant reductions in T-C, LDL-C and TG levels were seen

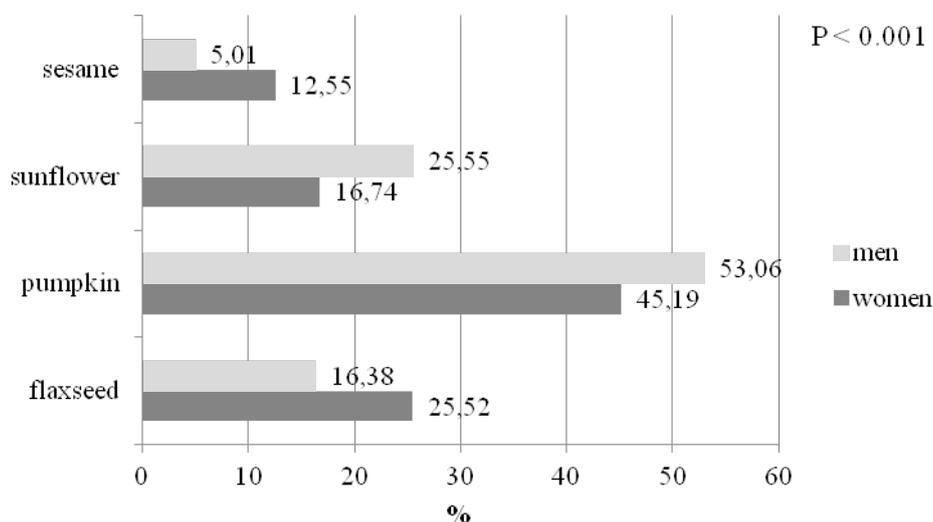


Figure 1. The most frequently consumed seeds (n=889)

Table 4. The mean serum lipids of participants based on species of seeds consumption

Species	n (%)	Lipid profile (mmol.L ⁻¹)			
		T-C	HDL-C	LDL-C	TG
Women (n=313)					
flaxseed	79 (25.52)	4.71 ± 1.28	1.49 ± 0.35	2.71 ± 1.08	1.48 ± 1.39
pumpkin	142 (45.19)	4.77 ± 1.11	1.49 ± 0.59	2.70 ± 0.94	1.68 ± 1.04
sunflower	53 (16.74)	4.73 ± 1.20	1.40 ± 0.41	2.81 ± 1.01	1.54 ± 0.69
sesame	39 (12.55)	4.19 ± 1.00	1.37 ± 0.45	2.53 ± 0.94	1.58 ± 0.74
P	-	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Men (n=576)					
flaxseed	94 (16.38)	4.70 ± 1.21	1.09 ± 0.24	2.99 ± 0.94	1.83 ± 0.96
pumpkin	306 (53.06)	4.69 ± 1.28	1.13 ± 0.45	2.95 ± 1.02	1.80 ± 1.00
sunflower	147 (25.55)	4.60 ± 1.18	1.15 ± 0.30	2.91 ± 1.04	1.57 ± 0.70
sesame	29 (5.1)	4.92 ± 1.34	1.17 ± 0.45	3.27 ± 1.16	1.55 ± 0.80
P	-	P > 0.05	P > 0.05	P > 0.05	P > 0.05

Data are expressed as average ± standard deviation (SD); *Tukey's post hoc* test; T-C, total cholesterol; HDL-C, high density cholesterol; LDL-C, low density cholesterol; TG, triglycerides

within each group after treatment with flaxseed oil and sunflower oil [1]. Flaxseed supplementation was well-tolerated and resulted in a consistent and significant decrease in T-C and LDL-C levels [29].

We also monitored the mean serum lipids of participants based on frequency of seeds consumption (Table 5). Women who consume seeds 1-2 times/month had significantly lower levels of T-C and LDL-C compared to women non-consuming seeds ($P < 0.05$). In men, we did not observe any changes in lipid profile.

Effect of the seeds consumption on body mass index

Nuts and seeds are energy dense foods that have been thought to be positively associated with increased body mass index. Despite the relatively high energy density, intake of nuts was actually

associated with less weight gain, lower risk of obesity, and lower risk of moderate weight gain in prospective studies [6, 9, 10]. Some of the reported health benefits derived from nut and seed consumption are control of body weight and blood pressure, reduction of coronary heart disease, and reduction of levels of blood cholesterol and triacylglycerols [30, 45]. Our results show that the different preference and frequency of seeds consumption did not cause changes in BMI. The mean BMI of participants based on species and frequency of seeds consumption is shown in Table 6. *Haghighatsiaret et al.* [26] demonstrated that incorporating whole flaxseed into the diet of hyperlipidemic and hypertensive patients along with healthy dietary recommendations for 8 weeks could reduce anthropometric measurements - waist circumference (WC) and waist to hip ratio

Table 5. The mean serum lipids of participants based on frequency of seeds consumption

Frequency	Parameter (mmol.L ⁻¹)				
	n (%)	T-C	HDL-C	LDL-C	TG
Women (n=313)					
1-2 times/week	75 (24.03)	4.67 ± 1.06	1.49 ± 0.31	2.84 ± 0.95	1.39 ± 0.58
1-2 times/month	114 (36.44)	4.77 ± 1.08 ^a	1.49 ± 0.64	2.52 ± 0.93 ^a	1.71 ± 1.10
never	124 (39.53)	5.05 ± 1.30 ^b	1.48 ± 0.49	2.94 ± 1.06 ^b	1.82 ± 1.51
P	-	$P < 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$
Men (n=576)					
1-2 times/week	103 (17.89)	4.58 ± 1.17	1.14 ± 0.50	2.85 ± 0.94	1.72 ± 0.95
1-2 times/month	217 (37.60)	4.61 ± 1.26	1.15 ± 0.37	2.88 ± 0.99	1.74 ± 0.83
never	256 (44.51)	4.60 ± 1.03	1.20 ± 0.37	2.81 ± 0.90	1.75 ± 0.84
P	-	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Data are expressed as average ± standard deviation (SD); *Tukey's post hoc* test, different superscript letters in the same column are significantly different; T-C, total cholesterol; HDL-C, high density cholesterol; LDL-C, low density cholesterol; TG, triglycerides

Table 6. The mean BMI of participants based on species and frequency of seeds consumption

Parameter	Women (n=313)		Men (n=576)	
	n (%)	BMI (kg.m ⁻²)	n (%)	BMI (kg.m ⁻²)
Species				
flaxseed	79 (25.52)	28.43 ± 3.42	94 (16.38)	30.04 ± 5.10
pumpkin	142 (45.19)	29.37 ± 3.92	306 (53.06)	29.74 ± 4.14
sunflower	53 (16.74)	28.89 ± 3.64	147 (25.55)	29.53 ± 3.58
sesame	39 (12.55)	29.95 ± 4.07	29 (5.1)	30.31 ± 5.89
P	-	$P > 0.05$	-	$P > 0.05$
Frequency				
1-2 times/week	75 (24.03)	28.31 ± 3.52	103 (17.89)	29.57 ± 3.55
1-2 times/month	114 (36.44)	29.49 ± 4.3	217 (37.60)	29.60 ± 4.51
never	124 (39.53)	29.74 ± 4.52	256 (44.51)	29.67 ± 5.28
P	-	$P > 0.05$	-	$P > 0.05$

Data are expressed as average ± standard deviation (SD); *Tukey's post hoc* test; BMI, body mass index

(WHR) and improve lipid profiles (TG, T-C, and LDL-C) of participants. In the study of *O'Neil et al* [40] nut consumption was significantly associated with beneficial effects in body mass index, waist circumference, blood pressure, insulin resistance, lower chance of obesity and overweight and increase in HDL-C levels. It is worth mentioning that, in general, an increase in body mass has not been observed with an increased consumption of tree nuts and legume seeds [22, 59]. However, considerable evidence from both epidemiological studies and clinical trials suggests that nuts are not associated with weight gain and may, in fact, help to lose weight when incorporated to an energy restricted diet [58].

CONCLUSION

In our study, we observed the associations between consumption of seeds, lipid profile and BMI in a group of randomly selected patients with CVD. Respondents indicated pumpkin seeds as the most commonly consumed seeds and their preference in women and men did not cause changes in lipid profile. Women who consume seeds 1-2 times/month had significantly lower levels of T-C and LDL-C compared to women non-consuming seeds. The lowest BMI in women was associated with flaxseed preference and in men with sunflower preference. Increasing frequency of seeds consumption of women and men did not cause changes of BMI.

Based on beneficial effects of seeds on cardiovascular risk factors, it seems that seeds consumption can be considered as a useful therapeutic approach for reducing lipid profile and body mass index.

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Conflict of interest

The authors declare no conflict of interest.

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LONG-TERM EFFECT OF ONE-TIME NUTRITIONAL EDUCATION IN SCHOOL ON NUTRITIONAL KNOWLEDGE OF EARLY SCHOOL-AGED CHILDREN

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ABSTRACT

Background. In the group of school-aged children nutritional education (NE) enables an early development of healthy eating habits, which can be transferred to the adult life.

Objective. The aim of the study was to assess the long-term effect of a one-time nutritional education, including also culinary workshops, on the level of nutritional knowledge (NK) of early school-aged children as well as to analyse the nutritional content of the children's books.

Material and methods. The study was conducted among second- and third-grade pupils from a primary school in Warsaw (n=76). The level of NK was determined using the same, self-administrated questionnaire at each of the three stages of the study.

Results. The level of NK was assumed as very good in 47% of respondents before the education, in 91% immediately after the education, and in 74% six months after it. Out of all 23.0 points (max.) on average 18.1 points were obtained at the first stage, 21.1 points at the second stage and 19.7 points at the third stage (p<0.001). Immediately after the NE and six months after it the level of NK increased significantly (comparing to the first stage) especially on these topics: the daily water requirements and the recommendations on physical activity (p<0.001 for both questions).

Conclusions. The results of the study confirmed the effectiveness of a one-time nutritional education on the level of nutritional knowledge. Moreover, results show that in the research group nutritional knowledge about importance of physical activity, adequate nutrition, eating varicoloured vegetables and fruits, screen time limits and distinguishing between healthy and unhealthy products was satisfactory, even at the baseline, but in the main source of fats in a diet, the role of milk products, the effect of having too little water in a diet and the sugar content in food children's knowledge was not enough.

Key words: *nutritional education, nutritional knowledge, school-based programs, children, primary schools*

STRESZCZENIE

Wprowadzenie. W grupie dzieci w wieku szkolnym edukacja żywieniowa (EŻ) pozwala wcześniej kształtować prawidłowe nawyki żywieniowe, które mogą być przeniesione w dorosłe życie.

Cel badań. Celem pracy była ocena długoterminowego wpływu jednorazowej edukacji żywieniowej (obejmującej również warsztaty kulinarne) na poziom wiedzy żywieniowej (WŻ) dzieci w wieku wczesnoszkolnym oraz analiza treści żywieniowych zawartych w podręcznikach uczniów.

Material i metod. Badanie przeprowadzono w grupie uczniów z klas II i III szkoły podstawowej w Warszawie (n=76). Do zbadania poziomu wiedzy żywieniowej na każdym z etapów badania wykorzystano ten sam, autorski kwestionariusz ankiety.

Wyniki. Bardzo dobry poziom wiedzy żywieniowej stwierdzono u 47% badanych na pierwszym etapie badania, u 91% badanych bezpośrednio po edukacji i u 74% badanych po sześciu miesiącach. Na 23,0 możliwe do uzyskania punkty respondenci uzyskali średnio: 18,1 punktów na etapie 1, 21,1 na etapie 2 i 19,7 na etapie 3, co świadczy o korzystnym wpływie jednorazowej edukacji żywieniowej na wiedzę żywieniową uczniów (p<0,001). Bezpośrednio po edukacji oraz sześć miesięcy później poziom WŻ znacząco wzrósł (w porównaniu do etapu pierwszego), szczególnie w zakresie: dziennego zapotrzebowania na wodę oraz zaleceń dotyczących aktywności fizycznej (p<0,001 dla obu pytań).

Wnioski. Otrzymane wyniki potwierdzają korzystny wpływ edukacji żywieniowej na wiedzę żywieniową uczniów. Ponadto w grupie badanej wiedza żywieniowa w zakresie: znaczenia aktywności fizycznej, prawidłowego żywienia,

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spożywania różnych warzyw i owoców, ograniczeń czasu spędzanego przed ekranem, a także rozróżniania produktów zalecanych i niezalecanych była zadowalająca, zaś jeżeli chodzi o zagadnienia takie jak: zalecane źródła tłuszczu w diecie, rola produktów mlecznych, niekorzystne efekty picia zbyt małej ilości wody oraz zawartość cukru w produktach, wiedza uczniów okazała się niewystarczająca.

Słowa kluczowe: *edukacja żywieniowa, wiedza żywieniowa, szkolne programy, dzieci, szkoły podstawowe*

INTRODUCTION

Nowadays, a high prevalence of childhood overweight and obesity is observed both in developed and developing countries [3, 24, 27]. According to the WHO, over 340 million children and adolescents aged 5-19, were obese or overweight in 2016, worldwide [37]. Among Polish children aged 8, the problem of overweight and obesity occurred in almost 1/3 of children (31%) - 32% of the surveyed boys and 29% of the surveyed girls based on WHO criteria [4, 7]. What is alarming, childhood obesity increases the chance of being obese in later childhood and leads to adulthood obesity as well [6, 11]. It has also been proven that overweight and obesity are one of the major risk factors for other noncommunicable, diet-related diseases such as hypertension, lipid disorders and type 2 diabetes [13, 14]. Although many of them appear mainly in adult life, they are associated with adverse nutritional behaviours, already present in childhood (such as insufficient consumption of vegetables and fruits, and excessive consumption of sweets and sweetened drinks) as well as lifestyle risk factors (such as sedentary lifestyle and insufficient physical activity) [20]. Nowadays, in the wake of the COVID-19 pandemic, adverse health behaviours have sharply increased, which resulted in weight gaining [1, 29]. Moreover, studies show that school-aged children are a nutritionally vulnerable group because of high nutritional requirements, intensified growth and development and high susceptibility to negative advertising, fashion and environmental patterns, created by their peers [5, 35, 36].

Therefore, there is no doubt that due to the high prevalence of adverse health behaviours among children and possible future consequences, it is important to implement evidence-based policies, such as nutritional education in schools, which will promote healthier dietary choices. Previous studies show that the increasing consumption of unhealthy foods in children is due to improper parents' attitudes toward unhealthy foods, social acceptance of eating unhealthy foods as well as due to the lack of knowledge and misunderstanding of the importance of healthy eating habits [16, 17, 28]. Moreover, the consequences of frequent unhealthy eating are delayed in time and as an effect seems not to be well-understood by children [16]. Based on previous studies' results, nutritional education has been

shown to be effective in increasing knowledge and appropriate dietary choices [8, 34, 35]. The efficacy of nutritional education is as high as the design and use of theories as well as a selection of educational methods which are more adapted to a given group [2, 17]. Moreover, it was stated that for nutritional education to be the most effective should last five or more months, should have support from school leadership and policymakers, and should involve not only children but also their parents [2, 22, 32].

Unfortunately, in most countries, health education (and as a component - nutritional education) in schools is not a priority. It rather remains on the periphery of the implemented program, has a low rank and is definitely underestimated by people, who create education and health policy [16, 40]. In Poland, non-governmental organisations, such as societies and foundations, are the ones which conduct nutritional education, which, as part of their activities include nutritional workshops with school children in the form of talks, discussions, games and cooking workshops [40, 41]. There are also governmental nutritional education programs, such as the *I know, what I eat (Wiem co jem)* campaign - a campaign of the Warsaw local government and the *Program for schools (Program dla szkół)* - an EU program, whose aim is to supply children with fruits and vegetables, milk and dairy products in schools. On the one hand, there are no available studies, which confirm the effectiveness of nutritional education in the group of early school children [15, 25]. On the other hand, interventional nutritional research shows that the effectiveness of education, even a one-time education, can be very high [31, 34]. Therefore, the aim of the study was to assess the long-term effect of one-time nutrition education among children in early school age. This effect was studied by a developed nutritional knowledge questionnaire after the analysis of nutritional content in children's' textbooks.

MATERIAL AND METHODS

Participants

The study was carried out on early school-aged children of a public primary school in Warsaw. The studied group was recruited using a procedure of purposive sampling. Parental written informed consent was obtained for the participation of their children. In total 147 children of early school-age

(1st-, 2nd- and 3rd-grade classes of primary school) were initially recruited (Figure 1). The inclusion criteria were as follows: headmaster's, class teachers' and children parents' written informed consent. The exclusion criteria were as follows: no parental written consent, as well as reading and spelling difficulties (1st-grade classes). In total 91 participants met the criteria. Due to non-participation in all stages of the study, 15 participants were excluded from the analysis. Finally, the study included 76 children. The number of children participating in the study differed at each of the three stages depending on children's presence as well as written informed consent possession.

Nutritional knowledge assessment

Nutritional knowledge was measured three times: before the nutritional education (at the baseline, S1 - first stage of the study), immediately after the nutritional education (S2 - second stage of the study), to measure the short-term effect of the education, and six months after the nutritional education (S3 - third stage of the study), to measure the long-term effect of the education (Figure 1). The main study was preceded by a pilot study (in October 2018), in which the understandability and accuracy of the questions were verified. As a result, the final version of the questionnaire was created and used in the main study. At each stage of the study, nutritional knowledge data was collected using a proprietary, self-administrated

questionnaire, completed by the participants at school. The questionnaire consisted of a total of 14 items. Three types of questions were used: closed-ended, true-false and ranking questions.

Every closed-ended question had three possible answers, from which only one was always correct (Q1-5, Q7-11; Table 1). Each of the 10 closed-ended questions was scored with 1 point if the chosen answer was correct or 0 points in case of 'I don't know' answer, wrong answers or missing data.

The questionnaire also contained one true-false question with 5 sentences which were scored with 1 point if the answer in the sentence was correct and 0 points if the answer was incorrect or in case of missing data (Q12; Table 1). The questionnaire also included one question with four items, which demanded a selection of a better cereal product from the given couples (Q6; Table 1). This type of questions was scored as follows: 0.5 points for each good choice or 0 points in case of each wrong answer or missing data.

The selection of products which should or should not be a part of a proper meal was also demanded in a question, where various products were shown on pictures and children were asked to choose which products are not recommended and to tick them. It was possible to get 0.5 points for ticking each of the inadvisable products (Q13; Table 1).

The last question was the ranking question, in which the respondents were asked to - using the

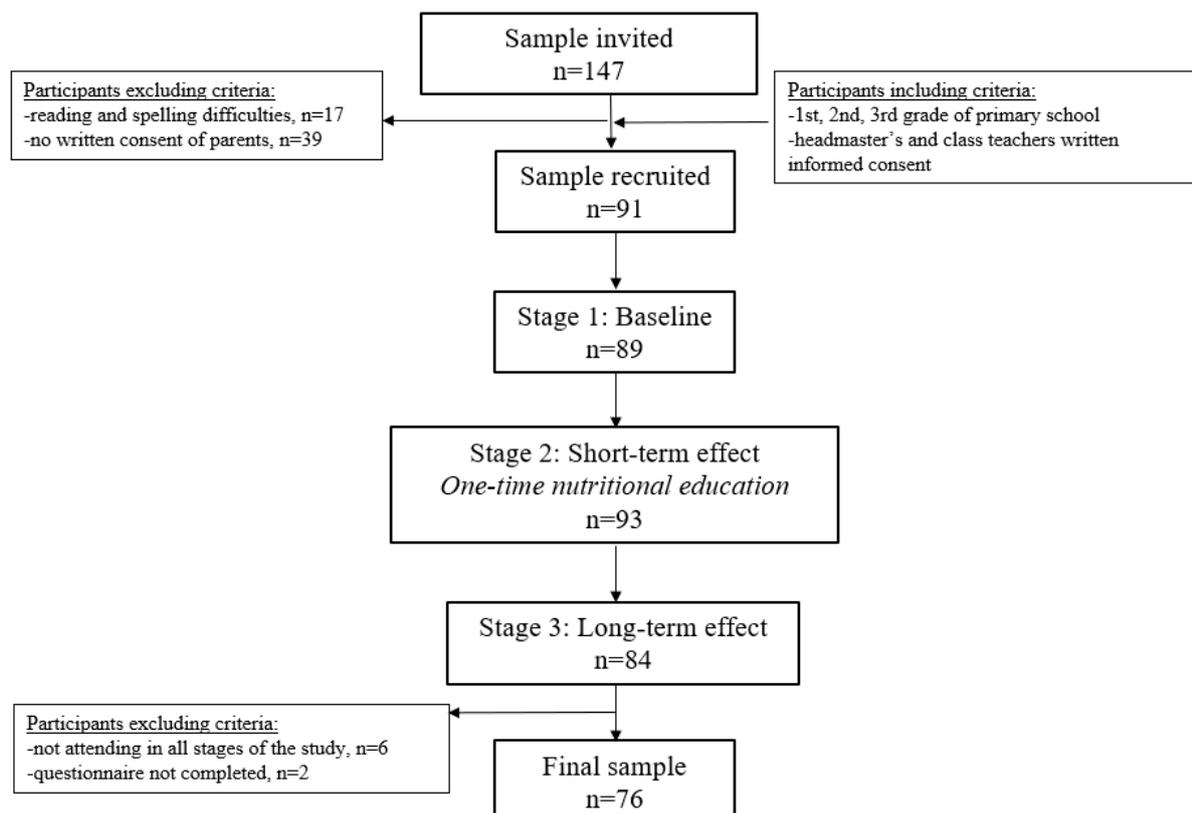


Figure 1. Study design and sample collection

Table 1. Questions and answers included in questionnaire

Questions	Answers
1. Is physical activity (sport, exercises) important?	Yes, because it improves condition and prevents obesity; Yes, because doing sports allows us to eat more sweets; No, because physical activity causes tiredness
2. Are there any screen time limits?	<2 h/d; 4h/d and more; There are no limits
3. Does adequate nutrition affect human health?	It has no effect on our health; It does not matter - we eat because we are hungry; It affects our health, strength and ability to learn
4. Why is it worth to eat varicoloured vegetables and fruit?	Because they look well together; Because they provide various ingredients; Colours do not matter
5. What should be the main source of fats in a diet?	Vegetables and fruit; Fish, nuts, plant-based oils; Grain products and dairy products
6. From each couple choose the product which is more beneficial for your health	Wheat bread <i>or</i> wheat-rye bread with grains; Kaiser roll <i>or</i> graham roll; Buckwheat <i>or</i> white rice; Oat flakes <i>or</i> chocolate flakes
7. Why are milk and milk products needed for proper human development?	Because they contain iron, which builds blood cells; Because they contain calcium and vitamin D, which build our bones and teeth; Because they contain vitamin C, which empowers immunity
8. Why is it worth to eat dairy products (e.g. kefir, cheese)?	Because they are a source of fiber; Because they are a source of 'good bacteria'; Because they contain vitamin C, which empowers immunity
9. Why is it better to eat wholemeal bread, groats and oat flakes instead of Kaiser rolls, white rice and chocolate flakes?	They are more colourful; They contain calcium, which build bones and teeth; They contain more necessary ingredients (e.g. fiber and vitamins)
10. What can drinking too little water cause?	Hiccups; Decreased disease resistance; Tiredness during lessons and difficulty concentrating
11. Should eating sweets and drinking sweet beverages be limited?	No, because they are tasty; Yes, because parents are saying that they should be limited; Yes, because in excess they can cause tooth decay or excess weight
12. Choose true sentences	6 glasses of water should be drunk daily; More fruits than vegetables should be eaten daily; 3-4 glasses of milk or dairy drinks should be drunk daily; It is better to eat a bar than fruit; Every child should be physically active for at least 60 minutes every day
13. Choose those products which should not be a part of proper meals	Bar; Donut; Fruit juice; Salty sticks; Chips; Pizza; Crisps; Hamburger
14. Rank the following products from the lowest to the highest amount of sugar	1-Tomato juice, 3-Flavoured yoghurt, 2-Banana, 4-Flavoured water

numbers from 1 to 4 - rank the products from the ones with the lowest to the ones with the highest amount of sugar. It was possible to get 1 point, if two products were ranked correctly, 2 points if four products were ranked correctly or 0 points in case of ranking 1 or 0 products correctly or in case of missing data (Q14; Table 1).

The points were summed up for each respondent, the maximum was 23 points. Based on number of obtained points, the respondents were divided into five nutritional knowledge levels, labelled as follows: very poor (0–4.5 points), poor (4.6–9.2 points), average (9.3–13.8 points), good (13.9–18.4 points), very good (18.5–23 points) (Table 2).

