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24 Chocimska Street, 00-971 Warsaw, Poland
e-mail: roczniki.pzh@pzh.gov.pl
Editor-in-Chief phone: +48 22 54 21 266; fax +48 22 849 35 13, e-mail: cwludwicka@pzh.gov.pl

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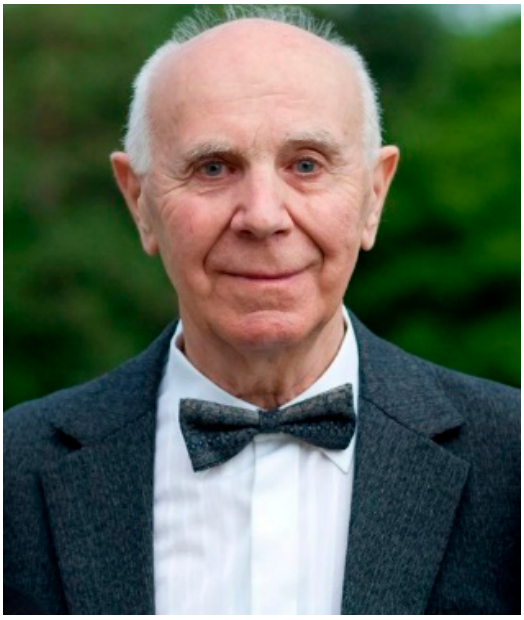
IN MEMORIAM: PROFESSOR STANISŁAW KAFEL (1927-2023)

Lucjan Szponar[1], *Ewa Rychlik*[1],

[1]National Institute of Public Health NIH – National Research Institute,
24 Chocimska str., 00-791 Warsaw, Poland



Professor Stanisław Kafel (1927-2023)

Professor of veterinary sciences Stanisław Kafel passed away on March 23, 2023 in Warsaw, Poland. He was a distinguished employee of the Institute of Food and Nutrition (IŻŻ) in Warsaw, incorporated in 2020 into the structures of the National Institute of Public Health - National Institute of Hygiene.

Professor Stanisław Kafel was born on July 1, 1927. In 1952 he graduated from the Faculty of Veterinary Medicine at the Maria Curie Skłodowska University in Lublin and was awarded a diploma in veterinary medicine. After graduation he started working at the Department of Animal Products Hygiene of the Institute of Veterinary Medicine in Puławy, obtaining the following scientific degrees of assistant, doctor and then doctor habilitated of veterinary sciences, where he was a head of the Laboratory of Microbiology of Animal Products. In addition, he worked at the Laboratory of the Veterinary Sanitary Inspection in Puławy acting as its manager. He was also a consultant on food hygiene for the Veterinary Department of the Ministry of Agriculture.

He has completed foreign fellowships at scientific institutions in the USA and Canada. He has actively participated in national and international congresses and scientific conferences, including in the USA and Canada, as well as in the meetings organised by FAO and WHO.

In 1971, as an expert in animal product hygiene, he was delegated to work for Food and Agriculture Organisation (FAO) of the United Nations in Rome, where he was involved in developing projects to improve food hygiene conditions and actively participated in their implementation in various regions of the world. After the end of this mission in 1976 he took a position at the Faculty of Veterinary Medicine at the Agro-Technical Academy in Olsztyn, where he was head of the Department of Animal Products Hygiene. There he developed research work in the

Corresponding author: Ewa Rychlik, National Institute of Public Health NIH – National Research Institute, 24, Chocimska str., 00-791 Warsaw, Poland tel +48 5509821, e-mail:erychlik@pzh.gov.pl

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field of food microbiology, enabling his assistants to go on scientific fellowships to the USA, Canada, England, Denmark and the Netherlands. In 1978 he was awarded the title of associate professor and in 1989 full professor of veterinary sciences.

In 1983, he began working at the Institute of Food and Nutrition in Warsaw, where he headed the Microbiology Laboratory. After leaving the Institute in 2001, he continued to maintain close relationships with it, including being a member of its Scientific Council.

Professor Stanisław Kafel, as an outstanding expert in meat hygiene, worked for many years in international forums with the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) in Geneva. On behalf of these organisations, he participated in many missions abroad, including Indonesia and India. He also participated in meetings of FAO/WHO expert groups where he provided opinions on food hygiene documents.

In his scientific work, he dealt with the issues of food hygiene, especially meat and meat products, laboratory testing methodology on food quality and health evaluation of food products. He also conducted scientific research on *Helicobacter pylori* and *Campylobacter jejuni* bacteria and factors inhibiting their growth. His scientific achievement include about 100 publications in national and international scientific journals. He supervised 6 completed doctoral dissertations.

The most important scientific achievement of Professor Stanisław Kafel was the development, together with a team of colleagues at the Institute of Food and Nutrition, of a urease test to detect the presence of *Helicobacter pylori* bacteria in the gastric mucosa. This test allows early diagnosis of risk factors for gastric cancer. The test was patented as the first of this kind on the Polish market. This test is still produced and used for diagnostic purposes to date, reducing the risk of premature deaths from stomach cancer.

Professor Stanisław Kafel, an outstanding scientist, will remain in the memory of his colleagues as a great leader and a warm-hearted colleague.

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REVIEW ARTICLE

FOLIC ACID – IMPORTANCE FOR HUMAN HEALTH AND ITS ROLE IN COVID-19 THERAPY

Katarzyna Kurowska[1], *Milena Kobylińska*[1], *Katarzyna Antosik*[1]

[1]Siedlce University of Natural Sciences and Humanities, Faculty of Medical and Health Sciences, Siedlce, Poland

ABSTRACT

Folic acid (folacin, B_9) is a vitamin that performs many very important functions in the human body, and its inadequate level - deficiency as well as excess, may contribute to an increased risk of developing many disease processes. The aim of this study was to analyze the available scientific literature on folic acid and its impact on human health. A systematic review of the studies, published until November 2022, was made on the basis of searching bibliographic databases such as: PubMed, Elsevier and Google Scholar. The following keywords and combinations were used: folic acid, folate, folic acid supplementation, folate deficiency. Folic acid, thanks to its high biological activity, has a direct and indirect effect on the metabolism of the human body cells. It plays a very important role, among others in the prevention of neural tube defects and megaloblastic anemia, the proper functioning of the nervous system, as well as reducing the risk of developing certain cancers. Currently, the important role of folic acid in maintaining the proper functioning of the immune system is also emphasized, which is of particular importance both in the prevention and in the situation of SARS-CoV-2 (COVID-19) infection.

The effects of deficiency and excess of vitamin B_9 may turn out to be dangerous to health and even life. There is a need for nutritional and health education of the society regarding the importance of folic acid for human health, due to the presence of large deficiencies in the population, which is particularly important for some social groups, such as, for example, women of procreation age, pregnant or breastfeeding, people with a nutrient malabsorption, and people who smoke or abuse alcohol.

Key words: *folic acid, folate, deficiency, supplementation, health*

STRESZCZENIE

Kwas foliowy (folacyna, B_9) to witamina, która pełni wiele bardzo ważnych funkcji w organizmie człowieka, a jej nieodpowiedni poziom – niedobór jak i nadmiar, może przyczynić się do zwiększonego ryzyka rozwoju wielu procesów chorobowych. Celem niniejszej pracy była analiza dostępnej literatury naukowej dotyczącej kwasu foliowego i jego wpływu na zdrowie człowieka.

Systematycznego przeglądu badań, opublikowanych do listopada 2022 r., dokonano na podstawie przeszukiwania bibliograficznych baz danych takich jak: PubMed, Elsevier oraz Google Scholar. Użyto następujących słów kluczowych i ich kombinacji: kwas foliowy, foliany, suplementacja kwasem foliowym, niedobór kwasu foliowego. Kwas foliowy, dzięki swojej wysokiej aktywności biologicznej ma wpływ bezpośredni, jak i pośredni na metabolizm komórek organizmu człowieka. Pełni bardzo ważną rolę m.in. w zapobieganiu powstawania wad cewy nerwowej, niedokrwistości megaloblastycznej, prawidłowym funkcjonowaniu układu nerwowego, jak również redukcji ryzyka rozwoju niektórych nowotworów. Obecnie, podkreśla się również istotną rolę kwasu foliowego w utrzymaniu prawidłowego funkcjonowania układu immunologicznego, co ma szczególne znaczenie zarówno w profilaktyce jak i w sytuacji zakażenia wirusem SARS-CoV-2. Skutki niedoboru, jak i nadmiaru witaminy B_9 mogą okazać się niebezpieczne dla zdrowia, a nawet życia. Istnieje potrzeba edukacji żywieniowo – zdrowotnej społeczeństwa w zakresie znaczenia kwasu foliowego dla zdrowia człowieka, ze względu na występowanie dużych niedoborów w populacji, co jest szczególnie ważne, dla niektórych grup społecznych takich jak np. kobiety w wieku prokreacyjnym, w ciąży czy też karmiące piersią, osoby z upośledzeniem wchłaniania składników odżywczych oraz osoby palące papierosy lub nadużywające alkohol.

Słowa kluczowe: *kwas foliowy, foliany, niedobór, suplementacja, zdrowie*

Corresponding author: Milena Kobylińska, Siedlce University of Natural Sciences and Humanities, Faculty of Medical and Health Sciences, Prusa Str. 14, 08-110 Siedlce, Poland, Tel: +48 256431262, e-mail: milena.kobylinska@uph.edu.pl

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INTRODUCTION

A well-balanced diet is a key factor for maintaining good health. One of vitamin that performs many very important functions in the human body is folic acid (folacin, vitamin B_9, pteroylmonoglutamic acid). Inadequate levels of this vitamin in the body - deficiency as well as excess - can contribute to an increased risk of developing many disease processes [18, 28].

In terms of chemical structure, folic acid consists of p-aminobenzoic acid, pteridine base, and glutamic acid (Figure 1). The biologically active form of folic acid is levomepholic acid, or L-5-methyl-tetrahydrofolate (5-MTHF) [18].

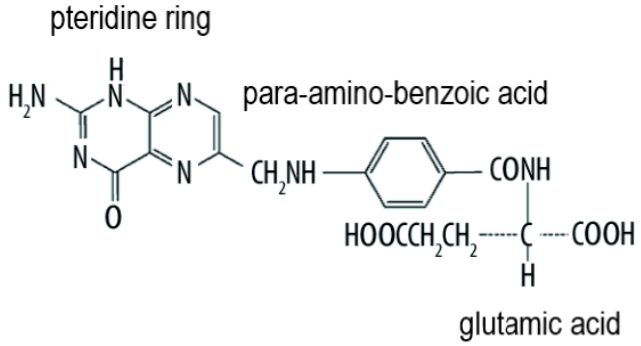

Figure 1. The structural formula of folic acid [1].

Folic acid, naturally occurs in food in the form of folates (salts of folic acid) - a group of compounds that differ in the number of glutamic acid residues and the oxidation state of the pteridine ring, which also includes folic acid derivatives such as dihydrofolate (DHF), tetrahydrofolate (THF) or methylfolate. It is worth noting that folic acid has the highest stability and bioavailability among the above-mentioned group of compounds [16, 32, 68].

It should be mentioned that the human body is not capable of synthesizing this vitamin, except in trace amounts, which are formed with the participation of the intestinal microbiota, mainly intestinal microorganisms of the genus *Bifidobacterium* (especially *B. asoledcentis* and *B. pseudocatenulatum*), *Lactobacillus, Bacterioidetes, Fusobacteria, Proteobacteria* and *Actinobacteria* [6]. Therefore, this vitamin should be supplied from exogenous sources, i.e. with food or appropriate supplementation [18, 23, 32, 39].

Sources of folate in the diet are both products of plant and animal origin. High in their content, among products of plant origin, are characterized by short-cooked and raw green leafy vegetables (Brussels sprouts, lettuce, broccoli, cabbage, spinach, asparagus, parsley leaf and root), legumes (peas, soybeans, beans), whole grain products, citrus fruits or nuts. Among products of animal origin high content of vitamin B_9 are: eggs, offal (mainly liver), soft ripened cheeses (brie, camembert) and some species of fish (e.g. salmon, tuna) [16, 51, 53, 41].

Folic acid, due to its high biological activity, has a direct and indirect effect on the metabolism of cells in the human body. It plays a very important role in, among other things, preventing neural tube defects [9, 50], reducing the risk of megaloblastic anemia [33, 41], proper functioning of the nervous system [9, 15, 41] and reducing the risk of developing certain cancers [6, 13, 63].

The purpose of this study was to review the current literature on the analysis of the impact of folic acid deficiency on the risk of fetal developmental disorders, megaloblastic anemia, neurodegenerative diseases, cancer, as well as to assess the importance of folic acid in maintaining the proper functioning of the immune system, especially in terms of prevention as well as in the situation of SARS-CoV-2 infection.

A systematic review of studies published up to November 2022 was done by searching bibliographic databases such as PubMed, Elsevier and Google Scholar. The following keywords and their combinations were used: folic acid, folate, folic acid supplementation, folic acid deficiency.

CAUSES AND EFFECTS OF FOLIC ACID DEFICIENCY IN THE ORGANISM

An important cause of folate deficiency in the human body is the dietary mistakes made, especially the insufficient intake of vegetables and fruits and whole grain cereal products each day - they are a valuable source of this vitamin [41, 78]. Low dietary folate content may also be due to high losses of folate during food processing and storage [17, 41]. Impaired utilization, absorption, and storage of folacin in the body's body cells can also be caused by drinking large amounts of tea or coffee [17], alcohol abuse [41], and smoking [17].

The concentration in the body of such vitamins and minerals as vitamin B_{12}, ascorbic acid, zinc, iron, among others, as well as proper levels of methionine, can have a significant impact on the human body's utilization of folate. Insufficient vitamin B_{12} in the body results in decreased folate retention in cells. Ascorbic acid deficiency, in turn, contributes to impairing the body's ability to maintain folate in a reduced, or metabolically active, state. Impaired folate absorption can also be caused by structural and functional disorders of the small intestine and a deficiency of conjugases that break down polyglutamine compounds [16].

Painkillers such as aspirin or ibuprofen, antiepileptic drugs, antibiotics, cortisone, sulfamides, as well as folic acid antagonists (e.g., trimethopim, methotrexate) also have a lowering effect on folate.

In addition, those at risk of folate deficiency include cigarette smokers, women who use oral contraceptives and alcohol abusers [55].

Folic acid deficiency in the human body leads to an increased risk of developing a number of disease processes, including megaloblastic anemia, which is characterized by: impaired concentration, tachycardia, chronic fatigue, headaches and dizziness, or pallor of the skin and mucous membranes (although there may also be foci of hyperpigmentation, especially of the dorsal surfaces of the fingers). Folic acid deficiency is also the cause of non-specific gastrointestinal complaints, manifested by: diarrhea or constipation, loss of appetite, burning tongue - the so-called "buffalo" tongue, which is characterized by a dark red color and smoothness. Symptoms of psychiatric disorders such as depression, cognitive function abnormalities, mood swings, delusions and dementia syndrome, thrombosis are attributed to hyperhomocysteinemia, which in turn is the result of folate inactivity [41, 58]. In the context of reproduction, folic acid deficiency significantly correlates with a higher incidence of neural tube defects. Deficiencies of this compound in the diet of pregnant women may increase the risk of cleft lip and palate in the child and the incidence of Down syndrome [64].

There is also scientific evidence that indicates that folic acid deficiencies may increase the risk of certain cancers, i.e. uterine cancer, ovarian cancer, breast cancer, colorectal cancer, lung cancer. What's more, there are also emerging scientific studies that confirm that not only deficiencies, but also excessive folate intake can contribute to accelerating the development of cancers such as prostate cancer and laryngeal cancer. However, the role and importance of folic acid in the process of cancer formation requires further research [60, 41].

Based on available scientific findings, it is indicated that with folic acid deficiency, neuropsychiatric symptoms may occur, including irritability, insomnia cognitive decline, fatigue, depression and psychosis [29, 41].

Folate deficiency can also affect genetic stability, as it is involved in the synthesis of purines and thymines. A reduction in the thymine pool needed for polymerization and DNA (deoxyribonucleic acid) repair is a consequence of folate deficiency. This can be followed by the misattachment of uracil in place of thiamine. The appearance of uracil, and on the opposite side of adenine, can lead to the conversion of base pairs during replication from a GC pair to an AT pair in the newly formed molecule. There is a high risk that such a disruption will have significant mutagenic consequences. When there is too little folate (which is also thymidine nucleotides) in the subsequent repair processes, complete genetic destabilization can occur, which is associated with chromosomal aberrations and DNA strand breakage [38]. Research indicates that the amount of defectively attached uracil to lymphocyte DNA decreases in women of childbearing age, which is related to the supply of 400 μg of folic acid per day, and this in turn confirms the need for folic acid supplementation during the procreative period. The genetic stability of the cell is also influenced by the level of methylation of the genome, where this compound is known to play an important role [26]. Thus, dietary folic acid deficiency contributes to the disruption of many very important metabolic processes, and can result in impaired cell growth and development. Among the best known functions of folic acid is the prevention of the development of neural tube defects and complications of pregnancy. Prevention of cardiovascular disease, neurodegenerative diseases, cancer and megaloblastic anemia are also cited [7, 16, 41].

FOLIC ACID AND FETAL DEVELOPMENT DISORDERS

Pregnant women are among those with a special need for folic acid. Deficiency of this component can cause many negative consequences. Folic acid is very important for the growth of cells of the developing fetus. The baby's spinal cord and brain grow from the neural tube - so the timing of its formation is very important. Statistics show that defects of the central nervous system (CNS) are one of the leading causes of neonatal deaths [9, 24, 50].

During pregnancy, folic acid deficiency leads to birth defects and complications such as miscarriage, fetal demise, premature placental separation, placenta previa, prematurity, low birth weight, and megaloblastic anemia [41, 50].

During pregnancy, the need for folate increases and can be met only partially through the supply of appropriate foods that are sources of these nutrients. It is estimated that the diet allows 50% of the daily requirement for these nutrients. For this reason, supplementation is recommended and plays a special role during the planning period of pregnancy and in its early stages. During pregnancy, the daily requirement for folic acid is 600 μg. The recommendations emphasize that all women of childbearing age should include folate-rich products in their diets, as well as fortified foods [71]. Women planning pregnancy should additionally supplement folic acid for at least 12 weeks before conception at 400 μg/day. During the first trimester of pregnancy - until the 12th week - folic acid should be supplemented at 400-800 μg/day. After the 12th week of pregnancy, women without additional risk factors should take folic acid supplementation at a dose of 600-800 μg/day [75]. Adequate intake of folic

acid prevents the development of neural tube defects. These are congenital disorders that arise in embryonic life and are very severe, affecting the child's nervous system. Neural tube defects manifest themselves as: brainlessness, microcephaly, spina bifida or hernias of the nervous system, causing, among other things, paralysis of the legs, rectal and bladder dysfunction, hydrocephalus, mental retardation, epilepsy, and in extreme cases can contribute to death. The neural tube is an organ that forms early in the pregnancy, during the embryonic period, roughly around its 4th week. Unfortunately, at this time very many women are unaware that conception has occurred. Folate deficiency during this period will adversely affect the formation of the baby's nervous system and the closure of the neural tube [22]. Based on the study, it was shown that women who gave birth to children with CNS defects had low plasma folate and elevated homocysteine levels [64].

All clinicians should remember that failure to educate pregnant women about the importance of folic acid can result in a medical malpractice lawsuit if the baby is born with a neural tube defect [41].

It should be mentioned, however, that scientific data suggest caution in the case of, for example, the presence of megaloblastic anemia, where the supply of folic acid may conceal the true cause of this condition, which may be vitamin B_{12} deficiency, and in the case of reduced metabolism of folic acid in the liver, where it is then recommended to supplement 5-MTHF instead of folic acid, since this compound does not require activation and is immediately available to the mother and fetus, not accumulating in the blood [24, 52].

A number of epidemiological studies have shown that folic acid supplementation of the mother's diet before pregnancy, as well as during the first weeks of pregnancy, can be an important element in the primary prevention of congenital vascular and cardiac defects in the child. Interpretations on selected types of these defects have proven that taking folacin supplements significantly reduces the risk of cardiac septal defects (primarily ventricular septal defect), but also cone defects, as well as vascular trunk defects (especially Fallot Syndrome and transposition of the great vessels) [78]. Women with obesity were found to have twice the risk of fetal neural tube defects, compared to women of normal weight. In addition, it was observed that folic acid supplementation reduced the risk of neural tube defects, which was particularly evident in women with a BMI >25 kg/m^2 compared to women whose BMI was <25 kg/m^2 [10]. In addition, it was found that nervous system defects in the fetus, significantly more often affect women who have type 1 or type 2 diabetes. An analysis by *Parker* et al. [57] found a lower rate of nervous system defects among a group of women with diabetes who supplemented with folic acid at a dose of more than 400 μg/day.

FOLIC ACID AND MEGALOBLASTIC ANEMIA

Megaloblastic anemia (MBA), otherwise known as macrocytic anemia, is a type of anemia characterized by both reduced numbers and the presence of unusually large and underdeveloped erythrocytes (megaloblasts) and their mature forms (macrocytes and megalocytes) [2, 69]. The underlying pathophysiology of this condition includes abnormalities in DNA synthesis and induction of apoptosis in pre-erythroblasts, which ultimately leads to pancytopenia [21, 41]. As indicated by the results of the study, the induction of apoptosis in cells, is associated with increased expression of the transcription factor - p53 protein, which may be due to low levels of vitamin B_{12}, as well as B_9, and high plasma homocysteine levels [33, 65]. In a study by *Yadav* et al. [79, 80], based on immunohistochemical analysis, p53 protein expression levels were found to be significantly higher in megaloblastic anemia patients who also showed very low levels of vitamin B_{12} and B_9, compared to controls.

According to research results, anemia associated with micronutrient deficiencies, including folic acid deficiency, is very common among pregnant women [30].

In infants as well as young children, megaloblastic anemia can also be caused by folic acid deficiency. Factors that cause this condition can be: increased need for folic acid (increased hemolysis of red blood cells, infections, rapid weight gain, prematurity); inadequate supply in food (severe malnutrition, use of goat's milk, exclusively milk feeding); interference with absorption from the gastrointestinal tract (cystic fibrosis, celiac disease); increased loss of folic acid (liver damage or abnormal tubular reabsorption in the neonatal period); inborn abnormal metabolism of folic acid; others (presence of parasites in the gastrointestinal tract, taking anticonvulsant drugs) [70].

Treatment of megaloblastic anemia caused by folic acid deficiency includes the use of a properly balanced diet, including the supply of foods rich in the folic acid component, and/or the use of folic acid in the form of oral, intravenous or subcutaneous preparations, usually in a dose of 1 to 5 mg/day [19, 48]. This dose exceeds the recommended daily supply of folic acid, which is 400 μg, thus allowing adequate supplementation of the deficiency of this component, even in the case of malabsorption syndrome. Treatment continues through the period of hematological recovery or until the cause of the deficiency is eliminated. For patients with malabsorption syndrome, treatment continues

indefinitely [69]. As the results of the study indicate, some foods, especially those intended for children, are fortified with folic acid, making folic acid deficiency extremely rare in children. In contrast, people who consume foods without folic acid fortification and with negligible amounts of vitamin B_9 have a higher risk of megaloblastic anemia [2].

FOLIC ACID AND THE DEVELOPMENT OF NEURODEGENERATIVE DISEASES

Folic acid plays an important role in the functioning of the nervous system, by, among other things, participating in the synthesis of neurotransmitters, i.e. epinephrine, norepinephrine and dopamine. Its deficiency in the body, can contribute to delayed development of the nervous system, increased risk of demyelination of the brain and peripheral nerves, deterioration of motor and behavioral abilities, as well as neurocognitive disorders [9, 15, 35, 42].

It should be mentioned that the occurrence of the above-mentioned dysfunctions concerning the nervous system, is primarily associated with high plasma levels of homocysteine, which, under conditions of folic acid deficiency, is not properly metabolized to methionine. According to research findings, homocysteine is an independent risk factor for both the development of cardiovascular [34] and cerebrovascular diseases, including *Alzheimer's* disease, *Parkinson's* disease and vascular dementia [40, 49, 59].

C*heng* et al. [14] conducted a study to analyze the effects of oral supplementation with folic acid, vitamin B_6 and B_{12} on cognitive function and plasma homocysteine levels, among the elderly. For this purpose, study participants were divided into two groups - an intervention group that received oral supplementation with 800 μg of folic acid, 10 mg of vitamin B_6 and 25 μg of vitamin B_{12}, for a period of 14 weeks, and a control group receiving a placebo. Patients' cognitive functions were assessed using the Basic Cognitive Aptitude Tests (BCAT). Based on the data obtained, an improvement in cognitive function was observed, as well as a significant reduction in homocysteine levels, with a concomitant increase in serum levels of folic acid, vitamin B_6 and B_{12} in subjects taking supplementation with the aforementioned vitamins.

Interesting results were also presented by *Ma* et al. [46], who analyzed the effects of 6 months of oral supplementation with folic acid (800 μg/day) or vitamin B_{12} (25 μg/day), or folic acid (800 μg/day) combined with vitamin B_{12} (25 μg/day), on cognitive function in people 65 years of age and older. Based on the data obtained, greater improvements in cognitive function, as assessed by the Wechsler Adult Intelligence Scale - Revised by China (WAIS-RC), were observed with a combination of folic acid and vitamin B_{12} supplementation than with oral supplementation with folic acid or vitamin B_{12} alone. Identical results were obtained for the improvement of pro-inflammatory cytokines, i.e. IL-6, TNF-α and MCP-1.

Folate deficiency not only contributes to an increase in plasma homocysteine levels, but also leads to inhibition of the synthesis of S-Adenosylmethionine (SAM), which plays an important role in both the synthesis and catabolism of catecholamines in the brain, resulting in a disruption of neurotransmission, symptoms of which can include cognitive decline and mood changes. Reduced levels of S-Adenosylmethionine (SAM) have been found both in depressed individuals and among those with *Alzheimer's* disease [18].

Alzheimer's disease, referred to as "senile" dementia, is the most common factor in dementia disorders in the elderly. It is a degenerative disease of the central nervous system that is characterized by progressive deficits in cognitive functions, especially memory, and behavioral disorders, such as apathy, agitation or psychotic symptoms. The presence of neurofibrillary degeneration and extracellular amyloid deposits in the form of amyloid plaques are neuropathological features of *Alzheimer's* disease [27].

A meta-analysis of studies conducted by *Wang* et al. [74] to evaluate the association between plasma homocysteine and folic acid levels and the risk of vascular dementia or *Alzheimer's* disease observed that a 5 μmol/L increase in plasma homocysteine levels was associated with a 9% increase in the risk of vascular dementia and a 12% increase in the risk of developing *Alzheimer's* disease.

A different conclusion was reached by *Ford* et al. [25], who in their study found no improvement in cognitive function, as assessed by the *Alzheimer's* Disease Assessment Scale - Cognitive Subscale (ADAS - Cog), between a group of subjects who took folic acid, vitamin B_6 and vitamin B_{12} supplementation (at doses of 2 mg, 25 mg, 400 μg per day, respectively) and a control group. Based on the results of a study by *Bae* et al. [8], it was concluded that not only hyperhomocysteinemia, but also hypohomocysteinemia can significantly increase the risk of dementia and *Alzheimer's* disease in the elderly. During a follow-up period of 5.5 years, both study participants with high serum homocysteine levels ≥10.6 μmol/L and low levels ≤8.9 μmol/L had a 4-5 times higher risk of developing dementia and *Alzheimer's* disease than those with serum homocysteine levels between 9.0 and 10.5 μmol/L. The study's authors suggest that the risk of dementia from overuse or misuse of vitamin preparations, including those containing folic acid, should be taken into account.

As already mentioned, too low levels of folic acid, as well as vitamin B_{12}, can result in increased homocysteine levels [41], in effect contributing to accelerated dopaminergic degeneration, a factor in cognitive impairment in *Parkinson's* disease. Patients with *Parkinson's* disease, with symptoms of cognitive decline, and who are being treated with levodopa should have their homocysteine levels measured annually, and should consider folic acid supplementation to lower their homocysteine levels if they reach levels higher than 10 μmol/L [31, 62].

A*namnart* and *Kitjarak* [3], analyzing the effect of folic acid and vitamin B_{12} supplementation on plasma homocysteine levels in *Parkinson's* disease patients treated with levodopa, showed that supplementation with the aforementioned vitamins was associated with a significant decrease in plasma homocysteine in the subjects.

Similar results were obtained by *Dong* and *Wu* [20], who, in a meta-analysis of 26 clinical-control studies, observed that patients with *Parkinson's* disease, were characterized by higher plasma homocysteine levels, with low levels of folic acid, as well as vitamin B_{12}, compared to control subjects. The results obtained by the above-mentioned authors, testify to the significant role of proper folic acid concentration in the body of people with *Parkinson's* disease.

FOLIC ACID AND CANCER RISK

Folate, plays an important role in the prevention of cancer, by influencing the proper course of DNA synthesis, transcription and repair. Deficiency of the aforementioned nutrient, can promote an increased risk of damage to genetic material, as well as hypomethylation, which can consequently initiate the process of carcinogenesis. Moreover, the results of the study indicate that folic acid deficiency, indirectly contributes to increased activity of protooncogenes, thus promoting the process of tumorigenesis [56, 82].

A meta-analysis of 16 prospective studies and 26 case-control studies examining the association between folic acid intake and breast cancer risk, conducted by *Chen* et al. [13], found that a dietary supply of folic acid at 153-400 μg per day was associated with a significantly lower risk of breast cancer among study participants than a dietary supply of less than 153 μg per day.

Folate has also been shown to have a preventive role in the early stages of colorectal carcinogenesis; however, concerns have been raised over its excessive consumption, which would in turn be a potential pro-cancer factor [47]. *Wang* et al. [73] conducted a study to analyze the association between increased folate intake and colorectal cancer risk among 86,320 women. Based on the data obtained, it was concluded that a high intake of folic acid, expressed as dietary folate equivalents, was not associated with an increased risk of colorectal cancer among the study population of women. The same results were also obtained by Australian researchers *van der Pols* et al. [71]. In contrast, a 2022 meta-analysis of studies, including randomized controlled trials, which aimed to evaluate the effect of folic acid supplementation on atrophic gastritis, intestinal metaplasia and endothelial neoplasia, which are referred to as gastric precancerous conditions (GPC), showed that a folic acid supplementation dose of 20-30 mg/day for 6 months was associated with a reduction and even a reversal of the progression of gastric precancerous conditions, which may have potential clinical application in the treatment of the above-mentioned abnormalities [44].

An interesting study was also conducted by *Yan* et al. (2022), who showed that maternal supplementation with folic acid and/or multivitamins, during pregnancy, was associated with a statistically lower risk of childhood and adolescent nasopharyngeal carcinoma in the offspring compared to the control group [81].

Depending on the type of cancer, various factors are distinguished that increase the risk of the disease. Increasing attention is being paid to disorders of the oxidation-antioxidation balance of the body. Oxidative stress is an important factor in the pathogenesis of many diseases including cardiovascular, pulmonary, neurodegenerative diseases, but also cancer. ROS (reactive oxygen species) significantly contribute to the acceleration of aging processes, the accumulation of oxidative damage products, which play an important role in the processes of carcinogenesis [36].

A systematic review and meta-analysis of studies aimed at evaluating the effects of folic acid supplementation on markers of oxidative stress showed that folic acid supplementation at a dose of 0.4-10 mg/day (mean 5.1 mg/day), applied over a period of 8 to 25 weeks, can significantly improve markers within the antioxidant defense system by increasing serum glutathione and total antioxidant capacity, and by reducing serum malondialdehyde (MDA) levels, which exhibit cytotoxic, mutagenic and carcinogenic properties [6]. In addition, as shown in a study by *Asbaghi* et al. [5], supplementation at doses ranging from 0.8-10 mg/day promoted a reduction in C-reactive protein (CRP) levels. Although the study did not show any side effects of high doses of folic acid supplementation, the authors of the study suggested caution when supplementing this nutrient at doses above 1 mg/day [5]. *Li* et al., in a recently published study in an animal model, found that a high-folate diet can significantly promote the development of hepatocellular carcinoma [45].

IMPORTANCE OF FOLIC ACID IN THE PREVENTION AND TREATMENT OF COVID-19

The processes affecting the body's effective immune response to infectious agents, such as respiratory viruses, among others, are extremely complex and still not entirely clear. However, it is known that the rate of innate immune responses, regulatory processes that prevent damage to the body's tissues, and the development of acquired adaptive responses are influenced by both environmental and lifestyle factors. Among lifestyle-related factors, the important role of dietary factors is particularly emphasized [11, 54].

Voelkle et al. [72] conducted a study in which they analyzed the prevalence of vitamin and mineral deficiencies, such as vitamin A, B_{12}, D, E, zinc, selenium, copper and folic acid, among 57 hospitalized patients with COVID-19, and analyzed the impact of deficiencies in the aforementioned components on the severity of the disease course. Based on the data obtained, it was found that higher serum levels of vitamin A, zinc, and folic acid were associated with a lower risk of severe progression of COVID-19 disease among the subjects.

Based on two, independent studies, conducted using computer simulations, it has been shown that folic acid can limit the replication of SARS-CoV-2. In one of these studies, *Sheybani* et al. [67], showed that folic acid can contribute to the inactivation of the furin endoprotease, which facilitates virus entry into the host cell, while in the second study, *Serseg* et al. [66], observed that folic acid inactivates the 3CLpro protease, which in turn has been shown to be essential in the replication of all coronavirus strains. *Acosta-Elias* et al. [1], summarizing the two aforementioned studies in their paper, however, believe that it is unlikely that folic acid can simultaneously inhibit two different proteases; nevertheless, they do not rule out a potential effect of folic acid on the inactivation of SARS-CoV-2, especially in pregnant women, who are mandatorily recommended to supplement the nutrient discussed in this paper. An increased supply of folic acid, the researchers noted, may have an impact on the lower rate of hospitalization of pregnant women for COVID-19 infections.

The results of another study to analyze the impact of nutraceutical compounds to achieve therapeutic targets against SARS-CoV-2, conducted by molecular docking, showed that folic acid alone or in combination with derivatives, i.e. tetrahydrofolic acid and 5-methylhydrofolic acid may be potential molecules against COVID-19 infection. This study, conducted by *Kumar* et al. [43], however, indicates a strong need to investigate the effects of folic acid and its derivatives against SARS-CoV-2 in *in vitro* and *in vivo* assays.

A complication of severe pneumonia is pulmonary hypertension, which studies have reported can also be a complication of severe pneumonia in COVID-19 infection. The primary mechanism underlying pulmonary hypertension is impaired function of the enzyme endothelial nitric oxide synthase (eNOS), thereby reducing local nitric oxide (NO) production [37]. It should be mentioned that folate supplementation has a direct effect on vascular endothelial function, eNOS (by increasing the availability of the tetrahydrobiopterin cofactor BH4), as well as increasing NO bioavailability. Based on available data, significantly lower serum folic acid levels have been reported among patients with severe COVID-19, which may significantly affect the increased risk of complications [12, 77].

The above-mentioned studies suggest that folic acid may be a potential prophylactic factor as well as an adjunct to the treatment of COVID-19 infection, as well as its complications, i.e. pulmonary hypertension. However, as this is a new issue, it requires further research to clarify the exact mechanism of the effect of folic acid and/or its derivatives on the reduced risk of SARS-CoV-2 infection [4].

CONCLUSION

In conclusion, based on the collected data, it can be said that folic acid has multidirectional biological effects and is an essential compound for maintaining the proper functioning of the body [68]. Both deficiency and excess of folic acid in the diet, is a factor that can promote an increased risk of cardiovascular disease, diseases of the central nervous system or certain cancers, among others [7, 9, 15, 16]. In order to reduce the risk of the aforementioned diseases, one should follow a well-balanced diet that includes foods that are rich sources of folic acid, such as green leafy vegetables, legumes, whole grains, citrus fruits and nuts, among others. If it is not possible to ensure a sufficient supply of folic acid with the diet, one should consider supplementation of the nutrient discussed in this paper [51].

According to the results of the study, folate supplementation, in particular, is recommended for people with megaloblastic anemia, neurodegenerative diseases, women in the preconception period, pregnant and breastfeeding women, people with elevated plasma homocysteine levels, as well as those taking certain medications (e.g. oral contraceptives, methotrexate). It should be emphasized that there is a need for further research to clarify both the optimal dose and length of supplementation that will provide measurable benefits [61].

Undoubtedly, there is a need for nutrition and health education of the public on the importance

of folic acid for human health, which would bring significant benefits not only for individuals, but also for the general public, contributing to improving public health.