Table 2. Nutritional knowledge categories

Nutritional knowledge categories	(% range (points))
Very poor	0-20 (0 – 4.5)
Poor	21-40 (4.6 – 9.2)
Average	41-60 (9.3 – 13.8)
Good	61-80 (13.9 – 18.4)
Very good	81-100 (18.5 – 23)

One-time nutritional education

In the term of November and December 2018 a one-time nutritional education program lasting two hours in each class was implemented. The classes were conducted in the form of talks and exercises

supporting children's (both individual and group) involvement (discussion as well as guesswork and singing a song about vegetables and fruits). The topics of the classes corresponded to the questions included in the questionnaire. The core components of the education included relaying knowledge of: the importance of healthy eating habits, hydration and physical activity on health, nutritional value of food products, sources of individual nutrients and screen time limits. The classes also included culinary workshops, in which a meal (cottage cheese and varicoloured pepper sandwiches) was prepared together. The aim of the culinary workshops was to show how to prepare a healthy, colourful and tasty meal covering the dietary guidelines. During the classes, the computer-based display responding to the research objectives was used.

Statistical analysis

To compare the frequency of possible answers selection at the three stages of the study, as well as to check the differences by gender and school class for the sum of obtained points in each stage of the study the Chi-square test was applied. Q-Cochran test was used to compare the frequency of choosing the correct answers in individual questions, over three stages of the study and McNemar's Chi^2 test was used to compare the frequency of choosing the correct answers in individual questions between the two stages of the study.

For quantitative variables, the differences between the three stages were compared using the Kruskal-Wallis test (sum of obtained points and categories of nutritional knowledge levels). The significance level of $p < 0.05$ was used. The following software was used: Statistica 13.0.

RESULTS

Characteristics of the studied group

The total number of children who participated in the study was 76 with 46 girls (61%) and 30 boys (39%) (Table 3). The mean age of the respondents was 8.4

± 1.1 years, with the age ranging from 7 to 10 years; 47% of the respondents were second-grade children, and 53% were third-grade children. Initial nutritional knowledge was assessed at an average of 18.1 points (79%) for the group, 18.5 points (80%) in girls, and 17.4 points (76%) in boys.

Analysis of the nutritional content of textbooks, used by the surveyed children

Second-grade children were following an educational program according to the *School Friends (Szkolni Przyjaciele)* textbook, in which they have hardly ever encountered any nutritional content before a one-time nutritional education. According to the nutritional textbooks content, over time between the second and the third stage of the study, a second-grade child should: understand the food-pyramid nutritional guidelines, know that eating varicoloured vegetables and fruits carries intake of numerous nutrients, and know some of the obesity causes.

Third-grade children were following an educational program according to the *New Hunters (Nowi Tropiciiele)* textbook in the first grade and in the second grade, and the *Our school (Nasza szkoła)* textbook – in third grade. In connection with the above, before the nutritional education, a third-grade student should know: which products could be a part of a healthy meal, the importance of nutrition for health, the nutritional value of various food groups and the importance of eating breakfast as well as how to prepare a healthy breakfast. After the time between the second and the third stage of the study, a third-grade student should know that the main role of dairy products is to build strong bones and teeth, because of the high calcium and vitamin D content.

Nutritional knowledge assessment

While analysing the mean nutrition knowledge score, it was stated that the results differed throughout the study ($p < 0.001$; Kruskal-Wallis test). The respondents gained on average: 18.1/23 (79%) points at the S1, 21.1/23 (92%) points at the S2 and 19.7/23 (86%) points at the S3 (Table 4). At the S1, 47% of

Table 3. Characteristics of the study group

Variables	Total sample n= 76 (100%)	Girls n= 46 (61%)	Boys n= 30 (39%)
	Mean \pm SD*	Mean \pm SD	Mean \pm SD
Age	8.4 \pm 1,1 (7.3-9.8)**	8.4 \pm 0,5 (7.6-9.8)	8.3 \pm 0.4 (7.3-9.1)
Class:			
Second grade	36 (47%)	24 (52%)	12 (40%)
Third grade	40 (53%)	22 (48%)	18 (60%)
NK initial	79%	80%	76%

*-standard deviation, **-range; NK -nutritional knowledge

the respondents had a very good level of nutritional knowledge, while 49% of the respondents had a good level of nutritional knowledge. The proportion of the respondents who had a very good level of nutritional knowledge at the S2 was higher than at the S3. Non respondent obtained as few points as to assess its level of nutritional knowledge as low or insufficient.

Detailed nutritional knowledge assessment

The mean nutrition knowledge score differed throughout the study (Table 5). Following the education (comparing the S1 and the S2), the level of NK increased significantly of these topics: better choice for the couple of Kaiser roll and Graham roll

as well as buckwheat and white rice, fermented milk products' function in human body, adverse health effect of drinking too little water, as well as daily water requirements, estimating sugar content in food and recommendations on physical activity ($p < 0.001$ for each question) (Table 5).

At each stage of the study only almost half of the respondents knew that good sources of fat are fish, nuts, and plant-based oils, but knowledge of this topic did not change significantly. The majority of the respondents knew, even at the baseline, the importance of healthy eating habits and physical activity, screen time limits, advantages of eating varicoloured vegetables and fruits, and which product from the

Table 4. Distribution of nutritional knowledge levels by stages of the study

Nutritional knowledge	% of respondents			p*
	Stage 1	Stage 2	Stage 3	
Categories				
Very poor	0	0	0	<0.001
Poor	4	0	0	
Average	49	9	26	
Good	47	91	74	
Very good				
Obtained points	mean (%)			<0.001
	18.1 (79%)	21.1 (92%)	19.7 (86%)	

*statistically significant (*Kruskal-Wallis* test); * $p < 0.05$

Table 5. The percentage of correct answers provided by the respondents to questions

Question	Stage 1	Stage 2	Stage 3	p ¹	p ²	p ³	p*
1. Importance of physical activity	97	99	99	ns	ns	ns	ns
2. Screen time limits	95	93	95				
3. Adequate nutrition impact on human health	99	95	99				
4. Worth to eat varicoloured vegetables and fruit	92	95	95				
5. The main source of fats in a diet	55	58	50				
6. Products more beneficial for health							
a. Wheat-rye bread with grains	92	95	96	ns	ns	ns	ns
b. Graham roll	88	97	92	0.023			0.004
c. Buckwheat	83	96	89	0.027			0.038
d. Oat flakes	100	100	100	ns			-
7. Role of milk and milk products	71	79	84	ns	0.044	ns	ns
8. Worth to eat dairy products (kefir, cheese)	20	68	33	<0.001	ns	<0.001	<0.001
9. Whole grain products as better option than non-whole products	80	79	87	ns	ns	ns	ns
10. Too little water effect	58	96	68	<0.001	ns	<0.001	<0.001
11. Sweets and sweet beverages limitation	87	95	95	0.004	ns	<0.001	ns
12. Choose true sentences							
a. 5-6 glasses of water should be drunk daily.	72	99	95	<0.001	<0.001	ns	<0.001
b. More fruits than vegetables should be eaten daily.	81	91	89	ns	ns	ns	ns

c. 3-4 glasses of milk or dairy drinks should be drunk daily.	73	92	84	0.005	ns	ns	0.008
d. It is better to eat a bar than fruit.	96	100	99	ns	ns	ns	ns
e. Every child should be physically active for at least 60 minutes every day.	80	99	97	<0.001	<0.001	ns	<0.001
13. Choose those products which shouldn't be part of proper meals							
a. Bar	97	100	100	ns	ns	ns	ns
b. Donut	99	99	100				
c. Fruit juice	80	87	89				
d. Salty sticks	93	93	96				
e. Chips	99	100	100				
f. Pizza	96	97	100				
g. Crisps	96	97	99				
h. Hamburger	96	99	99				
14. Ranking products from the lowest to the highest amount of sugar	25	92	34	0.004	ns	<0.001	<0.001

* $p < 0.05$ for the *Q-Cochran* test; p^1 - differences between the S1 and the S2 for the χ^2 McNemary test, p^2 - differences between the S1 and the S3 for the χ^2 McNemary test, p^3 - differences between the S2 and the S3 for the χ^2 McNemary test; ns- not statistically significant

given couple of cereal products is healthier (wheat-rye bread with grains and wheat bread, oat flakes, and chocolate flakes as well as fruit or a bar). Moreover, most of the surveyed children classified correctly bars, donuts, hamburgers, chips, crisps, salt sticks, and pizza to the group of items that should not be part of healthy meals. In these topics, nutritional knowledge did not change significantly after education (Table 5). A great majority of children at each stage of the study knew that eating sweets and drinking sweet beverages in excess should be limited, because of the greater probability of decay and overweight. Furthermore, at each stage of the study, almost a majority of respondents recognized healthy and unhealthy products, which were set on the pictures. For most respondents of the S1 (75%) and S3 (66%) the task of ranking products (tomato juice, flavoured cheese, banana, flavoured water) by increasing sugar content was too difficult. Only at S2 majority of children coped with this. NK in this topic differed significantly between all stages of the study ($p < 0.001$; *Q-Cochran* test).

DISCUSSION

At baseline, general nutritional knowledge, assessed by the implemented questionnaire was good (49% of respondents) or very good (47% of respondents) in this group. Immediately after the nutritional education the percentage of children, whose level of nutritional knowledge was assessed as very good doubled. Finally, the nutritional knowledge of 74% of pupils was classified as very good, which confirmed the effectiveness of the multi-comprehensive one-time nutritional education

(lessons with culinary workshops). The majority of the study group had knowledge of healthy lifestyle determinants (physical activity, screen time, and adequate nutrition). Taking into account specific content of nutritional knowledge, between baseline and the final stage, an increase of knowledge of the role of milk, milk products requirements as well as daily water requirements, the effects of drinking too little water, and recommendations on one-hour physical activity per day, was observed. Also, pupils recognized the content of sugar in the examples of food products better, however, only 34% of them coped with this task after six months. A low percentage of children knew the role of fermented milk drinks, both at the baseline (20%) and in the end line (33%) of this study. Moreover, the nutritional education did not change nutritional knowledge concerning fats, about 50% of pupils were able to classify the nuts, fish, and plant-based oils as fat sources in the diet.

Our results showed that a one-time program can be effective in increasing nutritional knowledge in children. It is consistent with *Grajek* and *Wanat* results [10]. The authors found that, after a one-time meeting with dieticians, awareness of recommended servings, according to the Food Pyramid, increased in the group of 4-6 grade children. Similarly, in the group of 164 adolescent girls from India the level of knowledge significantly improved after a one-time nutritional education using slides, charts and folders [26]. Unfortunately, there is very limited published research focusing on improving children's nutrition knowledge, attitudes and behaviours by a one-time nutritional education, while there is a lot of previous studies which indicated beneficial effects of long-term nutrition

education in the group of children and adolescents [12, 18, 19, 35]. For instance, in the study ABC Healthy Eating Program, with participation of 11-12 years-old children, who took part in education program with lasted 15 hours, it was observed that after nutritional education (including nutrition, dietary, sensory-consumer, hygiene and culinary topics) children from the educational group had a significantly higher increase in nutrition knowledge score than the control group [12, 33]. Even more optimistic, another study of 160 12-year-olds confirmed that nutritional education can also influence children's nutritional behaviours. Comparing the pre-test and post-test, an increase in the frequency of consumption of the breakfasts, milk and its products, fish and fish products and vegetables was observed after 8 hours of nutritional education [19]. In another study in a group of younger children (4-7 years), after classes in the nutrition and physical activity of children and their mothers, the variety of food and fruit and vegetable consumption, habitual drinking of water and the quality score of packaged lunches increased, and the consumption of sweet drinks decreased [18].

Our findings confirmed that even though children know the importance of physical activity, screen time limits, as well as the importance of adequate nutrition in maintaining human health, much more important, is implementing that into practice which is not common in both groups of children and adults. In the study with the participation of 884 adolescents from schools from Upper Silesia (Poland), huge discrepancies were observed between nutrition knowledge and its practical application of it in daily life, especially in the frequency of fish and vegetable consumption as well as a proper number of meals [30]. Similarly, among high school children, it was found that despite the level of nutritional knowledge was assessed as high, in daily life participants did not follow the guidelines of adequate nutrition [39]. It is also worth noticing that only a small percentage of 11-15 years-old children and adolescents from Poland were moderate physically active, both moderate (17.2%), at the recommended level as well as most of the surveyed children (79%) spent more than 2 hours on watching TV or movies on the computer, according to the Health Behaviour of School-age Children (HBSC) report [21]. The above data confirmed the need for further research focused both on children's nutritional knowledge and nutritional behaviours.

In the present study, one-time nutritional education increased the knowledge of the role of milk and milk products between the first and third stages of this study. We observed also an increase between the second and third stages of the study in pupils of grade 3 (75% of respondents and 90% of respondents knew the role of milk products, respectively; data not shown). It may be

linked with the content of textbooks in third-grades, where dairy products were mentioned, in the context of the human skeleton (information on the beneficial effects of milk and dairy products the proper structure of bones and teeth; it was also specified that this is due to the presence of calcium and vitamin D). It could be the explanation of the improvement between the second and third stage of the study. However, knowledge of the role of fermented milk products was not enough, and nutritional education in this topic caused only short-term effect.

In our study over 20% of children at the baseline were not aware of how much milk products should be eaten every day, as well as did not know the water requirements. The level of knowledge in this field slightly increased after nutritional education. Probably, for children who answered incorrectly, the answer 3-4 cups a day of milk products as well as 5-6 glasses of water could seem like a huge amount, while it might have been better to present it as pictures of portions, for a better understanding of the question. In contrast to the present study, at baseline much less adolescents from Poland (1/4 of them) knew how much milk and milk-based products should be eaten daily, while after nutritional education (lecture and presentation) 80% of adolescents were aware of that [34]. The above questions checked if children understand the dietary recommendations. In previous studies it was observed that understanding and legibility of recommendations in the form of Food Pyramid is insufficient, even in older age groups [9, 38]. Moreover, it was found that children aged 10-11 years were rather poorly recognizing the portion size [9]. In another study with primary school and junior high school pupils' participation, the poorest awareness was also observed in the field of food groups [23]. Therefore, based on previous studies and own findings, it seems necessary to constantly adapt the forms of conveying nutritional recommendations to children, for better understanding and, as a result, using them in everyday life.

CONCLUSIONS

This study shows that even a one-time nutritional education, including culinary workshops and various methods of activating children (discussion, guesswork, singing), can give measurable and long-lasting effects, in the form of improvement of children's nutritional knowledge level. The subsequent activities at the same educational stage should broaden issues such as: composing appropriate meals, eating breakfast, nutritional value of dairy products, the role of water in children's health, sources of sugar and fat in the diet. School health promotion activities, including nutritional education, should be more attractive to the young audience and focus on improving nutritional

literacy. Further research should focus on the effectiveness of nutrition education, both single and multiple, especially when combined with nutritional changes (the ability to apply knowledge in practice).

Conflict of interests

The authors declare no conflict of interest.

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NUTRITION HABITS AND FREQUENCY OF CONSUMPTION OF SELECTED FOOD PRODUCTS BY THE RESIDENTS OF URBAN AND RURAL AREA FROM THE SUBCARPATHIAN VOIVODESHIP

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ABSTRACT

Background. The dynamic development and change of rural lifestyle patterns towards urban patterns has been noticeable since the second half of the 20th century. Nutrition, as well as the lifestyle in urban and rural areas, has undergone a process of urbanization, which is evidenced by the following an increasing number of overweight people.

Objective. The aim of the study was to compare eating habits and frequency of consumption of selected groups of products between the urban and rural residents from the Subcarpathian voivodeship.

Material and methods. The survey was carried out on a sample of 279 persons from urban and rural areas of Subcarpathian voivodeship. In this study, the diagnostic survey method consisting of 3 (different) parts was used. To check the significance of differences, U *Mann-Whitney* and *Kruskal-Wallis* tests were used; whereas correlations between variables were determined by the *Spearman's* rank of correlation coefficient and *Pearson's* chi-quadrat test.

Results. In the part concerning eating habits, statistically significant differences occurred among others; for the circumstances of eating meals, the most commonly consumed snacks, food processing techniques and the most frequently selected fat. In the part concerning the frequency of consumption of individual product groups, significant differences occurred among others; for dairy products, grain products, fast food, alcohol. There was a correlation between the frequency of consumption of individual products and socioeconomic status.

Conclusions. The quality of the diet and eating habits of urban and rural residents of Subcarpathian voivodeship does not differ significantly. The detected differences in eating habits were more influenced by the education and socioeconomic status of the examined group than the place of residence.

Key words: eating habits, food consumption frequency, socio-demographic conditions, village, city, adults

STRESZCZENIE

Wprowadzenie. Dynamiczny rozwój i zmiana wiejskich wzorców stylu życia w kierunku miejskich wzorców zauważalne są już od drugiej połowy XX wieku. Sposób odżywiania podobnie jak styl życia na obszarach miejskich i wiejskich uległ procesowi urbanizacji czego dowodem jest m.in. zwiększająca się liczba osób z nadmierną masą ciała.

Cel. Celem badania było porównanie nawyków żywieniowych i częstotliwości spożycia wybranych grup produktów między ludnością miejską i wiejską z obszaru województwa podkarpackiego.

Material i metody. Badanie zostało przeprowadzone na próbie 279 osób z terenów miejskich i wiejskich województwa podkarpackiego. W pracy wykorzystano metodę sondażu diagnostycznego, której narzędziem była ankieta składająca się z 3 części. Do sprawdzenia istotności różnic użyto testów U *Manna-Whitneya* i *Kruskala-Wallisa*, korelacje między zmiennymi wyznaczono testami istotności współczynnika korelacji rang *Spearmana* oraz test chi-kwadrat *Pearsona*.

Wyniki. W części dotyczącej nawyków żywieniowych istotne statystycznie różnice wystąpiły m.in. dla okoliczności spożywania posiłków, najczęściej spożywanych przekąsek, technik obróbki potraw a także najczęściej wybieranego tłuszczu. W części dotyczącej częstotliwości spożycia poszczególnych grup produktów istotne różnice wystąpiły m.in. dla produktów mlecznych, zbożowych, dań typu fast food, alkoholu. Wykazano korelację pomiędzy częstością spożycia poszczególnych produktów a statusem społeczno-ekonomicznym.

Wnioski. Jakość diety i nawyki żywieniowe ludności zamieszkującej tereny miejskie i wiejskie województwa Podkarpackiego nie różnią się znacznie między sobą. Na wykryte różnice w sposobie odżywiania większy wpływ od miejsca zamieszkania miały wykształcenie i status socjoekonomiczny badanych osób.

Słowa kluczowe: nawyki żywieniowe, częstość spożycia produktów spożywczych, uwarunkowania socjodemograficzne, wieś, miasto, osoby dorosłe

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INTRODUCTION

The progress of civilization, which is noticeable today, was already visible in the second half of the 20th century. At that time, there was a transition from a traditional lifestyle to a modern model [23]. Research conducted among the rural population between 2000 and 2016, presented the changes in culture, leisure, recreation and modernization of homes that took place in these areas [7]. The progressive urbanization process in Polish villages and urban expansion are key factors affecting the quality and style of life [25].

The traditional diet of the rural population, characterized so far by a low intake of highly processed products and a high intake of natural products, is changing and acquiring more and more characteristics of an unhealthy diet, conducive to the development of overweight and obesity and civilization diseases. The diet of city dwellers quite rapidly diverged from that of country dwellers through industrialization, development of trade and catering in its broadest sense. This dietary pattern is characterized by a reduced intake of whole grains and legumes, and an increased intake of animal products, sugar, salt, fats and processed foods. The traditional way of eating in villages is approaching such a dietary pattern and the deterioration of health and increasing number of obese people in villages is the evidence of this [13].

Studies focusing on the progress of civilization and the accompanying changes in nutritional issues provide information within the nutritional transition with respect to place of residence. Low- and middle-income countries, as a result of the urbanization process, are characterized by an increasing consumption of processed foods and ready-to-eat meals. The development of cities and villages promotes more frequent eating out, which may result in a change in the regularity of meals and less control over calorie supply in the diet. Food consumption patterns in rural and urban areas may indirectly affect body mass index (BMI) and obesity prevalence [11, 21].

The differences in the quality of the diet of the rural and urban populations a few or a dozen years ago were clearly visible at the time. However, with the progress of civilization of both areas, the nature of the diet may change in a similar direction with similar dynamics. Unfortunately, in the literature there are few studies conducted among adults of both groups, taking into account dietary habits and the frequency of consumption of selected products, which allow to identify and justify possible differences in the diet.

In research, health inequalities in rural-urban status are observed, which may result in a higher prevalence of obesity among rural residents which may be caused by inappropriate dietary habits and frequency of consumption of specific food products.

Among the factors that may influence the prevalence of obesity among both populations, the study focused on dietary factors that may determine these issues [9].

The aim of the study was to compare dietary habits and frequency of consumption of selected groups of food products between inhabitants of cities and rural areas of Subcarpathian voivodeship.

MATERIAL AND METHODS

The study was conducted by trained members of the research team in April 2019, on a sample of 279 respondents in the age range 25-65 years (rural=160, urban=119), living in urban and rural areas of the Subcarpathian voivodeship. Information about the opportunity to participate in the survey was provided to residents through posters and flyers posted on information boards. The content of the announcements included basic information about the location and conduct of the study. With the approval of the management, the surveys were conducted in shopping malls and community centres, in specially designated areas. In the course of the study, the method of diagnostic survey with the use of questionnaire technique was applied. The research tool was a questionnaire consisting of 3 parts. Part I included questions on personal information, Part II was the author's questions on dietary habits and Part III included the validated FFQ-6 food frequency questionnaire.

The FFQ-6 questionnaire is an intermediate-level tool for obtaining data on the frequency of consumption of 62 assorted food groups, representing 8 food groups consumed in the past 12 months. Respondents can choose from 6 food consumption frequency categories: (1) never or almost never, (2) once a month or less often, (3) several times a month, (4) several times a week, (5) daily, (6) several times a day. Participation in the study was voluntary and anonymous.

Product consumption frequency categories, as qualitative data, were assigned ranks using conventional integers ranging from 1 to 6. The ranks assigned reflected the increasing consumption of the products (Table 1).

Table 1. Ranks assigned to frequency categories of consumption of products

Consumption frequency categories	Ranks assigned to frequency categories
Never or hardly ever	1
Once a month or less	2
Several times a month	3
Several times a week	4
Once a day / Daily	5
Several times a day	6

Qualitative variables (nominal and ordinal) were described by counts (n) and frequencies (%). Measurable variables were described by basic parameters: arithmetic mean, standard deviation, median and minimum and maximum value.

Nonparametric tests were used for statistical analysis. *Mann-Whitney U* test was used to test the significance of differences in the level of the measurable variable in two groups, *Kruskal-Wallis* test was used in at least three groups. Correlations between variables described at least on an ordinal scale were determined by *Spearman's* rank correlation coefficient significance test. Relationships between qualitative variables were examined by *Pearson's* chi-square and NW test of independence. A significance test of the difference between the two structure indices was used to compare the proportion of responses in the two groups.

The value of $p < 0.05$ was considered statistically significant. Statistical calculations were performed using the STATISTICA 10 PL statistical package.

RESULTS

Characteristics of the study group

Among the total number of subjects, women constituted 69.5% (194) and men 30.5% (85). Among the studied women, 60.3% (117) was rural dwellers and 39.7% (77) was urban women. Among the male respondents, 50.6% (43) was rural residents and 49.4% (42) was urban residents. The medium age in the study sample was 42 years (SD = 11.4). The medium age of women was 42.6 years (SD = 11), while among men this value was 40.8 (SD = 12.1). More than a half of the sample (52%) declared having higher education, 28% of respondents had secondary education, 15.8% had vocational education and 4.3% had primary education. Net income per family member varied greatly; in a small percentage of respondents (3.6%) it did not exceed 500 PLN, in 29.7% of people it was within the range of 500-1000 PLN, in 35.5% it reached from 1000 to 2000 PLN, while in 31.2% it was higher than 2000 PLN. Only a small proportion of those surveyed (5.7%) were receiving social security benefits. 81.4% (227) of working people participated in the survey.

Food habits

The second part of the questionnaire on eating habits included the times of eating the first and last meal, the circumstances of eating meals, the most frequently consumed snacks, the predominant food processing techniques, the most common fat product added to salads and the most frequently chosen fat for frying.

Residents of urban and rural areas consumed an average of 4 meals per day. A statistically significant

relationship occurred when the first meal was eaten. Urban residents (16%) were more likely than rural residents (10%) to eat a meal immediately after waking up or half an hour after waking up. However, rural residents (43.1%) were more likely to eat their first meal 1-2 hours after waking up than urban residents (31.9%).

Eating meals with the family at the table in the case of inhabitants of cities amounted to 43.7% and in the case of inhabitants of rural areas to 60.6%. Eating alone was indicated by 18.5% of urban residents and 13.8% of rural residents. Eating meals alone in front of a computer/television was declared by 19.3% of respondents from urban areas and 5% of respondents from rural areas. Additionally, there is a statistically significant correlation between the residence of the respondents and the circumstances of eating. Urban residents were more likely than rural residents to eat alone and in front of a computer/television. Rural residents were more likely than urban residents to eat meals with family around the table.

The most commonly consumed snacks by both urban (49.6%) and rural residents (51.9%) were fruit. On the other hand, the least frequently consumed snacks by urban residents (4.2%) and rural residents (3.1%) were vegetables. Consumption of salty snacks was declared by 14.3% of urban and 13.8% of rural residents. Snacking on sweets in those living in urban areas was reported at 23.5% and in those living in rural areas at 24.4%. The frequency of use of culinary techniques is shown in Figure 1.

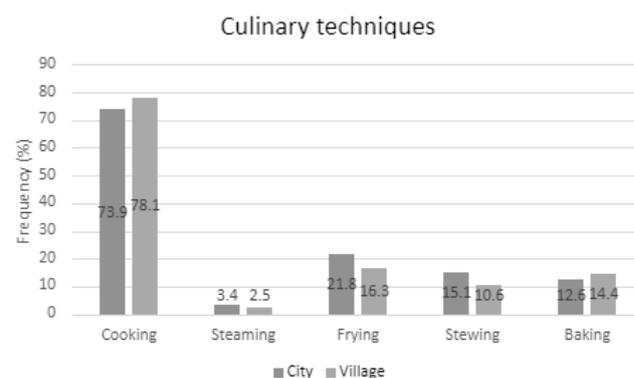


Figure 1. Place of residence of the persons surveyed and frequency of use of culinary techniques

The frequency of use of additions to salads is shown in Figure 2.

A statistically significant relationship exists between the place of residence of the respondents and the most frequently used frying fat. Urban residents (15.1%) were more likely to use olive oil than rural residents (3.8%). Rural residents were more likely to choose rapeseed oil and lard than urban residents. Rapeseed oil was chosen by 53.8% of urban residents and 68.1% of rural residents. The consumption of lard

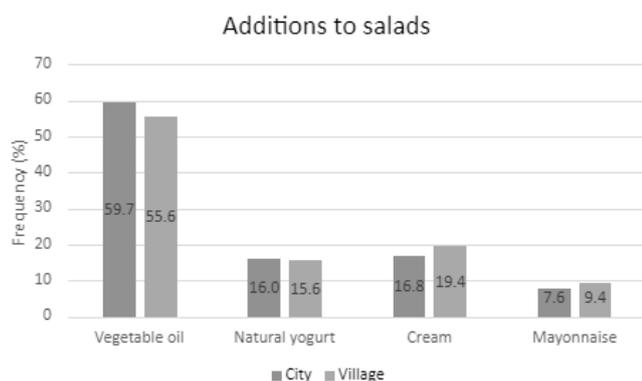


Figure 2. Place of residence of the persons surveyed and frequency of use of additions to salads

was 3.4% in people living in urban areas and 6.9% in people living in rural areas. The butter was indicated by 5.0% of urban residents and 1.3% of rural residents.

Frequency of food consumption

Between the place of residence and the frequency of consumption of the different product groups,

statistically significant differences occurred for: chocolate and chocolate products, dairy products, cereal products, groats, bananas, avocados, potatoes, nuts, cold cuts, canned fish, fast food dishes, powdered soups, fruit juices, total alcohol, beer and wine and drinks (Table 2).

Socio-economic status and frequency of consumption

The survey questions also include questions about net income per family member and education earned. The results are shown in Figure 3 and Figure 4.

An important correlation in the frequency of consumption of individual product groups by villagers and town residents was detected in the level of earnings and education of the subjects.

Statistically significant correlations between respondents' earnings and frequency of consumption occurred for white bread and potatoes. The higher the net income per family member, the lower the rate of consumption of these products by the subjects.

The level of education of the persons and the frequency of food consumption is shown in Table 3.

Table 2. Frequency of consumption of selected products (on a scale of 1-6) in persons living in urban and rural areas

No.	Products	Place of residence								U Mann-Whitney test	
		City n=119				Village n=160					
		M	SD	Min.	Max.	M	SD	Min.	Max.	Z	p
1.	Sweets and snacks (in total)	3.66	0.85	1	6	3.53	0.93	1	6	1.03	0.3026
2.	Sugar for sweetening	3.25	2.08	1	6	3.74	1.84	1	6	-1.68	0.0936
3.	Honey for dishes and sweetening	2.74	1.30	1	6	2.51	1.31	1	6	1.60	0.1102
4.	Chocolate products	3.38	1.02	1	6	3.07	1.09	1	5	2.35	0.0190*
5.	Dairy products (in total)	4.60	0.84	1	6	4.44	0.82	1	6	1.96	0.0499*
6.	Fermented dairy products	3.44	1.25	1	6	3.52	1.24	1	6	-0.21	0.8350
7.	Eggs and egg dishes	3.64	0.92	1	6	3.48	0.88	1	6	1.72	0.0863
8.	Natural cottage cheese	3.61	1.10	1	6	3.61	1.01	1	6	0.45	0.6501
9.	Cereal products (in total)	4.56	0.73	1	6	4.83	0.63	1	6	-3.01	0.0026*
10.	Wholemeal bread	3.87	1.18	1	6	3.81	1.37	1	6	0.04	0.9679
11.	White bread	3.45	1.29	1	6	3.68	1.38	1	6	-1.58	0.1137
12.	Groats (in total)	3.44	0.92	1	5	3.11	0.77	1	5	3.65	0.0003*
13.	Fats and oils (in total)	4.49	0.85	1	6	4.44	0.85	1	6	0.46	0.6425
14.	Oil	3.77	0.94	1	6	3.71	0.84	2	6	0.74	0.4566
15.	Butter	3.99	1.31	1	6	3.93	1.33	1	6	0.40	0.6917
16.	Cream	2.89	1.25	1	6	3.16	1.04	1	5	-1.67	0.0951
17.	Other animal fats	1.86	1.11	1	6	1.91	0.97	1	5	-1.01	0.3121
18.	Fruits (in total)	4.50	0.86	2	6	4.40	0.90	2	6	0.63	0.5290
19.	Stone fruits	3.14	0.95	1	5	3.13	1.11	1	6	0.39	0.7000
20.	Kiwi and citrus	3.40	0.88	1	6	3.39	1.06	1	6	0.02	0.9830
21.	Berry fruits	2.71	0.82	1	6	2.79	0.98	1	6	-0.40	0.6875
22.	Bananas	3.61	0.98	1	6	3.23	1.00	1	6	3.07	0.0022*
23.	Apples and pears	3.98	1.00	1	6	3.80	0.99	1	6	1.39	0.1653
24.	Avokado	1.86	0.99	1	5	1.61	0.84	1	5	2.14	0.0327*
25.	Vegetables (in total)	4.25	0.89	2	6	4.37	0.84	2	6	-1.24	0.2160
26.	Legume seeds	2.70	1.03	1	6	2.68	0.89	1	6	0.05	0.9624

27.	Potatoes	3.61	0.95	1	6	3.93	0.84	1	6	-2.91	0.0036*
28.	Nuts	2.95	1.12	1	5	2.65	1.02	1	6	2.27	0.0230*
29.	Meat (in total)	4.02	0.79	1	6	4.17	0.69	1	6	-1.86	0.0627
30.	Processed meats (in total)	2.51	0.96	1	6	2.56	0.83	1	5	-0.49	0.6243
31.	Cold cuts	3.61	1.15	1	6	3.91	0.95	1	6	-2.23	0.0255*
32.	Red meat	3.14	1.00	1	5	3.08	0.94	1	5	0.90	0.3696
33.	Poultry and rabbit meat	3.49	0.85	1	5	3.37	0.77	1	5	1.74	0.0812
34.	Fish (fatty and lean)	2.79	0.72	1	4	2.78	0.72	1	4	0.02	0.9861
35.	Canned fish	2.06	0.90	1	4	2.24	0.83	1	4	-2.06	0.0393*
36.	Fast food meals	2.23	0.86	1	6	1.91	0.83	1	4	3.05	0.0023*
37.	Ready to eat meals	1.72	0.89	1	6	1.60	0.78	1	5	1.03	0.3020
38.	Powdered soups	1.35	0.72	1	6	1.19	0.46	1	3	2.19	0.0286*
39.	Fruit juice	2.98	1.11	1	6	2.67	1.17	1	6	2.17	0.0297*
40.	Fruit nectars	2.20	1.05	1	6	2.06	1.15	1	5	1.41	0.1571
41.	Vegetable juices	2.10	1.20	1	6	1.88	1.04	1	5	1.42	0.1545
42.	Energy drinks	1.46	1.01	1	6	1.27	0.67	1	4	1.72	0.0853
43.	Alcohol (in total)	2.61	1.02	1	6	2.30	1.04	1	5	2.52	0.0117*
44.	Beer	2.32	1.06	1	6	1.97	1.05	1	5	2.94	0.0033*
45.	Wine and drinks	2.21	0.94	1	6	1.97	0.93	1	5	2.22	0.0263*
46.	Vodka and spirits	1.86	0.81	1	4	1.74	0.86	1	5	1.41	0.1578

M – mean, SD – standard deviation, Z – value of *Mann-Whitney* U test for large groups, p – the test probability level
* – statistically significant, $p < 0.05$

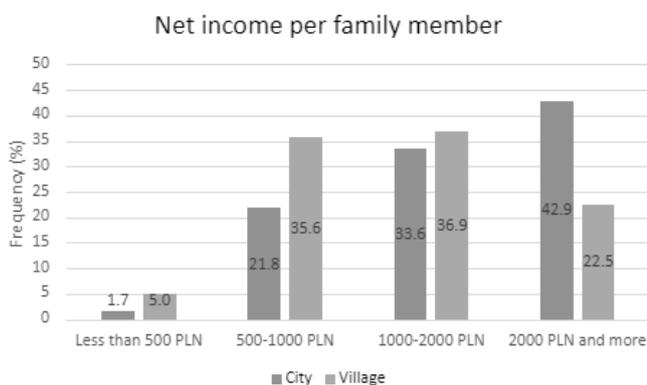


Figure 3. Place of residence of the persons surveyed and net income per family member

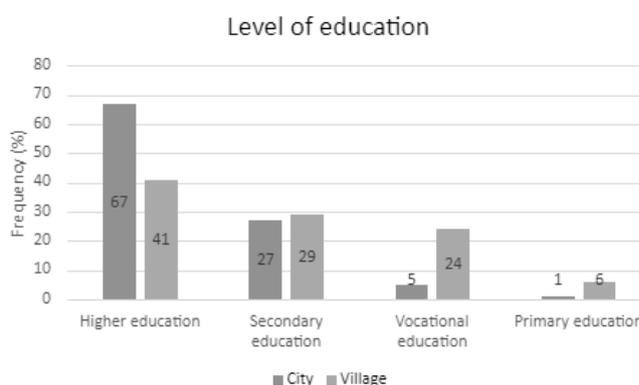


Figure 4. Place of residence of the persons surveyed and level of education

Table 3. Level of education and frequency of consumption of white bread, total processed meats, total vegetables, total fruit, total alcohol, lean fish and oily fish

Education and frequency of consumption of selected products	n	Rs	t(n-2)	p
White bread	279	-0.152	-2.56	0.0111*
Processed meat	279	-0.118	-1.98	0.0484*
Vegetables	279	0.103	1.72	0.0872
Fruits	279	0.108	1.82	0.0705
Alcohol	279	0.134	2.25	0.0252*
Lean fish	279	0.128	2.15	0.0327*
Oily fish	279	0.120	2.02	0.0448*

Education described as: primary, vocational, secondary, higher education

Rs – Value of R *Sperman's* coefficient for abundance n, t(n-2) – value of the t-statistic that checks the significance of the Rs factor for the number of degrees of freedom n-2, n – number of subjects surveyed, p – test probability level

* – statistically significant, $p < 0.05$

DISCUSSION

In the available literature there is very little scientific research carried out with adults, analysing their eating habits and frequency of consumption with the separation of urban and rural areas in Poland.

A 2018 study by the Statistics Poland (GUS) on the average monthly consumption of certain foods is an auxiliary reference element for the frequency of food intake shown in our study [3].

The first group was total cereal products and potatoes, which, as the results of our research showed, statistically is more consumed by the rural population. In the data submitted by the GUS, the urban population consumed 5.17 kg of bread and cereal products and 2.63 kg of potatoes, while the rural population consumed 5.96 kg of bread and cereal products and 3.52 kg of potatoes, which may confirm the results of our research.

There was no difference in sugar intake and in the GUS data the difference was 0.76 kg for the city and 1.21 kg for the village. The results of our study show a higher consumption of chocolate and chocolate products among city dwellers, while in the data presented by the GUS the results are similar: 0.20 kg city and 0.18 kg village. However, given the entire pool of products that is a source of simple sugars, the GUS data show the difference in consumption of these products (1.63 kg for the city and 1.96 kg for the countryside), which was not shown in our study.

Consumption of dairy products in our study turned out to be significantly higher for urban residents. According to the GUS data, yoghurts and cheese and cottage cheese (0.52 and 0.45 and 0.87 and 0.76 kg, in turn) had a slightly higher share of the diet of city dwellers (0.52 and 0.45 kg, in turn), while milk and cream contributed more to the diet of the villagers (3.25 and 2.74 and 0.37 and 0.34 kg). The difference in consumption of vegetables between the inhabitants of towns and villages was not significant. For GUS data it is also not very different, as for the city it was 3.79 kg and for the village it was 3.85 kg. There were no differences in consumption of fruit between villagers and city dwellers. According to GUS data, consumption of fruit in the city was 4.03 kg and in the countryside 3.32 kg. Consumption of meat did not differ significantly statistically. In the GUS study, urban consumption of meat was 4.93 kg and for rural residents it was 5.63 kg.

The data of the GUS mostly coincide with the correlations found in our survey and may constitute a certain indicator in the evaluation of the results of such studies, however, the average statistical consumption is not an ideal comparison to the data of the questionnaire frequency of consumption. However, in the absence of up-to-date studies comparing product

consumption among adults living in the countryside and in the city, such data might be helpful in analysing the study [3].

Another issue examined was the consumption of snacks between meals among urban and rural residents. The study by *Maruapula* et al. show that young people living in urban areas consumed significantly more snack servings and fewer servings of traditional foods compared to those living in rural areas. According to the authors, this may be due to the greater availability of outlets where snacks can be purchased in relation to rural areas [17]. When addressing consumption of fruit, our study did not show differences in consumption between residents of both areas. On the other hand, the analysis of the diet of rural and urban residents in a study conducted by *Utzig* shows a higher consumption of fruit among urban dwellers relative to the countryside [27]. *Cipora* et al. study among middle schoolers living in rural areas also confirms the frequency of consumption of fruit, which was eaten daily by almost half of those surveyed [8].

In the study *Calyniuk* et al. potatoes, in both the city and the countryside, were shown to be the most common addition to lunch, while our studies showed that potatoes were more often consumed by rural residents. A similar situation applies to the consumption of cold cuts. Our research indicates that these products were more often chosen by rural residents, while in this study they were the most popular sandwich product among both rural and urban residents. In addition, the *Calyniuk* et al. study raises the issue of eating the first breakfast among children, which reported that children from rural areas were more likely to eat breakfast, while our research has shown that city dwellers were more likely than rural residents to eat their first meal as soon as they woke up. In addition, the *Calyniuk* et al. study raises the issue of eating the first breakfast among children, which reported that children from rural areas were more likely to eat breakfast, while our research has shown that city dwellers were more likely than rural residents to eat their first meal as soon as they woke up [5]. *Karamnova* et al. study also touches on the issue of the consumption of cold cuts, which turned out to be higher among the inhabitants of the village. In addition, the results of the study show that the rural diet model was characterized by higher consumption of salt, sugar and butter. No such relationship was detected in our study [14].

When it comes to eating sweets in our study, snacking on products from this group in rural and urban residents was 24.4% and 23.5%. In the absence of sufficient studies on the consumption of sweets by adults living in rural areas, the *Cipora* et al. study can serve as a reference on the consumption of sweets

by middle schoolers living in rural areas. 22.6% of respondents declared daily consumption of sweets [8]. By contrast, in the *Suliburska et al.* study on the lifestyle and quality of diet of young adults from urban and rural areas, increased consumption of sweets among urban dwellers has been demonstrated in relation to rural residents [24].

The data from our study show a higher consumption of fast food by city dwellers than rural residents. *Cipora et al.* [8] also recorded a 53.0% intake of this type among rural middle schoolers for consumption less than once a week and 5.2% for daily consumption. These results suggest that rural residents relatively rarely consume fast food. Similar results are shown by the *Suliburska et al.* [24] study which also has an increased consumption of fast food among urban residents.

A study by *Chęcińska et al.* [6], which assessed the diet of urban and rural youth, showed that dairy products and groats were more likely to be consumed by city dwellers than rural residents. In rural areas, however, white bread was more often consumed. The results of this study confirm the results of our research. This study can only serve as a marginal comparative material due to the small amount of research among adults living in urban and rural areas. *Akaichi* [1], which shows an increased consumption of products such as fresh milk, milk powder, butter, margarine, yoghurts in urban and rural areas, also raises the issue of consumption of dairy products among the inhabitants of villages and cities.

Aziz and Malik's study attempts to analyse household consumption patterns in Pakistan between rural and urban households. In the study, consumption of cereal products was similar in both urban and rural areas and showed no correlation with socioeconomic status, as shown in our study. The authors also show that spending on vegetables and meat increased as income increased in rural areas. In the case of spending on fruits and milk and dairy products, higher income correlated with higher consumption among urban residents. Similar results were obtained in our study [2].

Martin et al. study carried out among women of childbearing potential living in urban

and rural areas show slight differences between the two populations in terms of eating habits and diet. Women in rural areas were found to have a slightly higher share of protein in their diet, fat, monounsaturated fats and cholesterol, iron and a higher share of meats in their diet than the women from urban areas. This study did not show significant differences in the frequency of consumption of these products in addition to cold cuts, which can be classified as both a source of protein and meat in the diet. *Martin et al.* also showed that higher annual

household income correlated with a higher diet value in all women. These results coincide with data from our survey, which show that higher respondents' income was linked to lower consumption of white bread and potatoes, products whose high consumption negatively affects the quality of eating habits [16].

Foreign literature also addresses the problem of differences in the nature and quality of diet of urban and rural residents. *Dean and Sharkey* note that for rural areas, the distance between the residence and the retail food outlet affects the frequency of fresh fruit and vegetable consumption. The greater the distance from the place of residence to a food retailer, the less frequently did rural residents consume fresh fruit and vegetables [12]. A study by *Mennen et al.* that assessed the habitual diet of rural and urban populations in Cameroon, Central Africa detected higher intakes of energy, fat and alcohol in rural men and women relative to those living in urban areas. Higher carbohydrate and protein intakes were also statistically significant for women living in rural areas [18]. In the study by *Colić-Barić et al.* [10] a higher consumption of fast-food products, soft drinks and alcohol was observed among urban residents compared to rural residents. The study by *Liebman et al.* conducted among rural populations in Wyoming, Montana and Idaho shows an association of excessive body weight with increased consumption of sweetened beverages and eating while watching television [15].

The level of food consumption, the quality and type of products selected are closely correlated with socioeconomic factors. Among the criteria of socioeconomic status, the authors of the study included education (primary, vocational, secondary, higher) and net income per family member (less than 500 PLN, PLN 500-1000 PLN, 1000-2000 PLN, 2000 PLN and more).

In our study, there were significant correlations between the education of the subjects and the frequency of consumption of the selected groups of products. Higher education respondents were much more likely to choose products such as fish and alcohol than those with lower levels of education. In contrast, people with lower education were more likely to consume white bread and processed meat. Significant results showed correlations between the level of earnings and the frequency of food consumed. Subjects with a higher income per family member were less likely to choose white bread and potatoes compared to people on lower incomes. The results obtained correlate well with the results of the *Niedźwiedzka et al.* study on the diversified consumption of food in the context of the socio-economic status of Polish elderly people. The higher socio-economic status of the subjects, i. e. higher education and high monthly income, increased food intake. These people were more

likely to consume products with beneficial health m. in fruits and vegetables, fish and fruit and vegetable juices [20]. Similar results were obtained in another study on the dietary styles of Poles. Respondents with higher education represented a “health-promoting style”, which included frequent consumption of raw vegetables and fruits, fish, wholemeal bread and reduced meat consumption. The level of earnings was not statistically significant [4].

The data of the GUS also provides information on the quantity of consumption of the products concerned in relation to the level of education. The results of our tests show the relationship between consumption of white bread, processed meat, alcohol and fish and education. It turned out that people with higher education were less likely to consume these types of products. The data from the GUS study did not separate bread into wholemeal and white, but there is a higher consumption of this product among people with lower education, which amounted to 3.10 kg for people with secondary education, 3.40 kg for people with vocational education and 3.90 kg for people with primary education. While, for those with higher education, it was 2.61 kg. For fish and seafood, consumption is represented at a similar level of 0.28 kg irrespective of the education of the test subjects. Consumption of processed meat shall be confirmed by the results of our tests. As the level of education declared by respondents increased, the amount of cold cuts and meat preparations they consumed decreased. They were: for people with a higher education of 1.64 kg, a secondary education of 2 kg, a vocational education of 2.17 kg and a primary education of 2.41 kg [3].

In the cross-sectional study *Mitra et al.* among 12000 people over the age of 35, 58 districts of Bangladesh showed that some socioeconomic factors correlated with the nutrition status of the study group. The authors of the study observed a positive correlation in overweight among the people in higher affluence, while negative for underweight. *Mitra et al.* showed no significant link between the state of nutrition and the level of education. On the other hand, the level of education may influence the dietary practice determining the quality of the diet, as demonstrated in our study [19].

The relationship of demographic and socioeconomic factors, and the glycemic load of the diet of adult Poles, was presented in the study of *Róžańska et al.* The authors observed a higher glycemic load of meals in the diet of rural residents and in people with lower education. A lower glycemic load of meals was recorded for city dwellers and those with higher education. Products and dishes with a higher glycemic load are a.o. boiled potatoes, white rice, white bread and pasta. In our study, a correlation was detected

between the increased consumption of cereal products and potatoes among the rural residents, which may confirm the results of the above-mentioned studies. In terms of education, our study showed that people with higher education were less likely to eat white bread, which may partly confirm the results of other researchers [22].

These studies and the results of our study may prove that the socioeconomic status of urban and rural residents and the place of residence may be linked to the quality of the diet and eating habits. Accessibility to health services in rural areas may also be important in terms of nutritional behaviour. A study conducted in rural areas in Poland on the availability of healthcare indicates a significant discrepancy in the level of availability of medical care in rural areas. Of the 16 provinces, the best access to health care was indicated in the Silesian and Subcarpathian voivodeship, while the smallest access was shown in the West Pomeranian, Warmian-Masurian and Pomeranian voivodeships. The authors conclude that access to health care is limited in 9 provinces, while in the remaining 7, access was good or very good [26].

The results of own study confirm that nowadays the quality of diet and eating habits of the population living in urban and rural areas do not differ significantly from one another. A significant impact on the frequency of consumption of individual product groups by the subjects of urban and rural areas had a level of income and their education. Also important were the circumstances of eating meals, from which it appears that rural residents were more likely than city dwellers to eat with their families at the table, indicating the traditional approach of the rural population in this area. Among certain groups of food products, significant differences were noted for the frequency of consumption depending on the place of residence of the subjects. The frequency of food consumption among the rural residents among others is higher for cereal products and cold cuts. By contrast, people living in urban areas had a higher consumption among other things for certain fruits, fast food and selected alcoholic beverages.

The correlations found in this study, regarding food intake, eating habits and socioeconomic factors, are only a slice of the diet characteristics of rural and urban populations, due to the pilot nature of the study. In the future, it is planned to repeat the research on more people and in more towns and villages, which will allow for a broader analysis of the detected compounds.

The present study was able to document differences and similarities in diet quality and eating habits between urban and rural sub-county populations. The strengths of the study were: weight and height in this study was directly measured by a team of healthcare

professionals who have a wide range of experience in collecting health information, this factor could mitigate the potential bias of recall information. There are also a number of potential limitations of the study that need to be taken into account when interpreting the results. This study was limited in geographic scope and should be replicated among a larger sample across more regions. Another limitation was the uneven gender distribution of the respondents due to the greater interest of women in participating in the study compared to men. Response bias, such as social desirability, is common in self-reported questionnaires, and might have led to underestimation or overestimation of the present associations. The food frequency questionnaire used in our study that does not give the exact amount of food eaten by respondents. In the future studies, it is planned to use a 24-hour Recall questionnaire. Due to the limited number of studies in this area, the authors plan to conduct future studies with more people covering a larger area.

CONCLUSIONS

1. When analysing the data provided in the own survey, it can be concluded that the quality of the diet and the frequency of consumption of food products are not only linked to the place of residence, but also to the education and economic status of the subjects.
2. The results collected may indicate the need for nutrition education in both urban and rural areas.
3. There is a significant need for more research into eating habits and food intake among adults living in urban and rural areas, which will allow for a broader assessment and analysis of the changes in diet in both areas and in different age groups.