Authors' contributions

K.K., M.K. analyzed the data, performed data interpretation and drafted the manuscript. K.A. designed and reviewed the manuscript. All authors revised and approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest

REFERENCES

1. *Acosta-Elias J., Espinosa-Tanguma R.*: The folate concentration and/or folic acid metabolites in plasma as factor for COVID-19 infection. Front Pharmacol 2020;11:1062 doi:10.3389/fphar.2020.01062.
2. *Albrecht K., Matysiak M.*: Zalecenia dietetyczne w profilaktyce i leczeniu nierozrostowych chorób układu krwiotwórczego [Dietary recommendations in the prevention and treatment of non-proliferative hematopoietic diseases]. Nowa Pediatr 2018;22(3):60-69 (in Polish) doi:10.25121/np.2018.22.
3. *Anamnart Ch., Kitjarak R.*: Effects of vitamin B_{12}, folate, and entascapone on homocysteine levels in levodopa – treated Parkinson's disease patients: a randomized controlled study. J Clin Neurosci 2021;88:226-231 doi:10.1016/j.jocn.2021.03.047.
4. *Asad D., Shuja S.H.*: Role of folate, cobalamin, and probiotics in COVID-19 disease management [letter]. Drug Des Devel Ther 202115:3709-3710 doi:10.2147/DDDT.S333295.
5. *Asbaghi O., Ashtary-Larky D., Bagheri R., Moosavian S.P., Nazarian B., Afrisham R., Kelishadi M.R., Wong A., Dutheil F., Suzuki K., Naeini A.A.*: Effects of folic acid supplementation on inflammatory markers: a grade--assessed systematic review and dose-response meta--analysis of randomized controlled trials. Nutrients 2021;13(7):2327 doi:10.3390/nu13072327.
6. *Asbaghi O., Ghanavati M., Ashtary-Larky D., Bagheri R., Kelishadi M. R., Nazarian B., Nordvall M., Wong A., Dutheil F., Suzuki K., Naeini A.A.*: Effects od folic acid supplementation on oxidative stress markers: a systematic review and meta-analysis od randomized controlled trials. Antioxidants 2021;10(6):871 doi:10.3390/antiox10060871.
7. *Badian M., Dzierżanowski T.*: Suplementacja witamin u chorych onkologicznych. Część I – witaminy z grupy B [Vitamin supplementation in oncology patients. Part I - B vitamins]. Med Paliat 2018;10(3):131-136 (in Polish) doi:10.5114/pm.2018.79835.
8. *Bae B.J., Han J.W., Song J., Lee K., Kim T.H., Kwak K.P., Kim B.J., Kim S.G., Kim J.L., Moon S.W., Park J.H., Ryu S.H., Youn J.Ch., Lee D.Y., Lee D.W., Lee S.B., Lee J.J., Jhoo J.H., Kim K.W.*: Hyperhomocysteinemia may increases the risk of dementia and *Alzheimer's* disease: a nationwide population-based prospective cohort study. Clin Nutr 2021;40(7):4579-4584 doi:10.1016/j.clnu.2021.05.034.
9. *Banyś K., Knopczyk M., Bobrowska-Korczak B.*: Znaczenie kwasu foliowego dla zdrowia organizmu człowieka [Importance of folic acid for the health of the human body]. Farm Pol 2020;76(2):79-87 (in Polish) doi:10.32383/farmpol/118863.
10. *Bomba-Opoń D., Hirnle L., Kalinka J., Mrozikiewicz--Seremak A.*: Folate supplementation during the preconception period, pregnancy and puerperium. Polish Society of Gynecologists and Obstetricians Guidelines. Ginekol Pol 2017;88(11):633–636 doi:10.5603/GP.a2017.0113.
11. *Calder P.C., Carr A.C., Gombart A.F., Eggersdorfer M.*: Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. Nutrients 2020;12(4):1181 doi:10.3390/nu12041181.
12. *Chalupsky K., Kracun D., Kanchev I., Bertram K., Gorlach A.*: Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia--induced pulmonary hypertension by recoupling endothelial nitric oxide synthase. Antioxid Redox Signal 2015;23(14):1076-1091 doi:10.1089/ars.2015.6329.
13. *Chen P., Li C., Li X., Li J., Chu R., Wang H.*: Higher dietary folate intake reduces the breast cancer risk: a systematic review and meta-analysis. Br J Cancer 2014;110(9): 2327–2338 doi:10.1038/bjc.2014.155.
14. *Cheng D., Kong H., Pang W., Yang H., Lu H., Huang Ch., Jiang Y.*: B vitamin supplementation improves cognitive function in the middle aged and elderly with hyperhomocysteinemia. Nutr Neurosci 2016;19(10):461-466 doi:10.1179/1476830514Y.0000000136.
15. *Chłopicka J.*: Czy dieta i suplementy mogą wspomóc pracę mózgu? [Can diet and supplements support brain function?]. Wszechświat [Universe] 2020;121(1-3):30-39 (in Polish).
16. *Cieślik G., Gębusia A.*: Skutki niedostatecznej podaży kwasu foliowego ze szczególnym uwzględnieniem znaczenia dla kobiet w wieku rozrodczym [Effects of inadequate supply of folic acid with special emphasis on the importance for women of childbearing age]. Hygeia Public Health 2011;46(4):431–436 (in Polish).
17. *Czarnowska M., Gujska E.*: Effect of freezing technology and storage conditions on folate content in selected vegetables. Plant Foods Hum Nutr 2012;67(4):401-406 doi:10.1007/s11130-012-0312-2.
18. *Czeczot H.*: Kwas foliowy w fizjologii i patologii [Folic acid in physiology and pathology]. Postepy Hig Med Dosw 2008;62:405-419 (in Polish).
19. *Devalia V., Hamilton M.S., Molloy A.M.*: British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol 2014;166(4):496-513 doi:10.1111/bjh.12959.
20. *Dong B., Wu R.*: Plasma homocysteine, folate and vitamin B_{12} levels in Parkinson's disease in China: a meta

– analysis. Clin Neurol Neurosurg 2020;188:105587 doi:10.1016/j.clineuro.2019.105587.
21. *Dzierzak E., Philipsen S.*: Erythropoiesis: development and differentiation. Cold Spring Harb Perspect Med. 2013;3(4):a011601 doi:10.1101/cshperspect.a011601.
22. *Ehmke vel Emczyńska-Seliga E.*: Suplementacja kwasem foliowym w ciąży i w okresie jejplanowania [Folic acid supplementation during pregnancy and pregnancy planning]. Available http://www.ncez.pl/ciaza-i-macierzynstwo/plodnosc-iciaza/suplementacjakwasem-foliowym-w-ciazy-i-okresie-jej-planowania (accessed: 13.10.2021) (in Polish).
23. *Engevik M.A., Morra Ch.N., Roth D., Engevik K., Spinler J.K., Devaraj S., Crawford S.E., Estes M.K., Kalkum M., Versalovic J.*: Microbial metabolic capacity for intesitnal folate production and modulation of host folate receptors. Front Microbiol 2019;10:2305 doi:10.3389/fmicb.2019.02305.
24. *Ferrazzi E., Tiso G., Di Martino D.*: Folic acid versus 5-methyl tetrahydrofolate supplementation in pregnancy. Eur J Obstet Gynecol Reprod Biol 2020;253:312-319 doi:10.1016/j.ejogrb.2020.06.012.
25. *Ford A.H., Flicker L., Alfonso H., Thomas J., Clarnette r., Martins R., Almeida O.P.:* Vitamins B_{12}, B_6, and folic acid for cognition in older men. Neurol 2010;75:1540–1547 doi:10.1212/WNL.0b013e3181f962c4.
26. *Gaszyńska J.*: Kwas foliowy – rola i zasadność suplementacji u różnych grup pacjentów. Praca poglądowa w ramach specjalizacji z farmacji aptecznej [Folic acid - role and validity of supplementation in different patient groups. Review paper as part of specialization in pharmacy pharmacy]. Poznań. 2018;1-14, https://www.woia.pl/page/5/biblioteka.html (dostęp: 13.10.2022) (in Polish).
27. *Gaweł M., Potulska-Chromik A.*: Choroby neurodegeneracyjne: choroba Alzheimera i Parkinsona [Neurodegenerative diseases: Alzheimer's and Parkinson's disease]. Post Nauk Med 2015;28(7):468-476.
28. *Głąbska D, Włodarek D.*: Witaminy [Vitamins]. In: *Włodarek D., Lange E., Kozłowska L., Głąbska D.*, eds. Dietoterapia [Diet therapy]. Warszawa, PZWL, 2015 (in Polish).
29. *Goluch-Koniuszy Z., Gięzek M.*: Stan odżywienia, skład ciała a sposób żywienia otyłych kobiet w wieku 60–85 lat, słuchaczek Stowarzyszenia Uniwersytetu Trzeciego Wieku w Szczecinie [Nutritional status, body composition and diet of obese women aged 60-85 years, listeners of the Association of the University of the Third Age in Szczecin]. Bromatol. Chem Toksyk 2015;48(4):724–735 (in Polish).
30. *Gopal M., Sunitha K., Arockiasamy J., Sibqathulla M.J., Yuvaraj J., Kalyanaraman S.*: Micronutirent deficiency in pregnancy: time to think beyond iron and folic acid supplementation. Indian J Community Med 2022;47(3):425-428 doi:10.4103/ijcm.ijcm_743_21.
31. *Gorzkowska A.*: Żywienie w chorobie Parkinsona [Nutrition in Parkinson's disease]. Aktualn Neurol 2017;17(4):199-207 (in Polish).
32. *Greenberg A.J., Bell J.S., Guan Y., Yu Y.H.*: Folic acid supplementation and pregnancy: more than just neural tube defect prevention. Rev Obstet Gynecol 2011;4(2):52–59.
33. *Hariz A., Bhattacharya P.T.*: Megaloblastic Anemia. StatPearls. 2020. https://www.ncbi.nlm.nih.gov/books/NBK537254/ (accessed: 13.10.2022).
34. *Hou H., Zhao H.*: Epigenetic factors in atherosclerosis: DNA methylation, folic acid metabolism, and intestinal microbiota. Clim Chim Acta 2021;512:7-11 doi:10.1016/j.cca.2020.11.013.
35. *Huang L., Zhao J., Chen Y., Ma F., Huang G., Li W.*: Baseline folic acid status affects the effectiveness of folic acid suplements in cognitively relevant outcomes in older adults: a systematic review. Aging Ment Health 2022;26(3):457-463 doi:10.1080/13607863.2021.187519.
36. *Igielska-Kalwat J., Gościańska J., Nowak I.*: Karotenoidy jako naturalne antyoksydanty [Carotenoids as natural antioxidants]. Postępy Hig Med Dosw 2015;69:418-428 (in Polish).
37. *Itelman E,, Wasserstrum Y,, Segev A., Avaky Ch., Negru L., Cohen D., Turpashvili N., Anani S., Zilber E., Lasman N., Athamna A., Segal O., Halevy T., Sabiner Y., Donin Y., Abraham L., Berdugo E., Zarka A. Greidinger D., Agbaria M., Kitany N., Katorza E., Shenhav-Saltzman G., Segal G.*: Clinical characterization of 162 COVID-19 patients in Israel: preliminary report from a large tertiary center. Israel Med. Assoc J 2020;22:271-274.
38. *Kalemba-Dróżdż M.*: Niedobór folianów w diecie i ich wpływ na stabilność genetyczną [Dietary folate deficiency and its impact on genetic stability]. In: *Dębska G., Jaśkiewicz J.*, eds. Interdyscyplinarne aspekty nauk o zdrowiu [Interdisciplinary aspects of health sciences]. Kraków, Oficyna Wydawnicza AFM 2011 (in Polish).
39. *Kapka-Skrzypczak L., Niedźwiecka J., Skrzypczak M., Wojtyła A.*: Kwas foliowy – skutki niedoboru i zasadność suplementacji [Folic acid - effects of deficiency and validity of supplementation]. Med Og Nauk Zdr 2012;18(1):65-69 (in Polish).
40. *Kaye A.D., Jeha G.M., Pham A.D., Fuller M.C., Lerner Z.I., Sibley G.T., Cornett E.M., Urits I., Viswanath O., Kevil Ch.G.*: Folic acid supplementation in patients with elevated homocysteine levels. Adv Ther 2020;37(10):4149-4164 doi:10.1007/s12325-020-01474-z.
41. *Khan K.M., Jialal I.*: Folic acid deficiency. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2022.
42. *Kretzschmar G.C., Targa A.D.S., Soares-Lima S.C., dos Santos P.I., Rodrigues LS., Macedo D.A., Pinto L.F.R., Lima M.M.S., Boldt A.B.W.*: Folic acid and vitamin B_{12} prevent deleterious effects of rotenone on object novelty recognition memory and Kynu expression in an animal model of Parkinson's disease. Genes 2022;13(12):2397 doi:10.3390/genes13122397.
43. *Kumar V., Kancharla S., Jena M.K.*: In silico virtual screening – based study of nutraceuticals predicts the therapeutic potentials of folic acid and its derivatives

against COVID-19. Virusdisease 2021;32(1):29-37 doi:10.1007/s13337-020-00643-6.
44. *Lei J., Ren F., Li W., Guo X., Liu Q., Gao H., Pang Y., He Y., Guo J., Zeng J.*: Use of folic acid supplementation to halt and even reverse the progression of gastric precancerous conditions; a meta-analysis. BMC Gastroeneterol 2022;22(1):370.
45. *Li J.T., Yang H., Lei M.Z., Zhu W.P., Su Y., Li K.Y., Zhu W.Y., Wang J., Zhang L., Qu J., Lu H.J., Chen Z.J., Wang L., Yin M., Lei Q.Y.*: Dietary folate drives methionine metabolism to promote cancer development by stabilizing MAT IIA. Signal Transduct Target Ther 2022;7(1):192 doi:10.1038/s41392-022-01017-8.
46. *Ma F., Zhou X., Li Q., Zhao J., An P., Du Y., Xu W., Huang G.*: Effects of folic acid and vitamin B_{12}, alone and in combination on cognitive function and inflammatory factors in the elderly with mild cognitive impairment: a single-blind experimental design. Curr. Alzheimer Res 2019;16:622–632 doi:10.2174/1567205016666190725144629.
47. *Mason J.B., Tang S.Y.*: Folate status and colorectal cancer risk: A 2016 update. Mol Aspects Med. 2017;53:73-79 doi:10.1016/j.mam.2016.11.010.
48. *Merrell B.J., McMurry J.P.*: Folic Acid. https://www.ncbi.nlm.nih.gov/books/NBK554487/ (accessed: 14.12.2022).
49. *Miwa K., Tanaka M., Okazaki S., Yagita Y., Sakaguchi M., Mochizuki H., Kitagawa K.*: Increased total homocysteine levels predict the risk of incident dementia independent of cerebral small-vessel diseases and vascular risk factors. J. Alzheimer's Dis 201649(2): 503–513 doi:10.3233/JAD-150458.
50. Muchacka R, Kukla M. Odżywianie kobiet w czasie ciąży [Nutrition for women during pregnancy]. Wałbrzych, Prace naukowe WSZiP 2017;42(3) (in Polish).
51. *Mziray M., Siepsiak M., Żuralska R., Domagała P.*: Znaczenie kwasu foliowego w diecie osób starszych. Zasadność suplementacji żywności [The importance of folic acid in the diet of the elderly. The legitimacy of food supplementation]. Pielęg Pol 2017;2(64):293-299 (in Polish) doi:10.20883/pielpol.2017.39.
52. *Naderi N., House J.D.*: Recent developments in folate nutrition. Adv Food Nutr Res 2018;83:195-213 doi:10.1016/bs.afnr.2017.12.006.
53. National Institutes of Health. Folate. Fact sheet of health professionals. https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/ (accessed: 14.10.2022).
54. *Netea M.G., Dominguez – Andreas J., Barreiro L.B., Chavakis T., Divangahi M., Fuchs E., Joosten L.A.B., van der Meer J.W.M., Mhlanga M.M., Mulder W.J.M., Riksen N.P., Schlitzer A., Schultze J.L., Sun J.C., Cavier R.J., Latz E.*: Defining trained immunity and its role in health and disease. Nat Rev Immunol 2020;20(6):375-388 doi:10.1038/s41577-020-0285-6.
55. *Okumura K., Tsukamoto H.*: Folate in smokers. Clin Chim Acta 2011;412(7-8):521-526.
56. *Olejnik A., Tomczyk J., Kowalska K., Grajek W.*: Rola naturalnych składników diety w chemoprewencji nowotworów jelita grubego [The role of natural dietary components in colorectal cancer chemoprevention]. Postepy Hig Med Dosw 2010;64:175-187 (in Polish).
57. *Parker E.S, Yazdy M.M., Tinker C.S., Mitchel A.A., Werler M.M.*: The impact of folic acid intake on the association between diabetes, obesity, and spina bifida. Am J Obstet Gynecol 2013;209(3):239.e1–239.e8 doi:10.1016/j.ajog.2013.05.047.
58. *Podolak-Dawidziak M., Solnica B.*: Badania w niedokrwistości [Research in anemia]. In: *Gajewski P.* eds. Interna Szczeklika Med Prakt. 2017 (in Polish).
59. *Price B.R., Wilcock D.M., Weekman E.*: Hyperhomocysteinemia as a risk factor for vascular contributions to cognitive impairment and dementia. Front Aging Neurosci 2018;10:350 doi:10.3389/fnagi.2018.00350.
60. *Przygoda B., Wierzejska R., Matczuk E., Kłys W., Jarosz M.*: Witaminy. [Vitamins]. In: *Jarosz M., Rychlik E., Stoś K., Charzewska J.*, eds. Normy żywienia dla populacji Polski i ich zastosowanie [Dietary Reference Values for the Polish population and their application]. Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny, Warszawa, 2020 (in Polish).
61. *Puga A.M., Ruperto M., de Lourdes Samaniego--Vaesken M., Monter-Bravo A., Paertearroyo T., Varela--Moreairas G.*: Effects of supplementation with golic acid and its combinations with other nutrients on cognitive impairment and Alzheimer's disease: a narrative review. Nutrients 2021;13(9):2966 doi:10.3390/nu13092966.
62. *Qiu F., Wu Y., Cao H., Liu B., Du M., Jiang H., Li S.*: Changes of peripheral nerve function and vitamin B_{12} level in people with Parkinson's disease. Front Neurol 2022;11:1-8 doi:10.3389/fneur.2020.549159.
63. *Reynolds E.H.*: Vitamin B12, folic acid, and the nervous system. Lancet Neurol 2006;5(11): 949–960 doi:10.1016/S1474-4422(06)70598-1.
64. *Ruta H., Kajdy A., Maksym B.R.*: Znaczenie metabolizmu kwasu foliowego dla płodności [Importance of folic acid metabolism for fertility]. Fides Ratio 2018;3(35):92-101 (in Polish).
65. *Schulz E., Arruda V.R., Costa F.F., Saad S.T.*: Cytoplasmic overexpression of p53 and p21(ras) in megaloblastic anemia. Haematologica 2000;85(8):874-875.
66. *Serseg T., Benarous K., Yousfi M.*: Hispidin and lepidine e: two natural compounds and folic acid as potential inhibitors of 2019-novel coronavirus main protease, (2019-ncovmpro), molecular docking and sar study. Curr. Comupter-Aided Drug Des 2020;16:1-11 doi:10.2174/1573409916666200422075440.
67. *Sheybani Z., Dokoohaki M.H., Negahdaripour M., Dehdashti M., Zolghadr H., Moghadami M., Masoompour S.M., Zolghadr A.R.*: The role of folic acid in teh management of respiratory disease caused by COVID-19. ChemRxiv 2020;1-15 doi:10.26434/chemrxiv.12034980.
68. *Shulpekova Y., Nechaev V., Kardasheva S., Sedova A., Kurbatova A., Bueverova E., Kopylov A., Malsagova K., Dlamini J.C., Ivashkin V.*: The concept of folic acid in health and disease. Molecules 2021;26(12):3731 doi:10.3390/molecules26123731.

69. *Socha D.S., DeSouza S.I., Flagg A., Sekeres M., Rogers H.J.*: Severe megaloblastic anemia: Vitamin deficiency and other causes. Cleve Clin J Med 2020;87(3):153-164 doi:10.3949/ccjm.87a.19072.
70. *Sułek K.*: Niedokrwistości megaloblastyczne [Megaloblastic anemias]. In: *Dmoszyńska A.*, eds. Wielka Interna – Hematologia [Great Internal Medicine – Hematology]. 2011. (in Polish).
71. *van der Pols J., Baade P., Spencer L.B.*: Colorectal cancer incidence in Australia before and after mandatory fortification od bread flour with folic acid. Public Health Nutr. 2021;24(8):1989-1992 doi:10.1017/S1368980021000562.
72. *Voelkle M., Gregoriano C., Neyer P., Koch D., Kutz A., Bernasconi L., Conen A., Mueller B., Schuetz P.*: Prevalence of micronutrient deficiennces in patients hospitalized with COVID-19: an observational cohort study. Nutrients 2022;14(9):1862 doi:10.3390/nu14091862.
73. *Wang F., Wu K., Li Y.*: Association of folate intake and colorectal cancer risk in the postfortification era in US women. Am J Clin Nutr 2021;114(1):49-58 doi:10.1093/ajcn/nqab035.
74. *Wang Q., Zhao J., Chang H., Liu X., Zhu R.*: Homocysteine and folic acid: risk factors for Alzheimer's disease – an updatet meta – analysis. Front. Aging Neurosci 2021;13:665114 doi:10.3389/fnagi.2021.665114.
75. *Weker H., Więch M., Strucińska M.*: Zasady żywienia kobiet w okresie ciąży [Principles of nutrition for women during pregnancy]. In: *Weker H.*, eds. Żywienie kobiet w okresie ciąży – teoria i praktyka [Nutrition of women during pregnancy - theory and practice]. Warszawa, PZWL, 2021 (in Polish).
76. *Wiesner A., Paśko P.*: Stosowanie suplementów u kobiet ciężarnych w świetle najnowszych rekomendacji Polskiego Towarzystwa Ginekologów i Położników [Use of supplements in pregnant women in the light of the latest recommendations of the Polish Society of Gynecologists and Obstetricians]. Farm Pol 2021;77(1):40-47 (in Polish) doi:10.32383/farmpol/133610.
77. *Wiltshire E., Pena A.S., MacKenzie K., Shaw G., Couper J.*: High dose folic acid is a potential treatment for pulmonary hypertension, including, when associated with COVID-19 pneumonia. Med Hypotheses 2020;143:110142 doi: 10.1016/j.mehy.2020.110142.
78. *Wiśniewska K.*: Pierwotna profilaktyka wrodzonych wad rozwojowych poprzez suplementację diety kwasem foliowym na terenie powiatu poznańskiego i miasta Poznania w latach 2005-2009. Rozprawa doktorska. Uniwersytet Medyczny im. Karola Marcinowskiego w Poznaniu [Primary prevention of congenital malformations by dietary supplementation with folic acid in the Poznań district and the city of Poznań in 2005-2009. Doctoral dissertation. Karol Marcinowski Medical University in Poznan]. 2014 (in Polish).
79. *Yadav M.K., Manoli N.M., Madhunapantula S.V.*: Comparitive assessment of vitamin – B_{12}, folic acid and homocysteine levels in relations to p53 expression in megaloblastic anemia. PLoS One 2016;11(10):e0164559 doi:10.1371/journal.pone.0164559.
80. *Yadav M.K., Manoli N.M., Vimalraj S. Madhunapantula S.V.*: Unmethylated promoter DNA correlates with p53 expression and apoptotic levels only in vitamin B_9 and B_{12} deificient megaloblastic anemia but not in non-megaloblasitc anemia controls. Int J Biol Macromol 2018;109:76-84 doi:10.1016/j.ijbiomac.2017.12.070.
81. *Yan Y., Xie Ch., Di S., Wang Z., Wu M., Li Y., Liu Y., Jiang R.*: Supplemental folic acid and/or mutlivitamins in pregnancy is associated with a decreased risk of childchood and adolescent nasopharyngeal cacinoma. J Dev Orig Health Dis 2022;13(5):550-555 doi: 10.1017/S2040174421000635.
82. *Zalega J., Szostak-Węgierek D.*: Żywienie w profilaktyce nowotworów. Część II. Składniki mineralne, witaminy, wielonienasycone kwasy tłuszczowe, probiotyki, prebiotyki [Nutrition in cancer prevention. Part II. Minerals, vitamins, polyunsaturated fatty acids, probiotics, prebiotics]. Probl Hig Epidemiol 2013;94(1):50-58 (in Polish).

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ORIGINAL ARTICLE

ENERGY EXPENDITURE DURING TRAINING AND OFFICIAL LEAGUE MATCH IN PROFESSIONAL FEMALE SOCCER PLAYERS – A PILOT STUDY

Hubert Dobrowolski[1], *Dariusz Włodarek*[2]

[1]Department of Functional and Organic Food, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (SGGW), Warsaw, Poland
[2]Department of Dietetics, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (SGGW), Warsaw, Poland

ABSTRACT

Background. The most important component of a well-balanced diet is the proper energetic value. However, adequate estimation of the body's energy needs is difficult for professional athletes, including soccer players. There is little research showing energy expenditure during training and lack of studies on the energy expenditure of professional female soccer players during a match.

Objective. The aim of our study was to estimate energy expenditure during training and official league match in female soccer players and comparing it.

Material and methods. Seven Polish professionally practicing soccer females (23.4±6,6 years old; 63.5±7.8 kg; 168.5±5.8 cm; 46±4.4 kg fat-free mass) participated in the study. The participants had their height and body mass measured. Energy expenditure during activities was measured by means of a SenseWear Pro3 Armband device. Body composition was assessed with Akern BIA 101 Anniversary Sport Edition device.

Results. Statistically higher energy expenditure was achieved in the study group during the match hour (452±55 kcal/ hour) compared to the training hour (353±28 kcal/ hour) as well as in the case of energy expenditure per hour of activity per kg of fat-free mass (match: 9.94±1.75 kcal/kg fat-free mass/hour; training: 7.71±0.8 kcal/kg fat-free mass/hour). During one hour of training, more time was spent on sedentary, light, and moderate activities, but the difference was statistically significant only for light activities. More time during the match hour than during the training hour was spent on vigorous and very vigorous activities.

Conclusions. In conclusion, the energy expenditure of the players during the match was greater than in the case of the planned intensive training, which was caused by the timeshare of more intense physical activities and going a longer distance during match.

Key words: *training, soccer match, league match, intensity of activities, physical activity, athletes*

STRESZCZENIE

Wprowadzenie. Najważniejszym elementem dobrze zbilansowanej diety jest jej odpowiednia wartość energetyczna. Jednakże odpowiednie oszacowanie potrzeb energetycznych zawodowych sportowców, w tym piłkarzy nożnych, jest niezwykle trudne. Niewiele jest badan wskazujących na wydatek energetyczny w trakcie treningu oraz brak jest badan wskazujących na wydatek energetyczny w trakcie meczu wśród piłkarek nożnych.

Cel. Celem naszych badań było oszacowanie wydatku energetycznego w trakcie treningu i oficjalnego meczu piłkarskiego w grupie kobiet trenujących piłkę nożną oraz porównanie tych wartości.

Materiał i metody. W badaniu wzięło udział siedem polskich profesjonalnych piłkarek nożnych (23,4±6,6 lat; 63,5±7.8 kg; 168,5±5,8 cm; 46±4,4 kg beztłuszczowej masy ciała) Zmierzono masę i wysokość ciała. Wydatek energetyczny w trakcie aktywności mierzono za pomocą opasek naramiennych SenseWear Pro3. Skład ciała oceniono przy użyciu analizatora Akern BIA 101 Anniversary Sport Edition.

Wyniki. Statystycznie wyższy wydatek energetyczny obserwowany był w trakcie godziny meczowej (452±55 kcal/godzinę) w porównaniu do godziny treningowej (353±28 kcal/godzinę) podobnie jak miało to miejsce w przypadku wydatku energetycznego w trakcie godziny aktywności w przeliczeniu na beztłuszczową masę ciała (mecz: 9,94±1,75 kcal/kg beztłuszczowej masy ciała/godzinę; trening: 7,71±0,8 kcal/kg beztłuszczowej masy ciała/godzinę). W trakcie godziny treningu więcej czasu poświęcone było na odpoczynek, aktywności lekkie i o umiarkowanym natężeniu, ale różnica była

Corresponding author: Hubert Dobrowolski, Department of Functional and Organic Food, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (SGGW), 159c Nowoursynowska Str., 02-776 Warsaw, Poland, tel. +48 22 593 70 47, email: hubert_dobrowolski@sggw.edu.pl

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istotna statystycznie jedynie w przypadku aktywności lekkich. Więcej czasu w trakcie godziny meczowej, niż w przypadku godziny treningowej, było poświęcone na aktywności o wysokim i bardzo wysokim natężeniu.
Wnioski. Wydatek energetyczny w trakcie meczu był wyższy niż podczas intensywnego treningu, co było skutkiem większej ilości czasu poświęconego na aktywności o wysokim natężeniu oraz dłuższe dystanse pokonywane w trakcie meczu.

Słowa kluczowe: *trening, mecz piłkarski, mecz ligowy, intensywność aktywności, aktywność fizyczna; sportowcy*

INTRODUCTION

Diet is undoubtedly an element affecting the athlete's exercise capacity. Adequate food and fluid supply before, during and after physical performance may help maximise exercise performance and accelerate recovery time [26].

The most crucial component of a well-balanced diet is the proper energetic value. An adequate energy supply increases the probability of appropriate macro- and micronutrient amounts in the diet. This can have an influence both on health aspects and the optimisation of exercise capacity. Therefore, correctly estimated energy needs are crucial for proper diet preparation. Deficient energy intake may lead to the development of the Relative Energy Deficiency in Sport syndrome (RED-S), which may cause serious health problems (impairment of immunological functions, menstrual-function disorder, osteoporosis, disorders of the growth, as well as endocrine, metabolic, hematological, psychological, gastrointestinal, and cardiovascular systems) and affect exercise capacities (increased injury risk, decreased training response, impaired judgment, decreased motor coordination and concentration, irritability, depression, decreased glycogen supplies, and decreased muscle strength and stamina) [21].

However, adequate estimation of the body's energy needs is difficult for professional athletes, including soccer players. Research shows that soccer players can perform 726 different actions, moves, and turns during a match, while time spent on individual activities varies considerably between players in various field positions [5]. Other studies also show differences in physical activity between players in different positions on the field – midfielders covered greater distances (even by 15%) than players playing in other positions [25]. Factors crucial to success in soccer are agility, strength, the ability to perform repetitive sprinting and stamina [24], which increase individual players` energy needs. Therefore, a unique approach to energy needs assessment is a crucial component of setting proper personal nutritional strategies for soccer players. These athletes should adjust their energy intake from diet to their activity level and aims, which are individual for every player [6]. Also, the energy supply adjustment depends on the training type, activities performed during it, and the training macro- and micro-cycle. Training to improve exercise capacity and prepare for matches may be full of activities with various loads. *Helgerud* et al. [11] showed that training with very high intensity (4 x 4 minutes of running at the level of 90-95% VO2max) resulted in improved performance, increased distance, increased number of sprints and contact with the ball during the match compared to control group, but did not adversely affect parameters such as jump height, strength, speed or precision of passing the ball. *Hoff* and *Helgerud* [13] indicated that the participation of soccer players in strength training with high loads improved their efficiency during a sprint and better jumping ability, with a simultaneous improvement in aerobic capacity, thanks to the progress of the economy of physical exertion . Even tactical training can be very strenuous. As *Hoff* et al. [14] demonstrated, specific football training with ball dribbling and mini-team games can be performed as interval aerobic training, the intensity of which can be as high as 91.7% and 84.5% VO2max, respectively. Therefore, knowing how to translate training into matches is crucial to planning appropriate nutritional strategies for soccer practitioners.

In addition, it should be pointed out that the number of women practising soccer continues to increase. In its plans, FIFA (fr. *Fédération Internationale de Football Association*) has set itself the task of increasing the number of women soccer players to 60 million by 2026 and doubling the number of affiliated members by that year [9]. Despite the growing number of female footballers and affiliated clubs, interest in women's soccer is already huge. In 2015, the Women's World Cup was watched via media by 750 million users, while 1.35 million fans watched the struggles on the pitch [9]. Despite the interest in the topic of women's soccer and development strategies indicating the need for interest in the topic from professional player care, the topic of women's soccer is still marginalised in research. Appropriate energy intake is essential because it supports optimal body function, determines the capacity for the information of macronutrients and micronutrients, and assists in manipulating body composition [1]. Energy deficiencies are often observed in athletes, especially women, resulting in reduced physical performance and growth and adversely affecting health [16]. Some studies examined the fulfilment of energy needs in a group of women training soccer. In their study, *Martin* et al. [20] showed the daily energy expenditure

of female soccer players at the level of 2154±596 kcal/day while the daily energy intake with a diet was 1904±366 kcal/day. *Gibson* et al. [10], in turn, in their study group, noted the energy value of the food ration of soccer players at the level of 2079±460 kcal/day, with the daily energy needs averaging 2546±190 kcal/day. *Mullinix* et al. [22] also reported insufficient energy intake with the diet (2014 kcal/day) compared to the needs of the study group (2716 kcal/day). Proper energy supply on training and match days is, therefore, a key to covering the energy needs of soccer players. However, the studies have generally observed too low energy intake from the diet in this group of athletes.

Considering the above, our study aimed to estimate the female soccer players' energy expenditure generated during regular training and official league matches and then compare the analysis of these expenses between activities and players' field positions. The aim of our research was also to examine whether it was necessary to carry out research during the start-up period (during official soccer matches) on a wider group and to test the applicability of the research tools described further on.

MATERIAL AND METHODS

Seven professionally practicing soccer females participated in this pilot study (23.4±6.6 years old). In the study participated: 4 defenders, 2 midfielders and one striker. All the female soccer players were players from one of the teams from the highest Polish women's league (extra league). Also, all the players had many years of training experience. Therefore, they are one of the best Polish female soccer players and should be considered professional. Additionally, it should be emphasised that such a group size, given the nature of the study and the measurement during official competitions, is a representative sample (especially given the pilot nature of the study), looking at the number of female players playing at this league level. The approval of participation in the research and conducting any measurements and tests used during the study was obtained from each participant or their legal guardians (if participants were underage). The study and all procedures used in it were accepted, and permission from the local Ethics and Scientific Research on Humans Commission was obtained (approval number: 24/2017, 19 June 2017).

Inclusion criteria include being under 35 years old, registered in the local Soccer Association and actively participating in training sessions. Exclusion criteria were long-term injuries (without permitting to train within the last six months), those who underwent metal implants operation or were diagnosed with any long-term disease.

Participants' body mass and height were measured twice by means. Height measurement was measured using a standard stadiometer (accurate to within 0.1 cm), and weight was measured using a standard scale, accurate to within 0.1 kg. During body-mass measurements, participants were asked only to wear underwear; during height measurements, no footwear or socks were allowed. From obtained data, a Body Mass Index was calculated.

The fat mass and fat-free mass content were measured by the bioelectrical impedance method (BIA), using the Akern BIA 101 Anniversary Sport Edition (Akern Srl., Italy) device. During the measurement, the participants were not during their period and did not suffer from any mental or physical stress. The analysis was carried out in a tetrapolar system. Before placing the electrodes, the contact points were cleaned with alcohol to remove the stratum corneum and dried. The participants were also asked to remove all metal items and jewellery. During the analysis, all the conditions of measurement correctness recommended by the manufacturer were used. BIA is a simple, reliable, valid body composition assessment tool [17, 18]. This method has a low predictive or standard error [17] and is a non-invasive and accurate [27].

After taking anthropometric and body composition measurements, the participants were put on armbands measuring energy expenditure. Participants wore the armbands for at least one hour before and after training and before and after an official league match. The training structure was not interfered with to avoid disturbing the naturally occurring exercises. However, it was agreed with the trainer that the monitored training would be filled with strenuous exercises of considerable intensity, intended to increase the motor skills and endurance of the players. According to the arrangements with the team's coach, this type of training is the most strenuous and intense for the players.