Conflict of interest

The authors declare no conflict of interest.

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AN EVALUATION OF THE NUTRITIONAL STATUS OF ELDERLY WITH THE USE OF THE MNA QUESTIONNAIRE AND DETERMINATION OF FACTORS CONTRIBUTING TO MALNUTRITION. A PILOT STUDY

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ABSTRACT

Background. In the elderly, nutrition significantly influences biological aging and physiological and pathological changes in the body. A balanced diet and physical activity are the key to good physical and mental health.

Objective. The aim of this study was: (1) to perform nutritional screening tests in senior citizens residing in eastern Poland and (2) to evaluate the risk of malnutrition in elderly people who live with their families, seniors who live independently, and residents of nursing homes.

Material and method. The Mini Nutritional Assessment (MNA) screening tool comprising a questionnaire with 22 questions and an abbreviated and survey-adapted version of the questionnaire (KomPAN) were used to investigate eating habits and self-perception of health and nutrition. A total of 398 correctly filled out questionnaires were selected and the resulting data were subjected to statistical analyses in the Statistica 10 program.

Results. The BMI values of most respondents were indicative of malnutrition or risk of malnutrition, regardless of gender (mean BMI 23.88 kg/m² ±5.08). Most overweight and obese respondents were female (p=0.0001). The observed decreased BMI values were significantly (p=0.0012, rs= 0.6714) correlated with lower food intake. Unintentional weight loss greater than 3 kg was most frequently noted in respondents living in nursing homes and living independently (p=0.0021). Eating difficulties also considerably influenced the respondents' nutritional status. The respondents' BMI values were significantly correlated (p<0.0001) with their MNA scores, the average MNA score was 21.0±4.4, (rs = 0.7293). Overweight and obese respondents were more likely to consume at least three meals daily (OR=1.87; 95% CI: 1.32-2.06; p<0.001).

Conclusions. The BMI values and MNA scores of the tested subjects indicate that the majority of the surveyed respondents were malnourished or at risk of malnutrition, regardless of gender. In the present study, the residential environment significantly influenced the patients' nutritional status, in particular in respondents with impaired motor abilities and eating difficulties.

Key words: malnutrition, MNA, unintentional weight loss, BMI, elderly people

STRESZCZENIE

Wprowadzenie. Sposób żywienia w wieku starszym wywiera istotny wpływ na biologiczne starzenie się oraz fizjologiczne i patologiczne zmiany organizmu. Prawidłowe odżywienie jest podstawą utrzymania zdrowia i kondycji fizycznej.

Cel. Celem pracy była 1) przesiewowa ocena stanu odżywienia osób starszych na terenie wschodniej Polski, 2) ocena czynników ryzyka występowania niedożywienia wśród osób mieszkających z rodzinami, samotnie lub w domach pomocy społecznej.

Material i metody. Badanie zostało przeprowadzone za pomocą ankiety opartej na kwestionariuszu Mini Nutritional Assessment (MNA) zawierającej metryczkę i 22 pytania oraz zmodyfikowanego kwestionariusza (KomPAN) do badania zwyczajów żywieniowych i sposobu żywienia. Uzyskano 398 prawidłowo wypełnionych kwestionariuszy, które poddano analizie w programie Statistica 10.

Wyniki. Zdecydowana większość badanych osób starszych bez względu na płeć cechowała się niedożywieniem lub miała ryzyko niedożywienia ocenione na podstawie wartości BMI (BMI średnie 23,88 kg/m² ±5,08). Wśród osób z nadwagą i otyłością statystycznie częściej występowały kobiety (p=0,0001). Spadek masy ciała był statystycznie istotnie związany z ograniczeniem przyjmowania posiłków u badanych (p=0,0012). Najczęściej niezamierzony spadek masy ciała powyżej 3 kg odnotowano u osób mieszkających samotnie lub w DPS (p=0,0021). Trudności w spożywaniu posiłków miały duży wpływ na stan odżywienia badanych. Zależności pomiędzy wartością BMI, a ilością punktów uzyskanych przez respondentów w teście MNA były istotne statystycznie (p<0,0001), wraz ze spadkiem wartości BMI występował spadek ilości punktów uzyskanych w teście MNA. Średnia ilość punktów, którą uzyskali respondenci w teście MNA wyniosła

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21,0±4,4. Osoby z nadmierną masą ciała istotnie częściej spożywały co najmniej 3 posiłki dziennie (OR=1,87; 95% CI: 1,32-2,06; $p<0,001$).

Wnioski. Zdecydowana większość badanych osób starszych bez względu na płeć cechowała się niedożywieniem lub miała ryzyko niedożywienia ocenione na podstawie wartości BMI i wyniku testu MNA. W przeprowadzonym badaniu środowisko zamieszkania miało istotny wpływ na stan odżywienia pacjentów, szczególnie w przypadku wystąpienia ograniczeń ruchowych lub zaburzeń w przyjmowaniu pokarmów.

Słowa kluczowe: *niedożywienie, MNA, niezamierzona utrata masy ciała, BMI, osoby starsze*

INTRODUCTION

The elderly population is growing rapidly around the world and is expected to increase from 739 million in 2009 to 2 billion in 2050 [33]. In the elderly, nutrition significantly influences biological aging and physiological and pathological changes in the body [19]. A balanced diet and physical activity are the key to good physical and mental health. Malnutrition decreases physical ability and quality of life, it prolongs hospitalization and contributes to higher mortality in the senior population [22, 29].

The elderly are particularly susceptible to malnutrition due to age-related changes in brain regulation of hunger and satiety, lower compensation for energy intake from food, changes in the gastrointestinal tract (lower metabolic rate) and weakening of the sense of smell and taste. Coexisting chronic diseases, dental problems, depression, impaired mobility or immobilization also contribute to malnutrition [3, 17]. The risk of malnutrition increases significantly in patients with cancer, gastrointestinal, cardiovascular, respiratory and neurological diseases [32].

Malnutrition in the elderly increases the risk of muscular atrophy and muscle weakness, it lowers psychomotor skills and bone mineral density, contributes to the risk of bone fracture, anemia, immune disorders, increases susceptibility to infections, respiratory disorders, water-electrolyte imbalance, orthostatic hypotension, bed sores, wound infections and prolongs healing of postoperative wounds [1, 3, 9]. Nutrition is often correlated with socioeconomic status. Older citizens with low socioeconomic status cannot afford wholesome food products [10]. Environmental factors also play a role in nutrition. Social isolation, loneliness and grieving contribute to malnutrition as well as protein, mineral and vitamin deficiencies [23]. Pharmacological treatment can also decrease appetite, promote nausea and impair intestinal absorption.

In geriatric patients, screening tests are recommended as the first step in diagnosing malnutrition. Simple, low-cost and rapid methods should be used to detect malnutrition in the elderly [12]. According to the European Society for Clinical Nutrition and Metabolism (ESPEN), nutritional status should be diagnosed with standard screening tools such as the Mini Nutrition Assessment (MNA), Nutrition Risk Screening 2002 (NRS) and the Malnutrition

Universal Screening Tool (MUST) [15, 16, 18]. Various screening tools are used in different countries. The Malnutrition Universal Screening Tool is a popular solution in Australia and the United Kingdom [14], whereas the NRS 2002 tool is more widely used in Western Europe [28]. In Poland, the MNA is one of the most popular nutrition screening tools. It involves a questionnaire for evaluating the risk of malnutrition in elderly patients in nursing homes, senior care centers and hospitals as well as in independently living seniors with normal albumin levels and BMI values. The MNA is a highly valuable tool which evaluates both physical and mental health. It accounts for the food intake, weight loss, mobility, psychological stress and acute physical illness in the three months preceding the test. Assessing malnutrition in older people with simple tools can be critical to early diagnosis and intervention. In addition, learning about the risk factors for malnutrition will help direct the attention of medical staff and nutritionists to the most at-risk groups. The obtained results will allow to find out the frequency of the problem and to develop a support strategy for the elderly at risk of malnutrition.

The aim of this study was to perform nutritional screening tests in senior citizens residing in eastern Poland and to evaluate the risk of malnutrition in elderly people who live with their families, seniors who live independently, and residents of nursing homes.

MATERIAL AND METHODS

Place and period of study

The study was carried out between March and June 2019 in the Lublin Region in eastern Poland.

Methods

The Mini Nutritional Assessment (MNA) screening tool comprising a questionnaire with 22 questions and an abbreviated and survey-adapted version of the questionnaire (KomPAN) were used to investigate eating habits and self-perception of health and nutrition. The inclusion criteria were age higher than 65 years, permanent residence in the Lublin Region and absence of mental or neurodegenerative diseases preventing direct contact and completion of the questionnaire. Persons with chronic and terminal illnesses were excluded from the study. The

participants gave their consent to participate in the study, were informed about its purpose and that the collected data would not make it possible to identify the participants. The questionnaire was filled out independently or with the assistance of a care worker by a total of 546 respondents. Anthropometric measurements were also performed. Participation in the study was voluntary and anonymous, and all respondents gave their consent to participate in the survey.

Four anthropometric assessments were taken by the surveyor while participants wore light clothing and were barefoot: height, weight, calf circumference (CC) and mid-arm circumference (MAC). Height was measured using a mechanical stadiometer; weight was measured with a digital weighing scale. The Body Mass Index was calculated as weight in kg / (height in m)². Calf circumference was measured on seated participants with an inextensible tape at several locations to find the maximal bare calf circumference. For mid-arm circumference, the participant's forearm was held in horizontal position to locate and mark the mid-distance between the acromial surface of the scapula and the olecranon process of the elbow.

The sum of the MNA score distinguishes between elderly patients with: 1) adequate nutritional status, MNA \geq 24; 2) protein-calorie malnutrition, MNA $<$ 17; 3) risk of malnutrition, MNA between 17 and 23.5. The obtained scores were characterized by 96% sensitivity, 98% specificity and 97% predictive value [16].

Statistical Analysis

A total of 398 correctly filled out questionnaires were selected, and the resulting data were subjected to mathematical and statistical analyses in the Statistica 10 program. Normality of the distribution of continuous variables was assessed with the *Shapiro-Wilk* test. For continuous variables, the data were presented as means with 95% confidence interval (95% CI) while categorical variables were presented as a sample percentage (%). The MNA tool was validated by calculating *Spearman's* rank correlation coefficients between the overall MNA score and each of the 18 items included in the MNA. BMI has been found to be a useful tool in clinical and public health practice for assessing the nutritional status of adults [6] and the elderly [8]. The prevalence of three nutritional categories—malnourishment, risk of malnourishment and normal nutritional status—were calculated to assess the respondents' nutritional status. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. The significance of ORs was assessed by *Wald's* statistics. The levels of statistical significance were set at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***)). The *Shapiro-Wilk* test

was used to test the normality of distributions. The average nutritional status of groups was compared by ANOVA, and frequency distributions were evaluated by *Pearson's* chi-squared (χ^2) test. Two-tailed p-values < 0.05 were considered statistically significant in all tests. Analyses were performed using Statistica software (version 13.1 PL; StatSoft Inc., Tulsa, OK, USA; StatSoft, Krakow, Poland).

RESULTS

The participants were 37.9% male and 62.1% female (Table 1) with a mean age of 70.6 years (minimum 65 years, maximum 88 years). Only urban residents lived in nursing homes, including 15 men and 24 women. Independently living seniors were more often female (19.0%) urban dwellers ($p=0.0023$), and only 8.6% of male respondents lived alone. Regardless of their place of residence, the majority of respondents lived with at least one family member, usually a spouse or children.

Table 1. Characteristic features of the surveyed population

Characteristic features	Number of respondents n (%)	p-Value
Female	247 (62.1)	<0.05
Male	151 (37.9)	
Place of residence		
Rural area	142 (35.7)	<0.05
Urban area	256 (64.3)	
Residential environment		
Living with family	299 (75.1)	<0.01
Living independently	60 (15.1)	
Living in a nursing home	39 (9.8)	
Chronic diseases		
Hypertension & cardiovascular diseases	109 (27.4)	<0.05
Diabetes	57 (14.3)	
Osteoporosis	29 (7.3)	
Asthma/allergy	21 (5.3)	
Other	182 (45.7)	
Nutritional status		
Overweight/obese	58 (14.6)	<0.01
Healthy weight	95 (23.9)	
Risk of malnutrition	247 (61.5)	

The respondents' nutritional status was evaluated based on a BMI scale designed specifically for the elderly. There are no evidence-based guidelines to assist clinicians in classifying elderly patients based on their BMI values. However, there is strong emerging evidence that BMI cut-offs may not be appropriate in seniors. Therefore, it is recommended that BMI

classifications are adjusted for people aged ≥ 65 years as follows: underweight ≤ 23.99 kg/m², healthy weight 24-29.99 kg/m² and overweight >30 kg/m² [5, 31].

The BMI values of most respondents were indicative of malnutrition or risk of malnutrition, regardless of gender (mean BMI 23.88 kg/m² \pm 5.08) (Figure 1). Most overweight and obese respondents were female ($p=0.0001$). The obtained scores were not differentiated by the respondents' place of residence or residential environment. A significantly ($p=0.0012$) higher number of respondents with healthy weight were urban dwellers who lived with their family or spouse. Gender was not a differentiating factor.

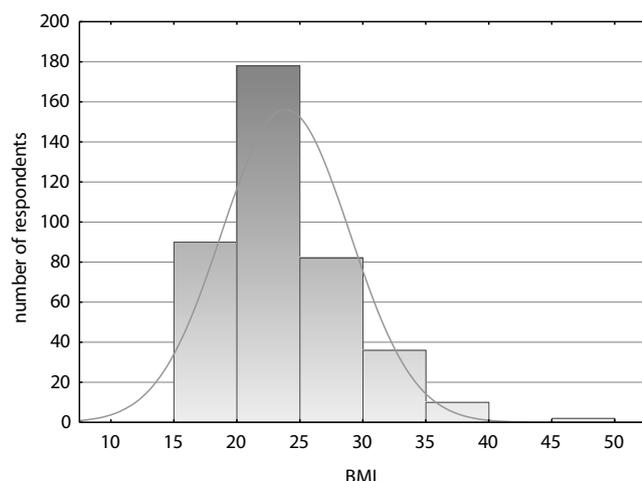


Figure 1. Evaluation of nutritional status based on BMI values, normal distribution

The respondents' BMI values were influenced by lifestyle and diet (Table 2). Patients whose food intake decreased significantly in the previous three months were characterized by a decrease in BMI values (mean BMI of 20.90 kg/m², $p<0.0001$). Respondents whose body weight decreased by more than 3 kg in the previous 3 months were also characterized by lower BMI (mean BMI of 22.25 kg/m², $p<0.0001$). The observed decreased BMI values was significantly ($p=0.0012$; $r_s = 0.6714$) correlated with lower food intake. Unintentional weight loss greater than 3 kg was most frequently noted in respondents living in nursing homes and living independently ($p=0.0021$). Eating difficulties also considerably influenced the respondents' nutritional status. Respondents who required assistance in eating were characterized by lower BMI (mean BMI of 20.95 kg/m², $p=0.0054$), and these seniors were significantly ($p=0.0001$) less likely to live with their families. Appetite did not exert a significant influence on BMI ($p=0.1411$).

The surveyed respondents suffered from chronic diseases. Their BMI values were most influenced by colorectal cancer (mean BMI of 18.10 kg/m²) and ischemic heart disease (mean BMI of 20.63 kg/m²). The BMI of respondents with diabetes was significantly higher (24.98 kg/m², $p=0.0016$). In elderly patients suffering from chronic diseases, pharmacological treatment can adversely influence nutritional status; however, these factors were not significantly correlated in the surveyed subjects ($p=0.2627$).

Table 2. Distribution of BMI values and the number of points in the MNA test depending on the characteristics of the population

Parameter	BMI [kg/m ²]			Points in MNA test	p-Value
	Mean/S	Minimum	Maximum		
Sex					*
Female	25.61 \pm 3.7	18.6	45.1	22.6	
Male	22.41 \pm 5.9	17.4	46.3	19.5	
Residential environment					**
living with family	23.9 \pm 2.4	18.7	46.3	23.3	
living independently	24.99 \pm 3.1	18.9	35.2	21.7	
living in nursing home	21.48 \pm 5.4	17.4	21.7	18.6	
Nutritional status					ns
overweight/obese	34.7 \pm 4.9	30.0	46.3	23.9	
healthy weight	26.4 \pm 2.6	24.2	29.9	23.1	
underweight	20.8 \pm 2.1	17.4	23.1	18.5	*
unintentional weight loss during the last 3 months	23.54 \pm 2.9	19.0	33.6	19.4	**
psychological stress in the past 3 months	24.3 \pm 5.1	18.3	38.5	16.2	***

Nutritional status BMI categorized with cut-offs according to: underweight BMI < 23.99 kg/m²; healthy weight BMI = 24.0 to 29.9 kg/m²; overweight/obesity BMI ≥ 30 kg/m², Statistically significant at * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, ns—not significant.

Motor skills were significantly ($p=0.0052$) correlated with BMI values. The majority of respondents did not have mobility problems (73.6%) and were characterized by the highest BMI (mean BMI of 24.30 kg/m^2). Bedridden patients accounted for 4.88% of the studied population, and they were characterized by the lowest BMI (21.54 kg/m^2) which was significantly correlated with lower food intake ($p=0.0002$). The place of residence significantly influenced the respondents' nutritional status. Rural residents were characterized by significantly ($p<0.0001$) lower BMI ($21.19 \text{ kg/m}^2 \pm 4.12$) than urban dwellers ($25.34 \text{ kg/m}^2 \pm 4.76$).

The respondents' BMI values were significantly correlated ($p<0.0001$) with their MNA scores (Figure 2). The average MNA score was 21.0 ± 4.4 . A strong positive ($r_s = 0.7293$) correlation was observed in Spearman's rank correlation test, indicating that a decrease in BMI values was correlated with a decrease in MNA scores.

fruit and vegetables less than once a week were at greater risk of malnutrition. These respondents were significantly more often residents of nursing homes or independently living seniors ($p=0.0002$) who had a sedentary lifestyle ($p=0.034$), experienced pain ($p=0.0001$) and eating difficulties ($p=0.0023$). Dairy products and meat were the most popular foods in the surveyed population, whereas legumes, fish and vegetables were least popular. A strong negative ($r_s = -0.863$) correlation between a sedentary lifestyle and the deficiency of nutritional foods in the respondents' diets was observed in Spearman's rank correlation test. In the studied population, 75% of the respondents, including patients with a history of hospitalization, had never been subjected to nutritional assessments. The respondents who had been assessed with the use of various questionnaires suffered from colorectal and pancreatic cancer, and they had been evaluated as part of their oncological treatment. In this group, 2/3 of the respondents had been assessed with the use of the

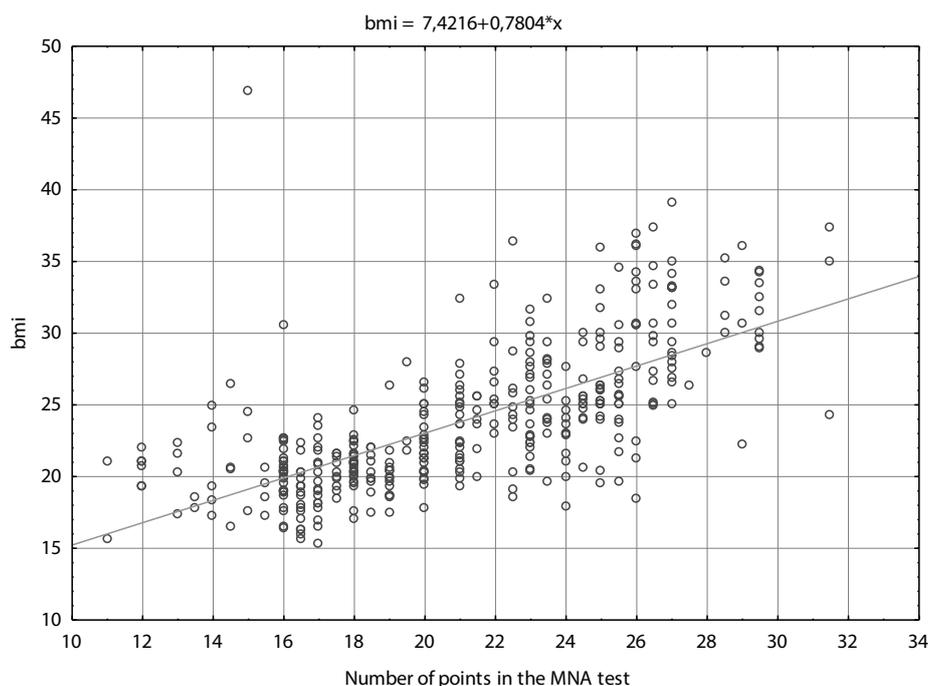


Figure 2. Correlation between MNA scores and BMI values in relation to nutritional status

The respondents' physical activity levels were correlated with their overall MNA scores, and persons with a sedentary lifestyle scored a significantly lower number of points in the MNA test ($p<0.05$). Respondents receiving more than 3 medications daily were characterized by lower MNA scores ($p<0.05$) and a higher risk of malnutrition.

The respondents' dietary preferences were also significantly correlated with malnutrition or the risk of malnutrition expressed by their MNA scores. People who consumed dairy products, legumes, meat, fish,

MNA tool, and the nutritional status of the remaining subjects had been diagnosed with custom-designed questionnaires or the SGA tool. Only 11 patients had been assessed more than once, and the remaining patients who had been diagnosed as malnourished or at risk of malnutrition had not been evaluated repeatedly.

Based on the results of anthropometric analyses, the studied population was divided into three groups: a) overweight/obese, b) at risk of malnutrition/malnourished, c) healthy weight (reference group). The presence of relationships between the risk of

malnutrition and overweight vs. health condition, nutritional status and, indirectly, the score in the MNA screening test was determined (Table 3).

by significantly lower MNA scores ($p < 0.05$). *Spearman's* rank correlation test revealed a strong negative correlation ($r_s = -0.841$) between the above

Table 3. Odds ratio (95% confidence interval). Relationships between malnutrition or excess body weight and factors influencing the score in the MNA questionnaire

	Overweight/obese (healthy weight reference group)	Risk of malnutrition (healthy weight reference group)
Weight loss in the last 3 months	0.94 (0.87-1.03)	1.73** (1.54-1.89)
Neuropsychological problems	0.93 (0.71-1.04)	1.09 (0.94-1.21)
Takes more than 3 prescription drugs per day	1.06 (0.91-1.19)	1.06 (0.89-1.07)
Pressure sores or skin ulcers	1.04 (0.82-1.15)	1.29* (1.19-1.59)
Number of full meals eaten daily	1.87** (1.32-2.06)	0.78* (0.66-1.03)
Consumptions markers for protein intake	1.13 (0.88-1.24)	0.77* (0.54-0.89)
Views self as being malnourished	1.09 (0.94-1.23)	1.35* (1.21-1.57)
Views self as having no nutritional problems	2.06*** (1.57-2.34)	0.69** (0.52-0.89)

Statistically significant at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

Unintentional weight loss (OR=1.73; 95% CI: 1.54-1.89; $p < 0.01$) and pressure sores or skin ulcers (OR=1.29; 95% CI: 1.19-1.59; $p < 0.05$) were significantly more prevalent in respondents who were classified as malnourished or at risk of malnutrition, which, combined with a low intake of protein products, can compromise motor skills and the quality of life. Overweight and obese respondents were more likely to consume at least three meals daily (OR=1.87; 95% CI: 1.32-2.06; $p < 0.001$). The study demonstrated that other-than-normal nutritional status was significantly more likely to contribute to negative self-perceptions of health and dietary problems.

DISCUSSION

According to epidemiological data, the prevalence of malnutrition reaches 30-65% among hospitalized seniors and 25-60% among residents of nursing homes. Malnutrition is least prevalent (2-32%) among elderly persons who live with their families [20]. In the present study, the residential environment was significantly correlated with BMI values ($p = 0.0014$). All nursing home residents were malnourished or at risk of malnutrition, and their BMI values ranged from 18.24 to 22.88 kg/m² (mean BMI 21.49 kg/m² \pm 2.95 vs. BMI=23.94 in respondents living with families and BMI=24.99 kg/m² in independently living seniors). The WOBASZ Senior study conducted with the use of an abridged MNA questionnaire revealed that 13% of Polish seniors were malnourished and 57% were at risk of malnutrition [30].

Many chronically ill seniors suffer from depression and chronic pain, which decreases their wellbeing and quality of life. The respondents affected by psychological stress and disease were characterized

factors. *Ahmadi* et al.[2] demonstrated the depression had a negative impact on the nutritional status of seniors assessed with the use of the Mini-MNA questionnaire ($r = -0.58$, $p < 0.0001$). Residents of large urban areas were also at significantly more susceptible to depression than rural inhabitants (39.5% vs. 3.9%) [2]. Similar results were reported in an Israeli study where malnourished seniors were more likely to be depressed and physically inactive [13].