To measure energy expenditure, the SenseWear Pro3 Armband device was used (Body Media Inc., Pittsburgh, PA, USA). The armband is equipped with five sensors: two skin galvanometers (which measure the electrical conductance of skin), a skin temperature sensor, a heat-flux sensor (that measures the rate at which the body dissipates heat relative to air temperature), and a 3-axial accelerometer (to calculate the motion and intensity of physical activity and the output of total energy expenditure resulting from the intensity of the physical activity). The structure of the device enables measurement reading, including total energy expenditure (TEE), activity energy expenditure (AEE), the average physical-activity level (PAL), the average METs (METs; one MET is the equivalent of aspirated oxygen in 3.5 mL O2/kg body mass/min), the time expenditure of different intensity

activities (divided into five categories: <1.5, 1.5–3.0, 3.0–6.0, 6.0–9.0, and >9.0 METs), and many others. Research conducted by using this method is marked by high-accuracy measurements [15, 28], repeatability [15] and a low mean error rate [4]. After collecting the armband results, data on the training unit and the match were separated. The data contained in this way was analysed and compared with each other. Analysis of the records was completed using SenseWear 8.1. software (Body Media Inc., Pittsburgh, PA, USA).

This study omitted the discussion of meeting the nutritional and energy needs of the study group. The intake of energy and other nutrients by study participants has been the subject of other studies and has been published elsewhere [8]. However, it is worth highlighting in the light of these data that the majority of the group studied did not cover their energy needs.

Statistical analysis was conducted using the SPSS v. 20 software (IBM Corp., USA). To verify the normality of distribution, the *Shapiro–Wilk* test was used. For comparison of data collected during training and a league match, in the case of the normal distribution, we used the *Student's* t-test. In the case of a distribution deviating from normality, the *Wilcoxon* test was used. Correlations were tested using the *Spearman* test. The study's defined significance level was set to α=0,05.

RESULTS

The mean body mass in the study group was 63.5±7.8 kg. The mean height in the study group was 168.5±5.8 cm. The average BMI in the study group was 22.3±1.7 kg/m^2. Body composition analysis showed an average body fat content of 27.3±4.3% (17.5±4.4 kg). This meant the lean body mass was 73±3.9% (46±4.4 kg).

Table 1 presents the results obtained during the measurement of energy expenditure. Due to the different amounts of time spent on the pitch during training and a soccer match among other players, the energy expenditure [kcal] was converted into an hour of physical activity. The average energy expenditure during the match was 628±101 kcal, and the average energy expenditure during training was 534±43 kcal. However, no statistically significant difference was observed (p>0.05). Energy expenditure per kilogram of body mass per hour of activity for the match and training was 6.54±0.8 kcal/kg bm/h and 5.6±0.66 kcal/kg bm/h, respectively, and did not differ significantly (p>0.05, *Wilcoxon* test). Statistically, substantially higher energy expenditure was achieved in the study group during the match hour (452±55 kcal/h) compared to the training hour (353±28 kcal/h) (p≤0.05). Significant differences were also found in the case of energy expenditure per hour of activity per kg

Table 1. Energy expenditure during training and a league match

	Energy expenditure [kcal]	Energy expenditure over time [kcal/h]	Energy expenditure depending on body mass [kcal/kgbm/h]	Energy expenditure depending on fat free mass [kcal/kgffm/h]
	Mean±SD (median)			
Match	628±101 (657)	452±55 (453)	6.54±0.8 (6.25)	9.94±1.75 (9.83)
Training	534±43 (540)	353±28 (354)	5.6±0.66 (5.34)	7.71±0.8 (7.36)
p*	0.063	0.018	0.063	0.018

kgbm – kg of body mass; kgffm – kg fat-free mass

* *Wilcoxon* test

Table 2. Number of steps and activity time of varying intensity during training and league match per hour

	Steps	S	L	M	V	VV
	Mean±SD (median)					
Match	5953±588 (5914)	0±0 (0)	0.14±0.37 (0)	18.53±15.42 (14.68)	29.21±9.8 (32.17)	13.12±17.0 (0.61)
Training	4213±363 (4263)	1.21±1.56 (0)	6.42±5.38 (3.33)	27.78±9.95 (22.89)	20.24±8.08 (19.33)	5.01±3.62 (4.33)
p*	0.018	0.109	0.023**	0.128	0.091	0.345

S – Sedentary; L – Light; M – Moderate; V – Vigorous; VV – Very vigorous

All values are based on the hour of activity.

* *Wilcoxon* test

** Due to the normal distribution of the data, the *Student's* t-test was used instead of the *Wilcoxon* test

of fat-free mass (9.94±1.75 kcal/kg FFM/h vs 7.71±0.8 kcal/kg FFM/h, for match and training, respectively).

The time spent on physical activity of varying intensity and the number of steps taken during the match and training per hour is shown in Table 2. Significantly more (p=0.018, *Wilcoxon* test) steps were taken during the match hour (5953±588) compared to the training hour (4213±363). During one hour of training, more time was spent on sedentary, light, and moderate activities (1.21±1.56, 6.42±5.38 and 27.78±9.95, respectively) compared to the match hour (0±0, 0.14±0.37 and 18.53±15.42, respectively). However, the difference was statistically significant only for light activities (p=0.023, t-test). In turn, more time during the match hour than during the training hour was spent on vigorous and very vigorous activities. Still, the observed difference was not statistically significant (p>0.05, *Wilcoxon'*s test).

A correlation was found between the energy expenditure during the match hour and the number of steps during the match hour (p<0.001, rho=0.964, *Spearman's* test), which was not observed during the training hour and the number of steps during the hour (p=0.180, rho=0.571, *Spearman's* test). The number of steps performed per hour of activity also correlated with the energy expenditure per kilogram of fat-free mass per hour of physical activity during the match (p=0.003, rho=0.929, *Spearman's* test) as well as training (p=0.007, rho=0.893, *Spearman's* test). However, these relationships were not observed compared to the expenditure per kg of body mass per hour of activity (p>0.05, *Spearman's* test).

Energy expenditure during the match hour was strongly correlated with the time devoted to very vigorous activities (p<0.001, rho=0.964, *Spearman's* test) and inversely correlated with moderate intensity activities (p=0.036, rho=-0.786, *Spearman*'s test). Similar relationships were observed in the expenditure per kilogram of fat-free mass per hour of activity (for vigorous activities: p=0.003, rho=0.927; for moderate intensity activities (p=0.036, rho=-0.786). The expenditure per kilogram of fat-free mass during training was, in turn, correlated with vigorous activities (p=0.007, rho=0.893, *Spearman's* test). Energy expenditure during the training hour, regardless of body mass, was inversely correlated with the activities of sedentary (p=0.028, rho=-0.808, *Spearman's test*) and light (p=0.039, rho=-0.778, *Spearman's* test) intensities.

DISCUSSION

According to our knowledge, this is the first study of this type to compare energy expenditure and intensity of activities during training and an official league match among women practicing soccer professionally. It is also the first study that uses measuring equipment to classify activity and measure energy expenditure during an official league match.

The energy expenditure during the match was higher than during the training. This applies to absolute values and time (per hour of activity) or per competitor's body mass (total and fat-free mass). However, statistical significance was demonstrated when calculating the energy expenditure per hour of exercise and depending on the athlete's fat-free mass. Energy expenditure per hour of activity is a better indicator for observing differences in expenditure than energy expenditure in absolute terms. This is due to the different periods of participation in the match and training. A comparison of a player who played only 45 minutes during the match and who participated 90 minutes in the training session with a player who played 85 minutes during the match and who participated 80 minutes in the training session would be inadequate. It could give false beliefs about activity intensity during the match and training. Therefore, it should be assumed that the energy expenditure during the match was significantly higher than in the case of training. This raises questions about the fulfilment of nutritional needs by the female participants in the study. As previous studies [8] have shown, female soccer players did not cover their energy needs on training days, when energy expenditure has been shown to be lower. Thus, even more so on a match day, energy shortages can be expected to be even greater than on training days. This, in turn, will result in an increased likelihood of negative health effects, as well as impaired performance.

Many factors may cause a higher energy expenditure during match activity. As indicated by *Olthof* et al. (2019) [23], the energy expenditure will be greater during the match due to the awareness of the need to win and the efforts that go with it, as well as technical and tactical interactions between teams. A study of physical activity and energy expenditure of male soccer players conducted by *Anderson* et al. [2] showed that each of the players covered a longer distance and had higher speed on match days compared to training days, which expressed as higher energy expenditure on match days. Also, the results of studies by *Djaoui* et al. [7] suggest higher energy expenditure during the match. Comparing the maximum sprint speed, the players achieved higher values during the match compared to training in the form of Small Sided Games. However, this study did not evaluate players' total distance travelled. Also, neither of these studies measured energy expenditure. Naturally, the energy expenditure is an individual matter, depending, for example, on the player's position on the pitch, as shown by studies involving men and women training soccer. To create nutritional recommendations for

soccer practitioners, knowing the energy expenditure during training and match is essential to cover their needs and ensure optimal physical performance [3].

It is clear that the amount of the energy expenditure of individual players in our study was a derivative of the amount of physical activity. It was shown that it depended on the number of steps taken during training and the match. Naturally, with the increase in the number of steps, the work of the muscles increases, and thus the energy expenditure associated with it is on the rise too. The more significant number of steps taken during the match compared to training for the same amount of time is not surprising. However, no correlation was found between the number of actions taken and the energy expenditure per kilogram of body mass per hour of activity. This may indicate a similar level of involvement in the game between players, regardless of their body weight, which proves a uniform method of preparation for the match and training. The other research showed greater distances during the match compared to training, which had to be connected to a more significant number of steps [2]. According to our knowledge, however, no studies are comparing the number of actions taken during a match and training among female soccer players.

The present study found that female soccer players had much more time during training on low-intensity activities than during a match, and high-intensity activity lasted longer during a match. Awareness of the result's significance during the match and the necessity to win could be a mobilising factor for the players, resulting in greater involvement and increased physical effort, intensity, frequent sprints, sudden turns, and very intense short ball plays. Compared to these activities, the intense training had to be full of breaks between individual exercises. High involvement in the game during the match will result in more time devoted to vigorous or very vigorous activities compared to training, which will include breaks of several minutes between exercises, breaks for hydration, discussing mistakes with the coach between exercises, and consequently - more time devoted to low-intensity physical activity. It was also observed that there was a positive correlation between the amount of energy expenditure during the match and the time of very vigorous physical activity (the more significant amount of which increased the spending) and a negative correlation with the time of moderate intensity physical activity (the more significant amount of which decreased it). The analysis of soccer players' energy expenditure showed higher spending during the match hour compared to the training hour due to the differences in the intensity of physical exertion.

However, it is worth paying attention at this point to the lack of correlation between the number of steps during the hour of the match and training and the energy expenditure per kg of body mass and the significant correlation between the number of steps during the hour of the match and training and the energy expenditure per kg of fat-free mass among the players. Total body weight comprises all components, not only the amount of muscle tissue but also the amount of adipose tissue. While the content of muscle tissue causes a higher amount of effort performed, and thus the amount of energy expenditure, the amount of adipose tissue, to a lesser extent. Naturally, the content of adipose tissue can translate into energy expenditure because greater body weight is associated with the need to perform more effort and greater involvement of muscle tissue in this effort. However, fat-free mass is the most metabolically active tissue and primarily affects the amount of energy expenditure, in particular resting metabolism rate [12]. It can be concluded from the observed relationship that while the energy expenditure incurred by each kilogram of body mass was not related to the activity - in this case, the number of steps - it could have been related to the presence of adipose tissue, which is less metabolically active and had not as much effect on the performance. On the other hand, the energy expenditure incurred by each kilogram of fat-free mass - as a metabolically active tissue, which is, i.a., responsible for the performance of activities - was related to the number of steps taken. The greater number of steps taken forced the muscle tissue to expend more energy so that the tissue could perform specific work and activity. Physical activity can have an impact on energy expenditure, for example by modulating body composition. The greater content of an athlete's lean body mass will result in higher energy expenditure than a person with similar body weight. In this study, however, no relationship was found between fat free mass and energy expenditure during the hour of training and the hour of the match ($p>0.05$, *Spearman* test). However, this may result from a small study group, making it difficult to observe such relationships. There are also hypotheses that training exercise directly influences resting metabolic rate [19]. This research indicates, however, that this will be more related to the energy expenditure done by fat-free mass rather than the overall energy expenditure. It emphasises how important it is to take care of the proper body composition in athletes.

Our study has both strengths and weaknesses. Undoubtedly, the strength is the direct measurement of energy expenditure during the match and training on the same people using the same method. This allows the comparison of various factors influencing energy expenditure and physical activity parameters between the two key forms of physical activity for athletes - competition and preparation. The direct measurement of physical activity and energy expenditure is a solid study point. Due to the nature of the competition

and the consent of the referee, coach and players, such measurements are extremely rare and have a significant cognitive value. Taking measures with the same players during training and the match is also essential. However, despite these strengths, limitations should also be highlighted. The small study group is undoubtedly a limitation. Too small a group does not allow for complete observation and statistical comparison of some parameters, which will prevent specific dependencies from being often observed. The small group, however, is the result of the nature of the study. Frequent changes in the pitch, injuries, and tactics, as well as the consent of the coaches to perform the measurement, severely limit the possibility of conducting the study on a larger, more representative group. The second limitation is the length of the study. The research was conducted only during a single match and training session. Matches differ by the necessity to involve players in particular activities and the game itself. A more demanding opponent will also result in a higher involvement of players in specific football positions compared to an opponent who will be dominated in a particular phase of the game. Also, the expenditure during training will depend on the training cycle. Although the measurements were taken during one of the more demanding training sessions to relate the results to a match, training of a different nature could provide different results. Long-term research would undoubtedly give more reliable results.

CONCLUSIONS

In conclusion, the energy expenditure of the players during the match was more significant than in the case of the planned intensive training. This was mainly due to the timeshare of more intense physical activities and taking more steps, which was associated with going a longer distance during the match than during training. More high-intensity actions resulted in higher energy expenditure during the match. In contrast, the time spent on vigorous activities during training was shorter in favour of moderate-intensity activities, reducing energy expenditure. In addition, the number of steps performed was related to energy expenditure per kilogram of fat-free mass, suggesting body composition's influence in shaping the body's energy expenditure. More studies involving more players and lasting longer are needed to accurately determine the energy expenditure during training and matches and the factors influencing this. This knowledge will help meet athletes' nutritional needs and thus may contribute to better results.

Given the pilot nature of the study, it is important to point out the need to conduct research on a larger group of women soccer professionals at different levels of training and on different levels of the league ladder. The methodology used allows for an adequate comparison of energy expenditure during the key activities for professional athletes - training and competition - and conducting a full study will allow clear conclusions to be drawn. Conducting further research is therefore strongly recommended.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Academy of Nutrition and Dietetics, American College of Sports Medicine, Dietitians of Canada: Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. J Acad Nutr Diet, 2016;116(3):501–528, DOI: 10.1016/j.jand.2015.12.006
2. *Anderson, L., Orme, P., Di Michele, R., Close, G. L., Morgans, R., Drust, B., & Morton, J. P.*: Quantification of training load during one-, two- and three-game week schedules in professional soccer players from the English Premier League: implications for carbohydrate periodisation. Journal of Sports Sciences, 2016;34(13):1250–1259, https://doi.org/10.1080/02640414.2015.1106574
3. *Bangsbo, J., Mohr, M., & Krustrup, P.*: Physical and metabolic demands of training and match-play in the elite football player. Journal of Sports Sciences, 2006;24(7):665–674, https://doi.org/10.1080/02640410500482529
4. Ber*ntsen, S., Hageberg, R., Aandstad, A., Mowinckel, P., Anderssen, S. A., Carlsen, K. H., & Andersen, L. B.*: Validity of physical activity monitors in adults participating in free-living activities. Br. J. Sports Med., 2010;44:657–664, https://doi.org/10.1136/bjsm.2008.048868
5. *Bloomfield, J., Polman, R., & O'Donoghue, P.*: Physical demands of different positions in FA Premier League soccer. J. Sports Sci. Med., 2007; 6(1): 63–70
6. *Burke, L. M., Loucks, A. B., & Broad, N.*: Energy and carbohydrate for training and recovery. Journal of Sports Sciences, 2006;24(7):675–685, https://doi.org/10.4324/9780203967430
7. *Djaoui, L., Chamari, K., Owen, A. L., & Dellal, A.*: Maximal Sprinting Speed of Elite Soccer Players During Training and Matches. J. Strength Cond. Res., 2017;31(6):1509–1517. https://doi.org/10.1519/JSC.0000000000001642
8. *Dobrowolski, H. & Włodarek, D.:* Dietary Intake of Polish Female Soccer Players. Int. J. Environ. Res. Public Health 2019; 16(7): 1134. https://doi.org/10.3390/ijerph16071134

9. Fédération Internationale de Football Association (FIFA): Women`s football strategy. Available:https://www.fifa.com/about-fifa/official-documents?filterId=34EnUsHdgRdtsiouywwJtx (Accessed 13.02.2023)
10. *Gibson, J. C., Stuart-Hill, L., Martin, S., & Gaul, C.*: Nutrition status of junior elite canadian female soccer athletes. Int J Sport Nutr Exerc Metab, 2011;21(6):507–514, https://doi.org/10.1123/ijsnem.21.6.507
11. *Helgerud, J., Engen, L. C., Wisløff, U., & Hoff, J.*: Aerobic endurance training improves soccer performance. Med Sci Sports Exerc, 2001;33(11):1925–1931, https://doi.org/10.1097/00005768-200111000-00019
12. *Henry, C. J. K.*: Mechanisms of changes in basal metabolism during ageing. Eur. J. Clin. Nutr., 2000;54: S77–S91, https://doi.org/10.1038/sj.ejcn.1601029
13. *Hoff, J., & Helgerud, J.*: Endurance and strength training for soccer players: Physiological considerations. Sports Med., 2004;34(3):165–180, https://doi.org/10.2165/00007256-200434030-00003
14. *Hoff, J., Wisløff, U., Engen, L. C., Kemi, O. J., & Helgerud, J.*: Soccer specific aerobic endurance training. Br. J. Sports Med. 2002;36(3):218–221, https://doi.org/10.1136/bjsm.36.3.218
15. *Liden C.B., Wolowicz M., Stivoric J., Teller A., Vishnubhatla S., Pelletier R., Farringdon J.*: Accuracy and Reliability of the SenseWear TM Armband as an Energy Expenditure Assessment Device. BodyMedia, 2002;1–15
16. *Loucks, A. B.*: Energy balance and body composition in sports and exercise. Journal of Sports Sciences, 2004;22(1):1–14, https://doi.org/10.1080/0264041031000140518
17. *Lukaski, H. C., Bolonchuk, W. W., Hall, C. B., & Siders, W. A.*: Validation of tetrapolar bioelectrical impedance method to assess human body composition. J. Appl. Physiol., 1986;60:1327–1332.
18. *Lukaski, H. C., Johnson, P. E., Bolonchuk, W. W., & Lykken, G. I.*: Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am. J. Clin. Nutr., 1985;41:810–817, https://doi.org/10.1093/ajcn/41.4.810
19. *Manore, M. M., & Thompson, J. L.*: Energy requirements of the athlete: assessment and evidence of energy efficiency. In: *Burke L. & Deakin V.* eds. Clinical Sports Nutrition (5th ed., pp. 115–139). Australia, McGraw-Hill Education Pty Ltd., 2015.
20. *Martin, L., Lambeth, A., & Scott, D.*: Nutritional practices of national female soccer players: Analysis and recommendations. J. Sports Sci. Med., 2006;5:130–137.
21. *Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R., & Ljungqvist, A.*: The IOC consensus statement: Beyond the Female Athlete Triad—Relative Energy Deficiency in Sport (RED-S). Br J Sports Med, 2014; 48: 491–497, https://doi.org/10.1136/bjsports-2014-093502
22. *Mullinix, M. C., Jonnalagadda, S. S., Rosenbloom, C. A., Thompson, W. R., & Kicklighter, J. R.*: Dietary intake of female U.S. soccer players. Nutr Res., 2003;23(5):585–593, https://doi.org/10.1016/S0271-5317(03)00003-4
23. *Olthof, S. B. H., Frencken, W. G. P., & Lemmink, K. A. P. M.*: When Something Is at Stake: Differences in Soccer Performance in 11 vs. 11 during Official Matches and Training Games. J. Strength Cond. Res., 2019;33(1):167–173, https://doi.org/10.1519/JSC.0000000000002936
24. *Owen, A. L., Wong, D. P., Dunlop, G., Groussard, C., Kebsi, W., Dellal, A., Morgans, R., & Zouhal, H.*: High-intensity training and salivary immunoglobulin a responses in professional top-level soccer players: Effect of training intensity. J. Strength Cond. Res., 2016;30(9):2460–2469. https://doi.org/10.1519/JSC.0000000000000380
25. *Rampinini, E., Coutts, A. J., Castagna, C., Sassi, R., & Impellizzeri, F. M.*: Variation in top level soccer match performance. Int. J. Sports Med. 2007;28(12):1018–1024, https://doi.org/10.1055/s-2007-965158
26. *Rodriguez, N. R., DiMarco, N. M., & Langley, S.*: Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. J. Am. Diet. Assoc. 2009;109(3):509–527, https://doi.org/10.1016/j.jada.2009.01.005
27. *Sun, S. S., Chumlea, W. C., Heymsfield, S. B., Lukaski, H. C., Schoeller, D., Friedl, K., Kuczmarski, R. J., Flegal, K. M., Johnson, C. L., & Hubbard, V. S.*: Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. Am. J. Clin. Nutr. 2003;77(2):331–340. https://doi.org/10.1093/ajcn/77.2.331
28. *Teller, A.*: A platform for wearable physiological computing. Interact. Comput., 2004;16:917–937. https://doi.org/10.1016/j.intcom.2004.08.004

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ORIGINAL ARTICLE

SELECTED PERSONAL RESOURCES AND NUTRITIONAL BEHAVIOURS OF POLISH HANDBALL PLAYERS

Maria Gacek[1]

[1]Department of Sports Medicine and Human Nutrition, University of Physical Education, Kraków, Poland

ABSTRACT

Background. Diet, as one of the factors influencing exercise capacity depends, among others, on individual conditions.
Objective. The aim of the study was to analyse the nutritional behaviours of Polish handball players depending on their level of generalised self-efficacy as well as disposable optimism and satisfaction with life.
Material and methods. The study was carried out among a group of 142 men, aged 20-34, professionally training handball, using the author's original nutritional behaviour questionnaire and the Generalised Self-Efficacy Scale (GSES), the Life Orientation Scale (LOT-R) and Satisfaction with Life Scale (SWLS). Statistical analysis of the results was conducted by estimating Spearman's signed rank correlation coefficients, assuming the significance level of $p<0.05$.
Results. To the highest degree, the handball players fulfilled recommendations regarding the regular consumption of at least 3 meals a day, adequate fluid supplementation in conditions of physical exercise and eating the most caloric meal before or after main training sessions. Along with the increase in sense of efficacy (GSES), the scale of reducing sweet and salty snacks increased ($p<0.05$). Increasing optimism was conducive to proper hydration ($p<0.05$) and avoidance of sweet and salty snacks ($p<0.05$). With the increase in sense of satisfaction with life, the implementation of recommendations regarding the consumption of dairy products and vegetable fats, as well as adequate fluid supplementation in conditions of physical exercise, increased ($p<0.05$).
Conclusion. In the studied group of handball players, a limited scale of implementing qualitative nutritional recommendations for athletes was demonstrated. Moreover, positive correlations were noted between the analysed personal resources and some rational nutritional behaviours of the athletes, especially in terms of avoiding non-recommended products and correct fluid replenishment.

Key words: *nutritional behaviours, handball players, feeling of generalised self-efficacy, dispositional optimism, satisfaction with life*

STRESZCZENIE

Wstęp. Sposób żywienia, jako jeden z czynników wpływających na zdolności wysiłkowe, zależy m.in. od cech osobniczych.
Cel. Celem badań była analiza związków pomiędzy nasileniem poczucia uogólnionej skuteczności, dyspozycyjnego optymizmu i satysfakcji z życia a zachowaniami żywieniowymi polskich piłkarzy ręcznych.
Materiał i metody. Badania przeprowadzono w grupie 142 mężczyzn w wieku 20-34 lata, wyczynowo trenujących piłkę ręczną, z zastosowaniem autorskiego kwestionariusza zachowań żywieniowych oraz Skali Uogólnionej Własnej Skuteczności (GSES), Skali Orientacji Życiowej (LOT-R) oraz Skali Satysfakcji z Życia (SWLS). Analizę statystyczną przeprowadzono poprzez oszacowanie współczynników korelacji rang Spearmana, na poziomie istotności $p<0,05$.
Wyniki. Piłkarze ręczni w najwyższym odsetku realizowali zalecenia dotyczące regularnego spożywania przynajmniej 3 posiłków dziennie, odpowiedniej podaży płynów w warunkach wysiłku fizycznego oraz spożywania najbardziej kalorycznego posiłku przed lub po głównym treningu. Wraz z nasilaniem się poczucia uogólnionej skuteczności zwiększała się skala ograniczania słodkich i słonych przekąsek ($p<0,05$). Wyższe nasilenie optymizmu było związane z prawidłowym uzupełnianiem płynów ($p<0,05$) oraz unikaniem słodkich i słonych przekąsek ($p<0,05$). Wraz z nasilaniem się poczucia satysfakcji z życia zwiększała się realizacja zaleceń dotyczących spożywania produktów mlecznych i tłuszczów roślinnych oraz prawidłowego uzupełniania płynów w czasie wysiłku fizycznego ($p<0,05$).
Wnioski. Wśród badanych piłkarzy ręcznych wykazano ograniczoną skalę realizacji jakościowych zaleceń żywieniowych dla sportowców oraz pozytywne związki pomiędzy analizowanymi zasobami osobistymi a racjonalnymi zachowaniami żywieniowymi, szczególnie w zakresie ograniczania produktów o niskiej gęstości odżywczej i prawidłowego uzupełniania płynów.

Słowa kluczowe: *zachowania żywieniowe, piłkarze ręczni, poczucie własnej uogólnionej skuteczności, dyspozycyjny optymizm, satysfakcja z życia*

Corresponding author: Maria Gacek, University of Physical Education, Al. Jana Pawła II 78, 31-571 Kraków, Poland; Phone: +48 12 683 11 45, e-mail :maria.gacek@awf.krakow.pl

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INTRODUCTION

One of the current models of sports nutrition is the Swiss Pyramid, which recommends adequate hydration and daily consumption of vegetables and fruits, whole grains, dairy products and vegetable fats. It also indicates other sources of complete proteins for consumption, including fish, as well as limiting animal fats and salty and sweet snacks [3].

A group with increased and specific nutritional needs comprises professional handball players, whose training effectiveness depends on the level of various motor characteristics, including speed, agility, strength and physical endurance [41, 42]. In handball, interval efforts and body contact occur, requiring players to, e.g. acquire high muscle strength [10]. Emotional state and coping with stress also play significant roles in the sports performance [25] and intellectual abilities of the athletes [46]. The significance of personal resources has also been confirmed, including a high level of disposable optimism, which increases the motivation to achieve success is sports [39].

Vigorous exercise during handball games necessitates meeting increased nutritional demands [24]. A rational diet (possibly enriched with supplementation) increases exercise capacity, and thus, the effectiveness of training and rate of post-exercise regeneration, while reducing the risk of injury [36]. Meanwhile, in a new systematic literature review, it was found that athletes performing team sports do not meet nutritional recommendations, especially in terms of energy and carbohydrate supply [5, 26].

Health behaviours, including those related to nutrition, are conditioned by numerous environmental and personality factors [37]. Among the psychological features essential in shaping health culture, personal resources occupy an important place, including sense of generalised self-efficacy and level of disposable optimism, as well as satisfaction with life. The construct of self-efficacy, developed as part of Bandura's social learning theory, expresses the belief in the ability to achieve intended goals, including those concerning health and sports, which are achieved, e.g. by rational eating behaviours [2, 8, 9, 28]. It is worth adding that the achieved success, including those sport-related, significantly strengthen sense of generalised self-efficacy [2]. Dispositional optimism is an expression of generalised expectations concerning the positive effects of an individual's activity [4, 43]. Therefore, it is an important individual resource that stimulates motivation, perseverance and determination in achieving goals, including sports objectives. Satisfaction with life, in turn, is a subjective measure of well-being related to the cognitive assessment of quality of life [11, 28]. Within this context, the above-mentioned personal resources are important personality traits with a potentially predictive role for the quality of health behaviours, including nutritional ones, important for the optimisation of sports training. In previous studies, relationships have been demonstrated between the various personal resources and eating habits of competitive athletes [13-17, 20], football referees [18] and physical education students [19]. Research in the field of psychological determinants of athletes' nutrition also concerned personality traits [21, 22].

Nutritional choices are important for the health and exercise capacity of handball players. The available studies concern nutrition of handball players competing at the Super -league level [7], the influence of nutritional education on the nutritional status indices and eating habits of these athletes [35], the use of sports supplements in professional handball depending on gender and level of competition [36], assessment of energy and nutrient supply in the diet of Slovenian semi-professional handball players [47], the relationships between diet and physical activity performance of juniors training handball [30] and determinants of knowledge level regarding nutrition among Turkish handball players [23]. The latest works of Spanish authors concern the relationship between adherence to the recommendations of the Mediterranean diet and the body composition and physical capacity of beach handball players [32, 40], between nutrient intake and body composition and physical capacity of female beach handball players [31] and eating disorders among elite beach handball players [33].

Due to the fact that a relationship between personal resources and nutritional behaviour of athletes who train professionally in team sports has been show in previous studies [13, 14, 15, 16, 20], research has been undertaken on selected individual determinants of food choices among handball players, who are a group under-researched in this area.

The aim of the study was to assess the nutritional behaviours of Polish handball players depending on basic personal resources (level of generalised self-efficacy, dispositional optimism and satisfaction with life) in relation to the recommendations of the Swiss Food Pyramid for athletes.

MATERIAL AND METHODS

The study was carried among a group of 142 men, aged 20-34 (22.9±4.2) and, training handball competitively (1st, 2nd, 3rd league and Super-league). The basic criterion for the open selection of participants was playing sports professionally for at least 3 years. The participants' professional sports experience was between 3 and 23 years (9.6±4.4). The studied players, in relation to the current classification of the level of

activity and sports abilities [34], can be assigned to Tier 3 (Highly Trained/National Level).

In the study, an author-created, validated nutritional behaviour questionnaire was used. It refers to the qualitative recommendations of the Swiss Food Pyramid for people demonstrating increased physical activity, presented in the literature [3]. The questionnaire consists of 22 items on rational eating behaviours, with a 5-point *Likert* response scale (1 to 5, from 'definitely not', 'rather not', 'difficult to say', 'rather yes' to 'definitely yes'). The scale is constructed in such a way that the higher the score, the more intense the rational eating behaviour. The questionnaire enquiries concerned: regular consumption of at least 3 meals a day, recommended frequency of consuming vegetables and fruits, whole grains, dairy products, other nutritional sources of protein, adequate hydration before, during and after training, preferred fats and limiting non-recommended products (sweets, fast-food products, carbonated and non-carbonated sweetened beverages and energy drinks). The questionnaire is a reliable research tool, which was confirmed via the validation procedure (high internal consistency, *Cronbach's α* coefficient totalling 0.79).

Sense of efficacy was measured using the Generalised Self-Efficacy Scale (GSES) by *Schwarzer, Jerusalem* and *Juczyński* [28]. The GSES, containing 10 items, is constructed in such a way that the higher the test result (within the range of 10-40 points), the higher the sense of generalised self-efficacy. The median of raw scores on the GSES scale of the examined handball players was 32 (M=31.6, SD=3.27). The level of disposable optimism was measured using the Life Orientation Test (LOT-R) by *Scheier, Carver* and *Bridges*, in the adaptation by *Poprawa* and *Juczyński* [28]. On the LOT-R scale, consisting of 10 statements (6 diagnostic), the higher the result (within the range of 0-24), the higher the level of optimism. The median of raw scores on the LOT-R scale for the studied handball players totalled 16 (M=16.12; SD=3.35). Satisfaction with life was measured via the Satisfaction with Life Scale (SWLS) by *Diener, Emmons, Larsen* and *Griffin*, in the adaptation by *Juczyński* [28]. On the SWLS scale consisting of 5 items, the higher the score (within the range of 5-35), the higher the level of satisfaction with life. The median of raw SWLS scores among the assessed handball players was 29 (M=28.6, SD=1.85).

Research has been conducted in accordance with the principles found in the Declaration of Helsinki, after obtaining informed consent from the participants.

Statistical analysis of the results was performed using the PQStat ver. 1.8.0.444. The relationships between the level of generalised self-efficacy, dispositional optimism as well as satisfaction with life and the level of rational food choices were analysed by estimating the *Spearman's* signed rank correlation coefficients. The test probability at the level of $p<0.05$ was considered significant, while $p<0.01$, highly significant.

RESULTS

The assessment of intensity regarding rational eating behaviours (on a scale of 1-5), according to the median value, allowed to confirm that the handball players under study fulfilled recommendations concerning the consumption of at least 3 meals a day, adequate fluid replenishment during exercise and the preference of water for hydration (Me=5.00 i.e. 'definitely yes'). They also largely complied with the recommendations for regularity of meals and the consumption of the most caloric meal before or after the main training (Me=4.00, i.e. 'rather yes'). Other recommendations were implemented to a lesser extent (Me=3.00, i.e. 'difficult to say') (Table 1).

Statistical analysis demonstrated significant correlations between the intensity of the analysed personality dimensions and some nutritional behaviours of athletes. It has been shown that along with the increase in sense of efficacy (GSES), the scale of rational food choices in terms of reducing sweet and salty snack consumption increased (R=0.21, $p<0.05$). It has also been indicated that with the increase in the level of disposable optimism (LOT-R), the implementation of recommendations on correct fluid replenishment during exercise also increased (R=0.17, $p<0.05$), as well as avoiding sweet and salty snacks (R=0.14, $p<0.05$). There was also a positive correlation between the intensity of the sense of satisfaction with life (SWLS) and the consumption of milk and dairy products at least twice a day (R=0.14, $p<0.05$), daily consumption of vegetable fats (R=0.16, $p<0.05$) and adequate hydration during exercise (R=0.21, $p<0.05$) (Table 2).

DISCUSSION

The discussed research allowed to demonstrated improper nutritional behaviours as well as correlations between the analysed personal resources and some aspects of nutritional behaviours among professional athletes training handball.

The revealed abnormalities concerned, in particular, the low implementation of the recommendations regarding the consumption of highly nutritional products (vegetables and fruit, cereals, including wholemeal products, dairy products, fish), restriction of products with low nutritional value (sweet and salty snacks) and the implementation of a varied diet. Also noteworthy is the limited level of implementing

Table 1. Implementing recommendations of the Swiss pyramid among athletes training handball (descriptive statistics)

Nutritional behaviours	Min	Max	Me	Q25	Q75
Consuming at least 3 meals a day	3.00	5.00	5.00	5.00	5.00
Regular meal consumption (every 3-5 hours)	1.00	5.00	4.00	3.00	4.00
Most caloric meal before or after main training	1.00	5.00	4.00	3.00	4.00
200 ml of vegetable or fruit juice every day	1.00	5.00	3.00	3.00	4.00
Vegetables with at least 2 meals a day	1.00	5.00	3.00	2.00	4.00
Raw vegetables at least once a day	1.00	5.00	3.00	2.00	3.00
2-3 servings of vegetables every day	1.00	5.00	3.00	2.00	3.00
1-2 servings of fruit every day	1.00	5.00	3.00	3.00	4.00
Cereal products with every main meal	1.00	5.00	3.00	3.00	3.00
Whole-grain cereal products at least twice a day	1.00	5.00	3.00	3.00	4.00
Milk or dairy products at least twice a day	1.00	5.00	3.00	2.00	3.00
Fish consumption 1-2 times a week	1.00	5.00	3.00	3.00	4.00
Limiting animal fats in diet	1.00	5.00	3.00	3.00	4.00
Vegetable fats every day (almost every day)	1.00	5.00	3.00	2.00	3.00
Adequate hydration during exercise	3.00	5.00	5.00	5.00	5.00
Adequate hydration after exercise	3.00	5.00	5.00	4.00	5.00
Preference of water for hydration	2.00	5.00	5.00	5.00	5.00
Avoiding sweet carbonated and non-carbonated beverages in diet	1.00	5.00	5.00	4.00	5.00
Avoiding energy drinks in diet	2.00	5.00	5.00	4.00	5.00
Avoiding fast-food products in diet	2.00	5.00	5.00	4.00	5.00
Limiting consumption of sweet and salty snacks	2.00	5.00	3.00	3.00	4.00
Varied diet	1.00	5.00	3.00	2.00	4.00

Scale: 1 – 'definitely not', 2 – 'rather not', 3 – 'difficult to say', 4 – 'rather yes', 5 – 'definitely not'

recommendations regarding the reduction of animal fats in favour of vegetable oils and nuts. On the other hand, positive trends were found in hydration behaviours during and after exercise, which is an important area of sports nutrition, directly related to health and exercise capacity. Among others, the preference of water and other unsweetened beverages was confirmed, as well as limiting hypertonic beverages (sweet carbonated and non-carbonated beverages and energy drinks), which indicates the correct selection of fluids for hydration.

Assessment of the described choices within the context of nutritional recommendations and nutritional value of individual product groups may indicate a potentially low supply of antioxidants, potassium and magnesium (low daily level of consuming vegetables and fruits), which is negative with regard to antioxidant status, in which a diet rich in, i.e. vegetables and fruits, is important for its regulations [12]. Excessively low compliance with the recommendations regarding the consumption of wholemeal cereal and dairy products may limit the supply of dietary fibres and calcium. The risk of the low intake of probiotic products (e.g. fermented milk) in the diet should also be emphasised because it creates a risk of developing dysbiosis in athletes [6]. In turn, a too low frequency of consuming sea fish, oils and nuts may reduce the supply of unsaturated acids, including omega 3, which positively regulate the blood lipid profile [44].

Improper nutritional decisions found in the studied group of handball players correspond with the trends described by other authors. Similar nutritional mistakes, related to the insufficient consumption of certain groups of food products with high nutritional density, including whole grain cereal and dairy products, as well as fish, have also been described among athletes training team sports [1, 13, 27, 38]. The obtained results can also be related to the study among handball players. In a trial on professional Spanish handball players (N=14), excessively low energy levels were also found, as well as low energy from carbohydrates but high from fats [35]. In another study on 2 Slovenian semi-professional handball teams (17 men and 9 women) from the 1st league, insufficient energy and carbohydrate intake as well as excessive fat intake were also found [47]. Similarly, in a study among handball players at the Superleague level, positive eating behaviours were noted with regard to the number and frequency of consuming meals, however, their diet was not balanced, as deficits

Table 2. Correlations between intensity of personal resources and national nutritional behaviours among handball players (*Spearman's* signed rank correlation coefficient value)

Nutritional behaviours	GSES	LOT-R	SWLS
Consuming at least 3 meals a day	0.02	0.13	0.10
Regular meal consumption (every 3-5 hours)	0.03	0.13	0.01
Most caloric meal before or after main training	0.04	0.13	0.09
200 ml of vegetable or fruit juice every day	0.12	0.01	0.01
Vegetables with at least 2 meals a day	0.03	0.02	0.03
Raw vegetables at least once a day	0.02	0.07	0.01
2-3 servings of vegetables every day	0.06	0.05	0.09
1-2 servings of fruit every day	0.02	0.01	0.01
Cereal products with every main meal	0.01	0.01	0.09
Whole-grain cereal products at least twice a day	0.07	0.02	0.09
Milk or dairy products at least twice a day	0.05	0.02	0.14*
Fish 1-2 times a week	0.09	0.06	0.01
Limiting animal fats in diet	0.06	0.12	0.12
Vegetable fats every day (almost every day)	0.09	0.12	0.16*
Adequate hydration during exercise	0.11	0.17*	0.21*
Adequate hydration after exercise	0.01	0.09	0.03
Preferring water for hydration	0.06	0.02	0.12
Avoiding sweet carbonated and non-carbonated beverages in diet	0.04	0.02	0.03
Avoiding energy drinks in diet	0.04	0.11	0.12
Avoiding fast-food products in diet	0.01	0.07	0.05
Limiting consumption of sweet and salty snacks	0.21*	0.14*	0.05
Varied diet	0.01	0.01	0.05

*- statistical significance at the level of $p<0.05$
GSES - Generalised Self-Efficacy Scale, LOT-R - Life Orientation Scale, SWLS - Satisfaction with Life Scale

in energy, protein, calcium, iron and potassium were found, as well as an excess of fat, phosphorus and sodium intake [7]. In another study regarding the relationships between diet and training effectiveness of junior handball players (N=57) from Târgu Mures, a correlation was confirmed between food consumption and exercise effectiveness. It has been noted that for young athletes, diet quality is crucial for achieving a high level of training [30]. Nutritional abnormalities associated with incomplete adherence to the Mediterranean diet have also been described among Spanish beach handball players [31, 32, 40]. Thus, the results of the author's study, as well as research carried out by other authors, allow to indicate qualitative and quantitative improper nutritional behaviours among athletes training handball, regardless of gender and sports level. The described incorrect nutritional behaviours noted among the examined handball players, which could reduce the nutritional value of their diets, confirmed the validity of monitoring and rationalising the diets of athletes, as a rational diet is one of the factors contributing to achieving professional success. Other authors also drew attention to the need for nutritional education in handball players [23, 35].

The discussed research conducted among handball players also allowed to show a relationship between the sense of self-efficacy and the level of optimism, as well as satisfaction with life, and the implementation of certain nutritional recommendations. The observed statistically significant positive correlations concerned the tendency towards more severe reduction of sweet and salty snacks along with an increase in self-efficacy and optimism, proper fluid replenishment with an increase in optimism and life satisfaction, and also the implementation of recommendations regarding the consumption of dairy products and vegetable fats with increasing life satisfaction. The demonstrated correlations indicate the predictive importance of the analysed personality dimensions for the diet quality of handball players and, in particular, the relationship between a high level of self-efficiency, optimism and life satisfaction with more correct nutritional choices of athletes. This correlation can be explained by psychological characteristics. A high level of self-efficiency, disposable optimism and satisfaction

with life increases the belief in the possibility of achieving the set goals, increasing motivation and determination to achieve objectives. Therefore, these features constitute important individual resources that stimulate pro-health behaviours, including those related to rational eating practices [2, 4, 28, 43]. In athletes, a rational model of nutrition is a key factor in maintaining health and optimising the effects of training [3].

Similar tendencies towards more rational nutritional choices among people with a higher intensity of the analysed personal resources (self-efficacy, optimism and satisfaction with life) were also obtained in other studies among athletes, both in Poland and in other countries. For example, the relationship between sense of self-efficacy and more rational eating behaviours has been described among Polish football (American football) players [13]. Also, research among Polish basketball players showed a correlation between higher level of self-efficacy and rational eating behaviours in terms of regularity of consuming meals, preferring non-sweetened beverages, the daily consumption of fruit and vegetables, and avoiding fast food and confectionery products [20]. Overall, the meta-analysis allowed to confirm the predictive significance of a high level of self-efficacy for promoting health-related behaviours, including those nutrition-related [45]. On the other hand, among Polish athletes training team sports (252 women and 266 men, 19-34 years old), it was shown that players with a high level of optimism and satisfaction with life, consumed fruit and vegetables significantly more often, and people with a high level of optimism consumed vegetable oils and other vegetable fats at a significantly higher frequency. The statistical analysis also allowed to confirm a significant positive relationship between the level of disposable optimism and the general index of rational eating behaviours among athletes [15]. Furthermore, in research among football players, a positive correlation was noted between the level of dispositional optimism and the frequency of consuming recommended products (fruit, whole grains, nuts), and a negative correlation with the frequency of consuming non-recommended products (e.g. sweets and confectionery products, energy drinks) [14]. Similarly, in the research by *Lipowski* [29], a positive correlation was demonstrated between the level of optimism and some pro-health behaviours of women practicing sports.

The significance of the presented work is related to addressing the underexploited research issues regarding selected determinants of food choices among handball players. The author is aware of the limitations of the work (questionnaire research, limitations of author-designed questionnaire on nutrition, small number of analysed factors), hence, referring to them, it should be noted that in subsequent research, a greater number of analysed variables (wider nutritional analysis, broader spectrum of psychological and environmental factors) should be taken into account.

CONCLUSIONS

In the studied group of handball players, a limited scale of implementing qualitative nutritional recommendations for athletes was demonstrated, particularly, in terms of the frequency of consuming high-nutritional products (vegetables and fruit, cereal products, including wholemeal and dairy products, fish) and applying a varied diet, which could have reduced the nutritional value of the diet.

In the studied group of handball players, the relationship between the analysed personal resources and some eating behaviours was demonstrated, with an indication of a tendency towards more rational choices along with the increase in level of self-efficacy, optimism and satisfaction with life. The positive trends concerned, in particular, limiting non-recommended products (sweet and salty snacks), consumption of recommended products (dairy and vegetable fats) and proper fluid replacement in conditions of physical exercise.

The results allow to suggest validity of the analysis concerning determinants of nutritional behaviour, as well as monitoring and rationalisation of the handball players' diet so that they could be supportive of the health and physical fitness of athletes.

Conflict of interest
None declared.

REFERENCES

1. *Abbey E.L., Wright C.J., Kirkpatrick C.M.*: Nutrition practices and knowledge among NCAA Division III football players. J Int Soc Sports Nutr 2017;14(13). doi: 10.1186/s12970-017-0170-2.
2. *Bandura A.*: Self-efficacy: The exercise of control. Freeman, New York 1997.
3. *Burke L.M.*: A food pyramid for Swiss athletes. Int J Sport Nutr Exerc Metab 2008;18(4):430-437.
4. *Carver C.S., Scheier M.F., Segerstrom S.C.*: Optimism. Clin Psychol Rev 2020;30(7):879-889. doi: 10.1016/j.cpr.2010.01.006.
5. *Castillo M., Lozano-Casanova M., Sospedra I., Norte A., Gutiérrez-Hervás A., Martínez-Sanz J.M.*: Energy and Macronutrients Intake in Indoor Sport Team Athletes: Systematic Review. Nutrients 2022;14:4755. doi.org/10.3390/nu14224755.
6. *Cronin O., O'Sullivan O., Barton W., Cotter P.D., Molloy M.G., Shanahan F.*: Gut microbiota: implications for sports and exercise medicine. Br J Sports Med 2017;51(9):700-701. doi: 10.1136/bjsports-2016-097225.

7. *Dymkowska-Malesa M., Swora-Cynar E., Grzymisławska M., Grzymisławski M.*: Evaluation of the Nutrition and Supplementation Handball Players of the PGNIG Womem Super League Energa AZS Koszalin Club. Med Sport 2016;4 (4):241-250. DOI: 10.5604/1232406X.1229083.
8. *Feltz D.L., Lirgg C.D.*: Self-efficacy beliefs of athletes, teams, and coaches. (In:) R.N. Singer, H.A. Hausenblas, C.M. Janelle (Eds.). Handbook of sport psychology (2nd ed., 340-361). Wiley & Sons, New York 2001.
9. *Feltz D.L., Short S.E., Sullivan P.J.*: Self-efficacy in sport. Human Kinetics 2008.
10. *Ferragut C., Vila H., Abraldes J.A., Manchado C.*: Influence of Physical Aspects and Throwing Velocity in Opposition Situations in Top-Elite and Elite Female Handball Players. J Hum Kinetics 2018;63:23-32. doi: 10.2478/hukin-2018-0003.
11. *Filbay S., Pandya T., Thomas B., McKay C., Adams J., Arden N.*: Quality of Life and Life Satisfaction in Former Athletes: A Systematic Review and Meta-Analysis. Sports Med 2019;49(11):1723-1738. doi: 10.1007/s40279-019-01163-0.
12. *Frączek B., Morawska M., Gacek M., Pigoń K.*: Antioxidant activity as well vitamin and polyphenol content inthe diet for athletes. Ital J Food Sci 2019;31(4):617-630. doi. http://dx.doi.org/10.14674/IJFS-1510.
13. *Gacek M.*: Association between self-efficacy and dietary behaviors of American football players in the Polish clubs in the light of dietary recommendations for athletes. Rocz Panstw Zakl Hig 2015;6(4):361-366.
14. *Gacek M.*: Poczucie dyspozycyjnego optymizmu a częstość spożywania wybranych grup produktów i środków ergogenicznych wśród piłkarzy nożnych [Sense of dispositional optimism vs. frequency of consuming selected groups of products and performance enhancers among soccer players]. Med Og Nauk Zdr 2017;23(2):95-99. https://doi.org/10.26444/monz/75336 (in Polish).
15. *Gacek M.*: Selected individual determinants of nutritional behaviours among Polish athletes performing team sports. Hygeia Public Health 2019;54(4):243-250.
16. *Gacek M.*: Selected personal resources vs eating habits of soccer players. Med Sport 2019;35(3):149-155. doi: 10.5604/01.3001.0013.5815.
17. *Gacek M.*: Sense of self-efficacy and the content of energy and nutrients in the diet of elite Polish basketball players. Rocz Panstw Zakl Hig 2022;73(2):183-189. doi: 10.32394/rpzh.2022.0203.
18. *Gacek M., Kosiba G.*: Sense of generalised self-efficacy and the nutritional behaviour of district-level football referees. Antropomotoryka. J Kinesiol Exerc Sci JKES 2019;86(29):53-58. doi: 10.5604/01.3001.0013.7807.
19. *Gacek M., Kosiba G., Wojtowicz A.*: Generalised sense of self-efficacy and the level of physical activity among Polish and Spanish physical education students. J Phys Edu Sport 2020;20(3):1461-1467. doi:10.7752/jpes.2020.03201.
20. *Gacek M., Wojtowicz A.*: Personal Resources and Nutritional Behaviour of Polish Basketball Players. J Phys Edu Sport 2021;21(1):130-139. DOI:10.7752/jpes.2021.01018.
21. *Gacek M, Wojtowicz A, Popek A.*: Personality Determinants of Diet Health Quality Among an Elite Group of Polish Team Athletes. Int J Environ Res Public Health 2022;19(24):16598. https://doi.org/10.3390/ijerph192416598.
22. *Gacek M, Wojtowicz A, Popek A.*: Personality Determinants of Eating Behaviours Among an Elite Group Of Polish Athletes Training Team Sports. Nutrients 2023;15(1):39. https://doi.org/10.3390/nu15010039.
23. *Gümüşdağ H., Kartal A.*: Examination Of Nutrition Knowledge Level Of Handball Players Playing In League And National Teams In Terms Of Some Variables. J Int Anatolia Sport Sci 2017;2(3):208-215. DOI: 10.22326/ijass.23.
24. *Heaton L.E., Davis J.K., Rawson E.S., Nuccio R.P., Witard O.C., Stein K.W., Baar K., Carter J.M., Baker L.B.*: Selected In-Season Nutritional Strategies to Enhance Recovery for Team Sport Athletes: A Practical Overview. Sports Med 2017;47(11):2201-2218. doi: 10.1007/s40279-017-0759-2.
25. *Ivaskevych D., Borysova O., Fedorchuk S., Tukaiev S., Kohut I., Marynych W., Petrushevskyi Y., Ivaskevych O., Mihăilă J.*: Gender differences in competitive anxiety and coping strategies within junior handball national team. J Phys Edu Sport 2019;19(2):1242-1246. DOI: 10.7752/jpes.2019.02180.
26. *Jenner S.L., Buckley G.L., Belski R., Devlin B.L., Forsyth A.K.*: Dietary intakes of professional and semi-professional team sport athletes do not meet sport nutrition recommendations - a systematic literature review. Nutrients 2019;11(5):1160. https://doi.org/10.3390/nu11051160.
27. *Jenner S.L., Trakman G., Coutts A., Kempton T., Ryan S., Forsyth A., Belski R.*: Dietary intake of professional Australian football athletes surrounding body composition assessment. J Int Soc Sports Nutr 2018;5(1):43. doi: 10.1186/s12970-018-0248-5.
28. *Juczyński Z.*: Narzędzia pomiaru w promocji i psychologii zdrowia [Measurement tools in promotion and health psychology]. Pracownia Testów Psychologicznych, Warszawa 2012.
29. *Lipowski M.*: Level of optimism and health behavior in athletes. Med Sci Monit 2012;18(1):39-43. doi: 10.12659/MSM.882200.
30. *Martin S.A., Tarcea M.*: The relationship between exercise parameters, body weight, and nutritional habits of junior handball players. Civiliz Sport 2015;16 (4):329-332.
31. *Martínez-Rodríguez A., Sánchez-Sánchez J., Martínez-Olcina M., Vicente-Martínez M., Peñaranda-Moraga M., Asencio-Mas N., Gonzálvez-Alvarado L., Matlosz P., Yáñez-Sepúlveda R., Cortés-Roco G., Sánchez-Sáez J.A.*: Quantitative Diet, Body Composition and Sprint Performance in Female Professional Beach Handball Players. Nutrients 2022;1(1):138. doi: 10.3390/nu15010138.
32. *Martínez-Rodríguez A., Sánchez-Sánchez J., Martínez-Olcina M., Vicente-Martínez M., Yáñez-Sepúlveda R.,*

Cortés-Roco G., Vázquez-Diz J.A., Sánchez-Sáez J.A.: Professional Male Beach Handball Players Performance Profile. Nutrients 2022;14(22):4839. doi: 10.3390/nu14224839.
33. *Martínez-Rodríguez A., Vicente-Martínez M., Sánchez-Sánchez J., Miralles-Amorós L., Martínez-Olcina M., Sánchez-Sáez J.A.*: Eating Disorders in Top Elite Beach Handball Players: Cross Sectional Study. Children (Basel) 2021;8(3):245. doi: 10.3390/children8030245.
34. *McKay A.K.A., Stellingwerff T., Smith E.S., Martin D.T., Mujika I., Goosey-Tolfrey V.L., Sheppard J., Burke L.M.:* Defining Training and Performance Caliber: A Participant Classification Framework. Int J Sports Physiol Perform 2022;17:317-331. doi: 10.1123/ijspp.2021-0451.
35. *Molina-López J., Molina J.M., Chirosa L.J., Florea D., Sáez L., Jiménez J., Planells P., Pérez de la Cruz A., Planells E.*: Implementation of a nutrition education program in a handball team; consequences on nutritional status. Nutri Hospital 2013;28(4):1065-1076. doi: 10.3305/nh.2013.28.4.6600.
36. *Muñoz A., López-Samanes Á., Domínguez R., Moreno-Pérez V., Sánchez-Oliver A.J., Del Coso J.*: Use of Sports Supplements in Competitive Handball Players: Sex and Competitive Level Differences. Nutrients 2020;12(11):3357. doi.org/10.3390/nu12113357.
37. *Pelly F.E., Thurecht R.L., Slater G.:* Determinants of Food Choice in Athletes: A Systematic Scoping Review. Sports Med. Open 2022;8:77. doi.org/10.1186/s40798-022-00461-8.
38. *Petri C., Mascherini G., Pengue L., Galanti G.*: Dietary habits in elite soccer players. Sport Sci Health 2016;12(1):113-119. doi: 10.1007/s11332-016-0264-2.
39. *Popovych I., Blynova O., Nosov P., Zinchenko S., Kononenko O.*: Psychological factors of competitiveness of the women's youth handball team. J Phys Edu Sport 2021;21(1):227-235. DOI:10.7752/jpes.2021.
40. *Romero-García D., Esparza-Ros F., Picó García M., Martínez-Sanz J.M., Vaquero-Cristóbal R.*: Adherence to the Mediterranean diet, kinanthropometric characteristics and physical performance of young male handball players. Peer J 2022;10:e14329. doi: 10.7717/peerj.14329.
41. *Saavedra J.M., Halldórsson K., Kristjánsdóttir H., Þorgeirsson S., Sveinsson G.*: Anthropometric Characteristics, Physical Fitness and the Prediction of Throwing Velocity in Young Men Handball Players. Kinesiol 2019;51(2):253-260. DOI: 10.26582/k.51.2.14.
42. *Sabido R., Hernández-Davó J.L., Botella J., Jiménez-Leiva A., Fernández-Fernández J.*: Effects of block and daily undulating periodization on neuromuscular performance in young male handball players. Kinesiol 2018;50 (1): 97-103. https://doi.org/10.26582/k.50.1.6.
43. *Scheier M.F., Carver C.S.*: Dispositional Optimism and Physical Health: A Long Look Back, a Quick Look Forward. Am Psychol 2018;73(9):1082-1094. doi: 10.1037/amp0000384.
44. *Shahidi F., Ambigaipalan P.*: Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. *Annu Rev Food Sci Technol* 2018;9:345-381. doi: 10.1146/annurev-food-111317-095850.
45. *Sheeran P., Maki A., Montanaro E., Avishai-Yitshak A., Bryan A., Klein W.M.P., Miles E., Rothman A.J.*: The impact of changing attitudes, norms, and self-efficacy on health-related intentions and behavior: A meta-analysis. Health Psychol 2016;35(11):1178-1188. doi: 10.1037/hea0000387.
46. *Strykalenko Y., Shalar O., Huzar W., Voloshinov S., Yuskiv S., Silvestrova H., Holenko N.*: The correlation between intelligence and competitive activities of elite female handball players. J Phys Edu Sport 2020;20(1):63-70. DOI:10.7752/jpes.2020.01008.
47. *Teraž K., Meulenberg C.*: Nutritional intake of Slovenian semi-professional handball players. Ann Kinesiol 2020;10(2):129-147. https://doi.org/10.35469/ak.2019.201.

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ORIGINAL ARTICLE

CONSUMPTION OF FOOD SUPPLEMENTS IN THE FES-MEKNES REGION, MOROCCO. PROFILES AND TYPOLOGY

Hamza El Finou[1], *Nadia Salhi*[1], *Abdelhamid Zaid*[1], *Lhoussaine El Rhaffari*[1]

[1]Department of Biology, Laboratory of Bioactives, Health and Environment, Moulay Ismail University of Meknes, Faculty of Sciences, Morocco

ABSTRACT

Background. The consumption of food supplements is increasing. This evolution is due to several factors, including nutritional deficiencies in the population, a sedentary lifestyle, and a decrease in physical activity. In addition, stress and an active lifestyle led to several dysfunctions (fatigue, deconcentration) that food supplements can help to overcome.

Objective. This study aimed to determine the profiles of food supplement consumers in the Fes-Meknes region (Morocco), distribution, and production of these products. In addition, this survey aimed to evaluate consumers' knowledge of food supplements as part of their self-medication.

Material and methods. The present study was conducted in the form of a survey using a questionnaire composed of two parts. The first part provides information on the socio-demographic status of respondents, including gender, age, and level of education. The second part concerned various information on the consumption of food supplements.

Results. The results obtained showed that of the 498 subjects, 68.88% declared that they had already consumed the food supplements. The study revealed the predominance of the female gender at 69.68% and the age group 21-30 years (80.32%). Among the reasons for consumption, improving general health comes first with 56.29%. Our results also showed high consumption of vitamins (44.04%) and minerals (24.79%), next come proteins and plant extracts at 16.62% and 14.54% respectively. The consumption of food supplements is most often done on the advice of a doctor or dietician with 43.60%, and the pharmacy and para-pharmacy remains the main distribution channel at 75.78%.

Conclusion. The present survey allowed us to update the current situation of food supplement consumption and a way of regulatory monitoring and more control for an organization of the sector.

Key words: *consumption, food supplements, health, minerals, vitamins, self-medication*

INTRODUCTION

The concept of nutrition is now part of our daily lives. Faced with the rise of sedentary lifestyles, the growth of food, and the emergence of alternative medicines, the food supplements (FS) market is booming in many developed countries [1]. The pharmaceutical and food industries offer a wide range of food supplements that include several products of various forms and uses. As these forms are very similar to drugs in their galenic form, the difference between a drug and dietary supplements is becoming less and less obvious. These forms represent today a real challenge to satisfy and retain the consumer.

The consumption of food supplements continues to grow both nationally and internationaly. This is due to several factors including the awareness of the need for these products for people who follow specific nutritional diets, as well as the development of the sports sector (gyms and fitness) that make their customers aware of the body discipline sometimes requiring the intake of food supplements [2]. Finally, stress and an active lifestyle led to several dysfunctions (fatigue, deconcentration) that nutritional supplements can help to overcome.

The COVID-19 pandemic has created widespread psychosis and anxiety. The consumption of FS increased (Vitamin C, A, and Zinc) due to the supposed role of micronutrients in strengthening immunity to cope with this pandemic. The purchase and consumption of aromatic and medicinal plants also increased during this period [3]. Faced with this situation, the practice of self-medication has increased. For this, many substances have been used without medical advice. Indeed, a recent study in Togo showed that about one out of three people had used this practice to prevent

Corresponding author: Hamza El Finou, Bioactives, Health and Environment Laboratory, Moulay Ismail University of Meknes, Faculty of Sciences, Department of Biological Sciences, 11201- Meknes, Morocco. Tel: + 212 6 26 51 66 46, e-mail: h.elfinou@edu.umi.ac.ma

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COVID-19 [4]. Another study in Bangladesh found that the prevalence of self-medication during the COVID-19 epidemic was 88.3% [5]. In Kenya, the prevalence of self-medication increased from 36.2% before the pandemic to 60.4% during the pandemic [6]. that the use of dietary supplements is part of self-medication worldwide. In our study, we focused on the population of the Fes-Meknes region (Morocco), but according to previous studies, it is evident that the world population has the same tendency to practice self-medication.

FS are typically healthy compounds that present no potential danger. This indicates that consumers can use them without risk. However, some studies of their physiological effects, have reported their potential risks on body functioning, especially in the absence of nutritional deficiencies [7]. Thus, the consumption of FS must not exceed the maximum recommended levels. As a result, excess consumption can be harmful to health in the long term. Any supplementation must be under the supervision of a health professional and any intake without supervision may present health risks [8].

Since food supplements are potential sources of nutrients and other bioactive compounds, it is important from a public health perspective to know who is consuming them, what types, and under what circumstances. For this, the main aim of this survey is to evaluate the profiles of FS consumers, and the production and distribution of FS, to update the current situation of FS consumption, and provide new data concerning this sector, particularly in the Fes-Meknes region in Morocco.

MATERIAL AND METHODS

Study context

The survey was carried out in the Fez-Meknes region during the 2020-2021 academic year. A network of students allowed us to distribute the questionnaire in the different cities of the region via the Internet and in the field, which allowed us to interview 498 subjects in 4 months. In addition, the second phase of our survey was carried out with 70 pharmacists to have information related to the production and distribution of food supplements.

The inclusion criteria for this study were the consumption of FS (343 subjects). While the non-consumers of FS were excluded from the analysis process (155 subjects).

SURVEY PROCEDURE

From February to May 2021, a cross-sectional, anonymous, and descriptive study was conducted online and in the field with 498 citizens from different cities in the Fez-Meknes region (Morocco). All participants accepted the use of their responses for publication and scientific studies. An email invitation to participate in the survey containing a link to the online questionnaire was sent to students, teachers, health professionals, and others. The first page of the questionnaire contained an implied consent statement, although participants were free to accept or decline participation as they saw fit. The survey gathered information on:

- General information on the socio-demographic status of participants, including gender, age, level of education, etc.
- The age has been classified into 4 classes: under 20 years; 21-30 years old; 31-40 years old; 41-50 years old and over 50 years old.
- The FS consumption mode section consisted of 10 questions. It discussed the different types of FS consumed by participants. These types have been grouped into standardized categories (Vitamins/minerals/proteins and FS from plants/plant extracts).
- In addition, the participants who previously or currently used food supplements (even a few years ago) are considered consumers, while those who answered never are considered non-users.
- For the variables that concerned the consumption of the FS, the analysis concerned only the consumers.
- It also included questions on the method of consumption (frequently (many times), occasionally (accidentally), and regularly (on an exact prescribed diet), the purchase recommendation (advised by a friend, medical prescription), places of purchase (Pharmacy/Para pharmacy, specialized surfaces, supermarkets, Internet), and consumer expectations (improve general health, filling a deficiency, coping with a disease).

Data collection

"Google Forms" was used to design and develop the questionnaire form. The answers that were added were automatically collected, arranged in a file, and then converted to Excel format. Preliminary to the survey's administration, a pilot study was conducted on a sample of 60 participants to confirm the reliability and validity of the questionnaire. As a result, changes were made to several of the survey questions to make it easier for respondents to understand the questions.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) Version 20 was used to conduct the statistical analysis. While qualitative variables were expressed as percentages (number of people that used the study modality/total population), quantitative data were expressed as means. The *Chi*-square test was conducted to see if any categorical differences merited further investigation, such as the association between FS consumption and certain variables. Statistical significance is defined as a p-value <0.05.

RESULTS

Socio-demographic data of consumers

The sociodemographic data of the participants and the mode of FS consumption data are presented in Table 1. Of the 498 participants, 68.88% reported consuming FS (343 subjects). Regarding the gender and age of consumers, we obtained a dominance of the female gender at 69.68% against 30.32% of men and the age group 21-30 years old at 61.81%. In addition, 69.97% of consumers had a university level of education.

Circumstances for buying food supplements

The consumption of food supplements is most often done on the advice of a doctor, dietician, or psychologist with a percentage of 43.60%, then the consumption of food supplements by own initiative of the consumer at 29.94%. While the consumption following recommendations from relatives presents only 16.57%, and the consumption after reading articles or television programs presents a percentage of 9.88% (Figure 1).

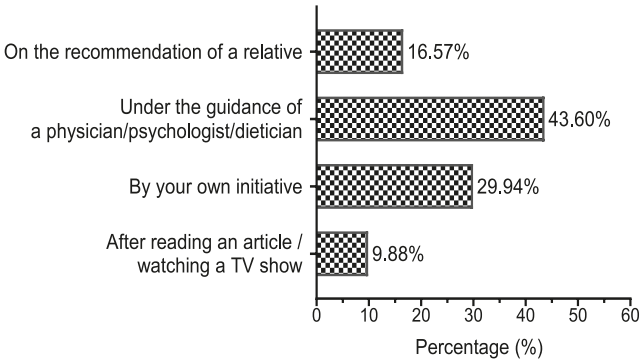

Figure 1. Circumstances for buying food supplements

Currently, the free sale in pharmacies/para-pharmacies, in specialized supermarkets, and on the Internet, has greatly facilitated consumers' access to food supplements. The results obtained showed that 75.78 % of consumers buy food supplements in pharmacies.

Most consumed components of food supplements.

The results obtained showed high consumption of vitamins (44.04%) and minerals (24.79%). Next, come proteins and plant extracts at 16.62% and 14.54% respectively (Figure 2). Among the consumer participants, 125 of them (36.44%), were able to give the exact names of the supplements they use. Table 2 shows a descriptive analysis of the composition and functions of some food supplements cited by consumers.

Table 1. Summary of the socio-demographic and FS consumption data

Variables	Percentage %
Gender	
– Male	30.32
– Female	69.68
Age	
– Under 20 years	02.92
– 21-30 years	61.81
– 31-40 years	18.95
– 41-50 years	04.37
– Over 50 years	11.95
Level of education	
– Illiterate	07.87
– Primary school	04.66
– High school	17.49
– University	69.97
Frequency of consumption	
– Frequently	13.31
– Occasionally	71.67
– Regularly	15.01
Food supplements acquisition places	
– Pharmacy/Para pharmacy	75.78
– Specialized surfaces	07.69
– Supermarkets	05.41
– Internet	05.41
– Stores	05.70
Reasons given for taking food supplements	
– Improve general health	56.29
– Filling a deficiency	25.89
– Coping with a disease	17.81
Symptoms treated with food supplements	
– Blood circulation	04.47
– Digestion and intestinal transit	14.78
– Reinforcing natural defences	16.84
– Weight loss and/or gain	20.27
– General tiredness	43.64
Consumer satisfaction after consuming food supplements	
– Satisfied	57.10
– Partially satisfied	32.24
– Unsatisfied	10.66
Average price of food supplements	
– Between 100 and 200 MAD	61.43
– Between 50 and 100 MAD	31.43
– More than 200 MAD	07.14
Evolution of the demand for food supplements in the last 5 years	
– No idea	07.14
– Growing	70.00
– Descending	04.29
– Almost stable	18.57

Table 2. Descriptive analysis* of some food supplements cited by consumers

Food Supplement	Category	Composition	Function
Supradine	Multi-vitamin	Vitamins (A, B_2, B_6, B_{12}, C, D, E, K) Minerals (Zn, Mg, Cu, Ca). Coenzyme Q10	Energizer
Docivox	Natural syrup	Natural active ingredients (Thyme fluid extract)	Soften the throat, soothe the respiratory tract and strengthen the natural defences.
Tardyferon	Micro-nutrients	Iron Ferrous Sulphate Folic acid	Treatment of anaemia and iron deficiency
Whey	Source of proteins	Amino acids	Protein for athletes
Nurax	Appetite stimulant	Fenugreek, dry extract of gentian, Iron, Wheat germ,12 vitamins	Appetite stimulant
Additiva	Multi-vitamin	Vitamins (A, B_1, B_2, B_6, B_{12},) Minerals, Pantothenic acid	Vitamin supply
Forcapil	Micro-nutrients	Zinc, Vitamin B_8 Vitamin B_9	Hair and nail care
Nuravit	Appetite stimulant	Vitamins (B_1, B_2, B_6, C)	Appetite stimulant
Omega-3	Fatty acids	Omega-3	Development and function of the retina, brain, and nervous system
Levure de bière	Appetite stimulant	Non-pathogenic microscopic fungi (*Saccharomyces cerevisiae* and *Candida utilis*)	Regulators of intestinal transit and loss of appetite
Relaxium	Micro-nutrients	Magnesium Vitamin B_6	Micro-nutrients

*: The descriptive analysis was based on the notices of each food supplements (consulted in pharmacies).

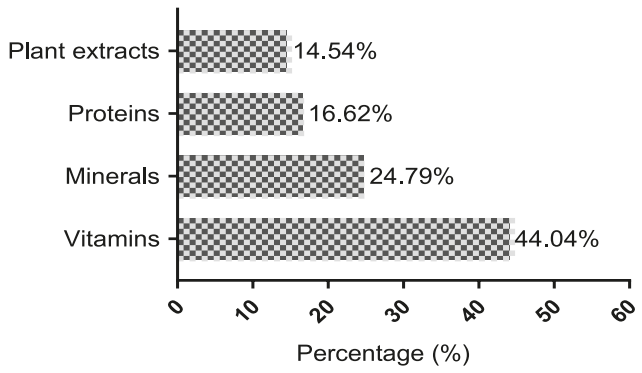

Figure 2. Most consumed components of food supplements

Production and distribution of food supplements

During the second phase of the survey, conducted among 70 pharmacies and para pharmacies in the Meknes region, the distribution and production of dietary supplements were identified. The results showed that the most marketed food supplements were intended for cosmetic and wellness use (32.2%) and skin care (46.6%). Then comes hair care at 21.2%. According to the producers interviewed, the food supplement market has developed over the past 5 years. The results show that the average prices of these products are between MAD 100 and MAD 200 at 61.43%, between MAD 50 and MAD 100 (31.43%), and 7.14% for products above 200 MAD (Table 2).

The raw material used for the production of these products is of both Moroccan and foreign origin. Supplier markets are European (56.23%), American (22.14%), Asian (16.72%), and African (4.91%). The growth in demand for food supplements has been observed over the past 5 years by 70% of producers interviewed. Interviews conducted at the pharmacy level showed that 82.9% of clients are young, confirming the results obtained with consumers (Table 2).

Association of FS consumption with some variables.

The *Chi*-square test was performed to reveal any association between the consumption of FS and other variables (Table 3). The results obtained showed that the consumption of FS varies depending on the age of participants (Chi^2=9.48; p=0.002), the education level

Table 3. Summary of the association of FS consumption with some parameters studied

Variables	Percentage %	*Chi* ^{2}	P-value
Gender			
– Male	30.32	3.84	0.441
– Female	69.68		
Age			
– under 20 years	02.92	9.48	0.002
– 21-30 years	61.81		
– 31-40 years	18.95		
– 41-50 years	04.37		
– Over 50 years	11.95		
Level of education			
– Illiterate	07.87	7.81	0.000
– Primary school	04.66		
– High school	17.49		
– University	69.97		
Frequency of consumption			
– Frequently	13.31	5.99	0.147
– Occasionally	71.67		
– Regularly	15.01		
Food supplements acquisition places			
– Pharmacy/Para pharmacy	75.78	9.48	0.976
– Specialized surfaces	07.69		
– Supermarkets	05.41		
– Internet	05.41		
– Stores	05.70		
Consumer satisfaction after consuming food supplements			
– Satisfied	57.10	5.991	0.000
– Partially satisfied	32.24		
– Unsatisfied	10.66		

(Chi^2=7.81; p<0.001), and the satisfaction of consumers (Chi^2=5.991; p<0.001). It was also observed that no significant association between the FS consumption and gender (Chi^2=3.84; p=0.441), the FS acquisition places (Chi^2=9.48; p=0.976), and the frequency of consumption (Chi^2=5.99; p=0.147).

The preferred design of the FS for consumers

Consumers preferred tablets first at 37.2% followed by capsules at 28.5%. In addition, some consumers preferred drinkable suspensions (15.6%), flavored drinks, and infusions (12.8%). While pastilles were the least used at 5.9% (Figure 3).