Depression in the elderly can often lead to malnutrition or dehydration, which, in turn, can induce various kinds of physical illnesses. Physical ailments, including those associated with malnutrition, can also induce depression in the elderly who are more psychologically vulnerable [27]. Senior citizens should receive psychiatric and nutritional assistance due to the strong relationship between the body and the mind in older age.

Lack or loss of appetite contributes to the risk of malnutrition. *Payett* et al. [21] demonstrated that healthy appetite increases energy intake ($p < 0.01$) and protein intake ($p < 0.05$). In a study by *Shahar* [26] more than 20% of the respondents suffered from poor appetite, and these patients were diagnosed as malnourished. In the current study, appetite was significantly correlated with the MNA score ($p < 0.05$), and a strong positive correlation ($r_s = 0.7854$) was noted in *Spearman's* rank correlation test. The average MNA score of respondents with normal appetite was 22.7 ± 3.3 , and it was higher than the MNA score of patients who suffered from lack of appetite or whose appetite had deteriorated significantly in the 3 preceding months (20.8 ± 2.7). Loss of appetite is highly implicated in unintentional weight loss and malnutrition. Similar observations were made by *Brabcova* in a study of seniors residing in the Czech

region of Budejovice, where unintentional weight loss, depression and decreased appetite were most highly correlated with malnutrition. Social and economic factors were less implicated in malnutrition than biological factors [7].

The taste and aroma of food stimulate appetite and contribute to the pleasure of eating. In this study, respondents with an impaired sense of taste and smell were characterized by significantly lower MNA scores (21.0 v 22.7 in respondents without such impairment; $p=0.0023$). Many pharmaceutical treatments can alter perceptions of taste and smell, including lipid lowering drugs, antihistamines, antibiotics, anti-inflammatory drugs, bronchodilators and other asthma drugs, antihypertensive drugs, *Parkinson's* disease treatments and antidepressants. The mechanism by which these drugs affect taste or smell remains unknown [11]. Eating difficulties associated with oral health problems, limited motor ability and digestive disorders contributed to malnutrition ($p=0.0012$). Research indicates that chewing problems can decrease food intake and increase the risk of malnutrition [13].

In the present study, the respondents' subjective evaluations of health status were significantly correlated with their MNA scores ($p=0.0012$). Persons who described their health status as good and very good, relative to their peers, scored a higher number of points in the MNA test ($p<0.05$), and most of them were women living with their families or living independently. Respondents who described their health status as poor or very poor, relative to their peers, scored significantly fewer points in the MNA test ($p<0.05$), and most of them were independently living men and women residing in nursing homes.

When the risk of malnutrition is diagnosed early in senior citizens, nutritional interventions can be introduced to increase energy and protein intake and, consequently, prevent nutrition-related diseases. According to ESPEN data, malnutrition can decrease physical activity, impair rehabilitation, prolong treatment and hospitalization of the elderly [24]. Late diagnosis of malnourished patients with protein and energy deficiencies and sarcopenia prolongs hospitalization and increases treatment costs [4]. The MNA tool supports rapid and simple evaluations of nutritional status without increasing treatment costs. The presented method is sensitive, it does not require specialist tests, it effectively diagnoses patients who are at risk of malnutrition, and it supports quick nutritional interventions, in particular in health care facilities [25].

Strengths and Limitations

The strength of the study was the anthropometric assessment and assessment of the nutritional status of a large group of elderly people living in various

environments. Measurements of height, weight and weight, calf circumference (CC), and mid-arm circumference (MAC) were made using validated equipment. An additional strength was the research using the MNA questionnaire, many people were assessed for the first time and thus diagnosed with the risk of malnutrition.

The limitation in the study was the difficulty in reaching people living alone, and this group is definitely more at risk of malnutrition than people living with their family.

CONCLUSIONS

1. The BMI values and MNA scores of the tested subjects indicate that the majority of the surveyed respondents were malnourished or at risk of malnutrition, regardless of gender.
2. In the studied population, the risk of malnutrition was not significantly correlated with the respondents' economic status.
3. Unintentional weight loss, depression, chronic pain and decrease in appetite were the main factors that contributed to malnutrition in seniors.
4. In the present study, the residential environment significantly influenced the patients' nutritional status, in particular in respondents with impaired motor abilities and eating difficulties.
5. The MNA questionnaire is a simple and sensitive tool which should be used to evaluate all patients older than 65 years in outpatient clinics, hospitals, nursing homes and sanatoriums.
6. Assessment of the nutritional status of the elderly should be included in screening tests carried out in primary health care facilities, in dietitian offices or in other medical facilities.

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Conflict of interest

The authors declare no conflict of interest.

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ATTITUDE AND ACCEPTANCE OF COVID-19 VACCINE AMONGST MEDICAL AND DENTAL FRATERNITY – A QUESTIONNAIRE SURVEY

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ABSTRACT

Background. The second wave of Novel Coronavirus disease (COVID-19, SARS-CoV-2) is proving more disastrous than the first because of the new mutant strains. Under these circumstances, vaccination is the only effective solution that can save millions of lives across the globe.

Objectives. The present study was conducted to assess the attitude and acceptance/willingness of health care professionals (medical and dental) towards COVID-19 vaccine.

Materials and Methods. An online questionnaire survey was conducted among medical and dental professionals working in different hospitals of two states of India. A total of 520 subjects constituted the final sample size. A self-constructed questionnaire (divided into 2 parts) containing 12 questions was administered to obtain information from the subjects regarding their attitude and willingness towards COVID 19 vaccine. Statistical analysis was done using chi-square test and multiple liner regression analysis. Odds ratio with 95% CI were also generated. Statistical significance was set at $p \leq 0.05$.

Results. Majority of the subjects (67% of dental and 73% of medical) had full confidence on the effectiveness of COVID-19 vaccine. Willingness to get vaccinated was shown by 63% of subjects and 65% had positive attitude towards vaccine. Some subjects (45.5% dental and 48.4% medical) showed concern regarding unforeseen effects of the vaccine. Willingness to get vaccinated was 3.45 higher in subjects who were involved in COVID duties. Subjects giving less preference to natural immunity over vaccine showed more willingness (OR: 2.98) towards getting the vaccine.

Conclusion. The findings of the study showed that acceptance and attitude of subjects regarding COVID 19 vaccine was suboptimal as there were various factors which contributed towards subjects' hesitancy to get vaccinated. There is an utmost need to address various issues regarding vaccine safety to promote high uptake.

Key words: COVID-19, safety, emergencies, health, vaccines

INTRODUCTION

In December 2019, the Novel Coronavirus disease (COVID-19, SARS-CoV-2) emerged from China, Wuhan, and is currently claiming thousands of lives everyday across the globe. Its RNA structure resembles that of the SARS-CoV-1 that triggered the Severe Acute Respiratory Syndrome (SARS) epidemic [7, 24]. World Health Organization (WHO) on March 12, 2020 classified COVID-19 as a pandemic [24]. Currently, there are 141 million active cases and 3.01

million deaths have been reported globally. India has reported the 2nd maximum number of cases of COVID-19 with 15.6 million active cases and 1,83,000 deaths [21].

Self-quarantine and social distancing are effective measures that can decrease the Novel Coronavirus disease spread. Development of herd immunity among certain populations might have contributed towards lower morbidity [15]. However, widespread vaccination is the most active way of monitoring and lowering infectious diseases [17]. While immunization

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has effectively lessened the universal problem of morbidity and mortality, public assurance regarding vaccines is being exaggerated by numerous factors [8].

Maintaining assurance regarding the vaccine is subject to the interface between providers and patients [13, 14]. Application and attitude of vaccination by healthcare authorities is a main concern that is dependably related with adherence to vaccination schedules, patient acceptance and vaccination which decreases aversion/reluctance [19]. Furthermore, vaccinated health professionals (medical and dental practitioners) have a perceptible aftereffect on patients' acceptance towards taking a vaccine.

Health authorities' tenacity to utilize and approve the vaccine to their patients can be predisposed by attitudes and knowledge concerning vaccines. It is documented that healthcare specialists who have negative attitude, hatred or reluctance concerning vaccinations, convey these approaches towards vaccination to patients which inclines them to adopt a negative towards the vaccine [2]. Moreover, vaccine doubtfulness detected in the widespread public has been associated with the level of vaccine uncertainty among health care professionals [1]. Public assurance in vaccines universally should be the primary goal for public health authorities for maximum vaccination coverage [5].

The development of an effective vaccine against COVID-19 has been a challenging task for vaccine makers worldwide. Many companies are still developing COVID-19 vaccines and many are in the final stages of trial whereas others have already completed this phase. India has already rolled out two vaccines- 'Covishield' (ChAdOx1, Oxford-Astra Zeneca vaccine) (January 2021) advanced in the UK and manufactured by Serum Institute of India's (SII) has second one is 'Covaxin' (March 2021) which is developed by Bharat Biotech Pharma company, India [20]. First preference for vaccination is being given to front line workers and engaging in COVID 19 duties and next priority is given to all health care workers.

The caliber, content and distribution of educational data about vaccines by healthcare professionals are valuable in educating patients into accepting vaccinations [16]. Moreover, health care workers are directly exposed to the disease because in their working environment. Therefore, the objective of this questionnaire survey was to assess the attitude and willingness to accept the COVID-19 vaccine amongst medical and dental health professionals.

MATERIALS AND METHOD

Ethical clearance

Ethical clearance to conduct the present study was obtained from concerned health authorities.

Participation in the study was voluntary and informed consent was obtained from those study subjects who were willing to participate. Moreover, any type of information disclosing the identity of the patient was not collected. The study was conducted in January 2021.

Study population and study sample

The present questionnaire based study was conducted among dental and medical professionals working in different hospitals of two states in India (Punjab & Uttar Pradesh). The following formula was used to calculate the required sample size:

$$n = \frac{Z^2 1-(\alpha / 2) \times S^2}{d^2}$$

where: Z is the standard normal score with 95% confidence interval (CI) ($\alpha=0.05$), S is the standard deviation of the variable, and d is maximum acceptable error. After applying the formula, 520 subjects constituted the final sample size after excluding the non-responders. Subjects from both the medical and dental specialties were enrolled in the study using systematic random sampling methodology.

Questionnaire/Research Instrument

A self-designed close-ended questionnaire written in English was constructed specifically for the study. The content of the questionnaire was verified by infectious and communicable disease specialist and it was pre-tested for validity and reliability. The reliability of the questionnaire was good (0.82). The questionnaire was split into two sections- A 'General Section' (Section A) which was made to collect socio-demographic details of the subjects (gender, occupation, experience, working profile etc.). Section B comprised of 12 questions on knowledge, attitude and willingness regarding COVID-19 vaccine. The questionnaire was made available to study subjects via email and WhatsApp (Social Media Application) and not handed over personally in order to minimize any contact with the subjects to prevent the spread of the virus. The subjects were given one week time to fill the questionnaire and return it. The response of subjects' (positive or negative) towards the questionnaire was assessed for attitude and willingness towards the vaccine.

Statistical Analysis

Data obtained from responses to the questionnaire was assessed using SPSS statistical package (SPSS, version 21.0, Chicago, IL, USA). Categorical measurements were done using number and percentages. Chi-square test as used to examine association between different variables. The significance level was set at <0.05 . Multivariate

logistic regression analysis was also performed to assess the effect of various independent variables on willingness to get vaccinated. Odds ratio with 95% CI were also generated.

RESULTS

Socio-demographic characteristics

The analysis of the general demographic data (Table 1) revealed that the age of the subjects ranged from 24-55 years and majority of the study subjects were in the age-group of 25-35 years (208, 40%) and 65.6 % (340) were males. The study population comprised more of dental professionals (54.4%) as compared to

medical health professionals (45.6%). Subjects having experience of more than 10 years were comparatively less (41.6%) as compared to subjects having more than 10 years' experience (58.4%). Of the total subjects, 35% (182) were doing COVID related duties at their respective workplace.

Response to the questionnaire on attitude regarding the vaccine

The responses of the subjects pertaining to attitude regarding vaccine are summarized in Figure 1. More than 85% of subjects were aware regarding the availability of COVID-19 vaccine in India by January end. Majority of the subjects (67% of dental and 73%

Table 1. Socio-demographic characteristics of the study population

Socio-demographic characteristic		Number	Percentage (%)
Age (in years)	25-35	208	40
	35-45	171	33.1
	45-55	141	26.9
Gender	Male	340	65.6
	Female	180	34.4
Type of health care worker	Medical	237	45.6
	Dental	283	54.4
Years of Experience	10	303	58.4
	More than 10	217	41.6
Working Profile	Governmental sector	192	37
	Private sector	328	63
Involved in Covid-19 duty	Yes	182	35
	No	338	65

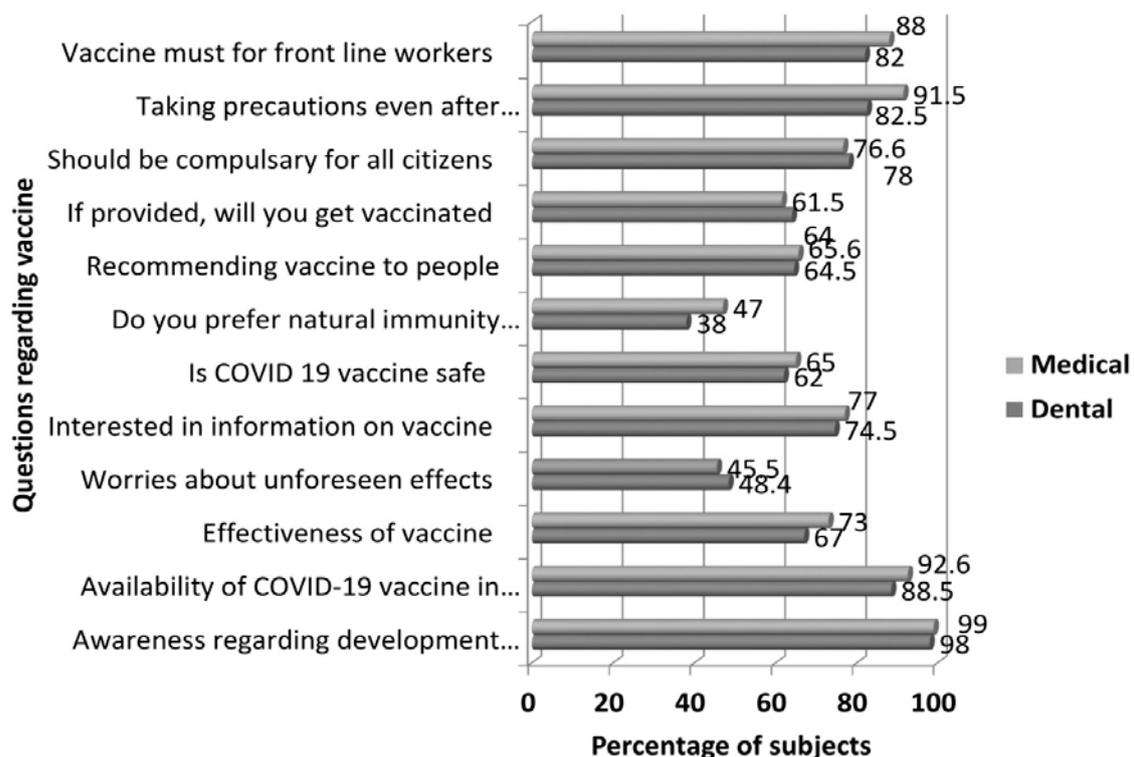


Figure 1. Subjects' response towards the questionnaire assessing attitude and willingness

of medical) had full confidence on the effectiveness of COVID-19 vaccine and more than 60% of subjects were of the opinion that it is safe. However, some of the subjects (45.5% dental and 48.4% medical) showed concern regarding unforeseen effects of the vaccine. More than 40% of the subjects preferred natural immunity over vaccines. When asked about their willingness to get vaccinated, 63% of subjects gave a positive response and 65% reported that they would recommend vaccine to others. Vaccine should be compulsory for all citizens of India were emphasized by 76.6% of medical and 78% of dental professionals. Overall, 65% of subjects (338) showed a positive attitude towards vaccination and the findings were significantly associated with the working profile of study subjects ($p=0.024$) (Table 2).

Multivariate logistic regression analysis

Multivariate logistic regression analysis was also performed to assess the effect of various independent variables on the willingness of subjects to get vaccinated (Table 3). Odds ratio with 95% CI were also generated. The odds of willingness to get vaccinated were 2.12 times greater in dental health professionals as compared to medical health professionals. Willingness to get vaccinated was 3.45 higher in subjects who were involved in COVID duties as well. Subjects giving less preference to natural immunity over vaccine showed more willingness (2.98 times) towards getting the vaccine. Odds of getting vaccinated were 2.78 times higher among those who were interested in gathering information about the vaccine. Moreover, subjects having perception that the vaccine should be

Table 2. Attitude towards Covid-19 vaccine

Attitude	Profile of subjects	No. (%)	p value
Positive	Medical	158 (66.7%)	0.024*
	Dental	180 (63.6%)	
Negative	Medical	79 (33.4%)	0.065
	Dental	123 (36.3%)	

*Chi-square test, $p \leq 0.05$ Statistically significant

Table 3. Multivariate logistic regression analysis on willingness to get vaccinated

Variable	OR (Odds Ratio)	95% CI	p value
Type of health worker			
Medical	1.00	0.54 - 3.56	0.023*
Dental	2.12		
Years of experience			
10	1.00	1.00 - 3.76	0.065
More than 10	2.65		
Working profile			
Government sector	1.32	0.67 - 2.87	0.071
Private sector	1.00		
Involved in COVID duties			
Yes	3.45	1.2 - 5.61	0.002*
No	1.00		
Do you prefer natural immunity over vaccine			
Yes	1.00	0.5 - 4.34	0.034*
No	2.98		
Is COVID 19 Vaccine safe			
Yes	2.46	0.72 - 3.78	0.045*
No	1.00		
Interested in information on vaccine			
Yes	2.78	1.45 - 3.23	0.045*
No	1.00		
Vaccine should be compulsory for all Indian citizens			
Yes	3.23	1.06 - 3.78	0.027*
No	1.00		

* $p \leq 0.05$, statistically significant

compulsory for all Indian citizens were more willing to get vaccinated (OR: 3.23).

DISCUSSION

High rates of vaccination against diseases in a population have been one of the most important advances for maintenance of public health around the globe. Since the start of the current pandemic, scientists all over the world have been working tirelessly to develop new and effective vaccines that can guard people from COVID-19 and its new variants [12]. More than 100 vaccine candidates are being developed world widely by different pharmaceutical companies. Some of the companies have already introduced their vaccines while others are in their last stages of clinical trials. Recent evidence suggests that people who are fully vaccinated for COVID-19 are less likely to be infected without showing symptoms and therefore are less likely to spread infection to others [3].

Although more than 95% of subjects were aware regarding the development of vaccine against COVID-19, more than one-third of subjects (37%) were unwilling to get vaccinated. This acceptance rate is lower as compared to some study conducted in another part of the world [6] but high as compared to a recent study conducted among health care workers in Saudi Arabia [18]. A possible explanation for this could be due to concerns regarding possible substandard vaccine quality and misinformation conveyed by certain social and mass media sources which have spread rumors regarding the safety of COVID-19 vaccine in people belonging to some race/ethnicity [4]. Vaccine hesitancy developed among medical and dental practitioners can impact their own decision to get vaccinated and to endorse the vaccine to their patients. Social media influencers and celebrities can play a significant role to normalize uptake of the vaccine and combat misinformation.

The results of the study revealed that 35% of the subjects had negative attitude towards COVID-19 vaccine and the findings were statistically significant when compared with working profile of subjects (medical & dental). More than 40% of subjects had some concerns regarding unforeseen effects of the vaccine as it is developed during an emergency within a span of few months (fast-track). Similar finding is observed in some other study conducted among health care workers in another country [23].

Results of multivariate analysis showed higher rates of acceptance for vaccination among dental professionals compared to medical professionals. This could be due to higher exposure risks for dentists during oral examination and treatment procedures. However, contrasting findings were reported by some other study conducted on medical and dental students

[11]. Moreover, subjects engaged in COVID-19 duties (dealing with COVID-19 patients) at their respective health care facilities were more willing to get vaccinated as compared to others. Data gathered from a Turkish study conducted on health professionals reported similar findings [10]. This could be due to the fact that subjects dealing with COVID-19 patients have inherent fear of catching the infection which can be passed on to their family.

There is a natural tendency in human beings to gather all information before adopting a new idea that propels us to take a particular decision. It was observed in our study that subjects who were interested in gathering information regarding the COVID-19 vaccine were 2.78 times more willing to get vaccinated as compared to other subjects. Similar findings were observed in a recent study conducted in Democratic Republic of Congo where subjects used to attend lectures and discussions to gather new information regarding vaccine [9]. Subjects who thought that COVID-19 vaccine should be made compulsory for India citizens were more likely to get vaccinated as compared to others. Our findings were in congruence with the findings of another study [18].

Healthcare professional's approval plays a significant part in their patients' vaccination conduct. They can provide significant evidence to the general population and their opinion can be a key factor in patients' judgement to be vaccinated. In this situation and with the moderate positive response for vaccination in our study subjects, there is a substantial need for addressing worries and creating alertness to advance chances for a higher rate of acceptance of the COVID-19 vaccine. The present study has some limitations also. It is not nationally representative as it is conducted in only one state of the country and it is an online survey. Secondly, the study involved a limited sample size as most of the health care professionals were actively engaged in COVID-19 related duties and therefore had limited time to participate in a survey. Moreover, as the country is witnessing a high surge of COVID-19 cases in the second wave (300,000 per day) from the last couple of weeks because of the 'new mutant' strain [22], some subjects who were previously hesitant or unwilling to take the vaccine might consider to opt for the vaccine now (the study was conducted in January 2021). Therefore the actual number of subjects willing to take the vaccine could be somewhat more in the current situation. All these things might have impact on the study's generalisability.

CONCLUSION

The study concluded that rate of acceptance (willingness) of COVID-19 vaccine among subjects was suboptimal though 65% of subjects showed positive attitude towards the vaccine. Health care

workers especially the front line workers are at greater risk of contracting the disease. It is because of this reason that Government of India gave utmost priority to front line workers to get vaccinated. While the expecting COVID-19 vaccine could be effective in protecting the healthcare workers, our data shows that uncertain attributes like safety, development of vaccine in an emergency, unforeseen effects are likely a hurdle for low acceptance.

Recommendations

Vaccine hesitancy, as a global threat, is affecting all countries. Developing personalised policies to address concerns recognized in the study to decrease vaccine doubtfulness will be crucial to successful widespread vaccination against COVID-19. Interventional educational programs through webinars and seminars should be conducted among health care workers to eliminate fears concerning the vaccine. Health care workers should be made aware of the fact that existing vaccines provide reasonable protection and are effective against many variants (emerging in different nations) of interest and concern. Government can engage eminent personalities and celebrities (spokespersons) to combat any misinformation regarding the vaccine through mass media communication channels. Social media monitoring should be done and any type of erroneous claims regarding COVID vaccine should be refuted. Science has provided us the vaccines, but it is our responsibility to use them to protect ourselves and our world.

Conflict of interest

The Authors declare no conflict of interest.

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APPRAISAL OF AWARENESS ON MEDICAL EMERGENCIES AND ITS MANAGEMENT AMONG DENTISTS IN BHUBANESWAR, INDIA*

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ABSTRACT

Background. Medical emergencies can be distressing for any dental professional, whether in a dental practice, hospital or other sites.

Objectives. To assess the knowledge and awareness of dental practitioners towards medical emergencies and its management in Bhubaneswar.

Material and Methods. In this cross-sectional investigation, a self-administered questionnaire which included demographic details and 19 questions regarding knowledge about medical emergencies, was disseminated to a random sample of 183 dentists working in their private dental clinic set up in Bhubaneswar. Chi-square test was used for statistical analysis.

Results. Total sample size was 183, out of which 79 (43.17%) were males and 104 (56.83%) were females. The mean age was found out to be 30.7 ± 5.38 years. Practically 96% of the practitioners were not certain enough to deal with the emergency conditions at dental office. Around 67% had not attended any workshop on emergency training. It was amazing to observe that larger part (90%) of the experts didn't possess first aid kit at their dental office. In instances of handling emergency situation at the dental chair no statistical significance ($p > 0.05$) with respect to age and gender was found.

Conclusion. This investigation showed that hypothetically dental professionals had better knowledge on medical emergencies yet at the same time they were not equipped efficiently to manage the same at their workplace.

Key words: dentist, medical emergencies, management, questionnaire

INTRODUCTION

Confronting medical emergencies in everyday dental practice is an unavoidable circumstance which can be hazardous. Obviously, a thorough and vivid knowledge about handling these situations is an unquestionable requirement for each dental specialist for their smooth practice. Dental professionals must be aware of the signs and challenges of the frequently occurring medical emergencies and effects of their interaction with various drugs [5]. Based on the study conducted by Malamed, the most frequent emergencies before, during, and right after the treatment include hyperventilation, convulsion, and hypoglycemia, followed by vasovagal syncope, angina pectoris, hypotension, hypersensitivity reactions, and adverse drug reactions [16]. Some other emergencies include cardiac arrest, fits and seizures, diabetic emergency, asthmatic assault, hypertensive emergency, and ingestion of a foreign body.

Local anaesthesia being a dental therapeutic intervention, when in addition to stress caused by dental treatment can result in clinical signs, various emergencies, or exacerbated systemic disease in patients [25]. Furthermore, it may also happen because of reaction with certain dental materials (resins, latex), or due to any invasive dental procedure [15]. With expanding age, presence of systemic diseases as well as increased drug consumption, there is pervasiveness of medical emergencies now a days. Through an itemized and intensive case history, a cautious assessment and altering some treatment strategies, dentists can manage to prevent medical emergencies in their everyday practice. In United Kingdom (UK), the Resuscitation Council has made it vital for all the dental clinics to have minimal emergency equipment that incorporates portable oxygen cylinder, oxygen face mask, set of oropharyngeal airways, device for inhaled bronchodilators, blood glucose measuring apparatus, defibrillator, suction and sterile syringes

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and needles [2]. Basic medications like oxygen, epinephrine, diphenhydramine, bronchodilators, glucose, aspirin and aromatic ammonia should be accessible in dental clinics.

It is critical for dental specialists to stay up to date with the most recent knowledge and information, and successfully practice the clinical abilities to oversee life-threatening circumstances in dental practices, in order to reduce the adverse medical emergency outcomes. Information on the predominance of medical emergencies and the competence of dentists in adequately managing medical emergencies in India is scarce. To our knowledge, this is the first study conducted in Bhubaneswar, Odisha that evaluates the awareness and knowledge about medical emergencies, the preparedness and competence of dentists to manage such situations, and compare medical emergencies encountered between dentists in relation to their gender, age group and qualification. Accordingly, the objective of this study was to assess the knowledge and awareness about medical emergencies and its management among the dentists in Bhubaneswar.

MATERIAL AND METHODS

A cross-sectional study was conducted from January to March 2019 among the dental practitioners of Bhubaneswar, India. The survey protocol was approved by Institutional Ethical Committee. Dentists having their own dental practice set up and also provided an informed consent were included in the study. Those who did not respond to the questionnaire after two successive attempts were excluded. Convenient sampling technique was used to contact the dental practitioners and a total of 200 questionnaires were distributed amongst them.

A self-administered structured questionnaire was developed in English and pretested on a group of ten dentists to assess the face validity that was detected to be 90%. Based on the thoughts stated by a panel of four academicians, mean content validity ratio was intended as 0.85. Test of reliability encompassed two components: Question-question reliability, which was calculated by percentage of agreement (89%) and internal reliability for the responses to questions, which was evaluated using Cronbach's alpha coefficient (0.86).

The final questionnaire consisted of two sections, the first of which included demographic information (age, sex and qualification). The second part included 19 questions regarding the dentists' knowledge and awareness about handling medical emergencies in the dental office.

The nature and purpose of study were explained to the dentists. Questionnaire (Annex 1) was distributed to dental practitioners by a single investigator, offered

adequate time to fill it and attempt was made to collect the filled questionnaires within three days. The investigator simultaneously requested the respondents to answer every question sincerely without any access to other sources. Voluntary nature was accentuated and confidentiality was guaranteed to the respondents.

For the purpose of analysis, proportion of scores was used to assess the level of knowledge and awareness among the participants. Correct responses of <40%, 40-60%, 60-80% and >80% were categorized as having poor, average, good and very good knowledge scores respectively. Descriptive statistics were used to summarize the data. Chi-square (χ^2) test was used to assess the relationship between categorical variables. For all tests, confidence interval and 'p' value were fixed at 95% and < 0.05 correspondingly.

RESULTS

Of the total 200 questionnaires distributed, 183 returned the completed questionnaires yielding a response rate of 91.5%. The age of the respondents ranged from 24 to 50 years (mean 30.7 ± 5.38). The sample comprised of 79 (43.2%) males and 104 (56.8%) females; 110 (60.1%) undergraduates and 73 (39.9%) postgraduates (Table 1).

Almost 96.2% dentists enquire about patient's medical history including medication and allergy. About 129 (70.5%) recorded filled history performa of the patient and 124 (67.8%) obtained the vital signs of the patient before commencing any treatment. When asked about attending any workshop on emergency training and management, 67.2% gave a positive response. Meanwhile 60.1% were confident enough to handle emergency situations while 39.9% lacked confidence. Higher confidence was reported in giving intramuscular injections (99.4%) in comparison with intravenous injections (56.8%) which were found to be statistically significant ($p < 0.001$). Meanwhile 110 (60.1%) dentists mentioned that they could handle any emergency situation at their office. About 56.8% had emergency kit handy at their office and the most available drugs in the dentist's office were oral glucose (97.8%, $p > 0.05$), followed by adrenaline (95.1%, $p < 0.03$) and hydrocortisone (68.9%, $p < 0.004$). It was motivating to note that all the dentists found it necessary to attend workshops on management of medical emergencies (Table 2).

Table 3 provides the responses for all the knowledge-based questions. It was appreciable to note that 94.5%, majority females (96.2%) and MDS graduates (95.9%) dentists were conscious about placing a patient in Trendelburg position and administering ammonia inhalant in case of syncope, but this was not found to be statistically significant ($p > 0.05$). Poor knowledge (30.6%) is reported for management of airway

Table 1. Demographic characteristics of the participants (n=183)

Characteristics	n (%)
Age groups (in years)	
≤25	10 (5.5)
26-30	101 (55.2)
31-35	37 (20.2)
36-40	25 (13.6)
>40	10 (5.5)
Gender	
Male	79 (43.2)
Female	104 (56.8)
Qualification	
BDS	110 (39.9)
MDS	73 (60.1)

obstruction during treatment due to aspiration which is not significant ($p>0.05$). In response to providing immediate action under emergency, nearly 40% only responded correctly demonstrating a poor to average knowledge which was found to be statistically significant ($p<0.001$).

In response to the question on extraction of tooth in patients with prosthetic heart valve 88.6% males and 75% females gave correct response which was found to be statistically significant ($p<0.019$). In addition to this, significance ($p<0.014$) was also found between the age groups where elderly persons gave more correct responses. Similarly, the next question about dental procedures in patients with prosthetic heart valve was answered correctly by more males (86.1%) than females (83.7%) but this association was not found statistically significant ($p>0.05$). Whereas, significance ($p<0.001$) was found between the age group in which a higher number of dentists from all the age groups gave correct responses.

More than 95% of dentists were aware about the full form of BLS which was not significant ($p>0.05$). Hardly 50% of the subjects responded correctly in regard to location of chest compression and males (59.5%) provided more correct response in comparison to females (42.3%) which was significant only in relation to gender ($p<0.035$). There were mixed responses about the knowledge on ratio of Cardiopulmonary Resuscitation (CPR) for a single rescuer in adult. Here, female dentists and elderly age group (>36 years and above) gave more correct responses and it was statistically significant ($p<0.05$). It was observed that the knowledge was poor (21.3%) in relation to rescue breathing in infants which was statistically significant only among age groups ($p<0.012$).

DISCUSSION

Consequently, being alert and ready for an emergency and realizing that facing an emergency can be a real possibility in a dental clinic is of utmost importance. Although medical emergencies incidence in dental clinics is less, but when it occurs it can be life threatening [24]. Hence recognition of "at-risk" patients and subsequent appropriate management is vital [25].

Our study also focussed on enquiring about the key points such as medical history, vital signs of the patients, and availability of emergency drug kits at their dental office. About 96.2% dentists enquired about the medical history of the patients, but only 70.5% dentists recorded a filled case history performa and 67.8% recorded vital signs. This was similar to the findings of *Varma et al* [24]. The above observations were high in comparison to studies of *Kumarswami et al* [14] and *Pandey et al* [20]. This may mainly be due to increase in the awareness regarding medical emergencies among dentists.

In our study around 67.2% of the practitioners recalled having received training in the management of medical emergencies during their course. This was almost similar to the results by *Varma et al* (74.1%) at Khammam [24] and *Atherton et al* [4] in Great Britain (75.2%) but was very high in comparison to *Kumarswami et al* (7.6%) [14] and *Gupta et al* (42.1%) [13]. Instructions on the management of medical emergencies in teaching institutions should be standardized in terms of course content, allocation of teaching hours, faculty members responsible, and the method of training which may enable the graduates to develop an orderly and confident approach to the diagnosis and handling of emergencies encountered in dental practice. However, it was surprising to note that 90.7% dental practitioners had attended workshops on

Table 2. Distribution of responses to questions on awareness in handling emergencies at dental office

Variables	Total n=183 n (%)	Gender n (%)		P value	Age groups (in years) n (%)						P value	Qualification n (%)		P value
		Male (n=79)	Female (n=104)		≤25 n=10	26-30 n=101	31-35 n=37	36-40 n=25	>40 n=10	MDS n=73		BDS n=110		
Q1.	Y - 176 (96.2) N - 7 (3.8)	74 (93.7) 5 (6.3)	102 (98.1) 2 (1.9)	0.1	10 (100) 0 (0)	99 (98.1) 2 (1.9)	37 (100) 0 (0)	22 (88) 3 (12)	8 (80) 2 (20)	0.001*	68 (93.2) 5 (6.8)	108 (98.2) 2 (1.8)	0.2	
Q2.	Y - 129 (70.5) N - 54 (29.5)	48 (60.8) 31 (39.2)	81 (77.9) 23 (22.1)	0.01*	6 (60) 4 (40)	79 (78.2) 22 (21.8)	29 (78.4) 8 (21.6)	11 (44) 14 (56)	4 (40) 6 (60)	0.001*	46 (63) 27 (37)	83 (75.5) 27 (24.5)	0.01*	
Q3.	Y - 124 (67.8) N - 59 (32.2)	55 (69.6) 24 (30.4)	69 (66.3) 35 (33.7)	0.6	2 (20) 8 (80)	67 (66.3) 34 (33.7)	28 (75.7) 9 (24.3)	22 (88) 3 (12)	5 (50) 5 (50)	0.001*	51 (69.9) 22 (30.1)	73 (66.4) 37 (33.6)	0.1	
Q4.	Y - 123 (67.2) N - 60 (32.8)	56 (70.9) 23 (29.1)	67 (64.4) 37 (35.6)	0.6	2 (20) 8 (80)	67 (66.3) 34 (33.7)	29 (78.4) 8 (21.6)	17 (68) 8 (32)	8 (80) 2 (20)	0.005*	59 (80.8) 14 (19.2)	64 (58.2) 46 (41.8)	0.003*	
Q5.	Y - 110 (60.1) N - 73 (39.9)	52 (65.8) 27 (34.2)	58 (55.8) 46 (44.2)	0.2	4 (40) 6 (60)	60 (59.4) 41 (40.6)	20 (54.1) 17 (45.9)	17 (68) 8 (32)	7 (70) 3 (30)	0.08	46 (63) 27 (37)	64 (58.2) 46 (41.8)	0.4	
Q6.	Y - 182 (99.5) N - 1 (0.5)	78 (98.7) 1 (1.3)	104 (100) 0 (0)	0.2	10 (100) 0 (0)	101 (100) 0 (0)	37 (100) 0 (0)	24 (96) 1 (4)	10 (100) 0 (0)	0.001*	72 (98.6) 1 (1.4)	110 (100) 0 (0)	0.5	
Q7.	Y - 104 (56.8) N - 79 (43.2)	62 (78.5) 17 (21.5)	42 (40.4) 62 (59.6)	0.001*	4 (40) 6 (60)	44 (43.6) 57 (56.4)	29 (78.4) 8 (21.6)	22 (88) 3 (12)	5 (50) 5 (50)	0.001*	51 (69.9) 22 (30.1)	53 (48.2) 57 (51.8)	0.006*	
Q8.	Y - 164 (89.6) N - 19 (10.4)	68 (86.1) 11 (13.9)	96 (92.3) 8 (7.7)	0.2	8 (80) 2 (20)	91 (90.1) 10 (9.9)	32 (86.5) 5 (13.5)	23 (92) 2 (8)	10 (100) 0 (0)	0.001*	68 (93.2) 5 (6.8)	96 (87.3) 14 (12.7)	0.001*	
Q9 Adrenaline	Y - 174 (95.1) N - 9 (4.9)	77 (97.5) 2 (2.5)	97 (93.3) 7 (6.7)	0.1	10 (100) 0 (0)	94 (93.1) 7 (6.9)	36 (97.3) 1 (2.7)	25 (100) 0 (0)	10 (100) 0 (0)	0.7	73 (100) 0 (0)	101 (91.8) 9 (8.2)	0.03*	
Q9 Oral Glucose	Y - 179 (97.8) N - 4 (2.2)	77 (97.5) 2 (2.5)	102 (98.1) 2 (1.9)	0.8	10 (100) 0 (0)	97 (96.3) 4 (3.7)	37 (100) 0 (0)	25 (100) 0 (0)	10 (100) 0 (0)	0.4	73 (100) 0 (0)	106 (96.4) 4 (3.6)	0.2	
Q9 Ammonia Inhalant	Y - 98 (53.6) N - 85 (46.4)	42 (53.2) 37 (46.8)	56 (53.8) 48 (46.2)	0.9	4 (40) 6 (60)	60 (59.4) 41 (40.6)	15 (40.5) 22 (59.5)	10 (40) 15 (60)	9 (90) 1 (10)	0.001*	29 (39.7) 44 (60.3)	69 (62.7) 41 (37.3)	0.002*	
Q9 Hydro- cortisone	Y - 126 (68.9) N - 57 (31.1)	57 (72.2) 22 (27.8)	69 (66.3) 35 (33.7)	0.4	8 (80) 2 (20)	69 (68.3) 32 (31.7)	24 (64.9) 13 (35.1)	18 (72) 7 (28)	7 (70) 3 (30)	0.004*	46 (63) 27 (37)	80 (72.7) 30 (27.3)	0.03*	
Q9 Atropine	Y - 111 (60.7) N - 72 (39.3)	51 (64.6) 28 (35.4)	60 (57.7) 44 (42.3)	0.3	2 (20) 8 (80)	63 (62.4) 38 (37.6)	21 (56.8) 16 (43.2)	16 (64) 9 (36)	9 (90) 1 (10)	0.001*	46 (63) 27 (37)	65 (59.1) 45 (40.9)	0.1	
Q10.	Y - 183 (100) N - 0 (0)	79 (100) 0 (0)	104 (100) 0 (0)	---	10 (100) 0 (0)	101 (100) 0 (0)	37 (100) 0 (0)	25 (100) 0 (0)	10 (100) 0 (0)	----	73 (100) 0 (0)	110 (100) 0 (0)	---	

Y = yes; N = No *P<0.05 statistical significance

Table 3. Distribution of responses to questions on knowledge/awareness in handling emergencies at dental office

Variables	Responses	Total n (%) n=183	Gender (n=183)		P value	Age groups (in years) (n=183)						P value	Qualification (n=183)		P value
			Male n (%) n=79	Female n (%) n=104		≤25 n (%) n=10	26-30 n (%) n=101	31-35 n (%) n=37	36-40 n (%) n=25	>40 n (%) n=10	MDS n (%) n=73		BDS n (%) n=110		
Q 11	Correct	173 (94.5)	73 (92.4)	100 (96.2)	0.22	10 (100)	91 (90.1)	37 (100)	25 (100)	10 (100)	0.72	70 (95.9)	103 (93.6)	0.74	
	Incorrect	10 (5.5)	6 (7.6)	4 (3.8)		0 (0)	10 (9.9)	0 (0)	0 (0)	0 (0)		0 (0)	3 (4.1)		7 (6.4)
Q 12	Correct	56 (30.6)	23 (29.1)	33 (31.7)	0.35	4 (40)	30 (29.7)	12 (32.4)	8 (32)	2 (20)	0.71	25 (34.2)	31 (28.2)	0.86	
	Incorrect	127 (69.4)	56 (70.9)	71 (68.3)		6 (60)	71 (70.3)	25 (67.6)	17 (68)	8 (80)		48 (65.8)	79 (71.8)		
Q 13	Correct	71 (38.8)	32 (40.5)	39 (37.5)	0.69	4 (40)	37 (36.6)	13 (35.1)	11 (44)	6 (60)	0.067	29 (39.7)	42 (38.2)	0.0001*	
	Incorrect	112 (61.2)	47 (59.5)	65 (62.5)		6 (60)	64 (63.4)	24 (64.9)	14 (56)	4 (40)		44 (60.3)	68 (61.8)		
Q 14	Correct	148 (80.9)	70 (88.6)	78 (75)	0.019*	4 (40)	75 (74.3)	36 (97.3)	23 (92)	10 (100)	0.014*	64 (87.7)	84 (76.4)	0.13	
	Incorrect	35 (19.1)	9 (11.4)	26 (25)		6 (60)	26 (25.7)	1 (2.7)	2 (8)	0 (0)		9 (22.3)	26 (23.6)		
Q 15	Correct	155 (84.7)	68 (86.1)	87 (83.7)	0.17	8 (80)	83 (82.2)	30 (81.1)	21 (96)	10 (100)	<0.001*	67 (91.8)	88 (80)	0.34	
	Incorrect	28 (15.3)	11 (13.9)	17 (16.3)		2 (20)	18 (17.8)	7 (18.9)	1 (4)	0 (0)		6 (8.2)	22 (20)		
Q 16	Correct	175 (95.6)	77 (97.5)	98 (94.2)	0.36	10 (100)	95 (94.1)	35 (94.6)	25 (100)	10 (100)	0.91	69 (94.5)	106 (96.4)	0.18	
	Incorrect	8 (4.4)	2 (2.5)	6 (5.8)		0 (0)	6 (5.9)	2 (5.4)	0 (0)	0 (0)		4 (5.5)	4 (3.6)		
Q 17	Correct	91 (49.7)	47 (59.5)	44 (42.3)	0.035*	4 (40)	39 (38.6)	23 (62.7)	17 (68)	8 (80)	0.06	42 (57.5)	49 (44.5)	0.22	
	Incorrect	92 (50.3)	32 (40.5)	60 (57.7)		6 (60)	62 (61.4)	14 (37.3)	8 (32)	2 (20)		31 (42.5)	61 (55.5)		
Q 18	Correct	75 (41)	24 (30.4)	51 (49)	0.043*	6 (60)	34 (33.7)	14 (37.8)	14 (56)	7 (70)	0.018*	29 (39.7)	46 (41.8)	0.08	
	Incorrect	108 (59)	55 (69.6)	53 (51)		4 (40)	67 (66.3)	23 (62.2)	11 (44)	3 (30)		44 (60.3)	64 (58.2)		
Q 19	Correct	39 (21.3)	21 (26.6)	18 (17.3)	0.3	2 (20)	21 (20.8)	2 (5.4)	12 (48)	2 (20)	0.012*	18 (24.6)	21 (19.1)	0.26	
	Incorrect	144 (78.7)	58 (73.4)	86 (82.7)		8 (80)	80 (79.2)	35 (94.6)	13 (52)	8 (80)		55 (75.4)	89 (80.9)		

*P<0.05 statistical significance

emergency training or management programs which is very high [12].

The results of our study established that 60.1% of dentists were confident enough to handle emergency situations independently. This was similar to the findings of *Alhamad et al* - (45%) [2] and *Stafuzza et al* - (66%) [22]. Contradictory findings of lower response by *Kumarswami et al* (14%) [14] and higher response by *Broadbent* and *Thomson* among 80% of British dentists were also reported [6]. This difference may be attributed to more of theoretical knowledge and lack of practical training/experience.

The varying degrees of confidence among the dentists with regard to giving intravenous injections was quite evident. Here, 43.2% dental practitioners gave a negative response in comparison with the study findings of *Gupta et al* [12], *Arsati et al* [3] and *Varma et al* [24] who reported negative responses of 72.2%, 61.4% and 28.24% respectively.

In our study 89.6% of the practitioners had emergency kits at the dental office, which was similar to the findings of *Alhamad et al* (75%) [2], *Varma et al* (87.2%) [24] and *Atherton et al* (80%) [4]. But in contrast only 24% were having emergency kit as found by *Kumarswami et al* [14] and 8.9% by *Gbotolorun et al* [11]. In light of the findings of these studies it is suggested that there is a need for strict regulations requiring an emergency medical kit and the specific items to be kept by dental practitioners.

The most commonly available drugs in the clinic set up were oral glucose (97.8%), followed by adrenaline (95.1%) and hydrocortisone (68.9%). These findings were similar with that by *Gupta et al* [13] where oral glucose (82.2%) was followed by adrenaline (65.8%), and with *Kumarswami et al* [14] where adrenaline (88%) and oral glucose (81.4%) were most commonly available drugs. *Pandey et al* [20] found oxygen at only 2% clinics which is a very critical point to be addressed. Based on a study on Australian general dental practitioners, oxygen was found to be most common emergency item [8]. Also in Great Britain it was found that oxygen was the most commonly available emergency drug followed by adrenaline and oral glucose [4].

Attending the workshops on management of emergencies is essential for dentists not only to refresh their knowledge but also to learn new concepts in medical evaluation. Likewise, it was good to know that all our respondents (100%) felt the need to routinely attend such programs. This finding was contradictory to studies of *Gupta et al* [12] and *Fast et al* [10] who reported the necessity among 30% and 16% dentists respectively.

Our study also reflected that the dentists had a poor knowledge (38.8%) about the emergency medical services (EMS). Activation of EMS is a most crucial

step for management of emergency situations arising in a dental office in addition of being aware of state dental practice acts. The dentist will become more familiar with the accepted treatment and protocols for handling emergencies that forms the basis for a legal standard of care. Failure to follow the right principles results in unintentional injury which can lead to tragic consequences and sometimes legal action [9,18].

Average number of dentists had knowledge about basic life support and CPR (49.7%). Similar results were seen in a study by *Sharma et al* [21] and *Chandrasekharan et al* [7] where the overall knowledge score was below 50% and *Akritia et al* [1] who also reported inadequacy of knowledge about BLS. Nevertheless, improvement of knowledge and skills of CPR after a BLS training was demonstrated by *Marsden* [17] and *Sudeep et al* [23]. But the training of the resuscitation skills is difficult because of the busy schedules and lack of teachers and resources in India. In addition, due to the updating of the guidelines every 5 years, repetitive training is needed to ensure the changes [19].

It was found that dentists from age group >36 years and above had better knowledge ($p < 0.05$) about CPR. This can be reasoned by interpreting the experience of the elderly age group dentists. The male dentists in Bhubaneswar had a better knowledge about the location of chest compression than the females.

To prepare for emergencies we need to involve personal, staff, and office preparation wherein under personal and staff preparation, an in-depth knowledge of signs, symptoms, and management of emergencies, basic life support (BLS) measures, and cardiopulmonary resuscitation (CPR) are some of the essentials to be included. Office preparation involves maintaining emergency equipment, emergency drugs, and backup medical assistance. Effective management of an emergency situation in the dental office is ultimately the dentist's responsibility. The lack of training and inability to cope with medical emergencies can lead to tragic consequences and sometimes legal action. Thus, need of the hour is framing of legislations and update of the emergency medical kits.

LIMITATIONS

Since the study was conducted in a single geographical area, generalization should be done with caution. Future studies can include a question regarding the most frequently encountered medical emergency situation faced by the dentist in their practice. Dental students and interns can also be assessed for their preparedness to handle emergencies as this group can effectively be trained during early stages of their career.

CONCLUSIONS

A detailed knowledge about the signs, symptoms and appropriate management of the emergencies is the only way to handle these kinds of situations. Our study findings suggest that a moderate number of dentists have knowledge about the emergencies whereas a majority remains unaware on how to deal with these situations. Awareness of EMS and CPR among dental practitioners is very minimal and needs improvisation. Besides that, attending continuing

dental education program consisting of workshops and hands-on courses in this field should be made mandatory.

Conflicts of interest

None.