DISCUSSION

Considering all the results obtained in this study, it is obvious that the consumption of dietary supplements is widespread in Morocco. Our results showed that of 498 participants, 68.88% were FS consumers. In addition, the dominance of females at 69.68% against 30.32% of men's gender and the age group 21-30 years old at 61.81%. In this context, *Jamal* [9] conducted a survey in 2015 in Morocco with 504 volunteers and found that 46% of the subjects were consumers [9]. Other studies have also confirmed this trend [10, 11]. Contrary to the results obtained by *Naqvi* et al. [12], who analyzed the food supplement intake in the public and private sector at the pharmacy teaching universities of Karachi (Pakistan), and found that 51% were male, and 47.3% were female. By age groups, the highest figure was reported in the age group 27-30 years [12]. The consumption of food supplements also varies according to region. Thus, it is in the northern and central regions (Casablanca, Rabat, Marrakech, and Tangier) that we have the largest number of consumers [9]. On the other hand, in the South, the demand is almost non-existent. Our study also confirmed this hypothesis by the large number of consumers found in the Fez-Meknes region. From the point of view of consumer satisfaction after taking food supplements, we obtained that only 32.24% proved great satisfaction; while the study of *Jamal* [9], showed that about 90% of subjects said that the consumption of food supplements met their expectations [9].

For the circumstances of purchases, our study revealed that the consumption of food supplements is

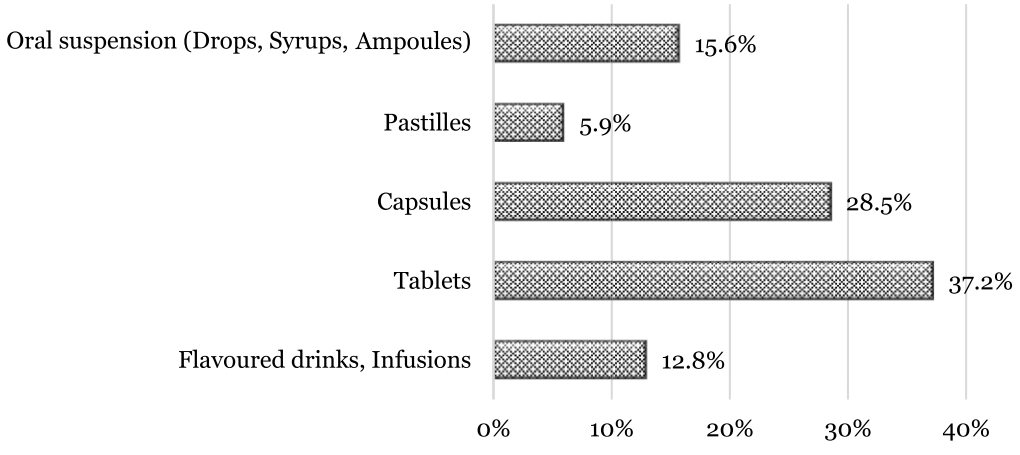

Figure 3. The preferred design of the FS for consumers

often done under the advice of a doctor or dietician with a rate of 43.60%, the taking of supplements by the consumer's initiative comes in second place at 29.94%. Another study conducted by *Pouchieu* et al. [13], showed an important part of self-medication, and nearly one consumer out of two took food supplements as self-medication, 21% bought supplements under medical advice, and 33% under medical prescription. A health professional generally recommends the use of food supplements [14].

According to the data obtained from the Syndicate of Dietetics and Supplements [15], the pharmacy remains the leading sales channel for food supplements in France, compared with direct sales on the internet, specialized stores, large and medium-sized retailers, and Para pharmacies. Our results also confirmed this tendency, from which we obtained that 75.78 % of consumers buy food supplements in pharmacies.

According to the results obtained in this study, it is most often a high consumption of vitamins, micronutrients, energizers, and minerals. Vitamins and minerals have a great variability in their complementary intakes, with the highest contributions concerning vitamin D, vitamin B_1, and vitamin C. In this context, several studies have also shown that the most commonly consumed nutrients in the form of supplements were magnesium, vitamin B_6, vitamin C, zinc, and iron, whereas omega-3 fatty acids and herbal supplements were consumed quite lowly [16]. Thus, according to *Nakhal* et al. [17], fish oil (omega 3) was the highest food supplement used at 18.02%, followed by Glucosamine, Chondroitin, Cranberry, and fiber with percentages at 12.11%, 11.83%, 9.29%, and 8.45%, respectively. In addition, 76% of the participants indicated that they recommend herbal food supplements to their friends/families, and patients [17].

The importance of applying rigorous regulations concerning the sale and purchase of FS is essential, because, As opposed to popular belief, excessive consumption of protein and amino acids can be potentially very harmful to our health as they can cause disorders of the bone system, impaired kidney function, increased risk of cancer and impaired liver function and can lead to coronary heart disease [18]. In addition, *Rutkowski* and *Grzegorczyk* [19] also reported the deleterious effects of excessive supplementation with antioxidants especially synthetic ones (Vitamin A, E, C, and β-carotene) which are often used in many preventive and curative medical treatments. However, they can cause hypervitaminosis and even a potentially toxic effect [19].

CONCLUSION

The present survey allowed us to bring new data concerning the food supplements sector in Morocco, in particularly the Fez-Meknes region, by studying several parameters related to the consumption, production, and distribution of these products. The FS supports a healthy diet and helps prevent disease, but uncontrolled consumption can be harmful and ineffectual in the absence of vitamin or mineral deficiencies. In this approach, the health professional remains a key player and must educate consumers on the benefits as well as the risks associated with taking food supplements. In this sense, the provision of clear and validated information is a very effective tool. In this study, the restrictions and sanitary measures concerning COVID-19 were a limit in front of the conduct of our survey. Thus, the participant's personal life was sometimes a major obstacle to access to complete information.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest regarding this article.

REFERENCES

1. *Tran S, Calabretto JP, Sorich M.* Consumer-pharmacist interactions around complementary medicines: Agreement between pharmacist and consumer expectations, satisfaction and pharmacist influence. International Journal of Pharmacy Practice 2013;21(6):378-385. https://doi.org/10.1111/ijpp.12027.
2. *Garthe I.* Dietary supplements and elite athletes: when nature becomes high risk. Curr Opin Endocr Metab Res. 2019;9: 66–73. https://doi.org/10.1016/j.coemr.2019.07.004.
3. *Boudia F, Dali Ali A, Mekaouche FZN, Fetati H, Senhadji I, Chaib H,* et al. Automédication et conseil pharmaceutique en période de pandémie de COVID-19 à Oran [Self-medication and pharmaceutical advice during the COVID-19 pandemic in Oran]. Algerian Journal of Health Sciences. 2021;3(2):30-37.
4. *Sadio AJ, Gbeasor-Komlanvi FA, Konu RY, Bakoubayi AW, Tchankoni MK, Bitty-Anderson AM,* et al. Assessment of self-medication practices in the context of the COVID-19 outbreak in Togo. BMC Public Health, 2021;21(1):1-9. https://doi.org/10.1186/s12889-020-10145-1.
5. *Nasir M, Chowdhury ASMS, Zahan T.* Self-medication during COVID-19 outbreak: a cross sectional online

survey in Dhaka city. Int J Basic Clin Pharmacol. 2020;9(9):1325-1330. https://doi.org/10.18203/2319-2003.ijbcp20203522.
6. *Onchonga D, Omwoyo J, Nyamamba D.* Assessing the prevalence of self-medication among healthcare workers before and during the 2019 SARS-CoV-2 (COVID-19) pandemic in Kenya. Saudi Pharm J. 2020;28(10):1149–54. https://doi.org/10.1016/j.jsps.2020.08.003.
7. *Bardou-Boisnier S, Caillaud K.* Les dispositifs informationnels sur les compléments alimentaires : une affaire de sante publique. Questions de Communication. [Information systems for food supplements: a matter of public health. Communication Issues]. 2015;(27):79-104. https://doi.org/10.4000/questionsdecommunication.9705.
8. *Ronis MJJ, Pedersen KB, Watt J.* Adverse Effects of Nutraceuticals and Dietary Supplements. An Rev Pharmacol Toxicol. 2018;58:583–601. https://doi.org/10.1146/annurev-pharmtox.
9. *Jamal FZ.* La consommation des compléments alimentaires au Maroc (2015). [The consumption of food supplements in Morocco (2015)]. Mohamed V University, Faculty of Medicine and Pharmacy. Doctoral dissertation. 2016.
10. *Dubecq C, Daniel Y, Aigle L, Bigard X.* Utilisation des compléments alimentaires à visée ergogénique chez les militaires français : Prévalence et modes de consommation lors d'une opération extérieure. [Use of ergogenic food supplements in the French military: Prevalence and consumption patterns during an external operation]. Sci Sports 2014;29(4):188–95. https://doi.org/10.1016/j.scispo.2014.06.001.
11. *Lacerda FMM, Carvalho WRG, Hortegal EV, Cabral NAL, Veloso HJF.* Factors associated with dietary supplement use by people who exercise at gyms. Rev Saude Publica. 2015;49(63). https://doi.org/10.1590/S0034-8910.2015049005912
12. *Naqvi AA, Ahmad R, Zehra F, Yousuf R, Kachela B, Nehal Nadir M.* Dietary Supplement Use Among Students of Pharmacy Colleges in the City of Karachi, Pakistan: Prevalence, Opinions, and Attitudes. J Diet Suppl. 2019;16(2):166–78. https://doi.org/10.1080/19390211.2018.1443191.
13. *Pouchieu C, Deschasaux M, Hercberg S, Druesne-Pecollo N, Latino-Martel P, Touvier M,* et al. Comportements alimentaires des patients atteints de cancer ou en rémission dans la cohorte NutriNet-Santé: focus sur la prise de compléments alimentaires. [Dietary behaviours of patients with cancer or in remission in the NutriNet-Santé cohort: focus on dietary supplementation]. In: Colloque Nutrition, microbiote, métabolisme et cancer NACRe. 2015.
14. *Hébuterne X, Gerber M.* La journée de réflexion sur les compléments alimentaires. Nutrition Clinique et Métabolisme. [The Food Supplement Retreat. Clinical Nutrition and Metabolism]. 2009;2(23):47–8. https://doi.org/10.1016/J.NUPAR.2009.04.002.
15. *Tsui B, Dennehy CE, Tsourounis C.* A survey of dietary supplement use during pregnancy at an academic medical center. Am J Obstet Gynecol. 2001;185(2):433–7. https://doi.org/10.1067/mob.2001.116688.
16. *Mestaghanmi H, Labriji A, Kehailou FZ, Sabri A, Barka CA, Bouzoubaa H,* et al. Study of the Association between the Consumption of Dietary Supplements and Lifestyle Factors in a Population of Moroccan Academics during the COVID-19 Health Crisis. OAlib. 2021;8(6):1-30. https://doi.org/10.4236/oalib.1107585.
17. *Nakhal SA, Domiati SA, Amin MEK, El-Lakany AM.* Assessment of pharmacy students' knowledge, attitude, and practice toward herbal dietary supplements. Journal of American College Health. 2022;70(6):1826-1830. https://doi.org/10.1080/07448481.2020.1825226.
18. *Delimaris I.* Adverse effects associated with protein intake above the recommended dietary allowance for adults. ISRN Nutr. 2013;2013:126929. doi: 10.5402/2013/126929
19. *Rutkowski M, Grzegorczyk K.*: Adverse effects of antioxidative vitamins. Int. J. Occup Med Ennviron Health. 2012;25(2):105-12.

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ORIGINAL ARTICLE

SOCIODEMOGRAPHIC, NUTRITIONAL AND HEALTH STATUS FACTORS ASSOCIATED WITH ADHERENCE TO MEDITERRANEAN DIET IN AN AGRICULTURAL MOROCCAN ADULT'S POPULATION

Rachida Moustakim[1], *Mohamed Mziwira*[2], *Mohammed El Ayachi*[1], *Rekia Belahsen*[1]

[1]Laboratory of Biotechnology, Biochemistry & Nutrition, Training and Research Unit on Nutrition & Food Sciences, Chouaib Doukkali University, Faculty of Sciences, El Jadida, Morocco
[2]Laboratory of Bio-Geosciences and Materials Engineering, Higher Normal School of Hassan II University, Casablanca, Morocco

ABSTRACT

Background. Numerous studies have demonstrated beneficial effects of adherence to the Mediterranean diet (MD) on many chronic diseases, including chronic kidney disease (CKD).

Objective. The aim of this study was to assess the adherence of a rural population to the Mediterranean diet, to identify the sociodemographic and lifestyle determinants and to analyze the association between adherence to MD and CKD.

Material and Methods. In a cross-sectional study, data on sociodemographic, lifestyle factors, clinical, biochemical parameters and diet were collected on a sample of 154 subjects. Adherence to MD was assessed according to a simplified MD score based on the daily frequency of intake of eight food groups (vegetables, legumes, fruits, cereal or potatoes, fish, red meat, dairy products and MUFA/SFA), using the sex specific sample medians as cut-offs. A value of 0 or 1 was assigned to consumption of each component according to its presumed detrimental or beneficial effect on health.

Results. According to the simplified MD score, the study data show that high adherence (44.2%) to MD was characterized by intakes high in vegetables, fruits, fish, cereals, olive oil, and low in meat and moderate in dairy. Furthermore, several factors such as age, marital status, education level, and hypertension status were associated with the adherence to MD in the study population. The majority of subjects with CKD have poor adherence to the MD compared to non-CKD with a statistically insignificant difference.

Conclusions. In Morocco, maintaining the traditional MD pattern play crucial role for public health. More research is needed in this area to precisely measure this association.

Key words: *Mediterranean diet, chronic kidney disease, simplified MD score, Morocco*

INTRODUCTION

The Mediterranean diet (MD), the diet of the populations residing along the Mediterranean Basin, is widely considered a healthy dietary model whose adherence presumed to have a beneficial effect on health and nutritional status of the individual [1, 2, 3] as well as on the environment [4]. The traditional Mediterranean diet is characterized by a high intake of vegetables, fruits, legumes, nuts, wholegrain and unrefined cereals, and a high intake of olive oil as the main source of dietary lipids, a moderate intake of fish and seafood, a low to moderate consumption of dairy products, and finally a low intake of red and processed meat [5, 6].

Numerous epidemiological studies have demonstrated the protective effect of Mediterranean diet against many chronic diseases, particularly diabetes mellitus, kidney disease, cardiovascular disease and metabolic syndrome [7, 8, 9, 10, 11, 12, 13]. In addition, several meta-analyses of cohort studies that examined the effect of MD on non-communicable diseases (NCDs) underlined that people with the highest levels of adherence to MD have decreased risk of developing diabetes by a 13–23% and that of developing cardiovascular disease (CVD) by 19–27% [14,15] compared those with low adherence levels.

Chronic kidney disease (CKD) is recognized as a serious public health problem around the world. It is associated with high cardiovascular morbidity and mortality and low quality of life [16, 17, 18, 19]. These metabolic and cardiovascular disease (CVD) factors, include obesity, diabetes, hypertension, and metabolic

Corresponding author: Rekia Belahsen, Laboratory of Biotechnology, Biochemistry & Nutrition, Training and Research Unit on Nutrition & Food Sciences, Chouaib Doukkali University, Faculty of Sciences, El Jadida, Morocco, e-mail: b.rekia@ gmail.com

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syndrome and are in continuous increase worldwide and in Morocco [20, 21, 22]. On the other hand, diet is thought to play a major role in the development of these diseases including CKD risks [23] and the adoption of a healthy diet is reported to protect against all forms of malnutrition and as well as NCDs [24]. One of diets recognized as healthy and of sustainable is Mediterranean diet [24].

The Mediterranean diet is indeed, a traditional reference model of healthy and balanced diet [3, 6, 25]. However, Morocco, like several other developing and Mediterranean countries, is undergoing a demographic, epidemiological and nutritional transition and therefore a deviation from MD model switching to westernized diet [26, 27]. The nutrition transition linked to the profound and rapid changes in eating habits is associated to increased urbanization, improvement of the economy and development of the food industry associated with globalization [28]. Several indexes or scores have been developed to evaluate adherence to MD. Among these, the Mediterranean diet score (MDS) is the most frequently used score to assess the degree of adherence to the traditional Mediterranean diet. It was first defined by *Trichopoulou* et al [3, 29], then expanded and updated later in 2003 with the addition of the fish component [30]. It is also a diet quality score based on heritage recommendations related to the traditional Mediterranean diet. The food components of this score include cereals, vegetables, fruits and nuts, pulses, milk and dairy products, meat and meat products, fish, and the ratio of monounsaturated fats to saturated fats. A high consumption of Mediterranean foods (favourable foods): cereals, legumes, fruits and nuts, vegetables, olive oil and fish was marked by a positive value «1» and a high consumption of non-Mediterranean foods (unfavourable foods): milk and dairy products and meat was marked with a negative or nul value «0». The total score varies therefore, from 0 (minimum adherence to MD) to 8 (maximum adherence to MD) and subjects with a higher score are considered more compliant with the traditional Mediterranean diet [3].

To our knowledge, no study has examined the relationship between compliance with MD and chronic kidney disease. The objective of the present study was therefore, on the one hand to examine the relationship between adherence to MD with sociodemographic and socioeconomic status and other lifestyle parameters, and on the other hand to evaluate the association of MD and CKD membership in a sample of the Moroccan adult population.

MATERIAL AND METHODS

Sample

The current study was carried out between January and December 2017 on a sample of 210 subjects aged 18 years and over, living in the agricultural province of Sidi Bennour in Morocco, and randomly selected from primary health care. The study was supported by the Moroccan ministry of higher education and research and the ministry of health of Morocco. Only people aged 18 years and older, with normal mental health were included. Pregnant women, patients with paralysis and persons with antecedent of kidney disease were excluded from this investigation.

Data collection

A questionnaire was used to collect data on sociodemographic and socioeconomic status (age, sex, marital status, area of residence, profession, monthly income and education level), personal and family health history (hypertension, diabetes and kidney disease) and lifestyle indicators (smoking, alcohol consumption, physical activity) and dietary habits. Blood pressure and anthropometric parameters (weight, height, waist and hip circumferences) were likewise carefully measured. All anthropometric and clinical measurements were performed by the same well-trained nurse in order to reduce subjective errors.

Anthropometric measurement

Weight was measured in light clothing and without shoes to the nearest 0.1 kg on a mechanical scale, and height was recorded to the nearest of 0.1 cm with a stadiometer with the subjects in a standing position, not wearing shoes and with shoulders in normal position. Body Mass Index (BMI) was calculated by dividing weight (kg) by the square of height (m^2), according to the World Health Organization (WHO) criteria, normal weight was defined as $18 \leq BMI < 25$ Kg/m^2, overweight as $25 \leq BMI < 30$ Kg/m^2 and overall obesity was defined as $BMI \geq 30$ Kg/m^2. Waist circumference (WC) in (cm) was measured at midway between the lowest rib and the iliac crest and the hip circumference (HC) at the level of the greater trochanter using a flexible tape and expressed in (cm) and the waist to hip ratio (WHR) was calculated as WC divided by HC. WC is a marker for central obesity and WHR for body fat distribution. According to the NCEP-ATP III reference, WC values larger than 88 cm for females and 102 cm for males are considered to be high and indicate abdominal obesity.

Laboratory measurements

Blood samples were collected by venipuncture after an overnight fast of at least 12 hr and all analyses were made on the day of blood collection. Serum creatinine

was measured according to the standard colorimetric Jaffe-Kinetic reaction method.

Chronic kidney disease

Estimated glomerular filtration rate (eGFR) was calculated using the Modification of diet in renal disease (MDRD) formula as follows [31–33]:

$$eGFR = 186 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African-American})$$

According to the national kidney foundation guidelines, the subjects were classified based on their eGFR levels, as without CKD when eGFR≥60 ml/min/1.73 m² and with CKD if eGFR<60 ml/min/1.73 m².

Mediterranean diet score

The food frequency questionnaire (FFQ) was completed for a sub sample of 154 participants in a face-to-face interview. The MDS is used to assess the degree of adherence to the MD. The calculation of this score was based on the frequency of the daily intake of each food group; the score is made up of eight components or food groups (vegetables, legumes, fruits, cereals, fish, meat and dairy products). To calculate the total frequency of each component, the frequency of the food items that belong to it is added, dairy products (milk, yogurt and cheese), cereals (bread, cereals, potatoes, rice, pasta and couscous) meats (meat red, white meat and processed meat), and the monounsaturated fatty acid to the saturated fatty acid ratio is computed for a fat intake. A value of 0 or 1 was contributed for each of the components with the use of the sex-specific median as the cut-off. For the beneficial components (vegetables, legumes, fruits, cereals, and fish), persons whose consumption was below the median were assigned a value of 0, and those with a consumption at or above the median were assigned a value of 1. For components presumed to be detrimental (meat and dairy products), persons that have a consumption below the median were assigned a value of 1, and those having a consumption at or above the median were assigned a value of 0. Thus, the total simplified MD score ranged from 0 (minimal adherence) to 8 (maximal adherence). This index is then used to classify subjects into two groups according to their adherence to the MD, "low" adherence to the MD (0 to 4 points), and "high" adherence to the MD (5 to 8 points) [26, 34].

Ethical consideration

The authorities were previously informed by the delegation of the Ministry of Health about the realization of the study, its objectives and terms. Also, the procedures and objectives of the study were clearly explained to the participants who provided written informed consent.

Statistical analyses

All calculations were performed using the SPSS statistics program version 24.0. Continuous variables were expressed by mean ± SD and categorical variables were reported by frequency and proportions. Student's t-test was used to compare differences in means and *Chi*-square test was used to compare differences in proportions. Analysis of variance (ANOVA) was used to determine the relationship between MDS categories and quantitative variables. In all statistical tests, p-value less than 0.05 is considered statistically significant.

RESULTS

Table 1 shows the mean values of the MDS according to the participants' characteristics. The mean MDS was 4.44 ± 1.56 with an interval of 1 to 8. The comparison of the mean MDS values according to the gender, area of residence, SES, anthropometrical status and prevalence of CKD did not show any significant difference. On the other hand, the comparison of this score according to the age groups, level of education and the incidence of hypertension indicated statistically significant differences.

Table 2 shows the association between all the sociodemographic and clinical characteristics of the population with the degree of adherence to Mediterranean diet. The table shows no significant difference in the degree of adherence to the MD between categories of gender, area of residence, SES, anthropometrical status, and prevalence of CKD. On the other hand, this adherence was significantly associated with age, marital status, level of education and the incidence of hypertension, and a negative correlation between MDS and hypertension has been noted (r =-0.268; p=0.001).

The consumption of food groups by gender is described in Table 3. Our results showed that the differences of medians of food groups consumption between the two gender were statistically significant except for meats and the MUFA/SFA fat ratio which were more frequently consumed by women. Otherwise, in our population 44.2% had high MDS while 55.8% had low MDS. The prevalence of adherence to the MD is relatively higher in men compared to women 47.7% vs. 42.7%.

Food groups' consumption according to the categories of adherence to the Mediterranean Diet is shown in the Table 4. According to table results, high adherence to the MD was characterized by high intakes of vegetables, fruits, legumes, fish, cereals, olive oil, and low meat and dairy consumption, whereas, a low

Table 1. Comparison of MDS values according to the study participants' characteristics

Characteristics	n (%)	MDS (mean ± SD)	P-value
Gender			
Male	44 (28.6)	4.43 ± 1.45	0.987
Female	110 (71.4)	4.44 ± 1.61	
Age categories (years)			
[18-29]	10 (6.5)	4.40 ± 1.83	**0.011**
[30-59]	86 (55.8)	4.76 ± 1.45	
≥60	58 (37.7)	3.97 ± 1.58	
Area of residence			
Urban	47 (30.5)	4.49 ± 1.33	0.777
Rural	107 (69.5)	4.41 ± 1.66	
Socio economic status			
Low	91 (59.1)	4.43 ± 1.52	0.777
Medium	45 (29.2)	4.36 ± 1.59	
High	18 (11.7)	4.67 ± 1.78	
Level of education			
Never attended	118 (76.6)	4.26 ± 1.53	**0.013**
Attended School	36 (23.4)	5.00 ± 1.54	
BMI categories			
Underweight	3 (1.9)	3.00 ± 1.00	0.298
Normal weight	39 (25.3)	4.26 ± 1.71	
Overweight	52 (33.8)	4.46 ± 1.48	
Obesity	60 (39.0)	4.60 ± 1.56	
Chronic kidney disease			
With CKD	6 (4.0)	3.67 ± 1.63	0.211
Without CKD	143 (96.0)	4.49 ± 1.56	
HTA			
Yes	48 (31.2)	3.81 ± 1.62	**0.001**
No	106 (68.8)	4.72 ± 1.46	

HTA: hypertension; MDS: Mediterranean diet score; CKD: Chronic kidney disease
The t test and 1-way ANOVA were used to compare the means of the MDS score according to the classes of the different characteristics studied.

Table 2. Characteristics of the population according to the degree of adherence to the Mediterranean diet

Charactetristics	Low MDS (1-4) n=86 (55.8%)	High MDS (5-8) n=68 (44.2%)	p-value
Gender			
Male	23 (52.3)	21 (47.7)	0.572
Female	63 (57.3)	47 (42.7)	
Age groups (years)			
18-29	6 (60.0)	4 (40.0)	**0.028**
30-59	40 (46.5)	46 (53.5)	
≥60	40 (69)	18 (31.0)	
Marital status			
Married	51 (51.0)	49 (49.0)	**0.099**
Not married	35 (64.8)	19 (35.2)	
Area of residence			
Urban	28 (59.6)	19 (40.4)	0.537
Rural	58 (54.2)	49 (45.8)	
SES			
Low	52 (57.1)	39 (42.9)	0.915
Medium	24 (53.3)	21 (46.7)	
High	10 (55.6)	8 (44.4)	
Education attainment			
Never attended	72 (61.0)	46 (39.0)	**0.019**
Attended school	14 (38.9)	22 (61.1)	

BMI categories			
Underweight	3 (100)	0 (0.0)	
Normal weight	22 (56.4)	17 (43.6)	0.470
Overweight	29 (55.8)	23 (44.2)	
Obesity	32 (53.3)	28 (46.7)	
CKD			
Yes	5 (83.3)	1 (16.7)	0.164
No	78 (54.5)	65 (45.5)	
HTA			
Yes	34 (70.8)	14 (29.2)	**0.012**
No	52 (49.1)	54 (50.9)	

The results are presented as n (%).
Chi^2 test was used to compare the distribution of the degree of adherence to the Mediterranean diet according to the different characteristics studied.

Table 3. Distribution of the daily consumption of the different food groups according to the degree of MD adherence (MDS)

Food variables	Total	Men n=44		p-value	Total	Women n=110		p-value
		Low MDS n=23 (52.3)	High MDS n=21 (47.7)			Low MDS n=63 (57.3)	High MDS n=47 (42.7)	
Vegetables								
Median	10.11			**0.007**	10.01			**0.000**
≥ Median		7 (30.4)	15 (71.4)			16 (25.4)	39 (83.0)	
< Median		16 (69.6)	6 (28.6)			47 (74.6)	8 (17.0)	
Legumes								
Median	0.31			**0.000**	0.17			**0.000**
≥ Median		5 (21.7)	18 (85.7)			18 (28.6)	41 (87.2)	
< Median		18 (78.3)	3 (14.3)			45 (71.4)	6 (12.8)	
Fruits								
Median	5.35			**0.007**	4.84			**0.000**
≥ Median		7 (30.4)	15 (71.4)			15 (23.8)	40 (85.1)	
< Median		16 (69.6)	6 (28.6)			48 (76.2)	7 (14.9)	
Fish								
Median	0.19			**0.000**	0.17			**0.000**
≥ Median		5 (21.7)	18 (85.7)			23 (36.5)	37 (78.7)	
< Median		18 (78.3)	3 (14.3)			40 (63.5)	10 (21.3)	
Cereals								
Median	4.59			**0.007**	4.40			**0.000**
≥ Median		7 (30.4)	15 (71.4)			21 (33.3)	35 (74.5)	
< Median		16 (69.6)	6 (28.6)			42 (66.7)	12 (25.5)	
MUFA/SFA								
Median	1.44			0.500	1.59			0.112
≥ Median		12 (52.2)	10 (47.6)			29 (46.0)	28 (59.6)	
< Median		11 (47.8)	11 (52.4)			34 (54.0)	19 (40.4)	
Meat								
Median	1.31			0.613	1.17			0.310
≥ Median		11 (47.8)	10 (47.6)			28 (44.4)	24 (51.1)	
< Median		12 (52.2)	11 (52.4)			35 (55.6)	23 (48.9)	
Dairy products								
Median	0.62			0.273	0.70			0.161
≥ Median		13 (56.5)	22 (50.0)			34 (54.0)	20 (42.6)	
< Median		10 (43.5)	22 (50.0)			29 (46.0)	27 (57.4)	

MDS: Mediterranean diet score; MUFA/SFA: Mono Unsaturated Fatty Acid/Saturated Fatty Acid ratio. The results are presented as n (%).
The *Chi*^{2} test was used to compare the distribution of the daily consumption of different food groups according to the degree of adherence to MD for both sexes.

Table 4. Food groups'consumption according to the categories of adherence to the MD

Adherence to MD	Low adherence n=86 (55.8%)	High adherence n=68 (44.2%)	p-value
Food groups			
Vegetables	8.75 (6.94 – 10.26)	11.94 (10.44 – 14.65)	0.000
Meat	1.23 (1.00 – 1.45)	1.17 (0.98 – 1.87)	0.484
Legumes	0.10 (0.00 – 0.18)	0.35 (0.20 – 0.53)	0.000
Fish	0.12 (0.06 – 0.20)	0.31 (0.20 – 0.53)	0.000
Cereals	4.01 (3.47 – 4.62)	4.97 (4.46 – 5.62)	0.000
Milk and dairy products	0.62 (0.23 – 1.24)	0.99 (0.39 – 2.19)	0.004
Fruits	3.60 (2.70 – 5.35)	6.71 (5.38 – 9.24)	0.000
MUFA/SFA	1.47 (1.05 – 1.84)	1.63 (1.25 – 2.16)	0.095

MD: Mediterranean diet; MUFA/SFA: Mono Unsaturated Fatty Acid/Saturated Fatty Acid ratio. The results are presented as the median (percentiles).
The *Mann-Whitney* test was used to compare the median daily consumption frequencies of the different food groups according to the degree of adherence to the Mediterranean diet.

adherence to MD was characterized by low intakes of vegetables, fruits, legumes, fish, cereals, olive oil, and high meat and dairy consumption. There were significant differences in the consumption of each food group according to the categories of adherence to Mediterranean diet ($P<0.05$), except for meats food group and the MUFA/SFA fat ratio.

DISCUSSION

The diet in Morocco is of the Mediterranean type. This diet has demonstrated a protective effect against the incidence of cardiovascular diseases as well as other chronic diseases such as diabetes, obesity, cancer or other metabolic disorders by numerous studies [7, 8, 14, 34–37]. Adherence to this diet has also been shown to play a key role in preventing several morbid conditions related to NCDs and cognitive health [7, 22, 37, 38–41]. However, the composition of the traditional Moroccan Mediterranean diet has actually undergone some variations over time, linked to a «westernization» of eating habits in addition to a sedentary lifestyle [26]. Few studies reported about the factors of adherence to the MD in the south of mediterranean basin populations. In Morocco, except the study by *El Rhazi* et al. [34], the present study revealed an association between the degree of adherence to the Mediterranean diet with some sociodemographic factors, such as age, the level of education and the marital status in Moroccan population. Indeed, a low adherence to MD was observed much more in the elderly individuals with a high level of education, and in no married persons. This can be explained by the fact that single person and those with a good level of education are more likely to adopt Western dietary patterns characterized by ready to take away and ready-to-eat foods. It reveals also the lack of nutritional education in the population. Whilst contradictory to observations of *Mohtadi* et al. and that of *O'Connor* et al [26, 37], this explanation is in line with that of other studies [34, 42, 43]. This highlights also the influence of the family potential on eating behavior, which is characterized by the preparation and sharing of cooked meals between different family members. However, no significant difference was recorded in this study concerning the other sociodemographic characteristics, namely: marital status, area of residence and socioeconomic status.

On the other hand, adherence to the Mediterranean diet is considered a protective factor against the risk of incidence of chronic kidney disease according to the literature data [7]. In the present study, The majority of subjects with CKD have poor adherence to the MD compared to non-CKD with a statistically insignificant difference ($p>0.05$). However, the association of MD adherence scores with hypertension, considered a cardiovascular risk factor of CKD in the study population, revealed a statistically significant difference. This result demonstrating that hypertension is inevitably associated with low adherence to the Mediterranean diet, is in agreement with that found by many other studies conducted on the Greek population [30, 44, 45]. Moreover, even if the association between CKD and the MD has not yet been extensively studied and more studies are required in this field, it is demonstrated that the high fiber content in MD improve GFR levels by decreasing nephron workload. Also, the antioxidant components of MD protect kidney function by improving endothelial function and protecting against major risk factors for CKD such as obesity and diabetes mellitus [7].

The findings showed that adherence to MD in the study population, was characterized by high intake of cereals, vegetables, fruits, legumes and fish, moderate

intake of milk and dairy products and low intake of meat. Other Moroccan studies have demonstrated similar results [26, 34]. Comparing the different food groups consumption according to gender and category of adherence to the MD, the results reported here, showed that men were more compliant than women with the MD, this finding was comparable to that obtained by other studies fulfilled in Morocco and Spain [26,42]. However, this finding is different from that obtained by the study of *El Rhazi* et al [34]. Also, in accordance with other studies carried out before on samples of the Moroccan and Italian populations, the analysis of the obtained results showed that there is no statistically significant association between the MDS and the anthropometrical status [26, 34, 46]. Furthermore, regarding the socio with economic status and even if not statistically significant, the present results showed that people belonging to households with a medium and a high socioeconomic status have relatively higher adherence to MD compared to those from disadvantaged households with a low socio-economic status. This result is similar to that found by *El Rhazi* et al and *Tong* et al [34, 47].

This current study has few limitations. Firstly, the dietary data was based on FFQ. Food consumption was not assessed with high accuracy and people might have overestimated the consumption of healthy nutrients and food items like vegetables, fruits or cereals, typical components of the MD and underestimated the consumption of unhealthy nutrients and food items like red meat and some fats. Secondly, like all other cross-sectional studies, we could not determine the causal-effect relationship between adherence to the MD and CKD. Despite these limitations, this seems to be the first study to evaluate the link between adherence to the MD and CKD among this Morroccan population.

CONCLUSIONS

The present study has focused on the Mediterranean diet which constitutes a culinary heritage and original diet of Morocco and other Mediterranean countries ; the diet tends to be abandoned in favor of a modern diet rich in saturated fats, salt and sugar. The study data found that high adherence to MD was characterized by high intakes of vegetables, fruits, fish, cereals, olive oil, low meats, and moderate milk and dairy products according to the used simplified MD score. Higher adherence to the MD in the surveyed adult sample was associated with several factors such as age, education level, and hypertension status. Finaly, although no significant difference was observed in the relationship between MD and the occurrence of CKD, maintaining the traditional MD model plays an essential role for public health and therefore, further research is needed to accurately measure and assess these associations.

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Conflict of interest

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REFERENCES

1. *Benhammou S, Heras-González L, Ibáñez-Peinado D, Barceló C, Hamdan M, Rivas A*, et al. Comparison of Mediterranean diet compliance between European and non-European populations in the Mediterranean basin. Appetite. 2016;107:521-6.
2. *Meybeck A, Redfern S, Hachem F, Capone R, Dernini S*, Food and Agriculture Organization of the United Nations, et al., éditeurs. Development of voluntary guidelines for the sustainability of the Mediterranean diet in the Mediterranean region: proceedings of a technical workshop, 14-15 March 2017, CIHEAM-Bari, Valenzano (Bari). 2017.
3. *Trichopoulou A, Martínez-González MA, Tong TY, Forouhi NG, Khandelwal S, Prabhakaran D*, et al. Definitions and potential health benefits of the Mediterranean diet: views from experts around the world. BMC Med. 2014;12(1):112.
4. *Serra-Majem L, Tomaino L, Dernini S, Berry EM, Lairon D, Ngo de la Cruz J*, et al. Updating the Mediterranean Diet Pyramid towards Sustainability: Focus on Environmental Concerns. Int J Environ Res Public Health. 2020;17(23):8758.
5. *Meybeck A, Suzanne R, Sandro Dernini, Roberto Capone, Fatima Hachem,*. Development of voluntary guidelines for the sustainability of the Mediterranean diet in the Mediterranean region. 2017; Publisher: FAO and CIHEAMISBN: 978-92-5-109832-5.
6. *Trichopoulou A, Costacou T, Bamia C, Trichopoulos D.* Adherence to a Mediterranean Diet and Survival in a Greek Population. N Engl J Med. 2003;348(26):2599-608.
7. *Chauveau P, Aparicio M, Bellizzi V, Campbell K, Hong X, Johansson L*, et al. Mediterranean diet as the diet of choice for patients with chronic kidney disease. Nephrol Dial Transplant. 2018;33(5):725-35.
8. *Grosso G, Mistretta A, Marventano S, Purrello A, Vitaglione P, Calabrese G*, et al. Beneficial Effects of the Mediterranean Diet on Metabolic Syndrome. Curr Pharm Des. 2014;20(31):5039-44.