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ANNEX 1. QUESTIONNAIRE

1. Do you enquire about medical history including medication and allergy? Yes/No
2. Do you obtain filled health history performa from the patients? Yes/No
3. Do you obtain the vital signs (blood pressure, pulse, respiration, and temperature) of the patients before commencing any treatment? Yes/No
4. Have you attended any workshop on emergency training or management programs? Yes/No
5. Do you think you can handle any emergency condition at your dental office? Yes/No
6. Can you give an intramuscular injection? Yes/No
7. Can you give an intravenous injection? Yes/No
8. Availability of emergency kits at dental office? Yes/No
9. Availability of emergency drugs. Yes/No
 - ADRENALINE
 - ORAL GLUCOSE
 - AMMONIA INHALANT
 - HYDROCORTISONE
 - ATROPINE
10. Do you think it is necessary to attend any workshop on management of medical emergencies? Yes/No
11. A patient suffered from syncope when you commenced a dental procedure. What would be your immediate action?
 - a. continue dental procedure
 - b. place patient in Trendelburg position and give ammonia inhalant
 - c. make patient to sit in upright position
 - d. not sure
12. A patient is cited with airway obstruction during dental treatment due to aspiration of foreign body what would you do?
 - a. attempt Heimlich/ Triple manoeuvrer
 - b. Examine mouth and local area
 - c. ask patient to cough
 - d. all of the above
13. If you confirm somebody is not responding to you even after shaking and shouting at him. What will be your immediate action?
 - a. start Cardio Pulmonary Resuscitation (CPR)
 - b. activate Emergency Medical Services (EMS)
 - c. put him in recovery position
 - d. not sure
14. How do you plan for extraction of a tooth in patients with prosthetic heart valve?
 - a. advise antibiotic prophylaxis
 - b. ask the patient to stop blood thinners
 - c. advise the patient to take consent from the general physician
 - d. all of the above

15. Which of the following procedures can be performed in patients with prosthetic heart valve without giving antibiotic prophylaxis?
 - a. Dental radiographs
 - b. Placement of orthodontic brackets
 - c. Placement of removable prosthesis and orthodontic appliances
 - d. All of the above
16. What is the abbreviation of BLS?
 - a. Best life support
 - b. Basic life support
 - c. Basic lung support
 - d. Basic life sciences
17. What is the location of chest compression?
 - a. Left side of the chest
 - b. Mid chest
 - c. Xiphisternum
 - d. Not sure
18. Ratio of CPR, single rescuer in adult?
 - a. 15:2
 - b. 30:2
 - c. 15:1
 - d. not sure
19. How do you give rescue breathing in infants?
 - a. Mouth to mouth with nose pinched
 - b. Mouth to mouth and nose
 - c. Mouth to nose only
 - d. Mouth to mouth without nose pinched

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IN VITRO EVALUATION OF ANTIMICROBIAL EFFICACY OF SILVER ZEOLITE AGAINST COMMON ORAL PATHOGENS

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ABSTRACT

Objectives. Antimicrobial efficacy and toxicity analysis of 5 different concentrations of silver zeolite (SZ) compound against 5 common oral pathogens *Streptococcus mutans*, *Streptococcus pyogenes*, *Lactobacillus sp.*, *Staphylococcus aureus*, *Candida albicans*.

Material and methods. The antimicrobial efficacy of 5 different concentrations of SZ was tested against 5 common oral pathogens using the agar well diffusion method and the MIC and MBC values were determined using the micro broth dilution method. The toxicity of all 5 different concentrations was evaluated using brine shrimp assay and lethal concentration (Lc50) value was determined.

Results. At 10 µg/mL the antimicrobial activity of SZ was almost negligible. The antimicrobial activity was observed in an increasing trend against all the test microorganisms as the concentration increased. At 75 & 100 µg/mL the zone of inhibitions was more than the control. Furthermore, MIC and MBC values of SZ with concentrations 25, 50, 75, and 100 µg/mL were determined and recorded. SZ was equally effective against all the test organisms. The LC25 (lethal concentration 25) value was 1.6 µg/mL, whereas the LC50 value was 1.77 µg/mL and the LC75 value was 1.90 µg/mL, calculated from the probit computational method.

Conclusion. SZ has the potential to change the ongoing system and bring about a revolution as an antimicrobial drug. However, the dose must be regulated as it can be toxic in higher concentrations. SZ compounds with the correct study of physicochemical properties and toxicity analysis can increase their pharmacological use and market value.

Key words: silver zeolite, oral pathogens, antimicrobial efficacy, toxicity analysis; pharmaceutical applications

INTRODUCTION

The oral cavity is an ambient location for the growth of microorganisms. Several pathogenic or nonpathogenic microbes find an excellent incubation area inside the mouth [10]. This growth of microbes exponentially increases in the presence of a prosthesis or other dental apertures [22, 23]. The use of a partial or complete removable prosthesis imposes a large problem as it provides a greater surface area for microbial adhesion to the oral tissues [18]. In the same way, the use of partial and complete fixed prosthesis poses a challenge and difficulty in maintaining proper oral hygiene and following the mouth cleaning protocol [18, 22]. This, in turn, results in increased chances of

microbial infection in the oral tissues and the remaining teeth. Various common pathogenic bacteria and fungi like *Streptococcus mutans*, *Streptococcus pyogenes*, *Lactobacillus sp.*, *Staphylococcus aureus*, *Candida albicans* are responsible for causing hard tissue as well as soft tissue infections in the oral cavity [4]. Dental caries and oral candidiasis are the most frequently occurring microbial infections in the oral cavity [27]. These clinical consternations resulted in the need to have a new and effective antimicrobial agent that is effective against all these common pathogens, thereby protecting the oral cavity of patients who are partially or completely edentulous.

The noble metals bioactivity as well as their uses are in the focus of interest amongst the researchers

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due to multiple factors such as high reactivity, superior compatibility and the non-toxic nature of the eukaryotic cells [2, 8]. Out of the wide range of noble metals, silver seems to be quite popular in the usage of the biomedical field because of its remarkable physicochemical properties and its cost-effectiveness [3, 6, 13]. Silver is generally non-toxic and has been used in several different forms in the field of medicine and pharmacology for treating various ailments and infections. Silver has been described to be "oligodynamic" because of its potential bactericidal activity even at minimal concentrations [3, 6, 13]. Several inorganic materials act as a carrier for silver particles such as phosphate, zeolite, titanium dioxide, activated carbon that increases its pharmaceutical properties and shelf life, without changing its basic physicochemical properties [3, 6, 13].

The molecule Silver-zeolite (SZ) has a wide range of antimicrobial properties and is rarely used in the field of dentistry. In recent times there has been an increased interest in the usage of these silver nanoparticles (AgNPs) for their properties [25]. The concept is based on integrating AgNPs in between the minute voids present in the crystalline structure of the zeolites and the carrier that is inorganic such as zirconium phosphate silicate and zirconium phosphate [25]. It has been hypothesized that the release AgNPs and the silver ions tend to restrict the growth of nearby microorganisms. Several antimicrobial action mechanisms have been hypothesized for AgNPs like inactivation of vital microbial enzymes, RNA replication interruption and alterations in cell wall permeability [7, 14, 15, 25]. SZ has been introduced as a crystalline aluminosilicate material with silver ions. SZ is known as a pharmacological product effective against various microbes but has not been used extensively in the dental sciences, instead, products like zinc-undecylenate have been used whose antimicrobial efficiency and coverage is far lesser than that of SZ [7, 14, 15, 25]. Therefore, this study aimed to evaluate the antimicrobial efficacy of 5 different concentrations of SZ against five common oral pathogens, *S. mutans*, *S. pyogenes*, *Lactobacillus sp.*, *S. aureus*, *C. albicans* in both *in vitro* along with its toxicity analysis.

MATERIAL AND METHODS

Collections of microorganisms

The test organisms were isolated from patient samples attending the Department of Oral Pathology and Microbiology of our institution and were identified in the Central Research Laboratory of our Institute using standard microbiological methods and safety guidelines.

Agar well diffusion method

The agar-well diffusion procedure was followed to evaluate the antibacterial activity. Blood agar plates were used for *S. mutans* and *S. pyogenes*, Muller Hinton agar was used for *S. aureus* and *Lactobacillus* and Sabouraud dextrose agar was used for *C. albicans*. The bacterial strains were spread with an L-shape spreader with a fully grown broth culture of respective bacteria. Further, 0.5mm wells were bored on each plate with the help of a sterile cork borer. Six wells per plate in equidistant were made. 50 µl of 5 different concentrations: 10, 25, 50, 75 and 100 µg/ml was added to the wells. In the 6th well, amoxiclav (against all bacteria) and amphotericin B (against *Candida*) was used as a positive control. Then the plates were incubated at 37°C for 18-24 hours. The diameter of the zone of inhibitions (ZI, in mm) was measured after the incubation period [16, 30].

Micro dilution method

Use of standardized bacterial colony numbers: Comparison of different microorganisms was difficult since they had different optical densities, growth timing, and requirements. Also, at a time, the number of bacteria of different bacterial species in their respective medium is different. Hence, a concentration of 5×10^5 CFU/ml of each test bacteria were used [16, 30].

Preparation of bacterial culture: Under aseptic conditions, a single colony was inoculated into a 5 ml test tube containing its respective broth, and incubated at 37°C. After incubation, 100µl of that culture was transferred into the 10 ml test tube containing its broth and further incubator until its OD₆₀₀ was obtained at 0.5. When the absorbance was 0.5, serial dilution was done. Here 10⁻⁵ dilutions were taken. In this way, all the strains were prepared for this assay [16, 30].

Preparation of plates: Sterile 96 well microtiter plate was coated with resazurin, media, drug, and bacteria under aseptic conditions inside the laminar airflow. A stock solution of 100 µg/ml was prepared. After that, 4 different lower concentrations were prepared i.e. 10, 25, 50 and 75 µg/ml. Then the wells were filled up with 50 µl of respective broth media, different concentrations of extracts, 10 µl resazurin indicator solution and 10µl of bacterial suspension (5×10^6 CFU/ml) was added to each well to attain 5×10^5 CFU/ml concentration. Two blank wells were made in which the first one no drug was there and in the second well there was no bacterial culture. Each plate was wrapped with aluminium foil and they were incubated overnight at 37°C. The colour change from purple to pink or colourless was recorded as the growth of bacteria. The lowest concentration at which colour does not change to pink colour and remain blue is considered as the MIC value of an extract. Amoxiclav,

a broad-spectrum antibiotic was taken as the standard for the bacteria and whereas amphoteric b was the control used against *C. albicans*. The Minimum Inhibitory concentration (MIC) calculation of the control for the 5 microbial strains was done separately [16, 30].

Brine shrimp toxicity assay

The 5 different concentrations of SZ compounds were routinely evaluated in a test for lethality to brine shrimp larvae as described by *Waghulde et al* [29]. Toxicities of compounds were tested at 10, 24, 50, 75 and 100 µg/mL in 10 mL sea-water solutions with 1% DMSO (v/v). Ten, nauplii were utilized in each test and survivors checked after 24 hours. Distilled water was used as a negative control. The observed percentage of the lethality of the different concentrations SZ was determined based on probit analysis. The log₁₀ concentration values were taken on X-axis and probits were taken Y-axis. The probit values were calculated from the probit computational table [21].

RESULTS

The antimicrobial activity was observed in an increasing trend against all the microorganisms as the concentration increased. At 10 µg/ml the antimicrobial activity of SZ was almost negligible. However, at 25 µg/ml there was a significant increase in the antibacterial activity where *S. aureus* was most sensitive to SZ with a ZI of 19 and against *C. albicans* the ZI was 12 mm. Similarly, the ZI was recorded against all the concentrations (Figure 1, Table 1). At 75 & 100 µg/ml the ZI was more than the control. Furthermore, MIC and MBC values of silver zeolite with concentrations 25, 50, 75, and 100 were determined and recorded. The minimum inhibitory concentration (MIC) was found at 10 µg/mL. Higher ZI or MIC levels do not generally mean, that those concentrations can be directly used in manufacturing drugs or incorporated into any other antimicrobial drug to enhance its activity. Therefore, the host toxicity of each concentration exhibiting antimicrobial activity should be done.

From the graph plotted (Figure 2), the LC₂₅, LC₅₀, and LC₇₅ values were determined along with probit values and their corresponding log₁₀ concentration values, respectively (Table 2). Antilog values of these log₁₀ concentration values are 39.81 (LC₂₅), 58.88 (LC₅₀) and 79.43 (LC₇₅), which are the computed LC values. The individual MIC and LC₁₀₀ values are directly interpreted from the experiments. The LC₂₅ value was 1.6 µg/mL, whereas the LC₅₀ value was 1.77 µg/mL and the LC₇₅ value was 1.90 µg/mL, calculated from the probit computational method (Table 2).

Table 1. Antimicrobial assay by agar well diffusion method and determination of MIC and MBC values of silver zeolite in 5 different concentrations against 5 common oral pathogens (zone of inhibition in mm)

Strain	10 µg/ml			25 µg/ml			50 µg/ml			75 g/ml			100 µg/ml			Control		
	ZI	MIC	MBC	ZI	MIC	MBC	ZI	MIC	MBC	ZI	MIC	MBC	ZI	MIC	MBC	ZI	MIC	MBC
<i>S. mutans</i>	05	-	-	18	15	24	23	15	24	26	12	21	28	9	18	24	10	19
<i>S. pyogenes</i>	-	-	-	15	18	25	22	18	25	26	10	18	27	6	12	23	8	13
<i>S. aureus</i>	04	-	-	19	15	22	23	15	22	23	10	21	25	6	15	25	8	17
<i>Lactobacillus sp</i>	07	-	-	18	21	25	20	21	25	21	12	18	22	9	21	22	10	19
<i>C. albicans</i>	-	-	-	12	18	22	22	18	22	24	12	20	24	12	23	24	11	20

ZI, Zone of inhibition; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

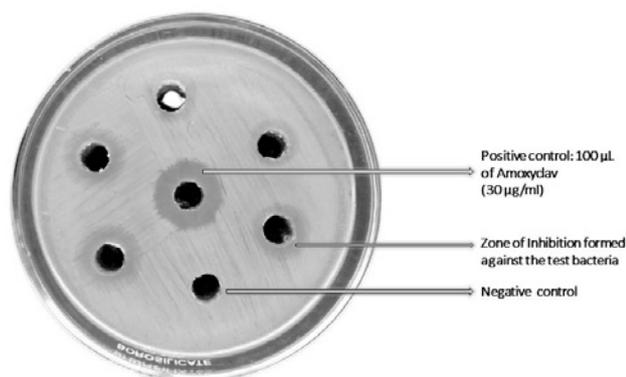


Figure 1. Determination of the antimicrobial activity of silver zeolite against different test organisms by Agar well diffusion method

Table 2. Lethality values during silver zeolite (SZ) toxicity to shrimp nauplii by probit computational method

SZ extract concentration	Log ₁₀ Concentrations	Percentage lethality	Probit values
0	-	0	-
10	1	6.67	3.5
25	1.4	16.67	4.0
50	1.7	43.34	4.8
75	1.9	70.0	5.5
100	2.0	99.94	8.1

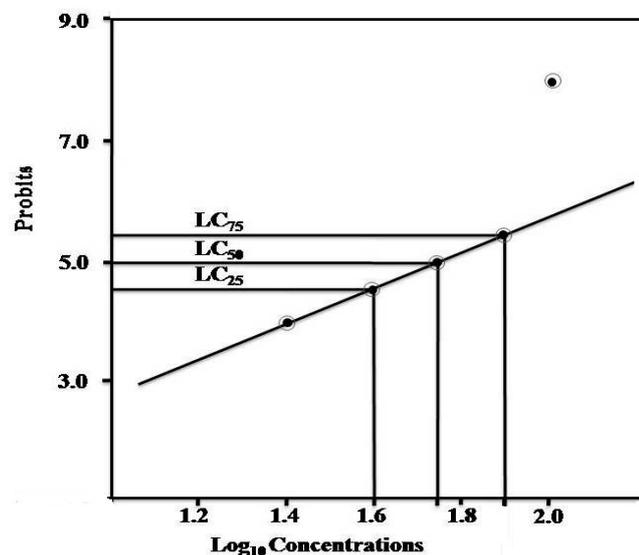


Figure 2. Graphical determination of LC₅₀ value

DISCUSSION

The continuous increase in oral infections has led to the search for noble antimicrobial agents which integrated into various daily use oral/dental products. The noble metals are well sought-after agents because of their therapeutic non-toxic nature towards eukaryotic cells. Out of the wide range of noble metals,

silver is being extensively used in the biomedical field because of its amazing physiochemical properties and its cost-effectiveness. The molecule SZ has a wide range of antimicrobial properties and is rarely used in the field of dentistry. The present *in vitro* study validated that SZ can inhibit the growth of 5 common oral pathogens. However, the increase in the toxicity level to nauplii cells, with an increase in concentration was a point of concern.

The antibacterial efficacy of SZ is similar to that of silver nitrate, where the inorganic salts and ion chelators generate reactive oxygen species, when they come in contact with the bacterial cell, resulting in cell wall damage [15]. In another study, the antibacterial activity of silver-loaded zeolite X was found effective in controlling pathogenic bacteria, such as *Escherichia coli*, *S. aureus*, and *Pseudomonas aeruginosa*, where zeolite X was synthesized and loaded with silver by ion-exchange method [12]. A study from the United Kingdom reported that silicone elastomers complex containing silver (14 wt%) & zeolite (2 wt%) was effective in limiting the growth of *E. coli* and *Staphylococcus epidermidis* [21]. Further, the study reported, the amalgamation of SZ with organo-silicanes, not only increased the mechanical strength of the elastomers, but they were also restricting the colonization of *C. albicans*, gram-positive and gram-negative bacteria, beyond a period of 24 hours incubation [5]. Similarly, a comparative study with polyvinyl chloride (PVC) tube containing SZ was effective in preventing the colonization of *S. epidermis* and *E. coli* for 5 and 20 days, respectively. In comparison, a simple plasticized PVC tube exhibited no antibacterial property [32]. Antimicrobial tests of silver in zeolite-loaded dental acrylic were active against *C. albicans*, *S. mutans* and *Fusobacterium nucleatum* [14]. An Indian study compared the antimicrobial effectiveness of SZ incorporated into two different types of softliners when used *in vivo* for 28 days in complete denture patients, where SZ showed a significant reduction in colony-forming units in both the soft liners [28]. Furthermore, the use of SZ was endorsed by many studies as an antifungal and antibacterial agent [11, 20]. Studies also confirmed, SZ acts as an ion pump, providing the controlled timely release of silver ions, and thus, the controlled release provides continuous antimicrobial protection [17]. The results obtained in our study also reported good antimicrobial activity.

A study with tissue conditioners reported conditioners incorporated with SZ reported slight toxicity to the dermal cells [1]. A cytotoxicity assay of SZ against lung carcinoma, human hepatocellular, colon carcinoma cell-line and breast adenocarcinoma revealed that SZ was nontoxic and silver substituted micronized zeolite-can be used as an antitumor

drug [31]. Further, Zeolite-silver-zinc nanoparticles biocompatibility was evaluated against pulmonary adenocarcinoma cells using MTT assay, where no cytotoxicity was reported [26]. However, a comet assay of SZ with silver nanoparticles against the MRC-5 cell line indicated DNA damage in results [19]. SZ has been incorporated into polymers, textiles, metal coatings, dental/medical materials, environmental/consumer products because of its high antimicrobial and less host toxicity [8, 9]. Our study also reported higher toxicity to the nauplii, with an increase in SZ concentrations. The limitations of this study was the exact physicochemical properties were not analyzed. Moreover, the toxicity study was done using an *in vitro* model, which cannot be accurate. Hence, additional efficient *in vivo* studies must be done using cell lines to confirm the toxicity levels of SZ.

CONCLUSIONS

With the opportunities for new therapeutic options, it is evident that one must opt for new trials and implications of unexplored zones of medicine. SZ has the potential to change the ongoing system and bring about a revolution. It has been proving to show its beneficial effects on its application in the field of dentistry. Various studies have shown that even in low concentrations of AgNPs it is effective on opportunistic microorganisms. However, the dose must be regulated as it can be toxic in higher concentrations. In the coming years, SZ compounds with the correct study of physicochemical properties and toxicity analysis can increase their pharmacological use and market value.

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None

Conflict of interest

The authors declare they have no conflicts of interest.

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DIETARY RECOMMENDATIONS DURING THE COVID-19 PANDEMIC. STATEMENT OF THE COMMITTEE OF HUMAN NUTRITION SCIENCE OF THE POLISH ACADEMY OF SCIENCES*

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ABSTRACT

During the COVID-19 pandemic, care for an adequate diet, well adapted to the body's needs and the current level of physical activity, becomes of particular importance. Many dietary compounds participate in the functioning of the immune system, while vitamins D, C, A (including beta-carotene), E, B6, B12, folic acid, zinc, copper, selenium, iron, amino acids, n-3 and n-6 polyunsaturated fatty acids and intestinal microbiota are crucial in various types of defence processes. There has been no evidence that consumed food and its compounds, including those with pro-/prebiotic properties, play a significant role in preventing SARS-CoV-2 infection or alleviating its course. However, in terms of the nutritional value of food and the prevention of dysbiosis, recommending a varied diet with a high proportion of plant-based foods and an adequate amount of animal-based foods has a sound scientific basis. Malnutrition, underweight and obesity are considered independent and prognostic risk factors of severe SARS-CoV-2 infection, which reduce a patient's chances of survival. Therefore, ensuring good nutritional status, including healthy body weight, is a reasonable approach in the prevention of viral infection SARS-CoV-2 or alleviating its course. The document is accompanied by two catalogues of practical nutritional recommendations during the COVID-19 pandemic, addressed to the general population and children.

Key words: *antiviral properties, breastfeeding, COVID-19, diet, food, immunity, nutrition, pandemic, SARS-CoV-2*

STRESZCZENIE

Podczas pandemii COVID-19 (ang. coronavirus disease 2019) troska o właściwą dietę, dobrze dostosowaną do zapotrzebowania organizmu i aktualnego poziomu aktywności fizycznej, nabiera szczególnego znaczenia. W funkcjonowaniu układu odpornościowego bierze udział wiele składników pochodzących z diety, a znaczenie w różnego typu procesach obronnych mają witaminy D, C, A (w tym beta-karoten), E, B6, B12, kwas foliowy, cynk, miedź, selen,

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żelazo, aminokwasy, wielonienasycone kwasy tłuszczowe n-3 i n-6 oraz mikrobiota jelitowa. Obecnie nie ma dowodów, aby spożywana żywność i zawarte w niej składniki, w tym o właściwościach pro-/prebiotycznych, odgrywały istotną rolę w zapobieganiu zakażeniu SARS-CoV-2 (ang. severe acute respiratory syndrome coronavirus 2) i łagodzeniu jego przebiegu. Jednak biorąc pod uwagę wartość odżywczą żywności i zapobieganie dysbiozie, rekomendowanie urozmaiconej diety, z dużym udziałem różnorodnej żywności pochodzenia roślinnego oraz odpowiednią ilością żywności pochodzenia zwierzęcego, ma dobre podstawy naukowe. Niedożywienie, niedowaga i otyłość są rozważane jako niezależne i prognostyczne czynniki ryzyka ciężkiego przebiegu zakażenia SARS-CoV-2, które zmniejszają szanse pacjenta na przeżycie, dlatego dbałość o dobry stan odżywienia, w tym prawidłową masę ciała, jest rozsądnym podejściem w profilaktyce zakażenia SARS-CoV-2 i łagodzeniu jego przebiegu. Do dokumentu dołączono dwa wykazy praktycznych rekomendacji żywieniowych podczas pandemii COVID-19 – skierowane do populacji generalnej oraz do dzieci.

Słowa kluczowe: COVID-19, dieta, karmienie piersią, odporność, pandemia, SARS-CoV-2, właściwości przeciwwirusowe, żywienie, żywność

INTRODUCTION

The global pandemic resulting from the infection with SARS-CoV-2 virus has changed all aspects of everyday life, as it has brought about a change in the dietary behaviour and lifestyle for most people, accompanied by an increase in stress experienced due to isolation, drastically reduced social interactions and concerns about contracting the virus, and the loss of employment and income [3,12,15,31]. Adequate nutrition is important to maintain health and, during the COVID-19 pandemic, taking care to follow a balanced diet which is well adapted to the body's needs and the current physical activity level, becomes particularly important [8,9,22,47].

Sources of information on nutrition and infection with the SARS-CoV-2 virus

Identification of nutritional factors associated with a mild, moderate, and severe course of infection with the SARS-CoV-2 virus is the starting point for the development of “good nutritional practices” and the formulation of principles of the prevention and treatment of the infection [42]. There is no doubt that the world has been taken by surprise by the scale of the pandemic and its consequences for human health and lives. Experts were expected to indicate methods for treating the infection and preventing its consequences. However, gathering strong scientific evidence (from randomised experimental studies) takes time, and it is difficult to obtain it within the timeframe of the pandemic.

The dietary recommendations have been developed through modifying the existing, well-documented dietary recommendations [11,16,45,56] and adapting them to the changes in Poles' diets and lifestyles observed during the COVID-19 pandemics and are also based on the following:

- knowledge of physiology and pathophysiology concerning the effects of food and nutrition on the nutritional status of the body and its functioning, including the functions of the immune system.

- evidence gathered prior to the pandemic for other respiratory diseases (e.g. pneumonia) and other viruses (e.g. influenza A and B virus),
- knowledge derived from few experimental studies conducted during the pandemic, usually single-centre studies with small sample sizes, or observational studies that are less powerful in explaining the correlations between the diet, SARS-CoV-2 virus infection, the course of infection and mortality; several large experimental studies in this field are underway, but their results and conclusions have yet to be published.