9. *Hariharan D, Vellanki K, Kramer H*. The Western Diet and Chronic Kidney Disease. Curr Hypertens Rep. 2015;17(3):16.
10. *Kiortsis DN, Simos YV*. Mediterranean Diet for the Prevention and Treatment of Metabolic Syndrome: Is it Worth It? Angiology. 2014;65(1):5-8.
11. *Martínez-González MÁ, Fuente-Arrillaga C de la, Nunez-Cordoba JM, Basterra-Gortari FJ, Beunza JJ, Vazquez Z,* et al. Adherence to Mediterranean diet and risk of developing diabetes: prospective cohort study. BMJ. 2008;336(7657):1348-51.
12. *Moustakim R, Mziwira M, El Ayachi M, Belahsen R*. Association of Metabolic Syndrome and Chronic Kidney Disease in Moroccan Adult Population. Metab Syndr Relat Disord. 2021;19(8):460-8.
13. *Zappalá G, Platania A, Bellia MA, Ragusa R, Marranzano M*. Eating habits and food intake in relation to adherence to the mediterranean diet, in adults living in the Island of Sicily. Mediterr J Nutr Metab. 2019;12(3):271-81.
14. *Galbete C, Schwingshackl L, Schwedhelm C, Boeing H, Schulze MB*. Evaluating Mediterranean diet and risk of chronic disease in cohort studies: an umbrella review of meta-analyses. Eur J Epidemiol. 2018;33(10):909-31.
15. *Aridi YS, Walker JL, Roura E, Wright ORL*. Adherence to the Mediterranean Diet and Chronic Disease in Australia: National Nutrition and Physical Activity Survey Analysis. Nutrients. 2020;12(5):1251.
16. *Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B*, et al. Chronic kidney disease: global dimension and perspectives. The Lancet. 2013;382(9888):260-72.
17. *Moustakim R, Mziwira M, El Aaychi M, Belahsen R*. Dietary diversity score and the incidence of chronic kidney disease in an agricultural Moroccan adults population. Rocz Państw Zakładu Hig 2022;73(3):293-301. doi: 10.32394/rpzh.2022.0221.27
18. *Moustakim R, El Ayachi M, Mziwira M, Belahsen R*. Undiagnosed chronic kidney disease and its associated risk factors in an agricultural Moroccan adult's population. Néphrologie Thérapeutique. 2020;16(3):147-52.
19. *Moustakim R., Mziwira M., El Ayachi M., Belahsen R.*. Assessment of nutritional status, dietary intake and adherence to dietary recommendations in hemodialysis patients. GSC Adv Res Rev. 2020;3(2):009-19.
20. *Belahsen R. R, Bermudez O.I. OI, Mohamed M. M, Fatima F. F, Newby P.K. PK, Tucker L.K. KL*. Obesity and related metabolic disorders are prevalent in Moroccan women of childbearing age. Int J Diabetes Metab. 2005;13(3):159-66. DOI: https://doi.org/10.1159/000497585
21. *Grundy SM*. Metabolic Syndrome Pandemic. Arterioscler Thromb Vasc Biol. 2008;28(4):629-36.
22. *Mziwira M, El Ayachi M, Lairon D, Belahsen R*. Mediterranean Diet and Metabolic Syndrome in Adult Moroccan Women. J Res Obes. 2015;1-18.
23. *Anderson CAM, Nguyen HA, Rifkin DE*. Nutrition Interventions in Chronic Kidney Disease. Med Clin North Am. 2016;100(6):1265-83.
24. FAO and WHO. Sustainable healthy diets. 2019.
25. *D'Alessandro A, De Pergola G*. The Mediterranean Diet: its definition and evaluation of *a priori* dietary indexes in primary cardiovascular prevention. Int J Food Sci Nutr. 2018;69(6):647-59.
26. *Mohtadi K, Msaad R, Benalioua N, Jafri A, Meftah H, Elkardi Y*, et al. Sociodemographic and Lifestyle Factors Associated with Adherence to Mediterranean Diet in Representative Adult Population in Casablanca City, Morocco: A Cross-Sectional Study. J Nutr Metab. 2020;2020:1-9.
27. *Trichopoulou A, Lagiou P*. Healthy Traditional Mediterranean Diet: An Expression of Culture, History, and Lifestyle. Nutr Rev. 1997;55(11):383-9.
28. *Belahsen R*. Nutrition transition and food sustainability. Proc Nutr Soc. 2014;73(3):385-8.
29. *Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E*, et al. Diet and overall survival in elderly people. BMJ. 1995;311(7018):1457-60.
30. *Trichopoulou A, Costacou T, Bamia C, Trichopoulos D.* Adherence to a Mediterranean Diet and Survival in a Greek Population. N Engl J Med. 2003;348(26):2599-608.
31. *Farhadnejad H, Asghari G, Mirmiran P, Yuzbashian E, Azizi F*. Micronutrient Intakes and Incidence of Chronic Kidney Disease in Adults: Tehran Lipid and Glucose Study. Nutrients. 2016;8(4):217.
32. *Levey AS, Coresh J*. Chronic kidney disease. The Lancet. 2012;379(9811):165-80.
33. *Vinhas J, Gardete-Correia L, Boavida JM, Raposo JF, Mesquita A, Fona MC*, et al. Prevalence of Chronic Kidney Disease and Associated Risk Factors, and Risk of End-Stage Renal Disease: Data from the PREVADIAB Study. Nephron Clin Pract. 2011;119(1):c35-40.
34. *El Rhazi K, Nejjari C, Romaguera D, Feart C, Obtel M, Zidouh A*, et al. Adherence to a Mediterranean diet in Morocco and its correlates: cross-sectional analysis of a sample of the adult Moroccan population. BMC Public Health. 2012;12(1):345.
35. *Godos J, Rapisarda G, Marventano S, Galvano F, Mistretta A, Grosso G*. Association between polyphenol intake and adherence to the Mediterranean diet in Sicily, southern Italy. NFS J. 2017;8:1-7.
36. *Martínez-González MA, Gea A, Ruiz-Canela M*. The Mediterranean Diet and Cardiovascular Health: A Critical Review. Circ Res. 2019;124(5):779-98.
37. *O'Connor LE, Hu EA, Steffen LM, Selvin E, Rebholz CM*. Adherence to a Mediterranean-style eating pattern and risk of diabetes in a U.S. prospective cohort study. Nutr Diabetes. 2020;10(1):8.
38. *Davis C, Bryan J, Hodgson J, Murphy K*. Definition of the Mediterranean Diet; A Literature Review. Nutrients. 2015;7(11):9139-53.
39. *Murphy KJ, Parletta N*. Implementing a Mediterranean-Style Diet Outside the Mediterranean Region. Curr Atheroscler Rep. 2018;20(6):28.
40. *Nissensohn M, Román-Viñas B, Sánchez-Villegas A, Piscopo S, Serra-Majem L*. The Effect of the Mediterranean Diet on Hypertension: A Systematic

Review and Meta-Analysis. J Nutr Educ Behav. 2016;48(1):42-53.e1.
41. *Sofi F, Macchi C, Abbate R, Gensini GF, Casini A*. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. Public Health Nutr. 2014;17(12):2769-82.
42. *Hu EA, Toledo E, Diez-Espino J, Estruch R, Corella D, Salas-Salvado J*, et al. Lifestyles and Risk Factors Associated with Adherence to the Mediterranean Diet: A Baseline Assessment of the Predimed Trial. Ruiz JR, éditeur. PLoS ONE. 2013;8(4):e60166.
43. *Papadaki A, Wood L, Sebire SJ, Jago R.* Adherence to the Mediterranean diet among employees in South West England: Formative research to inform a web-based, work-place nutrition intervention. Prev Med Rep. 2015;2:223-8.
44. *Gouveri E, Diamantopoulos EJ.* Chapter 29 - The Mediterranean Diet and Metabolic Syndrome. Mediterr Diet. 2015;313-23.
45. *Tzima N, Pitsavos C, Panagiotakos DB, Skoumas J, Zampelas A, Chrysohoou C*, et al. Mediterranean diet and insulin sensitivity, lipid profile and blood pressure levels, in overweight and obese people; The Attica study. Lipids Health Dis. 2007;7.
46. *Bonaccorsi G, Lorini C, Santomauro F, Sofi F, Vannetti F, Pasquini G,* et al. Adherence to Mediterranean diet and nutritional status in a sample of nonagenarians. Exp Gerontol. 2018;103:57-62.
47. *Tong TYN, Imamura F, Monsivais P, Brage S, Griffin SJ, Wareham NJ,* et al. Dietary cost associated with adherence to the Mediterranean diet, and its variation by socio-economic factors in the UK Fenland Study. Br J Nutr. 2018;119(6):685-94.

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ORIGINAL ARTICLE

FACTORS INFLUENCING COOKING METHOD, FREQUENCY, AND DURATION OF MEAL PREPARATION IN MOROCCAN HOUSEHOLDS

Imane Barakat[1,2], *Mohammed Elayachi*[1], *Rekia Belahsen*[1]

[1]Laboratory of Biotechnology, Biochemistry & Nutrition, Training and Research Unit on Nutrition & Food Sciences, Chouaib Doukkali University, Faculty of Sciences, El Jadida, Morocco
[2]Higher Institute of Nursing Professions and Health Techniques of Marrakech, Morocco

ABSTRACT

Background. The construction of the consumer's identity is dependent on how they prepare their meals.
Objective. Study the cooking methods, frequency, and duration of meal preparation in Moroccan households as well as the associated factors.
Materials and methods. This work is a part of a study with a validated conceptual and methodological framework that was conducted in 507 households in the region of Rabat-Salé-Kenitra in Morocco. The characteristics of the population and data on the cooking methods, frequency, and duration of meal preparation were collected by a survey. Associations between variables were studied by univariate logistic regression with a significance level of $p<0.05$.
Results. The majority of the population was aged between 35 and 65 years (76%) and lived in urban areas (70%). The univariate analysis showed that the urban area was a factor that hindered stewing ($p=0.009$), while the work status ($p=0.04$) and the marital status "Married" ($p=0.04$) were favorable factors; the household size ($p=0.02$) is a factor favoring steaming method; urban area ($p=0.04$), work status (p 0.03), nuclear family type ($p<0.001$), and household size ($p=0.02$) are factors hindering the use of oven cooking; urban area ($p=0.02$) and higher education level ($p=0.04$) are factors favoring the use of fried food, age category [20-34] years ($p=0.04$), higher education level ($p=0.01$) and work status ($p=0.01$) were factors that favored the use of grilling; nuclear family type, ($p=0.04$) and household size ($p=0.03$) were factors that hindered the preparation of breakfast; urban area ($p=0.03$) and Arab ethnicity ($p=0.04$) are factors hindering snack preparation; urban area ($p<0.001$) is a factor favoring dinner preparation; household size ($p=0.01$) and use of stewing at least four times a week ($p=0.002$) are factors hindering meal preparation time, while use of baking ($p=0.01$) is a favoring factor.
Conclusion. The study results point towards the implementation of a nutritional education strategy based on combining habits, preferences, and good cooking practices.

Key words: *cooking methods, frequency of meal preparation, duration of meal preparation, Morocco*

INTRODUCTION

The culinary environment, a phenomenon even necessary for human evolution, is a factor in the construction of consumer identity [1]. The change of this environment by different factors and to different degrees shapes the culinary pattern of traditional populations. These factors specific to a population can in turn impact its state of health. This is the case of the traditional Mediterranean food model, the evolution of which has led to the emergence of diseases that have hitherto characterized modern societies. The abandonment as well as the beneficial effects of traditional food models of populations health and the environment are impacted by changes in the food system and the acceleration of the pace of life [2]. The culinary environment encompasses, among other things, cooking methods, the frequency and duration of meal preparation.

Cooking is, technically a process consisting in irreversible transformation of raw food to their cooked shape [3], allowing the digestibility of food nutrients such as starch, plant proteins, collagen in meat, and cellulose and woody structures in plants and leading at the same time to a composition different from that of the initial food items and thus promoting the diversification of available foods [4, 5, 6]. Cooking uses several traditional or modern methods, that may improve or alter the food quality and respectively be beneficial or detrimental to the food and therefore

Corresponding author: Rekia Belahsen, Laboratory of Biotechnology, Biochemistry and Nutrition, Faculty of Sciences, Chouaib Doukkali University, El Jadida – 4000, Morocco, e-mail: b.rekia@gmail.com

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to health [7, 8]. Among these methods, one can cite cooking in water, in a pressure cooker, steaming, baking, grilling, and frying [9, 10].

In addition, the frequent home meal preparation promotes appropriate dietary intake and weight status [11] with long-term health benefits [12]. Indeed, a high frequency of home meal preparation has already been reported to be associated with higher intakes of vegetables, fruits, dietary fiber, folic acid and vitamins, and low-fat intake [13]. This is also a characteristic demonstrated in populations that are still adhering to traditional healthy diets such as Mediterranean dietary model [2]. Furthermore, scientific evidence has shown that meals require different amounts of time to be prepared, depending on the cooking method and the number of guests, and that spending more time preparing meals is associated with better nutritional quality [14, 15]. It is the case of a study on middle-aged women showing that spending more time on meal preparation had a lesser cardiovascular and metabolic risk profile [16].

The current work aimed, therefore to study the cooking methods, frequency, and duration of meal preparation and the associated factors. As such, it has a theoretical scope, as it contributes to the construction of a culinary identity for the Moroccan population; and an empirical scope, as it opens up perspectives for both quantitative and qualitative research on meal preparation methods.

MATERIAL AND METHODS

This work is a part of a study with a validated conceptual and methodological framework [17] that involved 507 households in the region of Rabat, Salé, Kenitra (RSK) in Morocco.

Data Collection

One member per household with a primary role in meal preparation was included in this study as a household representative (HR). Thus, data were collected from 507 HR through a survey on socio-demographic characteristics, cooking methods used, the frequency, and the duration of (time spent on) meal preparation within the households.

Variables of the study

(1) Socio-demographic characteristics: age, area of residence, education level, work status, marital status, family type (nuclear or extended), household size, and ethnicity.

(2) Frequency of use of cooking methods, including stewing, steaming, baking, frying, and grilling. This frequency is categorized as 1) <4 times per week; 2) ≥4 times per week.

(3) The frequency of preparation of meals and snacks is categorized as 1) <5 times per week; 2) ≥5 times per week.

(4) Daily preparation time for all meals and snacks is categorized as 1) <3 hours per day, 2) ≥3 hours per day; breakfast and snack preparation time is categorized as 1) <30 min, 2) ≥30 min; lunch preparation time is categorized as 1) <2 hours, 2) ≥2 hours; and dinner preparation time is categorized as 1) <1 hour, 2) ≥1 hour.

Statistical analysis

Statistical analysis was performed by SPSS software for Windows (Statistical Package for the Social Sciences) version 21 and Microsoft Office Excel 2007. The univariate analysis was used to investigate the factors associated with the type of cooking methods, frequency, and duration of meal preparation with a statistical significance level of $p<0.05$.

RESULTS

Characteristics of the population

Socio-demographic characteristics

Table 1 shows that 70% of the households are urban, 51.5% are composed of 5 to 12 members, 62% of the families are nuclear, 76% of the HR are aged between 35 and 65 years, 13% have a higher education, 76% are inactive, 80% are married, and 80% are of Arab ethnic origin.

Frequency of use of different cooking methods

The households surveyed use stewing (88%), frying (49%), steaming (41%), baking (27%), and grilling (18%) at least 4 times per week (Figure 1).

Frequency of preparing meals and snacks

The majority of surveyed households prepare breakfast (78%), lunch (84%), and afternoon snacks (87%) at least five times per week, while just 45% prepare dinner at this frequency (Figure 2).

Duration of meal preparation

72% of households spend a minimum of three hours per day in preparing meals. Figure 4 shows that 64% of the households surveyed spent less than 30 minutes preparing breakfast, 78% spent two hours or more preparing lunch, 57% spent less than 30 minutes preparing snacks, and 62% spent less than one hour preparing dinner (Figure 3).

Factors associated with meal preparation

Factors associated with the cooking method used

Table 2 shows that based on univariate analysis, urban area (OR=0.4; CI [0.18-0.7], p=0.009) is a factor

Table 1. Characteristics of households and their representatives (n=507)

Characteristics *	Values (%)	CI 95%
Age groups (years)		
[20-34]	122 (24)	[20.1-27.8]
[35-65]	385 (76)	[72.2-79.9]
Area of residence		
Urban	355 (70)	[66-74]
Rural	152 (30)	[26-33]
High education level		
Yes	67 (13)	[10.7-16.4]
No	440 (87)	[83.6-89.3]
Professional occupation		
Active	122 (24)	[20.3-27.6]
Inactive	385 (76)	[72.4-79.7]
Marital status ‚married'		
Yes	405 (80)	[76.6-83]
No	102 (20)	[17-23.7]
Family type		
Nuclear	316 (62)	[58-67]
Composed	191 (38)	[33-42]
Household size		
2-4 members	246 (48.5)	[43.8-53.1]
5-12 members	261 (51.5)	[46.9-56.2]
Ethnic origin		
Arabic	403 (80)	[76-83]
Berber	104 (20)	[17-24]

* = Expressed in size (%); CI = Confidence interval

hindering stewing at least four times a week, while work status (OR=2.2; CI [1.02-4.8]; p=0.04) and the Marital status "Married" (OR=1.8; CI [1.02-3.4]; p=0.04) are factors promoting it. The same table also shows that a household size of 2 to 4 members (OR=1.5; CI [1.05-2.1]; p=0.02) is a factor favoring steaming at least four times a week. In addition, the urban area (OR=0.6; CI [0.43-0.9]; p=0.04), work status (OR=0.5; CI [0.43-0.9]; p=0.03), nuclear family type (OR=0.3; CI [0. 21-0.5]; p<0.001), and household size of 2-4 members (OR=0.6; CI [0.42-0.9], p=0.02) are factors hindering the use of baking at least four times a week. Further, urban area (OR=1.6; CI [1.43-1.93], p=0.02) and higher education level (OR=1.6; CI [1.1-2.8]; p=0.04) are factors favoring the use of fried food at least four times a week. Finally, the age category of [20-34] years (OR=1.6; CI [1-2.7]; p=0.04), higher education level (OR=2.1; CI [1.2-3.75]; p=0.01) and work status (OR=1.8; CI [1.1-3.01], p=0.01) were factors that favored the use of fried food at least four times a week.

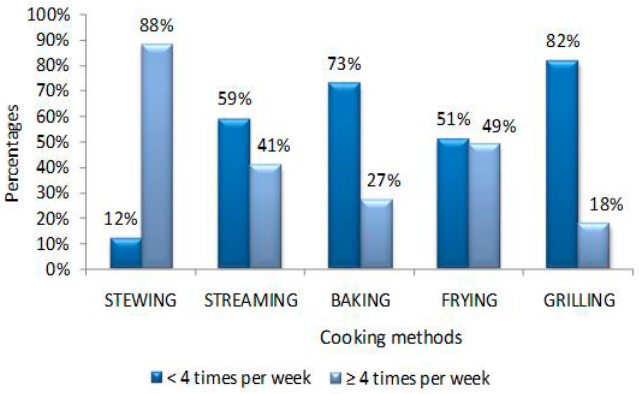

Figure 1. Distribution of households by frequency of use of cooking methods (n=507)

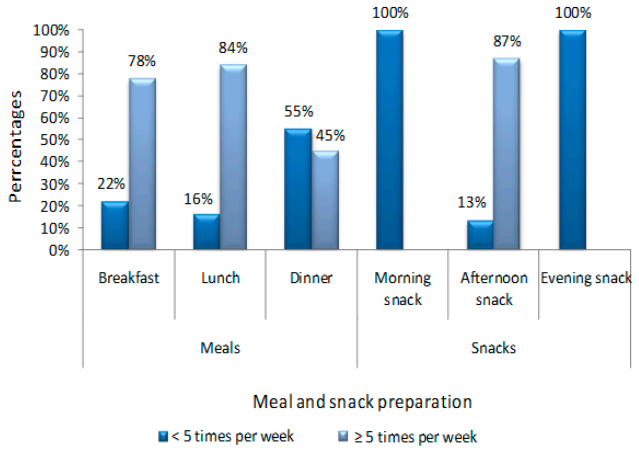

Figure 2. Distribution of households by frequency of meal and snack preparation (n=507)

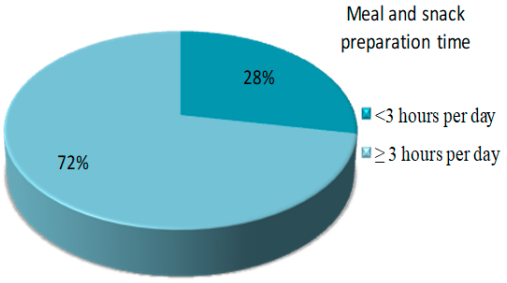

Figure 3. Distribution of households according to daily meal and snack preparation time (n=507)

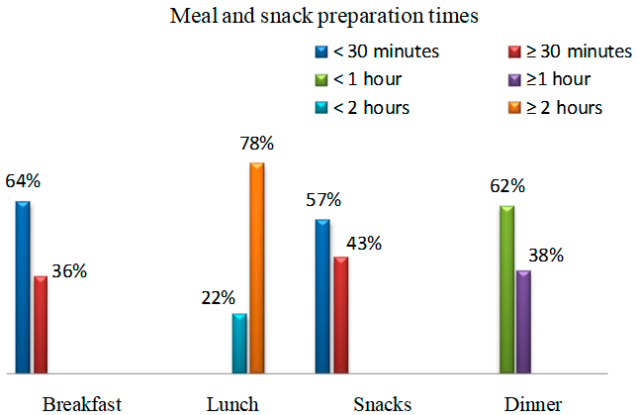

Figure 4. Distribution of households by meal preparation time (n=507)

Table 2. Factors associated with the use of cooking methods ≥4 times per week (n=507)

Characteristics	Stew			Steaming			Oven			Frying			Grilling		
	OR	IC (95%)	P*	OR	IC (95%)	P*	OR	IC (95%)	P*	OR	IC (95%)	P*	OR	IC (95%)	P*
Age groups (years)															
20-34	0.7	[0.39-1.3]	0.2 NS	1.008	[0.66-1.5]	0.9 NS	0.6	[0.42-1.1]	0.1 NS	0.4	[0.5-1.09]	0.1 NS	1.6	[1-2.7]	**0.04****
35-65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Area of residence															
Urban	0.4	[0.18-0.7]	0.009**	0.9	[0.65-1.4]	0.8 NS	0.6	[0.43-0.9]	0.04**	1.6	[1.43-1.93]	0.02**	1.4	[0.8-2.34]	0.2 NS
Rural	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High education level															
Yes	2.3	[0.8-6.5]	0.1 NS	1.1	[0.66-1.8]	0.6 NS	0.6	[0.31-1.2]	0.1 NS	1.6	[1.1-2.8]	0.04**	2.1	[1.2-3.75]	0.01**
No	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Professional occupation															
Active	2.2	[1.02-4.8]	0.04**	1.2	[0.8-1.8]	0.3 NS	0.5	[0.35-0.9]	0.03**	1.3	[0.9-1.9]	0.2 NS	1.8	[1.1-3.01]	0.01**
Inactive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marital status «married»															
Yes	1.8	[1.02-0.4]	0.04**	0.8	[0.57-1.3]	0.6 NS	1.06	[0.65-1.7]	0.8 NS	0.7	[0.5-1.13]	0.1 NS	0.6	[0.4-1.14]	0.1 NS
No	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Family type															
Nuclear	1.5	[0.8-2.6]	0.1 NS	1.1	[0.79-1.6]	0.4 NS	0.3	[0.21-0.5]	<0.001**	0.7	[0.5-1.06]	0.1 NS	1.2	[0.75-1.9]	0.4 NS
Composed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Household size															
2-4 members	0.7	[0.4-0.18]	0.2 NS	1.5	[1.05-2.1]	0.02**	0.6	[0.42-0.9]	0.02**	1.2	[0.88-1.8]	0.2 NS	0.8	[0.5-1.29]	0.4 NS
5-12 members	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethnic origin															
Arabic	0.8	[0.4-1.7]	0.6 NS	0.8	[0.57-1.3]	0.6 NS	0.8	[0.5-1.3]	0.4 NS	1.08	[0.7-1.66]	0.7 NS	0.8	[0.5-1.5]	0.6 NS
Berber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*= Univariate analysis with significance level p<0.05; **= Significant; NS = Not Significant. OR = Odds Ratio; CI = 95% confidence interval.

Factors associated with meal preparation frequency

Table 3 shows that based on univariate analysis, nuclear family type (OR=0.7; CI [0.35-0.95]; p=0.04) and household size of 2-4 members (OR= 0.6; CI [0.45-9.31]; p=0.03) are factors hindering the preparation of breakfast at least 5 times a week. This table also shows that urban area (OR=0.5; CI [0.3-0.9]; p=0.03) and Arab ethnicity (OR=0.5; CI [0.23-0.91]; p=0.04) were factors that hindered the preparation of snacks at least 5 times a week. Finally, the urban area (OR=2; [CI 1.4-3]; p<0.001) is a factor that favors the preparation of lunch at least 5 times a week.

Factors associated with meal preparation time

Table 4 shows that based on univariate analysis, household size of 2-4 members (OR=0.6; CI [0.4-

Table 3. Factors associated with meal preparation frequency ≥ 5 times per week (n=507)

Characteristics	Breakfast			Lunch			Snaks			Dinner		
	OR	IC (95%)	P*	OR	IC (95%)	P*	OR	IC (95%)	P*	OR	IC (95%)	P*
Age groups (years)												
20-34	1.07	[0.65-1.7]	0.7 NS	1.1	[0.6-1.9]	0.7 NS	1.04	[0.5-1.9]	0.9 NS	1.1	[0.8-1.7]	0.5 NS
35-65	-	-	-	-	-	-	-	-	-	-	-	-
Area of residence												
Urban	0.9	[0.56-1.4]	0.6 NS	1	[0.6-1.6]	0.9 NS	0.5	[0.3-0.9]	0.03**	2	[1.4-3]	<0.001**
Rural	-	-	-	-	-	-	-	-	-	-	-	-
High education level												
Yes	0.6	[0.4-1.2]	0.2 NS	1.2	[0.6-2.6]	0.5 NS	0.8	[0.3-1.6]	0.5 NS	0.9	[0.5-1.5]	0.6 NS
No	-	-	-	-	-	-	-	-	-	-	-	-
Professional occupation												
Active	0.8	[0.5-1.4]	0.4 NS	1.5	[0.8-2.9]	0.1 NS	1.2	[0.6-2.1]	0.6 NS	0.7	[0.5-1.1]	0.1 NS
Inactive	-	-	-	-	-	-	-	-	-	-	-	-
Marital status «married»												
Yes	0.8	[0.5-1.4]	0.4 NS	1.4	[0.8-2.4]	0.2 NS	1.2	[0.7-2.3]	0.5 NS	0.8	[0.5-1.3]	0.6 NS
No	-	-	-	-	-	-	-	-	-	-	-	-
Family type												
Nuclear	0.7	[0.35-0.95]	0.04**	1.2	[0.7-1.9]	0.4 NS	1.1	[0.7-1.9]	0.6 NS	0.9	[0.6-1.4]	0.7 NS
Composed	-	-	-	-	-	-	-	-	-	-	-	-
Household size												
2-4 members	0.6	[0.45-9.31]	0.03**	1.2	[0.7-2.3]	0.3 NS	1.2	[0.6- 2]	0.5 NS	0.99	[0.7-1.4]	NS
5-12 members	-	-	-	-	-	-	-	-	-	-	-	-
Ethnic origin												
Arabic	1.05	[0.6-1.7]	0.8 NS	1.2	[0.7-2.2]	0.4 NS	0.5	[0.23-0.91]	0.04**	0.97	[0.6-1.5]	0.9 NS
Berber	-	-	-	-	-	-	-	-	-	-	-	-

*= Univariate analysis with significance level p<0.05; **= Significant; NS = Not Significant. OR = Odds Ratio; CI = 95% confidence interval.

Table 4. Factors associated with meal preparation time ≥3 hours per day (n=507)

Characteristics	Meal preparation time ≥3 h/d		
	OR	IC (95%)	P*
Age groups (years)			
20-34	0.7	[0.48-1.2]	0.2 NS
35-65	-	-	-
Area of residence			
Urban	0.6	[0.43-1.05]	0.08 NS
Rural	-	-	-
High education level			
Yes	0.7	[0.43-1.3]	0.3 NS
No	-	-	-
Professional occupation			
Active	0.7	[0.46-1.1]	0.1 NS
Inactive	-	-	-
Marital status «married»			
Yes	1.3	[0.8-2.13]	0.2 NS
No	-	-	-
Family type			
Nuclear	0.9	[0.62-1.4]	0.7 NS
Composed	-	-	-
Household size			
2 - 4 members	0.6	[0.4-0.88]	0.01**
5 - 12 members	-	-	-
Ethnic origin			
Arabic	0.9	[0.58-1.55]	0.8 NS
Berber	-	-	-
Stewing			
<4 times/week	-	-	-
≥4 times/week	0.5	[0.35-0.79]	0.002**
Steaming			
<4 times/week	-	-	-
≥4 times/week	0.8	[0.55-1.2]	0.3 NS
Baking			
<4 times/week	-	-	-
≥4 times/week	1.8	[1.14-2.9]	0.01**
Frying			
<4 times/week	-	-	-
≥4 times/week	0.9	[0.5-1.7]	0.8 NS
Grilling			
<4 times/week	-	-	-
≥4 times/week	0.8	[0.53-1.43]	0.6 NS

* = Univariate analysis with significance level p<0.05; ** = Significant; NS = Not Significant. OR = Odds Ratio; CI = 95% confidence interval; h/d= hours per day.

0.88], p=0.01) and use of stewing at least four times per week (OR=0.5; CI [0.35-0.79], p=0.002) were factors hindering meal preparation time greater than or equal to three hours per day, whereas the use of baking at least four times per week (OR=1.8; CI [1.14-2.9], p=0.01) was a contributing factor.

DISCUSSION

There is no single national cuisine of a country as a whole. Traditional cuisine, for example, is actually made up of many regional cuisines depending on several factors including the climate, history and customs of a region, while having characteristics common to all the cuisine of a country. This includes the basic ingredients and cooking methods used in the preparation of regional recipes [18].

In this study, the stew is the frequently used method of cooking in the majority of the investigated population while almost half of it uses fried food frequently (at least 4 times a week). This result corroborates a previous study conducted in the province of El Jadida [19]. In fact, the food consumption pattern in Morocco is largely dominated by basic dishes, notably vegetable and/or legume-based stews, prepared with or without meat, and by fried potatoes, which become very popular among young people [20]. Regarding the frequency of meals and snacks preparation, breakfast and lunch are the main meals prepared at least 5 times a week by the population surveyed, while only a minority prepares dinner at this same rate. The snack most frequently prepared by the studied population is the afternoon snack. Snacks have an important part in Moroccan dietary habits and tend to replace dinner in some households given their frequency as well as the diet and nutritional composition.

Moreover, the beneficial implications of the frequency of meal preparation on the health status of individuals have been the subject of several studies [11, 12]. In addition, home meals preparation have been correlated with less frequent consumption of fast food, less fat use, and adequate intake of calcium, and whole grain vegetables, all of which promote healthy eating [21]. Furthermore, spending a significant amount of time preparing meals has been associated with better diet quality, including significantly higher consumption of vegetables, salads, fruits, and fruit juices [22]. On the other hand, the majority of the present study population spent three or more hours per day preparing meals and snacks, including less than 30 minutes for breakfast and snacks, two or more hours for lunch, and less than one hour for dinner. These durations are longer than those reported in a consumer survey conducted in Belgium, which found that the average time for the preparation of breakfast is 7 minutes, 15 minutes for lunch, and 33

minutes for dinner [23]. In this regard, a correlation of high cooking time with low cardiovascular and metabolic risks has been reported [16]. This could be related to the fact that home food preparation involves the use of primary foods that are beneficial in terms of nutritional value and quality far superior to processed foods known for their high contents in calories, saturated fatty acids, and sodium associated with health problems [14, 24].

Statistical analysis showed that the frequency of stewing method use is related to the urban area, work status, and married status. Among the many factors likely to influence the use of stewing as a method of cooking, includes indeed the lack of availability due to other social occupations including work. Similarly, marital status generally results in food choices that are appropriate for the entire family. In the Moroccan context, stew whether prepared in a tagine, in a pot, or in a pressure cooker, represents generally a dish that suits the dietary habits of the family. Further, the area of residence was also revealed as a factor associated with this cooking method. In this respect, the urban areas would be a hindering factor and the rural areas would be a factor favoring the use of this cooking method. The study also revealed that urban areas and high education level were associated with the use of fried foods, while another study showed that, further to urban areas, young age was associated with the use of this cooking method [19]. These last two factors have been recognized as associated with the modernization of food practices [25, 26]. Taking into account the frequency of different used cooking methods, it appears that generally, despite urbanization and work status, the study population adopts heterogeneous cooking methods, marked by a return to the traditional.

The statistical analysis also showed that urban area, household size, nuclear family type, and ethnic origin are factors associated with the frequency of meal preparation. Urban residency could have an impact on the frequency of meal preparation, given the specific living and working conditions that may lead to certain unavailability and lack of time, which may reduce the frequency of meals preparation. One of the most common obstacles to meal preparation is the lack of time. The type of family and the household size represent factors favoring the preparation of meals at home, on the one hand. This is related to the convivial aspect of this practice, which will be further promoted by the number of participating people. On the other hand, it is related to the low economic cost that this practice generates compared to out-of-home dining. In addition, ethnicity could promote food of good quality in households. Accordingly, a previous study, reported that participants declared that home-cooked meals were often prepared using traditional ingredients and that food preparation behavior is learned from family [27]. Furthermore, it has been reported that societies' choice of food practices may be influenced by their culture and identity [28].

In addition, statistical analysis has shown that among the factors associated with daily meal preparation time; there are household size, use of stewing, and use of baking. Indeed, the larger is the size of a household, the more food will be prepared, and the more time will be required for cooking. Baking is also a time-consuming process, especially for certain protein foods and vegetables that are rich in cellulose. It should be noted that the cooking time depends on the desired effect of the temperature on the different components of the food such as the increase of their digestibility [29, 30], the softening of the cellulosic and woody structures of the plants [31], or the guarantee of the hygienic aspects [32].

Although subjects with less education level and lower incomes have been reported to spend more time in preparing meals daily than those with more education and higher incomes [33], the present study did not reveal any association between education level and standard of living with meal preparation time. Likewise, while not in accordance with previous research data reporting that spending the least amount of time on meal preparation are generally working individuals who value convenience [22], the present study data did not find any association between work status and meal preparation time.

CONCLUSION

This work investigated factors influencing the cooking method, frequency, and duration of meal preparation in Moroccan households. The findings point to the necessity of the implementation of a nutritional education strategy based on combining habits, preferences, and good cooking practices.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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REFERENCES

1. *Stengel K.*: Hérédités alimentaires et identité gastronomique: suis-je réellement ce que je mange? L'Harmattan 2014;123.
2. *Serra-Majem L., Tomaino L., Dernini S., Berry EM., Lairon D., Ngo de la Cruz J.*, et al.: Updating the Mediterranean Diet Pyramid towards Sustainability: Focus on EnvironmentalConcerns. Int J Environ Res Public Health 2020;17(23):8758.
3. *Jeantet R., Croguennec T., Schuck P., Brule G.*: Handbook of Food Science and Technology 2: Food Process Engineering and Packaging. John Wiley & Sons 2016;346p.
4. *Raber M., Baranowski T., Crawford K., Sharma SV., Schick V., Markham C.*, et al.: The Healthy Cooking Index: nutrition optimizing home food preparation practices across multiple data collection methods. J Acad Nutr Diet 2020;120(7):1119-32.
5. *Buratti S., Cappa C., Benedetti S., Giovanelli G.*: Influence of Cooking Conditions on Nutritional Properties and Sensory Characteristics Interpreted by E-Senses: Case-Study on Selected Vegetables. Foods 2020;9(5):607.
6. *Nagy R., Máthé E., Csapó J., Sipos P.*: Modifying Effects of Physical Processes on Starch and Dietary Fiber Content of Foodstuffs. Processes 2021;9(1):17.
7. *Xu Z., Leong SY., Farid M., Silcock P., Bremer P., Oey I.*: Understanding the Frying Process of Plant-Based Foods Pretreated with Pulsed Electric Fields Using Frying Models. Foods Juill 2020;9(7).
8. *Asokapandian S., Swamy GJ., Hajjul H.*: Deep fat frying of foods: A critical review on process and product parameters. Crit Rev Food Sci Nutr 2020;60(20):3400-13.
9. *Choi YS., Hwang KE., Jeong TJ., Kim YB., Jeon KH., Kim EM., et al.*: Comparative Study on the Effects of Boiling, Steaming, Grilling, Microwaving and Superheated Steaming on Quality Characteristics of Marinated Chicken Steak. Korean J Food Sci Anim Resour 2016;36(1):1-7.
10. *Feng Q., Jiang S., Feng X., Zhou X., Wang H., Li Y., et al.*: Effect of different cooking methods on sensory quality assessment and in vitro digestibility of sturgeon steak. Food Sci Nutr 2020;8(4):1957-67.
11. *Taillie LS., Poti JM.*: Associations of cooking with dietary intake and obesity among SNAP participants. Am J Prev Med 2017;52(2 Suppl 2):S151-60.
12. *Méjean C., Lampuré A., Si Hassen W., Gojard S., Péneau S., Hercberg S., et al.* :Influence of food preparation behaviors on 5-year weight change and obesity risk in a French prospective cohort. Int J Behav Nutr Phys Act 2018;15(1):120.
13. *Ducrot P., Méjean C., Aroumougame V., Ibanez G., Allès B., Kesse-Guyot E., et al.*: Meal planning is associated with food variety, diet quality and body weight status in a large sample of French adults. Int J Behav Nutr Phys Act 2017;14:12.
14. *Chu YL., Addo OY., Perry CD., Sudo N., Reicks M.*: Time spent in home meal preparation affects energy and food group intakes among midlife women. Appetite. 2012;58(2):438-43.
15. *Zick CD., Stevens RB., Bryant WK.*: Time use choices and healthy body weight: a multivariate analysis of data from the American Time Use Survey. Int J Behav Nutr Phys Act 2011;8:84.
16. *Appelhans BM., Segawa E., Janssen I., Nackers LM., Kazlauskaite R., Baylin A.*, et al.: Meal preparation and cleanup time and cardiometabolic risk over 14 years in the Study of Women's Health Across the Nation (SWAN). Prev Med 2015;71:1-6.
17. *Barakat I., Elayachi M., Belahsen R.*: Validation of conceptual and methodological framework for the study of dietary practices and nutritional status of an adult population. Rocz Panstw Zakl Hig 2022;73(2):199-207. DOI: https://doi.org/10.32394/rpzh.2022.0211.
18. *Xavier Medina F.*: Food Culture in Spain. Food culture around the world. ISSN 1545-2638. Éditor: Greenwood Publishing Group, 2005. ISBN: 0313328196, 9780313328190.
19. *Barakat I, Kalili A., Moustakim R.* et. al.: Food Consumption Trends and Associated Factors in an Agricultural Community in Morocco. OAJBS 2020;2(2).ID.000160. DOI: https://doi.org/10.38125/OAJBS.000160.
20. *Benjelloun S.* : Profil nutritionnel marocain Organisation des Nations Unies pour l'alimentation et l'agriculture. Population (French Edition) 2011;5(4):764.
21. *Larson NI., Perry CL., Story M., Neumark-Sztainer D.*: Food preparation by young adults is associated with better diet quality. J Am Diet Assoc 2006;106(12):2001-7.
22. *Monsivais P., Aggarwal A., Drewnowski A.*: Time spent on home food preparation and indicators of healthy eating. Am J Prev Med 2014;47(6):796-802.
23. ISP (Belgique): Enquête de Consommation Alimentaire 2014-2015. Report n° D/2016/2505/51. Belgique: Institut Scientifique de Santé Publique 2016; p. 406.
24. *Potvin L.*: Les aliments ultra-transformés, un concept utile pour la santé publique. Can J Public Health 2019;110(1):1-3.
25. *Bigot R., Piau C.*: Les jeunes sont aujourd'hui favorables à la mondialisation. Centre de recherche pour l'étude et l'observation des conditions de vie 2003;1-4.
26. *Lahlou S.*: L'enquête du Crédoc sur les Comportements Alimentaires des Français. 2011;16.
27. *Smith TM.*, *Dunton GF., Pinard CA., Yaroch AL.*: Factors influencing food preparation behaviours: findings from focus groups with Mexican-American mothers in southern California. Public Health Nutr 2016;19(5):841-50.
28. *Reddy G., van Dam RM.*: Food, culture, and identity in multicultural societies: Insights from Singapore. Appetite 2020;149:104633.
29. *Lee SW., Rhee C.*: Effect of heating condition and starch concentration on the structure and properties of freeze-dried rice starch paste. Food Res Int. 2007;40(2):215-23.
30. *Sagum R., Arcot J.*: Effect of domestic processing methods on the starch, non-starch polysaccharides and in vitro starch and protein digestibility of three varieties

of rice with varying levels of amylose. Food Chemistry 2000;70(1):107-11.
31. *Kutoš T., Golob T., Kač M., Plestenjak A.*: Dietary fibre content of dry and processed beans. Food Chemistry 2003;80(2):231-5.
32. *Dupin H., Cuq JL., Malewiak MI., Leynaud-Rouaud C., Berthier AM.*: Alimentation et nutrition humaines. Paris (France) ESF 1992.
33. *Méjean C, Si Hassen W, Gojard S, Ducrot P, Lampuré A, Brug H, Lien N, Nicolaou M, Holdsworth M, Terragni L, Hercberg S, Castetbon K.* Social disparities in food preparation behaviours: a DEDIPAC study. Nutr J. 2017 Sep 20;16(1):62. doi: 10.1186/s12937-017-0281-2.

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ORIGINAL ARTICLE

BENEFITS OF SEA BUCKTHORN JUICE CONSUMPTION IN WOMEN OF PRODUCTIVE AGE WITH HYPERCHOLESTEROLEMIA

Jana Kopčeková[1], *Jana Mrázová*[1], *Katarína Fatrcová-Šramková*[1], *Marta Habánová*[1], *Martina Gažarová*[1], *Petra Lenártová*[1]

[1]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Nitra, Slovak Republic

ABSTRACT

Background. Sea buckthorn contains almost 200 nutrients and bioactive substances, including phenolic compounds such as flavonoids, vitamins, proteins, amino acids, minerals, alkaloids, chlorophyll derivatives, amines, organic acids, fatty acids and phytosterols. Human and animal studies suggest that sea buckthorn may have a variety of beneficial effects: cardioprotective, antiatherogenic, antioxidant, anticancer, immunomodulatory, antibacterial, antiviral and anti-inflammatory.

Objective. The aim of this study was to evaluate the effect of regular consumption of 100% sea buckthorn juice on the risk factors of cardiovascular diseases in women of productive age with hypercholesterolemia.

Material and Methods. A clinical study involved 19 women with a mean age of 54.06 ±2.97 years who consumed 50 mL of sea buckthorn juice daily for 8 weeks. Anthropometric and biochemical parameters in blood serum were monitored before the start of sea buckthorn consumption and after 8 weeks of consumption. Body composition was determined using an multifrequency analyzer InBody720. Routine biochemical analyzes were performed by standard methods in an accredited laboratory of the University Hospital by automatic biochemical analyzer BioMajesty JCA-BM6010/C. Statistical comparison between individual measurements was performed using a paired t-test, using Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA).

Results. We observed significant decrease of body weight, body mass index ($P<0.05$), body fat and visceral fat ($P<0.001$) after 8 weeks of consumption of 100% sea buckthorn juice. In this intervention study, we observed a significant decrease in low-density cholesterol ($P<0.05$) and a significant increase in high-density cholesterol ($P<0.001$). The level of triglycerides was similar at the end of the study ($P>0.05$). After the intervention, we observed decrease of orosomucoid, immunoglobulin A, immunoglobulin G, immunoglobulin M ($P<0.001$) and C-reactive protein ($P<0.01$).

Conclusions. The obtained results support the hypothesis that the daily consumption of sea buckthorn juice for eight weeks could contribute to the beneficial effects of reducing the risk of cardiovascular diseases, such as the reduction of body and visceral fat, LDL-C, CRP and the increase of HDL-C.

Key words: *sea buckthorn juice, cardiovascular diseases, hypercholesterolemia, obesity, cholesterol*

INTRODUCTION

Cardiovascular diseases (CVD) have emerged as a major cause of mortality and morbidity worldwide [47]. Dyslipidemia is a major modifiable contributor to cardiovascular diseases [31], elevated blood cholesterol accounts for nearly one third of ischemic heart diseases [13, 47]. Reduction in excess calories and improvement in dietary composition may prevent many primary and secondary cardiovascular events [56]. In recent years, research has focused primarily on natural products with beneficial effects, and increased attention is being paid to foods with demonstrable and effective antioxidant activity [41].

Many studies demonstrate that the fruits and leaves of some berry plants biosynthesize phytochemicals with antioxidant activity and can be used as a natural source of free radical scavenging compounds [26, 27, 40]. A diet rich in berries is thought to play an important role in preventing metabolic diseases associated with obesity [42]. The beneficial effects of berries may be mediated by the antioxidant and anti-inflammatory properties of polyphenols [23].

Sea buckthorn (*Hippophae* in Latin) belongs to the *Elaeagnaceae* family [54]. Around 150 species, subspecies and varieties of sea buckthorn have been identified within Europe and Asia. They differed in the habitat of the bush, the appearance of the berries

Corresponding author: Jana Mrázová, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, phone: +421 37 641 4223, e-mail: jana.mrazova@uniag.sk

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and their utility value [7]. The most important and widespread in Europe is the sea buckthorn *Hippophae rhamnoides* [55]. *Hippophae* fruits are called third generation fruits [18]. Sea buckthorn fruits, leaves, oil and other products are a source of many bioactive substances, including phenolic compounds, such as flavonoids, i.e. rutin, quercetin, kaempferol, or myricetin [6], vitamins (tocopherols, carotenoids, ascorbic acid, folate, vitamins B_1, B_2 and K), proteins, amino acids and minerals (Fe, Ca, P and K) [2, 14, 24, 59]. In addition, the plant contains organic acids (quinic acid, malic acid, oxalic acid and tartaric acid) [5, 20], fatty acids, especially unsaturated fatty acids (oleic acid, linoleic acid, linolenic acid) and phytosterols [5, 32]. The main group of phenolic compounds are flavonols, a group of flavonoids that have been identified in fruits, with an average content of 311.5 mg/100 g fresh weight [46]. One of the most interesting features is that sea buckthorn contains high concentrations of vitamin C [50]. The content of vitamin C per 100 g of sea buckthorn fruit is 600 mg, which is significantly more than in rose hips (250-800 mg), black currants (120-215 mg) or raspberries (15-30 mg) [24]. Chemical compounds in different parts of *Hippophae rhamnoides* may vary depending on climatic conditions, their origin and extraction methods [35]. Sea buckthorn fruits are interesting not only from a chemical point of view, but also from a biological and therapeutic point of view for their antioxidant, antitumor, hepato-protective and immunological properties [59]. Human and animal studies suggest that sea buckthorn may have a variety of beneficial effects: cardioprotective, antiatherogenic, antioxidant, anticancer, immunomodulatory, antibacterial, antiviral, and anti-inflammatory [4, 51]. The antioxidants present in sea buckthorn activate the transcription factor nuclear factor E2 (Nrf-2) and inhibit the nuclear factor kappa B (NF-kB) redox signaling pathway, which in turn activates antioxidant enzymes that are responsible for antioxidant activity and are considered one of the mechanisms of action antioxidant activity of sea buckthorn [19]. The anti-inflammatory activity of sea buckthorn can be attributed to ursolic acid, oleanolic acid, citric acid derivatives and flavonoids. Its anti-inflammatory mechanism of action may be related to the inhibition of the expression of pro-inflammatory cytokines and the reduction of the production of pro-inflammatory mediators [51]. *Jaśniewska* and *Diowksz* [17] report that sea buckthorn flavonoids help lower cholesterol levels, improve cardiac function, and protect endothelial cells from damage caused by oxidized low-density lipoproteins. Sea buckthorn polyphenols suppress the expression of cyclins, thereby arresting the cell cycle in the G1 phase and affecting the further proliferation of colon cancer [53]. Biochemical and histopathological studies have shown that sea buckthorn flavonoid extract significantly improves biomarkers including triglycerides aspartate aminotransferase and alanine aminotransferase in serum and liver [51]. Recent attention has focused on the use of fruit juices as a concentrated source of antioxidants. Drinking juice is an effective way to promote fruit and vegetable consumption and is very popular in many countries [3, 10, 43, 45]. A high amount of evidence shows that juice, as part of a balanced diet, contributes to a significant reduction in the risk of many diseases, such as cancer, neurodegenerative diseases and cardiovascular diseases [3, 33, 38].

The aim of this study was to evaluate the effect of regular consumption of 100% sea buckthorn juice (SBJ) on the risk factors of cardiovascular diseases in women of productive age with hypercholesterolemia.

MATERIAL AND METHODS

The study included 19 hypercholesterolemic women of productive age from 50 to 61 years old, with a mean age of 54.06 ± 2.97 years, who participated in an 8-week intervention program. Body composition (body weight – BW, body fat mass – BFM, body mass index – BMI, visceral fat area – VFA, skeletal muscle mass – SMM, fat-free mass – FFM, waist to hip ratio – WHR, intracellular water – ICW, extracellular water – ECW and total body water – TBW), blood pressure (systolic blood pressure – SBP and diastolic blood pressure – DBP), lipid profile (total cholesterol – T-C, low density cholesterol – LDL-C, high density cholesterol – HDL-C, triglycerides – TG), inflammation markers (C-reactive protein – CRP, interleukin-6 – IL-6, orosomucoid – ORM, immunoglobulin A – IgA, immunoglobulin G – IgG and immunoglobulin M – IgM), kidney and liver markers (alanine aminotransferase – ALT, aspartate aminotransferase – AST, gamma glutamyl transferase – GGT, urea, creatinine and uric acid) were monitored before the start of SBJ consumption and after 8 weeks of consumption.

Ethical aspects

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee at the Specialized Hospital St. Zoerardus Zobor, NPO Nitra, Slovak Republic (protocol number 3/101921/2021).

Dietary intervention

Volunteers consumed 50 mL of commercial 100% SBJ as recommended by the manufacturer for 8 weeks as part of their regular diet. The juice was provided by ZAMIO Ltd., Trhovište, Slovakia. Juice composition (g/100 mL): fats – 3.2; of which saturated fatty acids –

0.7; carbohydrates – 5.1; of which sugars – 4.7; proteins – 1.0. Total phenolic content – 1.56 mg gallic acid equivalents (GAE)/g, vitamin C – 385 mg/100 g and total carotenoid – 64.79 mg/100 g. The participants were instructed to maintain their normal eating habits during the study, to refrain from consuming dietary supplements and not to modify their physical activity.

Anthropometric parameters

Body height was measured on a Tanita WB-300 ambulatory electronic scale in an upright position, without shoes. We used multi-frequency bioelectrical impedance analysis (MFBIA) – InBody 720 (Biospace Co. Ltd., Seoul, Korea) to diagnose body composition. Blood pressure was determined with a digital electronic sphygmomanometer Omron M7 Intelli IT, HEM-7361T-EBK (Omron Healthcare, Tokyo, Japan).

Biochemical parameters

Venous blood was collected in the morning after 8 hours of fasting in a standard way. After blood serum separation, routine biochemical analyzes were performed in an accredited laboratory of the University Hospital with a BioMajesty JCA-BM6010/C automatic biochemical analyzer using commercial DiaSys kits (Diagnostic Systems GmbH, Holzheim, Germany) according to the manufacturer's instructions.

Statistical analysis

Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA) and MS Excel 2007 (Microsoft Corporation, Redmond, WA, USA) were used for statistical analysis. All data were expressed as mean ± standard deviation (SD). Statistical comparison between individual measurements was performed using a paired t-test, a value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Characteristics of study participants

The observed group consisted of 19 women of productive age from 50 to 61 years, with an average age of 54.06 ± 2.97 years. All women had an elevated level of total cholesterol, the average 6.41 ± 1.08 mmol.L^{-1}. From the individual values the basic statistical characteristics of the probands were calculated (Table 1).

Effect of SBJ consumption on anthropometric and biochemical parameters

Anthropometric measurements

Worldwide, the burden of morbidity and mortality from diet-related chronic diseases is increasing, driven by poor diet quality and overconsumption of calories. Fortunately, shifting current global dietary patterns towards high-quality, plant-based diets could alleviate these health and environmental burdens [15, 37] not only because of the low content of saturated fat and cholesterol, but also because of the considerable amount of micronutrients and bioactive compounds [12, 48]. Obesity is induced by chronic low-grade inflammation, which can act synergistically with oxidative stress. Thus, intake of fruits and plant extracts high in antioxidant phytochemicals has important anti-obesity activity [48]. *Huang* et al. [16] found that a vegetarian diet can be of considerable importance in weight reduction. The results of anthropometric characteristics and body structure after consumption of SBJ are shown in Table 2. Body weight and BMI of women significantly decreased during the study ($P<0.05$). The body mass index

Table 1. Basic characteristics of study participants

Characteristic	Average ± SD	Min. – max.
Age (yrs)	54.06 ± 2.97	50 – 61
BW (kg)	72.44 ± 14.59	49.2 – 100.8
BMI (kg.m^{-2})	26.13 ± 5.27	19.96 – 38.41
WC (cm)	93.10 ± 14.57	72.40 – 120.60
T-C (mmol.L^{-1})	6.41 ± 1.08	5.06 – 8.33
GLU (mmol.L^{-1})	4.92 ± 0.34	4.30 – 5.40

Data are expressed as average ± standard deviation (SD), min. – max.; BW, body weight; BMI, body mass index; WC, waist circumference; T-C, total cholesterol; GLU, glucose

Table 2. Anthropometric characteristics and blood pressure of study participants

Parameter	Baseline	Week 8	*p*-value
BW (kg)	72.43 ± 14.59	71.95 ± 14.72	0.0450
BFM (kg)	25.17 ± 11.08	24.26 ± 11.07	<0.001
BMI (kg.m^{-2})	26.13 ± 5.27	25.96 ± 5.32	0.0400
VFA (cm^2)	103.08 ± 39.90	99.46 ± 40.25	<0.001
SMM (kg)	25.89 ± 2.51	26.17 ± 2.63	0.0080
FFM (kg)	47.26 ± 4,33	47.69 ± 4,52	0.0130
ICW (kg)	21.39 ± 1.91	21.59 ± 2.03	0.0139
ECW (kg)	13.26 ± 1.29	13.36 ± 1.29	>0.05
TBW (kg)	34.65 ± 3.18	34.96 ± 3.31	0.0140
WHR	0.94 ± 0.08	0.93 ± 0.08	>0.05
SBP (mmHg)	130.60 ± 14.01	130.87 ± 14.40	>0.05
DBP (mmHg)	85.33 ± 9.62	85.80 ± 9.50	>0.05

Data are expressed as average ± standard deviation (SD); SBJ, sea buckthorn juice; BW, body weight; BFM, body fat mass; BMI, body mass index; VFA, visceral fat area; SMM, skeletal muscle mass; FFM, fat-free mass; ICW, intracellular water; ECW, extracellular water; TBW, total body water; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure

does not express the distribution of fat in the body. In the assessment of anthropometric indicators, we also focused on the assessment of visceral fat (VFA). We consider VFA as another indicator of health and obesity status. It is one of the important factors in the assessment of cardiometabolic risk, which correlates with the components of the metabolic syndrome in men and women, even with a normal BMI indicating the absence of obesity [1]. Abdominal obesity was observed in 7 women (36.8%) in the monitored group. In the whole group, after consumption of SBJ there was a significant decrease of VFA ($P<0.001$). Likewise, a WHR index higher than 0.85 reflects a risk for the development of metabolic diseases. In our monitored group, the WHR index was similar after 8 weeks of nutritional intervention ($P>0.05$). Weight reduction is necessary for the treatment of obesity. However, some treatments may cause a reduction on the muscular mass, reducing metabolic waste, and compromising weight loss and/or maintenance of the lost weight [29]. The current study demonstrated that taking SBJ for 8 weeks caused a significant increase of SMM and FFM ($P<0.05$). From the results of *Lehtonen* et al. [22], who observed the effect of consumption of different sea buckthorn fractions (equivalent to 100 g of fresh berries/day), showed that sea buckthorn supplementation for 33–35 days had a positive effect on the occurrence of metabolic diseases in overweight and obese women. *Larmo* et al. [21] also reported a significant effect of sea buckthorn fruit consumption on the metabolic profiles of overweight women. Recent studies have classified high systolic blood pressure (SBP) as the leading risk factors for death and disability worldwide, as well as hypertension being the largest risk factor for cardiovascular disease [44, 57]. The phytochemical and bioactive compounds of berries such as flavonoids, ellagitannins, and anthocyanins are potentially showing better effects in the risk reduction of various CVD such as reduce blood pressure, decreased endothelial dysfunction, which ultimately increases cardiovascular and brain health in an individual [50]. In our study, consumption of SBJ resulted in similar SBP and DBP values at the end of the study ($P>0.05$).

Kidney and liver markers

In this intervention study, volunteers consumed 50 mL of 100% bio commercial SBJ every day for 8-week period. In general, despite its bitter and sour nature, the juice was well accepted. SBJ consumption was well tolerated by participants and did not show changes ($P>0.05$) in liver and kidney function in serum following 8 weeks of SBJ intake (Table 3).

Lipid profile

The effect of 100% SBJ consumption on the lipid profile is shown in Table 4. Although there was no significant decrease in T-C in this intervention study, on the other hand there was a significant decrease in LDL-C ($P<0.05$) and a significant increase in HDL-C ($P<0.001$). The level of TG was similar at the end of the study ($P>0.05$). *Eccleston* et al. [11] found that SBJ with a flavonoid concentration of 1180 $mg.L^{-1}$ had protective properties against hypertension and coronary heart disease. The results of a study with 229 volunteers consuming 28 g of sea buckthorn berries for 3 months indicated increased blood concentrations of quercetin and isohamnetin, but consumption of sea buckthorn berries did not affect T-C, LDL-C, HDL-C and TG [21].

Inflammation markers

Increasing evidence demonstrates that inflammation plays an important role in the development of atherosclerosis [9, 34, 39]. Mainly, the crucial role of inflammation in atherosclerosis is particularly reflected by the overexpression of nuclear factor kappa B, C-reactive protein (CRP),

Table 3. Kidney and liver markers of study participants

Parameter	Baseline	Week 8	*p*-value
ALT ($\mu kat.L^{-1}$)	0.28 ± 0.10	0.32 ± 0.15	>0.05
AST ($\mu kat.L^{-1}$)	0.32 ± 0.06	0.34 ± 0.06	>0.05
GGT ($\mu kat.L^{-1}$)	0.36 ± 0.16	0.43 ± 0.27	>0.05
Urea ($mmol.L^{-1}$)	4.88 ± 1.45	4.98 ± 1.39	>0.05
Creatinine ($\mu mol.L^{-1}$)	64.75 ± 9.66	67.63 ± 9.63	>0.05
Uric acid ($\mu mol.L^{-1}$)	285.00 ± 63.04	302.67 ± 72.07	>0.05

Data are expressed as average ± standard deviation (SD), SBJ, sea buckthorn juice; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase

Table 4. Lipid profile of study participants

Parameter	Baseline	Week 8	*p*-value
T-C ($mmol.L^{-1}$)	6.49 ± 1.07	6.45 ± 1.06	0.6894
HDL-C ($mmol.L^{-1}$)	1.70 ± 0.24	1.82 ± 0.27	<0.001
LDL-C ($mmol.L^{-1}$)	3.91 ± 1.32	3.38 ± 0.58	0.0257
TG ($mmol.L^{-1}$)	1.29 ± 0.70	1.27 ± 0.69	0.1857

Data are expressed as average ± standard deviation (SD); SBJ, sea buckthorn juice; T-C, total cholesterol; HDL-C, high density cholesterol; LDL-C, low density cholesterol; TG, triglycerides

interleukin-6 (IL-6), IL-18, tumor necrosis factor-alpha (TNF-α), and other inflammatory mediators [30]. The CRP is a nonspecific positive acute-phase protein that immediately rises after initiating an inflammatory state [8] and it could be an indicator of endothelial dysfunction that is also linked to a higher risk of CVD [58]. Our results (Table 5) show, that regular 8 week-long consumption of 50 mL of SBJ per day resulted significant reduction of CRP, IgA, IgG, IgM, orosomucoid ($P<0.001$) and similar values of IL-6 ($P>0.05$). In the study of *Larmo* et al. [21] the participants daily took 28 g of frozen sea buckthorn puree or placebo product similar in appearance, taste and smell to the active product and consumption of sea buckthorn berries reduced serum concentrations of CRP. Flavonoids and vitamin C are likely to be among the bioactive compounds responsible for the anti-inflammatory effect of fruit, possibly through synergetic mechanisms [25, 28, 36, 52].

Table 5. Inflammation markers of study participants

Parameter	Baseline	Week 8	*p*-value
CRP ($mg.L^{-1}$)	5.72 ± 2.39	4.75 ± 1.60	0.0038
IL-6 ($ng.L^{-1}$)	7.88 ± 0.93	7.32 ± 0.78	>0.05
ORM ($g.L^{-1}$)	0.89 ± 0.22	0.56 ± 0.17	<0.001
IgA ($g.L^{-1}$)	1.71 ± 0.68	1.57 ± 0.69	<0.001
IgG ($g.L^{-1}$)	10.52 ± 2.65	9.99 ± 2.66	<0.001
IgM ($g.L^{-1}$)	1.25 ± 0.63	1.15 ± 0.60	<0.001

Data are expressed as average ± standard deviation (SD);
SBJ, sea buckthorn juice; CRP, C-reactive protein; IL-6, interleukin-6;
ORM, orosomucoid; IgA, immunoglobulin A;
IgG, immunoglobulin G; IgM, immunoglobulin M

CONCLUSION

Sea buckthorn fruits are called fruits of the third generation. One of the most interesting properties is that sea buckthorn contains high concentrations of vitamin C, carotenoids, tocopherols and other bioactive compounds. The aim of this study was to evaluate the effect of regular consumption of 100% sea buckthorn juice on the risk factors of cardiovascular diseases in women of productive age with hypercholesterolemia. The obtained results support the hypothesis that the daily consumption of SBJ could contribute to the beneficial effects of reducing the risk of cardiovascular diseases, such as the reduction of body and visceral fat, LDL-C, inflammation markers and the increase of HDL-C. However, longer studies with a larger number of volunteers are needed to more thoroughly investigate the consumption of SBJ in the prevention and treatment of cardiovascular diseases.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Babiarczyk B., Turbiarz A.*: Body Mass Index in elderly people – the reference ranges matter? Progress in Health Sciences 2012;2(1):58-67.
2. *Bekker N.P., Glushenkowa A.I.*: Components of certain species of *Elaeagnaceae* family. Chem Nat Compd 2001;37:97-116.
3. *Bhardwaj R.L., Nandal U., Pal A., Jain S.*: Bioactive compounds and medicinal properties of fruit juices. Fruits 2014;69:391-412 doi:10.1051/fruits/2014027.
4. *Chandra S., Zafar R., Dwivedi P., Shinde L.P., Prita B.*: Pharmacological and nutritional importance of sea buckthorn (*Hippophae*). The Pharma Innovation Journal 2018;7(5):258-263.
5. *Chong M.F.F., Macdonald R., Lovegrove J.A.*: Fruit polyphenols and CDV risk: a review of human intervention studies. Br J Nutr 2010;104:S28eS39 doi:10.1017/s0007114510003922.
6. *Christaki E.*: *Hippophae rhamnoides* L. (Sea buckthorn): a potential source of nutraceuticals. Food Pub Health 2012;2:69-72 doi:10.5923/j.fph.20120203.02.
7. *Ciesarová Z., Murkovic M., Cejpek K., Kreps F., Tobolková B., Koplík R., Belajová E., Kukurová K., Daško Ľ., Panovská Z., Revenco D., Burčová Z.*: Why is sea buckthorn (*Hippophae rhamnoides* L.) so exceptional? A review. Food Res Int 2020;133: 109170 doi:10.1016/j.foodres.2020.109170.
8. *Dallmeier D., Larson M.G., Vasan R.S., Keaney J.F., Fontes J.D., Meigs J.B. et al.*: Metabolic syndrome and inflammatory biomarkers: a community-based cross-sectional study at the Framingham Heart Study. Diabetology and metabolic syndrome 2012;4(1):28.
9. *Del Pinto R., Ferri C.*: Inflammation-Accelerated Senescence and the Cardiovascular System: Mechanisms and Perspectives. Int J Mol Sci 2018;19:3701 doi:10.3390/ijms19123701.
10. *Drossard C., Fröhling B., Bolzenius K., Dietrich H., Kunz C., Kersting M.*: Liking of anthocyanin-rich juices by children and adolescents. Appetite 2012;58(2):623-628 doi:10.1016/j.appet.2012.01.002.

11. *Eccleston C., Baoru Y., Tahvonen R., Kallio H., Rimbach G.H., Minihane A.M.*: Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. J Nutr Biochem 2002;13(6):346-354 doi:10.1016/s0955-2863(02)00179-1.
12. *Eilat-Adar S., Goldbourt U.*: Nutritional Recommendations for Preventing Coronary Heart Disease in Women: Evidence Concerning Whole Foods and Supplements. Nutr Metab Cardiovasc Dis 2010;20:459-466 doi:10.1016/j.numecd.2010.01.011.
13. *Feigin V.L., Krishnamurthi R.V., Parmar P., Norrving B., Mensah G.A., Bennett D.A.* et al.: Update on the global burden of ischemic and hemorrhagic stroke in 1990-2013: the GBD 2013 study. Neuroepidemiology 2015;45(3):161-176 doi:10.1159/000441085.
14. *Gao X., Ohlander M., Jeppsson N., Bjork L., Trajkovski V.*: Changes in antioxidant effects and their relationship to phytonutrients in fruits of Sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J Agric Food Chem 2001;48:1485-1490.
15. *Hemler E.C., Hu F.B.*: Plant-Based Diets for Personal, Population, and Planetary Health. Adv Nutr 2019;10:S275-S283 doi:10.1093/advances/nmy117.
16. *Huang R.Y, Huang C.C., Hu F.B., Chavarro J.E.*: Vegetarian diets and weight reduction: a meta-analysis of randomized controlled trials. J Gen Intern Med 2016;31(1):109-116 doi: 10.1007/s11606-015-3390-7.
17. *Jaśniewska A., Diowksz A.*: Wide Spectrum of Active Compounds in Sea Buckthorn (*Hippophae rhamnoides*) for Disease Prevention and Food Production. Bioavailability and Bioactivity of Plant Antioxidants 2021;10(8):1279 doi:10.3390/antiox10081279.
18. *Ji M., Gong X., Li X., Wang C., Li M.*: Advanced Research on the Antioxidant Activity and Mechanism of Polyphenols from *Hippophae* Species – A Review. Molecules 2020;25(4):917 doi:10.3390/molecules25040917.
19. *Kashyap Pyiush P. et al.*: Antioxidants in Fruits: Properties and Health Benefits. Singapore, Springer Nature Singapore Pte Ltd. 637, 2020. ISBN 978-981-15-7285-2.
20. *Kumar R., Kumar G.P., Chaurasia O.P., Singh S.*: Phytochemical and pharmacological profile of Seabuckthorn oil: a review. Res J Med Plant 2011;5:491-499 doi:10.3923/rjmp.2011.491.499.
21. *Larmo P.S., Yang B., Hurme S.A., Alin J.A., Kallio H.P., Salminen E.K., Tahvonen R.L.*: Effect of a low dose of sea buckthorn berries on circulating concentrations of cholesterol, triacylglycerols, and flavonols in healthy adults. Eur J Nutr 2009;48:277-282 doi:10.1007/s00394-009-0011-4.
22. *Lehtonen H.M., Suomela J.P., Tahvonen R., Yang B., Venojärvi M., Viikari J., Kallio H.*: Different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases on overweight and obese women. Eur J Clin Nutr 2011;65(3):394-401 doi: 10.1038/ejcn.2010.268.
23. *Loo B.M., Erlund I., Koli R., Puukka P., Hellström J., Wähälä K., Mattila P., Jula A.:* Consumption of chokeberry (Aronia mitschurinii) products modestly lowered blood pressure and reduced low-grade inflammation in patients with mildly elevated blood pressure. Nutr Res 2016;36(11):1222-1230 doi: 10.1016/j.nutres.2016.09.005.
24. *Malinowska P., Olas B.*: Sea buckthorn-valuable plant for health. Kosmos 2016;65(2):285-292.
25. *Middleton E.J., Kandaswami C., Theoharides T.C.*: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673-751.
26. *Mushtaq M., Wani S.M.*: Polyphenols and human health – A review. International Journal of Pharma and BioSciences 2013;4:338-360.
27. *Nazir A., Wani S., Gani A., Masoodi F.A., Haq E., Mir S.A., Riyaz U.*: Nutritional, antioxidant and antiproliferative properties of persimmon (*Diospyros kaki*) – a minor fruit of J&K India. International Journal of Advanced Research 2013;1:545-554.
28. *Nijveldt R.J., Van Nood E., Van Hoorn D.E., Boelens P.G., Van Norren K., Van Leeuwen P.A.*: Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001;74:418-425. doi:10.1093/ajcn/74.4.418.
29. *Nuttall F.Q.*: Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr. Today 2015;50:117-128 doi:10.1097/nt.0000000000000092.
30. *O'Morain V.L., Ramji D.P.*: The Potential of Probiotics in the Prevention and Treatment of Atherosclerosis. Mol Nutr Food Res 2020;64:e1900797.
31. *Pan L., Yang Z., Wu Y., Yin R.X., Liao Y., Wang J. et al.*: The prevalence, awareness, treatment and control of dyslipidemia among adults in China. Atherosclerosis 2016;248:2-9 doi:10.1016/j.atherosclerosis.2016.02.006.
32. *Patel C.A., Divakar K., Santani D., Solanki H.K., Thakkar J.H.*: Remedial Prospective of Hippophae rhamnoides Linn. (Sea Buckthorn). ISRN Pharmacol 2012;1-6 doi: 10.5402/2012/436857.
33. *Peluso I., Villano D.V., Roberts S.A., Cesqui E., Raguzzini A., Borges G., Crozier A., Catasta G., Toti E., Serafini M.*: Consumption of mixed fruit-juice drink and vitamin C reduces postprandial stress induced by a high fat meal in healthy overweight subjects. Curr Pharm Des 2014;20(6):1020-1024 doi:10.2174/13816128 2006140220144802.
34. *Prabhu S.D., Frangogiannis N.G.*: The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis. Circ Res 2016;119:91-112 doi:10.1161/circresaha.116.303577.
35. *Pundir S., Garg P., Dviwedi A. et al.*: Ethnomedicinal uses, phytochemistry and dermatological effects of *Hippophae rhamnoides* L. J. Ethnopharmacology 2021;266:113434 doi:10.1016/j.jep.2020.113434.
36. *Rein D., Schijlen E., Kooistra T., Herbers K., Verschuren L., Hall R., Sonnewald U., Bovy A., Kleemann R.*: Transgenic flavonoid tomato intake reduces C-reactive protein in human C-reactive protein transgenic mice more than wild-type tomato. J Nutr 2006;136:2331-2337 doi:10.1093/jn/136.9.2331.
37. *Rodríguez-García C., Sánchez-Quesada C., Toledo E., Delgado-Rodríguez M., Gaforio J.J.*: Naturally Lignan-