Changes in the diet and lifestyle during the COVID-19 pandemic

The available study results [11,18,21,31] show that in Poland, during the three initial months of the pandemic, many adults increased their food intake (34%) and the time spent in front of a screen (49%), while reducing their physical activity (43%). This resulted in an increase in the prevalence of overweight, and in the near future, it may trigger a further increase in the rates of obesity and many of its co-morbidities, e.g. cardiovascular diseases, diabetes and other metabolic diseases and certain types of cancer [39]. However, some adults have reduced their food intake (14%), including those with previously normal or too low body weight, thus increasing the problem of the prevalence of underweight, malnutrition and anorectic behaviour.

Such changes in food consumption and lifestyle must be considered a major hazard to public health, especially in the context of overweight and obesity. Before the pandemic, 4-5% of children aged 2-6 years, 8% of girls and 14% of boys of school age were obese (data from the National Health Programme for 2017-2020). Moreover, depending on the age, 4-28% of adults or the elderly were obese (data from the European Health Interview Survey, 2014) and two to three times as many Poles were overweight [34,35,46].

The diet, nutritional status and immunity of the body

The foods we eat and the components they are made of determine the body's nutritional status and can affect its ability to prevent and combat infections and promote recovery. Food is a source of approx. 70 nutritional compounds (e.g. amino acids, polyunsaturated fatty acids, glucose, vitamins, and minerals) and many biologically active components (e.g. polyphenols with antioxidant properties) but also anti-nutrients (e.g. phytates, oxalates, and ascorbic acid oxidase, i.e. an enzyme that breaks down ascorbic acid) or even harmful contaminants (e.g. arsenic, benzo(a)pyrene), which together are responsible for the body's health status. Good nutritional status (adequate saturation of tissues with nutrients) is essential for maintaining health and for the proper functioning of all systems and organs of the body, including the immune system.

The immune system protects the body against pathogens (bacteria, viruses, fungi, and parasites) through complex mechanisms in which a variety of specialised cells and regulatory substances are involved [7]. The immune system is active at all times and its activity increases during infection. Increased activity of the immune system is accompanied by an increased metabolic rate and higher consumption of energy and substrates needed for the synthesis of substances and cells involved in defence processes.

Many components derived from the diet are involved in the functioning of the immune system, with the special role in a variety of defence processes served by vitamins D, C and A (including beta-carotene), E, B6, B12, folic acid, minerals: zinc, copper, selenium, and iron, amino acids and polyunsaturated *n-3* and *n-6* fatty acids [7,42]. The most attention is given to vitamins D, C, and A and to zinc, selenium and polyunsaturated *n-3* fatty acids, as they play a crucial role in maintaining the integrity and functions of the immune system, including the activation, differentiation and proliferation of immune cells and in maintaining the stability of cell membranes as the immune response of the body is determined by their interaction at many stages of this process [7,50,51,58,62]. These key nutrients have been demonstrated to be of particular importance in supporting the anti-viral and anti-bacterial defence mechanisms of the body, alleviating infection symptoms and reducing the risk of a severe course of "regular" (i.e. non-covid) respiratory infections [5,7,23-25,28,29,36,40,41,52,53,60,66]. On this basis, their beneficial effect on the course of SARS-CoV-2 virus infection can be predicted and the ongoing clinical trials will help explain the role of nutrients in the treatment of this viral infection [2,7]. Currently, a discussion is underway on the possibility of the therapeutic use of vitamins C, D and A, as well as zinc

and polyunsaturated *n-3* fatty acids, in amounts not exceeding the upper tolerable intake levels (including the components derived from the diet) as a low-cost, pharmacological way to potentially help prevent infection with the SARS-CoV-2 virus or reduce its effects [42-44,48,63]

It is also the bacteria and other microorganisms found in the large intestine of humans (the so-called intestinal microbiota) that serve an important role in regulating immune system functions. The presence of a large number of probiotic microorganisms (e.g. *Bifidobacterium*, *Lactobacillus*) has a beneficial effect on many functions in the intestine and the whole body, including through the formation of a physical and chemical barrier that protects the large intestine against colonisation by pathogenic microorganisms and the probable strengthening of the body's innate immunity [7,32,37,42,59]. Diet is one of the major factors determining the composition of the intestinal microbiota. A disturbed composition of the intestinal microbiota (dysbiosis) is seen in people with a high intake of animal-based foods and a low intake of plant-based foods. Conversely, a favourable composition of the intestinal microbiota is found in people who eat more plant-based foods, including those regularly consuming prebiotic foods that contain short-chain oligosaccharides (e.g. onion, garlic, legumes, whole-grain cereal products from oats or wheat) and fermented foods containing lactic acid bacteria (e.g. yoghurt, kefir, acidophilus milk and fermented vegetables) [7,32,37,42,59,64]. Two systematic reviews with meta-analysis [37,64] have recently confirmed that probiotic microorganisms in adults enhanced the body's immune response to seasonal influenza vaccination. Moreover, a disturbed composition of the intestinal microbiota (dysbiosis) has been demonstrated in patients with a severe course of infection with the SARS-CoV-2 virus.

At present, there is no evidence that the foods consumed and the components they contain, including those with pro- and prebiotic properties, serve an important role in preventing infection with the SARS-CoV-2 virus and alleviating its course [7,49,59]. However, **taking into account the nutritional value of foods and the prevention of dysbiosis, recommending a diversified diet containing a high proportion of plant-based foods and an appropriate amount of animal-based foods has a solid scientific foundation.**

Malnutrition, obesity and infection with the SARS-CoV-2 virus

Nutritional deficiencies (overt or hidden malnutrition), generalised or relating to single components (e.g. vitamins C and D, B-group vitamins, zinc, selenium, iron, protein), reduce the body's

immunity and can therefore increase the risk of falling ill and worsen the course of several diseases. Thus, it is logical that any weakening of the immune system's defensive function, including malnutrition, is conducive to infection with the SARS-CoV-2 virus. On the contrary, a balanced diet that fully covers the human body's requirement for all nutrients can support the functioning of the immune system, including in the course of infection with the SARS-CoV-2 virus.

This view is supported by the results from observational studies. It was found that deficiencies of zinc, selenium and vitamin C or D were several times more common in patients with a severe course of infection with the SARS-CoV-2 virus than in non-infected individuals or patients with a mild course of infection with this virus [7,42]. Almost 50% of patients hospitalised for COVID-19 infection were malnourished [4,42]. Furthermore, in patients with general malnutrition, primary or secondary (resulting from a disease, hospitalisation or therapy applied), a more severe course of infection and higher mortality due to COVID-19 have been demonstrated. An analogous observation concerned underweight or obese patients, which indicates the need to maintain a sustainable energy balance and a normal body weight regardless of the need for adequate saturation of the tissues with nutrients. Experts of the European Society for Clinical Nutrition and Metabolism [4] (ESPEN) published (in 2020) their position containing an unequivocal recommendation stating that the prevention, diagnosis and treatment of malnutrition should be routinely applied in the treatment of patients with COVID-19 and that elderly patients and those with multiple other diseases should be given special care to ensure their adequate nutritional status.

Obesity is considered an independent and prognostic risk factor for a severe course of infection with the SARS-CoV-2 virus, which reduces a patient's chances of survival [4,38,42,65]. Coexistent malnutrition may be a cause of poor prognosis for obese people, as excessive body fat does not exclude the occurrence of nutrient deficiencies, particularly in people with an unhealthy diet or those who have repeatedly followed popular but harmful weight-loss diets. Obesity and infection with the SARS-CoV-2 virus share some similarities, as they exacerbate inflammatory processes in the body, which may take a particularly drastic course in infection with this virus. In a severe course of the SARS-CoV-2 virus infection, a critical response of the body occurs, referred to as the "cytokine storm", resulting from the activation of specific defence cells (macrophages) found in greater numbers in the adipose tissue [7,42,38,65]. Such a scenario has been described in the infection with the H1N1 influenza virus and is currently being considered for the infection with the SARS-CoV-2 virus [7,26].

Irrespective of the results of future research, **avoiding overweight and obesity should undoubtedly be considered a reasonable approach in the prevention of infection with the SARS-CoV-2 virus and alleviating its course.**

Food, its components and infection with the SARS-CoV-2 virus

In many products that are documented to be historically consumed by humans and have been used in traditional medicine, the presence of substances with anti-inflammatory, anti-fungal, anti-bacterial or anti-viral properties was demonstrated, e.g. vitamin C in fruits/juice of raspberries or elderberries, hesperidin, kaempferol and methylglyoxal in bee honey, allicin in garlic and onions, gingerols in ginger, curcumin in turmeric, piperine in pepper, etc [1,6,10,13,27,54,55,57].

There is, however, no strong scientific evidence, let alone systematic literature reviews with meta-analysis, indicating that specific foods (including herbs, herbal infusions and spices) or specific food components with anti-viral, anti-bacterial, and anti-inflammatory properties, or dietary supplements are responsible for the prevention, alleviation of symptoms or treatment of the SARS-CoV-2 virus infection, including the severe covid pneumonia, acute respiratory syndrome, the "cytokine storm", blood clotting disorders and organ failure leading to death [4,7,38,42,65]. **Currently, such an association is considered to be potential and is a valuable hypothesis** that requires evidence from the highest-quality experimental studies and awaits a quantitative summary in systematic literature reviews with meta-analysis.

In view of the above and of the likely health benefits, despite the lack of strong evidence, there is currently **no justification for advising against the consumption of foods containing substances with anti-inflammatory, anti-bacterial or anti-viral properties.** However, daily or frequent consumption of these foods should not lead to drastic changes in the diet composition and to worsening of the diet balance due to having excluded other foods containing crucial nutrients.

Therefore, the previously developed nutritional recommendations referring to the general population and population groups with special needs remain valid. Given that children, adolescents, the elderly and pregnant or breastfeeding women are at greater risk of nutrient deficiencies and the resulting adverse health effects, ensuring the good nutritional status of these population groups is a reasonable approach in the prevention of infection with the SARS-CoV-2 virus.

COVID-19 pandemic and general dietary recommendations

During the COVID-19 pandemic, care must be taken to achieve and maintain optimal nutritional status of the body, including a normal body weight, by providing with the diet all the nutrients needed by the body in amounts appropriate to the age, sex, physiological state and physical activity, in accordance with the dietary recommendations [30], if the individual needs of the body are not known.

The general principle of balanced nutrition is to consume a varied diet based on available foods. A key nutritional strategy for supporting the body and immune system functions should be a diet containing an adequate amount of water, a high proportion of plant-based foods (vegetables, fruit, whole-grain cereal products, legumes, nuts, and seeds), and an adequate amount of foods of animal origin (fish, dairy products, eggs and meat products) [8,9,22,42,47].

A variety of fresh and non-processed or minimally processed food products that provide vitamins, nutrients, dietary fibre, protein, polyunsaturated *n-3* and *n-6* fatty acids and antioxidant components (e.g. polyphenols, carotenoids) should be consumed daily. Foods high in sugar, salt and saturated fatty acids

should be avoided, to significantly reduce the risk of overweight, obesity, cardiovascular diseases, diabetes and certain types of cancer

GENERAL DIETARY RECOMMENDATIONS DURING THE COVID-19 PANDEMIC

1. Ensure that the energy value of the diet is adjusted to the current level of physical activity
2. Half of the daily dietary intake should be based on vegetables and fruit
3. Maintain adequate hydration of the body
4. Make reasonable food purchasing
5. Reduce sugar, salt and fat intake
6. Avoid drinking alcohol
7. Follow the principles of hygiene and safe food handling
8. Consume home-cooked meals in company as a method of stress relief
9. Ensure regular physical activity
10. Use dietary supplementation if the diet fails to meet the body's requirements
11. Use reliable sources of nutrition information

Table 1. General dietary recommendations during the COVID-19 pandemic

1. Ensure that the energy value of the diet is adjusted to the current level of physical activity
<ul style="list-style-type: none"> • Eat a variety of foods, as each food product has a different nutritional value. When choosing food, it is advisable to be guided by the "Healthy eating plate" and to find the right balance between the plant-based and animal-based foods • The daily diet should contain a wide variety of vegetables, fruit, whole-grain cereal products, legumes, nuts, and seeds and an adequate amount of fish, dairy products, eggs and meat products • The diet should be based on low-energy foods with low fat and sugar contents • It is advisable to choose foods with a low glycaemic index¹ (GI<50), which can prolong the feeling of satiety and reduce the energy value of meals while contributing to the normalisation of blood glucose levels and having a positive effect on the regulation of fat metabolism • If you are a sedentary worker, do not overeat or go on a fasting diet in order to maintain or achieve a normal body weight • If you are underweight or overweight, do not skip meals, eat regularly, try to regulate your daily rhythm, and ensure adequate length and quality of sleep
2. Half of the daily diet should be based on vegetables and fruit
<ul style="list-style-type: none"> • Eat at least five servings of vegetables and fruit every day, with a predominance of vegetables (3-4 servings of vegetables, and 1-2 servings of fruit) • Choose raw vegetables and fruit, or minimally processed vegetables (e.g. briefly cooked or steamed) from a variety of colours
3. Maintain adequate hydration of the body
<ul style="list-style-type: none"> • Drink at least 8-10 glasses of unsweetened beverages every day • Preferably, drink either water or fruit or herbal infusions • To improve the flavour of your water, add a piece of fresh, aromatic fruit or some fresh herbs • Eating fresh fruit and vegetables also helps maintain adequate hydration • Reduce your intake of caffeine-containing beverages, as too much caffeine intake (e.g. more than 3-4 cups of coffee a day) can promote dehydration and impair the quality of sleep

4. Make reasonable food purchasing
<ul style="list-style-type: none"> • Food shopping should be well planned and done according to a pre-prepared list of products that are actually needed • The food shopping list should include products that have run out or which need to be replenished, with attention being paid to their shelf-life • It is recommended to buy fresh products and those with a shorter shelf-life, e.g. vegetables, fruit and dairy products • Where it is necessary to use foods requiring longer storage, the best solution is frozen foods whose nutritional value is very close to that of fresh products • Freezing can also be a good way to make reasonable use of the food bought or prepared in too large quantities
5. Reducing sugar, salt and fat intake
Sugar
<ul style="list-style-type: none"> • Choose fresh, frozen or dried fruit without added sugars rather than sweets • If you do eat sweets, choose those with a lower sugar content and added fruit rather than cream and limit consumption • Pay attention to the fat content in sweets – read the nutrition information on labels • Reduce the amount of sugar or honey added to dishes and drinks and substantially reduce or give up the consumption of sweetened beverages
Salt
<ul style="list-style-type: none"> • Reduce or eliminate adding salt to dishes when cooking and, at the table, remove the saltshaker from the table • Salt can be successfully replaced with herbs and spices, or alternatively, with low-sodium salt • Reduce the intake of ready-made cold cuts and meat products. Instead, eat fish and use lean meats prepared by yourself at home, with spices and a reduced amount of salt • Limit or avoid the use of ready-made sauces (e.g. soy and fish sauces), stocks, soups and instant meals in favour of self-prepared dishes seasoned with little salt • Choose unsalted nuts and seeds for snacks
Fat
<ul style="list-style-type: none"> • Choose lean meats and cold cuts and trim excess fat off fatty meats • Limit traditional frying, or replace it with other cooking techniques that require no addition of fat, or low-fat techniques (e.g. baking or low-fat stewing) • Reduce your intake of processed, high-fat dairy products, e.g. processed cheese, cream cheeses and spreads • Remember that vegetable and animal fats have various nutritional values but the same energy value (9 kcal/gram), so, regardless of the type of fat, a high fat intake is conducive to obesity
6. Avoid drinking alcohol
<ul style="list-style-type: none"> • Alcohol impairs the functioning of the nervous system and reduces the immune system's response to infection • Isolation and limited social interactions can contribute to abnormal habits associated with alcohol drinking – avoid situations that encourage drinking alcoholic beverages • Increased alcohol consumption, especially by people who are overweight or obese, or have cardiovascular diseases or diabetes, increases the likelihood of a severe course of COVID-19 infection • Alcohol consumption increases the symptoms of depression and anxiety • Alcohol can reduce the efficacy, or increase the effects and toxicity of certain medications • Do not consume alcoholic beverages during the prevention or treatment of COVID-19 infection
7. Follow the principles of hygiene and safe food handling
<ul style="list-style-type: none"> • Wash food product packages after bringing them home, if possible • Thoroughly wash foods intended for raw consumption, particularly vegetables and fruit • Follow basic rules of hand, kitchen and dish hygiene • Keep raw and cooked foods separately at safe temperatures, preferably below 5°C • Thermally process (at above 60°C) foods that require it
8. Consume home-cooked meals in company as a method of stress relief
<ul style="list-style-type: none"> • Try to prepare meals together with your household members or friends using instant messaging • Have meals together with your household members or friends using instant messaging, at fixed times, in a calm and relaxed atmosphere • If you eat home-delivered, ready-made meals, pay attention to proper hygienic conditions during their transport and delivery and observe the “use by” date
9. Ensure regular physical activity
<ul style="list-style-type: none"> • If possible, try to take a walk every day • Do physical exercises adapted to the abilities and conditions on a regular basis • When doing sedentary work, do short relaxing exercises to change your body position

10. Use dietary supplementation if the diet fails to meet the body's requirements
<ul style="list-style-type: none"> • The use of dietary supplements should be targeted at supplementing the body's deficiencies in the nutrient that is lacking in the diet • If a routine vitamin D supplementation is recommended, adhere to the regimen • Using other dietary supplements containing vitamins or minerals, especially those containing selenium, should always be consulted with and supervised by your doctor • Certain vitamins and minerals, if taken in excess, can have an adverse effect on health (more information to be found in the Position of the Committee of Human Nutrition Science of the Polish Academy of Sciences on dietary supplements)
11. Use reliable sources of nutrition information
<ul style="list-style-type: none"> • During the pandemic, a lot of unconfirmed or distorted information on the effects of dietary factors on infection with the COVID-19 virus and the severity of its course has emerged, with the World Health Organization (WHO) referring to this problem of media disinformation as an "infodemic" • When searching for nutrition information, make sure that it is written by expert teams, preferably linked to academic circles, scientific societies or recognised institutions (e.g. WHO) and that it is confirmed by documented references to the results of scientific studies that have been published in renowned scientific journals • In situations requiring nutritional advice, seek help from a specialist: a dietitian or nutritionist.
<p>¹The glycaemic index is used to describe the body's response and the increase in blood glucose levels after eating food or a meal containing carbohydrates. For example, natural muesli, milk, natural yoghurt, fruit and vegetable juices, nuts, seeds, vegetables, fresh and dried legume seeds, and most fresh and dried fruit have a low glycaemic index (GI<50).</p>

The COVID-19 pandemic and dietary recommendations for children

During the COVID-19 pandemic, children and adolescents are subject to the same nutritional requirements as adults. Periods of their growth and development, in which potential malnutrition can have long-term health consequences, are considered to be crucial [14,17]. It is therefore important to provide the growing body with an adequate amount of all nutrients, with particular emphasis on vitamin D [30,56].

For infants up to six months of age, exclusive breastfeeding should be promoted as optimal to their health and development [56]. To date, no presence of replicating SARS-CoV-2 has been demonstrated in breast milk, while protective antibodies have been found in it [19,33,61]. SARS-CoV-2 virus infection confirmed in a mother is not a contraindication for breastfeeding the child. However, since recommendations on breastfeeding in the context of SARS-CoV-2 virus infection may change as new data become available, it is important to check them regularly, e.g. on the website of the Polish Neonatal Society (www.neonatologia.edu.pl).

As new food products are introduced to a young child's daily diet, it is essential to offer a variety of fresh and non-processed or minimally processed food

to provide water, all nutrients and dietary fibre in amounts appropriate to the child's age and needs [56].

A particular concern with of Polish children at various ages is the continuous increase in the prevalence of obesity [34,35], which is expected to rise, particularly during the pandemic due to lower physical activity resulting from online learning, extended time spent in front of a computer screen and the restrictions on movement and social interactions. Therefore, for the sake of physical, mental and social health, it is essential to promote daily physical activity within the limits permitted by epidemiological regulations [12,15,20].

DIETARY RECOMMENDATIONS FOR CHILDREN DURING THE COVID-19 PANDEMIC

1. Breastfeeding
2. Make sure to drink enough water every day
3. Ensure regular physical activity
4. Provide fresh and unprocessed or minimally processed foods
5. Provide fats of adequate quality
6. Reduce salt and sugar intake
7. Prepare home-cooked meals
8. Use psychosocial support and help from specialists

Table 2. Dietary recommendations for children during the COVID-19 pandemic

1. Breastfeeding
<ul style="list-style-type: none"> • Breastfeeding should be initiated as early as possible, preferably within two hours after birth and continued for the first six months of the child's life as exclusive feeding; then, until at least two years of age, complementary foods with no sugars or salt added should be introduced systematically during the second half of the year of the child's life, in accordance with the current guidelines of the national team of paediatric experts • If COVID-19 infection is confirmed or suspected in a mother, wear a face mask near the baby, wash your hands thoroughly with soap or disinfectant before and after contact with the baby and routinely clean and disinfect all the surfaces being touched • During a severe course of the COVID-19 infection in a mother, or in the case of complications that restrict direct breastfeeding, breastmilk can be expressed to ensure the baby has a continuous supply of breast milk if the mother's health allows it • If, however, breastfeeding is not possible, consideration should be given to the possibility of resuming breastfeeding after a pause or using breast milk from a milk bank
2. Make sure to drink enough water every day
<ul style="list-style-type: none"> • A child should drink several cups of water every day (the recommended daily water intake is 1-2.5 litres, including water from foods) • Water is the most suitable liquid, but other drinks (e.g. unsweetened milk) and vegetables and fruit containing a lot of water (e.g. cucumbers, tomatoes, spinach, melons, broccoli, Brussels sprouts, oranges, apples and berries) can be given to children as well • Avoid giving children sweetened beverages (e.g. fruit drinks, syrups, fruit juice concentrates, fizzy and still drinks) as they contain sugar(s) • Fruit juices can be given to children over one year of age, but in limited amounts (no more than ½-1 glass depending on the child's age)
3. Ensure regular physical activity
<ul style="list-style-type: none"> • Ensure that your child takes daily walks and plays outdoors • Exercise together with your child on a regular basis, adapting the physical exercises to the abilities and conditions • Control the amount of time your child spends in front of a computer or TV screen and limit it to the necessary minimum, especially if learning takes place in front of a screen
4. Provide fresh and non-processed or low-processed foods
<ul style="list-style-type: none"> • Every day, provide your child with vegetables, fruit, legumes (e.g. lentils, beans), low- or non-processed cereal products (e.g. coarse groats, oat and wheat flakes, maize), potatoes, nuts, and animal-based foods (e.g. meat, fish, eggs, dairy products) in quantities adapted to his/her age and nutrient requirements • If you use ready-made meals (e.g. jarred or canned) or dried vegetables and fruit, choose those with no or little salt and sugars added • Give your children raw vegetables and fresh fruit as snacks
5. Provide fats of adequate quality
<ul style="list-style-type: none"> • A child should eat unsaturated fats, e.g. found in fish, avocado, nuts, olive oil, rapeseed, sunflower and maize oil rather than saturated fats, e.g. found in fatty meat, lard and coconut oil • Give your child dairy products in the recommended quantities (e.g. milk, fermented milk drinks, butter, cream, cheeses and cottage cheese), as they are a source of vitamins D, A, and B2 as well as beneficial fatty acids and cholesterol which is essential during the early stages of development • Provide your child with various types of lean meat and fish rather than ready-made cold cuts that contain lots of fat and salt • Do not give your child highly processed foods (e.g. fast food meals, ready-to-eat snacks, fried dishes, frozen pizzas, cakes, cookies or cubed margarine), as they contain industrially produced trans-fatty acids
6. Reduce salt and sugar intake
<ul style="list-style-type: none"> • Reduce daily salt intake from any sources to less than 5 g (approx. 1 teaspoonful) and use iodised salt • When preparing meals for your child, reduce the amount of salt and high-sodium condiments (e.g. soya or fish sauces) • Do not give your child high-salt or sugar snacks (e.g. salty sticks, salted nuts, cookies or sweets) • Do not give your child drinks with a high sugar content (e.g. fruit drinks, fruit concentrates and syrups, flavoured milk and milk drinks)

7. Prepare home-cooked meals
<ul style="list-style-type: none"> • Prepare meals at home to improve the quality of your family's diet, as home-cooked food tend to be healthier and more nutritious for young and growing children than restaurant meals or industrially prepared food which may contain excessive amounts of salt, fats and sugars and can be high in calories • Eating out during the COVID-19 pandemic increases contact with other people and the chance of being infected • Involve children in choosing food and preparing meals together
8. Use psychosocial support and help from specialists
<ul style="list-style-type: none"> • If you feel insecure or overwhelmed by the responsibilities involved in feeding your children, seek psychosocial support from family and friends and specialist advice from a psychologist, dietitian or nutritionist

SUMMARY

This document is supplemented by two lists of recommendations addressed to the general population (Table 1) and to children (Table 2), which provide a detailed overview of practical nutritional recommendations for the population in Poland during the COVID-19 pandemic.

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