Rich Foods: A Dietary Tool for Health Promotion? Molecules 2019;24:917 doi: 10.3390/molecules24050917.
38. *Rodriguez-Roque M.J., Rojas-Grau M.A., Elez-Martinez P., Martin-Belloso O.*: *In vitro* bioaccessibility of health-related compounds as affected by the formulation of fruit juice- and milk-based beverages. Food Res. Int. 2014;62:771-778 doi:10.1016/j.foodres.2014.04.037.
39. *Ruparelia N., Choudhury R.*: Inflammation and atherosclerosis: what is on the horizon? Heart 2020;106:80-85 doi:10.1136/heartjnl-2018-314230.
40. *Sacchetti G., Maietti S., Muzzoli M., Scaglianti M., Manfredini S., Radice M., Bruni R.*: Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 2005;91(4):621-632 doi:10.1016/j.foodchem.2004.06.031.
41. *Sadef Y., Javed T., Javed R., Mahmood A., Alwahibi M.S., Elshikh M.S., AbdelGawwa M.R., Alhaji J.H., Rasheed R.A.*: Nutritional status, antioxidant activity and total phenolic content of different fruits and vegetables' peels. PLoS One 2022;17:e0265566. doi: 10.1371/journal.pone.0265566.
42. *Sikora J., Broncel M., Markowicz M., Chałubiński M., Wojdan K., Mikiciuk-Olasik E.*: Short-term supplementation with Aronia melanocarpa extract improves platelet aggregation, clotting, and fibrinolysis in patients with metabolic syndrome. Eur J Nutr 2012;51(5):549-556 doi:10.1007/s00394-011-0238-8.
43. *Singh G.M., Micha R., Khatibzadeh S., Shi P., Lim S., Andrews K.G., Engell R.E., Ezzati M., Mozaffarian D.*: Correction: Global, Regional, and National Consumption of Sugar-Sweetened Beverages, Fruit Juices, and Milk: A Systematic Assessment of Beverage Intake in 187 Countries. PLoS One 2019;14(3):e0214344 doi:10.1371/journal.pone.0214344.
44. *Stanaway J.D., Afshin A., Gakidou E., Lim S.S., Abate D., Abate K.H., Abbafati C., Abbasi N., Abbastabar H., Abd-Allah F. et al.*: Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: A systematic analysis for the global burden of disease study 2017. Lancet 2018;392:1923-1994.
45. *Starek M., Guja A., Dabrowska M., Krzek J.*: Assay of β-carotene in dietary supplements and fruit juices by TLC-densitometry. Food Anal Methods 2015;8(5):1347-1355 doi:10.1007/s12161-014-0019-0.
46. *Teleszko M., Wojdyło A., Rudzińska M., Oszmiański J., Golis T.*: Analysis of Lipophilic and Hydrophilic Bioactive Compounds Content in Sea Buckthorn (*Hippophae rhamnoides* L.) Berries. J Agric Food Chem 2015;63(16):4120-4129 doi:10.1021/acs.jafc.5b00564.
47. *Tripathy J.P., Thakur J.S., Jeet G., Chawla S., Jain S., Pal A. et al.*: Burden and risk factors of dyslipidemia-results from a STEPS survey in Punjab India. Diabetes Metab Syndr 2017;11(Supp.1):S21-S27 doi:10.1016/j.dsx.2016.08.015.
48. *Turner-McGrievy G., Mandes T., Crimarco A.*: A plant-based diet for overweight and obesity prevention and treatment. J Geriatr Cardiol 2017;14(5):369-374 doi:10.11909/j.issn.1671-5411.2017.05.002.
49. *Vilas-Franquesa A., Saldo J., Juan B.*: Potential of sea buckthorn-based ingredients for the food and feed industry – a review. Food Prod Process and Nutr 2020;2(1) doi:10.1186/s43014-020-00032-y.
50. *Wal A., Wal P., Roohi T.F.*: A review on role of berries and its bioactive compounds in treating hypertension. Asian Journal of Pharmaceutical and Clinical Research 2020;13(8):3-12 doi:10.22159/ajpcr.2020.v13i8.37932.
51. *Wang Z., Zhao F., Wei P., Chai X., Hou G., Meng Q.* 2022. Phytochemistry, health benefits, and food applications of sea buckthorn (*Hippophae rhamnoides* L.): A comprehensive review. Frontiers in Nutrition 2022;9:1-20 doi:10.3389/fnut.2022.1036295.
52. *Wannamethee S.G., Lowe G.D., Rumley A., Bruckdorfer K.R., Whincup P.H.*: Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. Am J Clin Nutr 2006;83:567-574 doi: 10.1093/ajcn.83.3.567.
53. *Wu H., Li Ch., Cui M., Guo H., Chen S. et al.*: Polyphenols from Hippophae rhamnoides suppressed colon cancer growth by regulating miRNA-mediated cell cycle arrest and apoptosis in vitro and in vivo. Journal of Functional Foods 2021;87:104780 doi:10.1016/j.jff.2021.104780.
54. *Xing J., Yang B., Dong Y., Wang B., Wang J., Kallio H.P.*: Effects of sea buckthorn (*Hippophae rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. Fitoterapia 2002;73(7-8):644-650 doi:10.1016/s0367-326x(02)00221-6.
55. *Yang B., Kallio H.*: Supercritical Co-extracted sea buckthorn (*Hippophae rhamnoides*) oils as new food ingredients for cardiovascular health. Proc Health Ingred 2002;17(19):7.
56. *Yu E., Malik V.S., Hu F.B.*: Cardiovascular Disease Prevention by Diet Modification: JACC Health Promotion Series. J Am Coll Cardiol 2018;72(8):914-926 doi:10.1016/j.jacc.2018.02.085.
57. *Yusuf S., Joseph P., Rangarajan S., Islam S., Mente A., Hystad P., Brauer M., Kutty V.R., Gupta R., Wielgosz A. et al.*: Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): A prospective cohort study. Lancet 2020;395:795-808 doi:10.1016/s0140-6736(19)32008-2.
58. *Zaha D., Vesa C., Uivarosan D., Bratu O., Fratila O., Mirela Tit D., Pantis C., Diaconu C., Bungau S.*: Influence of inflammation and adipocyte biochemical markers on the components of metabolic syndrome. Exp Ther Med 2020;20:121-128 doi:10.3892/etm.2020.8663.
59. *Zeb A.*: Chemical and nutritional constituents of sea buckthorn juice. Pak J Nutr 2004;3:99-106 doi:10.3923/pjn.2004.99.106.

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ORIGINAL ARTICLE

CROSS SECTIONAL STUDY OF VITAMIN B_{12} SUPPLEMENTATION IN SLOVAK AND CZECH VEGANS

Radek Latal[1], Marta Habanova[1], Eliska Selinger[2], Maros Bihari[1], Jadwiga Hamulka[3]

[1]Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovakia
[2]Third Faculty of Medicine, Charles University, Prague, Czech Republic and Centre for Public Health Promotion, National Institute of Public Health, Prague, Czech Republic
[3]Institute of Human Nutrition Sciences, Department of Human Nutrition, Warsaw, Poland

ABSTRACT

Background. The number of vegans in the world is growing and in Slovakia and the Czech Republic they make up 1% of the population. Vegan diet excludes all foods of animal origin and vegans who do not use vitamin B_{12} supplements are at risk of the vitamin B_{12} deficiency.

Objective. The aim of this study was to determine what proportion of Czech and Slovak vegans use vitamin B_{12} supplements regularly, irregularly or not at all and what is their supplemental cobalamin intake.

Materials and methods. The research involved 1337 self-identified vegans from Slovakia and the Czech Republic who were interviewed using the CAWI (Computer-Assisted Web Interview) method. Participants were recruited by posts in veganism-themed social media groups.

Results. Out of 1337 vegans 55.5% supplemented cobalamin regularly, 32.54% irregularly and 11.97% were not supplementing. Rate of not supplementing individuals was 5.04% higher in Slovaks than in Czechs. Short-term vegans had a significantly higher rate of not supplementing individuals (17.99%) compared to medium-term (8.37%) and long-term vegans (7.50%). Mean weekly cobalamin intake from supplements was 2938.34±2566.60 µg in regularly supplementing vegans compared to 1630.31±1949.27 µg in irregularly supplementing vegans, particularly due to the lower weekly supplementation frequency among irregularly (2.93) compared to regularly supplementing vegans (5.27).

Conclusions. The rate of supplementation in Slovak and particularly Czech vegans was higher than in other countries. The number of not supplementing individuals was significantly higher among short-term vegans, indicating that there is still a need for education on the importance of adequate and regular cobalamin supplementation, especially in new vegans. Our results support the hypothesis that the reason for higher rate of cobalamin deficiency in irregularly compared to regularly supplementing vegans is the lower cobalamin intake caused by lower supplementation frequency.

Key words: *veganism, vegan, vitamin B_{12}, vitamin B_{12} deficiency, vitamin B_{12} supplementation, vitamin B_{12} intake, Czech vegan, Slovak vegan*

INTRODUCTION

The number of vegans and the popularity of plant-based diets in the world are increasing [1, 2] and with them also the need to study and recognize nutritional challenges of these dietary patterns. Despite not being on the forefront of the trend, Slovakia and the Czech Republic already have a small vegan population [3]. Vegans comprise approximately 1% of all Slovaks and Czechs, with the highest rates being among young people. As the growing body of literature indicates, even exclusively plantbased diets can be healthful and nutritionally adequate, provided they are appropriately planned [4, 5]. This includes also supplementation of the vitamin B_{12}. Due to the exclusion of all products of animal origin, such as meat, dairy, eggs and fish, the only remaining reliable non-prescription sources of vitamin B_{12} for vegans are B_{12} fortified foods and supplements. Despite growing availability of the fortified foods and limited evidence supporting their use as a vitamin B_{12} source [6], regular supplementation is still recognized as the most reliable way to ensure sufficient vitamin B_{12} intake in vegans [7].

Current data on the vitamin B_{12} supplementation status in vegans from Slovakia are missing. Latest available data are from the year 2000, when researchers evaluated vitamin B_{12} status of 32 Slovak vegans and 62 Slovak lacto- and lacto-ovo vegetarians, with the

Corresponding author: Marta Habanova, Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovakia, tel. +42 1904665196, e-mail: marta.habanova@uniag.sk

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result of 78% of vegans being deficient [8]. Czech vegans were studied in the 2019 study, which involved 151 vegan participants. This research has demonstrated that the cobalamin deficiency was associated with the supplementation status of vegans and not with the duration of the vegan diet. By measuring the serum methylmalonic acid, holo-trans-cobalamin and homocysteine, researchers have found that the incidence of the cobalamin deficiency in the regularly supplementing vegans was only 5.56%, compared to 17.9% in irregularly supplementing vegans and 52.9% in not supplementing vegans [7]. We hypothesized that the reason for a higher rate of cobalamin deficiency in irregularly supplementing vegans compared to regularly supplementing vegans in the study by *Selinger* et al. has been the insufficient supplemental cobalamin intake. The aim of our study was therefore to: (i) identify, how prevalent is the regular and irregular supplementation in Slovak and Czech vegans (ii) identify their supplemental doses, frequency of supplementation and total supplemental cobalamin intake.

MATERIAL AND METHODS

Study design and participants

The study was designed as a cross-sectional study among self-reported vegans from Slovakia and Czech Republic. The sample was recruited through the social media posts in veganism-themed groups in February 2021. Data about their vitamin B_{12} supplementation habits were collected using the CAWI (Computer-Assisted Web Interview) method. Participants received information about the anonymity of the study, the voluntary nature and the possibility to stop their participation at any study stage.

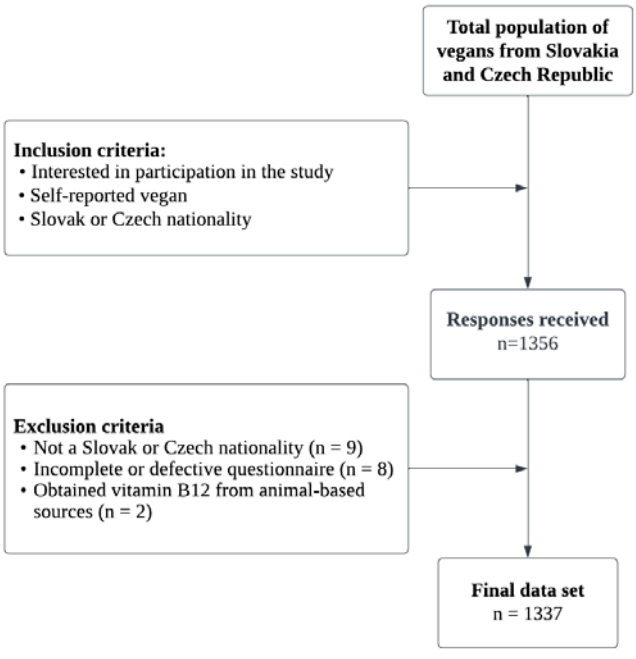

Figure 1. The inclusion and exclusion criteria and completion of participant selection

The inclusion criteria were willingness to participate in the study and being Czech or Slovak self-identified vegan. Exclusion criteria were reported animal-based foods among vitamin B_{12} sources and submission of the incomplete or defective questionnaire. From the total number of 1356 respondents, 1337 were included in the study. Total 19 questionnaires were excluded from the study for the reasons highlighted in Figure 1.

Questionnaire

Questionnaire consisted of 23 questions, covering basic demographic details, vitamin B_{12} sources, supplement use and information sources. Detailed questionnaire can be found in the Annex. Questions 1. to 6. inquired participants about the duration of their vegan diet, age, education level, nationality, sex and in the case of women also about pregnancy and breastfeeding.

The main question of the research was question number 7., asking whether participants used vitamin B_{12} supplements regularly, irregularly or not at all. This question was modeled after the research of *Selinger* et al. [7], in order to follow-up on their results. Respondents, who have selected regular or irregular use of vitamin B_{12} supplements, were further led to the series of questions 8. - 11., focused on the type of the supplement, supplementation frequency, used dose and the cobalamin form.

Respondents who indicated that they did not use vitamin B_{12} supplements, were instead led to the questions 12. and 13. Here they were asked about their reasons for not taking the vitamin B_{12} supplements and if they relied on any other sources instead. All participants then answered questions 14. and 15., inquiring about medication and medical conditions, which may interfere with the vitamin B_{12} absorption or status.

Questions 16. asked all participants if they informed their doctor about being vegan, followed up by questions 17. and 18., asking if they were ever tested for cobalamin deficiency and results of the test. Questions 19. to 23. were focused on what information sources vegans used to learn about vegan nutrition and availability and quality of information sources in Slovak and Czech languages.

Statistical analysis

We have divided participants by the duration of their veganism and their supplement use using the same methods as *Selinger* et al. Respondents were therefore classified as “short-term vegans” for those who were vegan for <3 years; “medium-term vegans” for 3–7 years and “long-term vegans” for >7 years. A Chi^2

test and *Cramér's* V were used to assess the statistical significance and intercorrelation between the nominal variables, including supplementation status and duration of veganism, sex, pregnancy/breastfeeding status, nationality, education level and other factors. To assess statistical significance between nominal and metric variables, two-tailed t-test for independent samples was used. For all analyses, a p-value <0.05 was considered statistically significant.

Supplemental doses were evaluated using mean with standard deviation (SD), median, minimum, maximum and quartiles. Supplemental doses of daily and weekly supplementing participants were compared to the recommendations for the daily and weekly intake respectively in separate tables. Supplemental doses of remaining participants, who supplemented 2 – 6x times a week, were converted to the cumulative weekly intake and compared to the recommendations for weekly intake in a separate table. Cumulative weekly intake was calculated by multiplying the dose by weekly frequency. For example, supplementation of 100 µg of cobalamin three times a week would result in cumulative weekly intake of 300 µg. Basic data processing and descriptive statistics were performed using MS Excel (MS Office Professional Plus 2019). Further statistical analysis, including hypothesis testing by two tailed t-test, *Chi*^{2} test and *Cramér's* V, were performed using DATAtab online statistics calculator https://datatab.net/.

RESULTS

Participants were on the vegan diet on average for 4.05±3.57 years, with the median value of 3 years. Men were on the vegan diet on average longer than women, 4.7±3.64 and 3.8±3.53 years respectively. As identified by the two-tailed t-test for the independent samples (equal variances assumed), this relation was statistically significant (p<0.001, 95% CI [-1.35, -0.45]).

Women comprised 76.59% (n = 1024) of all respondents and 9.75% (n = 98) of them were currently pregnant or breastfeeding. Proportion of the male vegans was increasing with the duration of the vegan diet, up to 31.88% in the group on vegan diet for over 7 years. Most vegans in the study were from the age group of 25-34-year-olds, who comprised 46.45% of all respondents. Oldest and youngest participants were 13 and 70 years old respectively. Of all participants, 53.7% had bachelor's or higher degree. Full demographic data are shown in the Table 1.

Vitamin B_{12} supplementation

Of all 1337 participants, 55.5% were using vitamin B_{12} supplements regularly and 32.54% irregularly. There was a statistically significant relationship between vitamin B_{12} supplementation and the duration of vegan diet ($\chi^2(4) = 33.09$, p<0.001, *Cramér's* V = 0.11). The highest rate of vegans, who did not use vitamin B_{12} supplements (17.45%), was identified in the group, who followed the vegan diet "short-term" (<3 years). In contrast only 8.31% of "medium-term" and 7.50% of "long-term" vegans were not supplementing at all.

Compared to women, men were more likely to use vitamin B_{12} supplements and supplement regularly, with the exception of the pregnant or breastfeeding women, who had the highest rates of supplementation of all groups. We have identified that there was a statistically significant relationship between the vitamin B_{12} supplementation and pregnancy or breastfeeding status ($\chi^2(2) = 18.82$, p<0.001, *Cramér's* V = 0.14).

Table 1. Demographic data

Sex (n = 1337)		
Male	313	76.59%
Female	1024	23.41%
Pregnancy or breastfeeding (n = 1005)[1]		
Yes	98	9.75%
No	907	90.25%
Nationality (n = 1337)		
Czech	518	38.74%
Slovak	819	61.26%
Duration of veganism (n = 1337)		
Under 3 yrs	556	41.59%
3-7 yrs	621	46.45%
Over 7 yrs	160	11.97%
Age (n = 1337)		
0-14	3	0.22%
15-24	319	23.86%
25-34	621	46.45%
35-44	265	19.82%
45-54	99	7.40%
55-64	26	1.94%
65+	4	0.30%
Education level (n = 1337)		
Lower secondary education or lower	39	2.92%
Upper secondary education without maturity diploma	55	4.11%
Upper secondary education with maturity diploma	525	39.27%
Bachelor's degree or equivalent	256	19.15%
Master's degree	436	32.61%
Doctorate	26	1.94%

[1] 19 out of 1024 female participants have not answered this question

There was a statistically significant difference in the vitamin B_{12} supplementation between Czech and Slovak vegans ($\chi^2(2) = 6.23$, p=0.044, *Cramér's* V = 0.07). Among Slovak vegans there was 5.04% higher rate of not supplementing individuals compared to Czech vegans. Supplementation rate was also higher in the group of participants with the bachelor's or higher degree, than in the group of those with high school diploma or lower education, but this result did not reach statistical significance. Supplementation rates for all groups can be found in the Table 2.

Supplemental doses

Out of 1173 respondents, who regularly or irregularly used vitamin B_{12} supplements, 690 provided valid details on both supplementation frequency and used dose and were supplementing on at least weekly basis. The mean dose taken by these participants was 616.53±536.82 μg and the most common frequency of supplementation was daily (40.72%). Doses used by vegans in specific frequencies are visualized in Figure 2 and the full data are available in Table 3.

Mean cobalamin intake in regularly supplementing vegans was 55.48% higher than in irregularly supplementing vegans, as shown in the Table 4. A two-tailed t-test for independent samples (equal variances not assumed) showed that this result was statistically significant, t(340.92) = 6.88, p = <0.001, 95% confidence interval [931.78, 1684.26].

Cobalamin intakes were compared to the recommended daily intake by EFSA for general population [9] and cobalamin intake targets for vegans proposed by different authors [10, 11, 12] in the Tables 5, 6 and 7. Out of 690 participants who used supplements on at least a weekly basis and provided a supplemental dose 42 (6.09%) reported one or more risk factors for the cobalamin malabsorption.

Table 2. Supplementation rates among Slovak and Czech vegans

Total	Supplement regularly		Supplement irregularly		Do not supplement		*p*-Value
	739	55.27%	434	32.46%	164	12.27%	
Q1 How long have you been vegan? (n = 1337)							
Under 3 years	303	54.50%	153	27.52%	100	17.99%	<0.001
3-7 years	349	56.20%	220	35.43%	52	8.37%	
Over 7 years	87	54.38%	61	38.13%	12	7.50%	
Q3 What is your highest level of education? (n = 1337)							
No college	318	51.37%	216	34.89%	85	13.73%	0.026
Some college	421	58.64%	218	30.36%	79	11.00%	
Q4 Nationality (n = 1337)							
Czech	297	57.34%	172	33.20%	49	9.46%	0.044
Slovak	442	53.97%	262	31.99%	115	14.04%	
Q5 Sex (n = 1337)							
Women	553	54.00%	338	33.01%	133	12.99%	0.171
Men	186	59.42%	96	30.67%	31	9.90%	
Q6 Are you currently pregnant or breastfeeding? (n = 1005)							
Yes	73	74.49%	18	18.37%	7	7.14%	<0.001
No	467	51.49%	314	34.62%	126	13.89%	
Q16 Have you informed your doctor about your vegan diet? (n = 1337)							
Yes	362	58.01%	208	33.33%	54	8.65%	0.001
No	377	52.88%	226	31.70%	110	15.43%	
Q17 Have you ever been tested to see if you get enough vitamin B_{12}? (n = 1337)							
Yes	241	57.11%	139	32.94%	42	9.95%	0.211
No	498	54.43%	295	32.24%	122	13.33%	
Q18 Have you ever been diagnosed with insufficient vitamin B_{12} intake? (n = 417)							
Yes	31	57.41%	20	37.04%	3	5.56%	0.389
No	207	57.02%	117	32.23%	39	10.74%	
Q19 Have you ever consulted your doctor or nutritionist about your vegan diet? (n = 1337)							
Yes	175	60.76%	86	29.86%	27	9.38%	0.72
No	564	53.77%	348	33.17%	137	13.06%	

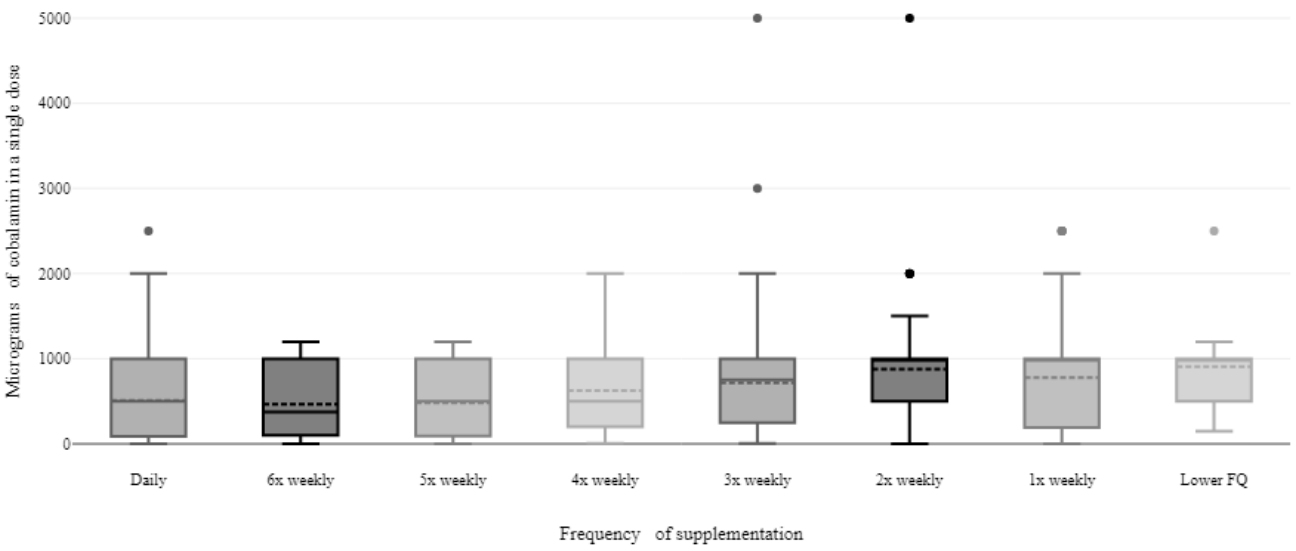

Figure 2. Supplemental doses by frequency of supplementation

Table 3. Supplementation rates and doses among Slovak and Czech vegans in micrograms)

Frequency	N (%)	Mean dose (SD)	Median	Min	Max
Daily	281 (40.72)	509.71±428.20	500	0.50	2500
6x weekly	29 (4.20)	466.22±429.63	375	2.50	1200
5x weekly	66 (9.57)	481.24±399.75	500	2.00	1200
4x weekly	53 (7.68)	625.08±491.49	500	2.80	2000
3x weekly	117 (16.96)	716.09±635.58	750	3.00	5000
2x weekly	82 (11.88)	875.27±643.55	1000	1.80	5000
Weekly	62 (8.99)	777.64±644.80	1000	1.00	2500
Total	690 (100)	616.53±536.82	500	0.50	5000

Table 4. Cobalamin supplementation in regularly and irregularly supplementing vegans (in micrograms)

	Regularly supplementing	Irregularly supplementing
N	739	434
Mean weekly intake (SD)	2938.34±2566.60	1630.31±1949.27
Median weekly intake	2500	1000
Mean single dose (SD)	607.34±471.57	646.99±712.53
Average weekly frequency	5.27	2.93

Out of 690 participants, who included cobalamin dose and supplemented at least weekly, 537 also included information on the vitamin B_{12} form in their supplements. The most frequently used cobalamin form was cyanocobalamin, with methyl-cobalamin being the second most popular.

Reasons for not supplementing

Out of 1337 respondents, 12.27% (n = 164) did not supplement vitamin B_{12}. To the question 12 *"What are your reasons for not supplementing vitamin B_{12}?",* where it was possible to select more than one answer, most frequently selected option was: *"I have not addressed it yet"* and in the second place: *"I get vitamin B_{12} from other sources"*. Among the open answers under the *"Other"* option, respondents the most frequently stated that they were tested for vitamin B_{12} adequacy. Detailed data are shown in Figure 3.

Respondents who have claimed that they obtain vitamin B_{12} from other sources were able to specify these alleged sources in question 13. The most frequently cited alleged sources of vitamin B_{12} were various types of algae, plant foods and mushrooms, which cannot be considered reliable sources of vitamin

Table 5. Cobalamin intake in daily supplementing participants (in micrograms)

Malabsorption risk	N	%	Mean daily dose (SD)	Mean weekly intake (SD)	Target	Within target	
No	265	94.31	513.84±431.13	3596.91±3017.94	4 µg [9]	257	96.98%
					10 µg [12]	245	92.45%
					50 µg [12]	214	80.75%
Yes	16	5.69	441.31±382.01	3089.19±2674.10	100 µg [11]	13	81.25%

Table 6. Cobalamin intake in weekly supplementing participants (in micrograms)

Malabsorption risk	N	%	Mean weekly dose (SD)	Target	Within target	
No	59	95.16	723.96±597.15	350 µg [10]	38	64.41%
				2000 µg [12]	3	5.08%
Yes	3	4.84	1833.33±763.76	2000 µg [11]	2	66.67%

Table 7. Cobalamin intake in two- to six-times weekly supplementing participants (in micrograms)

Malabsorption risk	N	%	Mean weekly intake (SD)	Target	Within target	
No	324	93.37	2233.97±1903.87	350 µg [10]	269	83.02%
				2000 µg [12]	187	57.72%
Yes	23	6.63	1892.78±1707.14	2000 µg [11]	11	47.83%

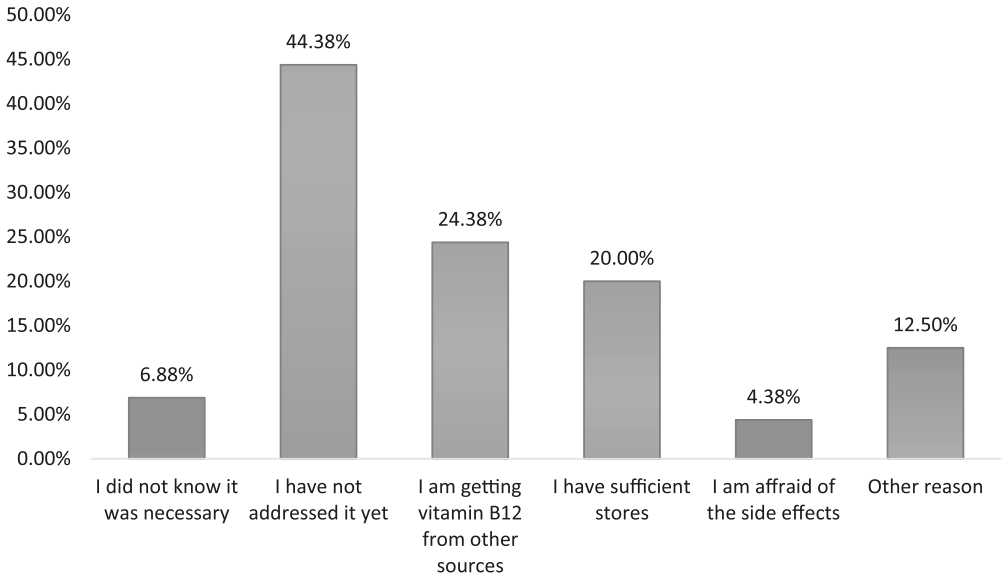

Figure 3. What are your reasons for not supplementing vitamin B_{12}?

Table 8. Cobalamin forms and averaged daily intake (in micrograms)

	N	%	Mean daily intake (±SD)	Median	Min	Max
Cyano	288	41.74	354.42±353.11	286	0.14	2143
Methyl	175	25.36	412.43±364.82	286	1	2500
Hydroxo	2	0.29	1250±1060.66	1250	500	2000
Adenosyl	2	0.29	82±8.49	82	76	88
Multiple	70	10.14	448.89±337.73	429	4	1429
Unknown	153	22.17	336.05±335.54	286	1	1400

Table 9. Claimed sources of vitamin B_{12} among not supplementing vegans

Claimed source	No of answers
Chlorella and other seaweed	12
Various kinds of vegetables, fruit and mushrooms	11
Fortified foods	6
Unwashed vegetables, contaminated with vit. B_{12}	4
Fermented foods	2
Vitamin B_{12} enriched toothpaste	1
Vitamin B_{12} injections	1

B_{12}. Potentially effective sources of vitamin B_{12}, such as vitamin B_{12} fortified toothpaste and intramuscular injections, have also been mentioned, as highlighted in the Table 9 [13, 14].

DISCUSSION

First aim of our study was to determine, what proportion of Czech and Slovak vegans use vitamin B_{12} supplements regularly, irregularly or not at all. Our research has found that most vegans used vitamin B_{12} supplements, 55.27% (n=739) regularly and 32.46% (n= 434) irregularly. Only 12.27% (n=164) of the participating vegans did not supplement vitamin B_{12} at all. This scale has been used in order to allow for comparison with the research by *Selinger* et al., who have presented the same question to a smaller sample of Czech vegans (n=72). In their study 11.64% vegans have not used vitamin B_{12} supplements, but the rate of regular supplementation was higher at 61.64% [7].

With regards to the duration of the vegan diet, the highest number of not supplementing individuals was found among short-term vegans (17.99%) in comparison to medium-term vegans (8.37%) and long-term vegans (7.50%). This result is consistent with findings by *Selinger* et al. who have found that it was the short-term vegans, who had the highest prevalence of lowered cobalamin levels and supports their conclusion that it is the presence of cobalamin supplementation rather than the duration of veganism that is associated with increased risk of cobalamin deficiency [7].

There was a statistically significant difference in supplementation rate between Slovak and Czech vegans, with 14.04% of Slovak and 9.46% of Czech vegans not supplementing. In comparison, in the study of similar sample size (n = 1530) conducted in Australia, 25.3% of vegans did not use any vitamin B_{12} supplements [15]. Other studies on cobalamin supplementation in vegans also included laboratory testing and therefore used smaller sample size, similarly to the Czech study by *Selinger* et al. These studies were conducted in Germany (31.03% not supplementing (n = 58)) [16], Spain (29.63% not supplementing (n = 54)) [17] and Denmark (37.14% not supplementing (n = 70)) [18] and also on the Czech vegan children (10.14% not supplementing (n = 69)) [19]. These results suggest that the vitamin B_{12} supplementation rates in Czech and Slovak vegans identified in our study are comparatively high.

Higher rate of overall supplementation as well as regular supplementation was found in male compared to female vegans in our study. However pregnant and breastfeeding women had the highest rate of supplementation of all groups, with 74.49% supplementing regularly and 18.37% irregularly. This is a positive result given since the majority of vegans in our study were women in the reproductive age (55.57% women under 35) and the need for the appropriate cobalamin supplementation in vegans is the most urgent during pregnancy and breastfeeding and in children. Only 7 out of 98 pregnant or breastfeeding vegan women (7.14%) did not supplement vitamin B_{12} at all. This is consistent with findings by *Světnička* et al., where only 6.5% of Czech vegan women did not use cobalamin supplements during pregnancy or breastfeeding [19]. Gynecologists and pediatricians can be instrumental in ensuring that vegans are supplementing vitamin B_{12} in these critical periods. However, as our results show, most vegans (53.33%) did not inform their doctor about being vegan.

Second aim of our study was to determine what supplemental doses vegans use. Vegans in our study used a wide variety of supplemental doses, ranging from 0.5 to 5000 μg of cobalamin in a single dose. The most popular frequency of supplementation in our study was daily (40.72%), compared to only 26.3% in Australian vegans [15]. Over half (50.29%) of our supplementing study participants used supplements two- to six-times a week and 8.99% supplemented once a week. Our results show that the irregularly supplementing vegans had 44.52% lower mean supplemental cobalamin intake compared to the regularly supplementing vegans. This was primarily caused by 44.40% lower supplementation frequency, rather than the supplemental doses, which were actually on average 6.53% higher among irregularly supplementing vegans. These results may explain the higher incidence of cobalamin deficiency among irregularly supplementing (17.90%) compared to regularly supplementing Czech vegans (5.56%) in the study by *Selinge*r et al [7].

Adequate daily intake of 4 μg recommended by the EFSA for the general adult population [9] was met or exceeded by 96.98% of daily supplementing vegans without risk factors for cobalamin malabsorption. This recommendation is however based on the assumption

that the cobalamin is obtained multiple times a day in small doses from food. In such a scenario active transport is utilized multiple times a day and the overall absorption rate can exceed 40%, resulting in absorption of 1.5 μg of cobalamin [9]. However, in order to absorb the same amount from a single daily supplemental dose, higher doses of 10 or even 50 μg may be required [12]. Thresholds of 10 μg and 50 μg were achieved or exceeded by 92.45% and 80.75% of daily supplementing vegans without risk factors for cobalamin malabsorption respectively.

Various conditions and medication, as well as advanced age, may increase the risk for cobalamin malabsorption, particularly affecting the ability to utilize active transport. In such cases oral supplementation may still be effective, as 1-2% of the supplemental dose is absorbed by passive non-specific mechanisms [9]. The oral dose of 1000 μg was found to be effective in the treatment of cobalamin deficiency even in the individuals suffering from pernicious anemia [20]. However, as suggested by *Rajan* et al., even 100 μg daily may be sufficient for the maintenance of already adequate cobalamin status in people with malabsorption [11]. This threshold was reached or exceeded by 81.25% of daily supplementing vegans with one or more risk factors for cobalamin malabsorption.

As an alternative to the daily supplementation, a weekly dose of 2000 μg has been proposed [12]. This target was however only reached by 5.08% of weekly supplementing vegans without risk factors for malabsorption. However as demonstrated by *Del Bo* et al., even a weekly dose of 350 μg may be sufficient for the maintenance of the adequate cobalamin status in vegans without the risk factors for malabsorption [10]. Weekly target of 350 μg was reached or exceeded by 64.41% of weekly supplementing participants without the risk factors for malabsorption. It is important to stress out that the cobalamin intake thresholds for vegans are still based on limited evidence and other factors, such as cobalamin form or supplement type, may affect the individual absorption rate.

Two most popular cobalamin vitamers used by study participants were cyanocobalamin and methyl-cobalamin. Cyanocobalamin is the most researched vitamer of the vitamin B_{12} and it proved to be reliable and the most stable cobalamin form [21]. Due to their lower stability, *Koeder* and *Perez-Cueto* [12] have suggested that the higher recommended doses may be required for vitamers other than the cyanocobalamin. Our results showed that the mean supplemental cobalamin intake among methyl-cobalamin users was 16.37% higher than that of the cyanocobalamin users. Currently there is however insufficient evidence to confirm whether such difference would be sufficient to compensate for lower stability.

The main strength of our research lies in the high number of participants, which allowed us to reach statistically significant results for the supplementation habits in Czech and Slovak vegans. It is at the same time a limitation, since we were not able to perform laboratory tests to evaluate actual cobalamin status of participants.

We can see several avenues future research can take to advance our understanding of vitamin B_{12} intake and metabolism in people. Apart from vegans there are other groups which may be at risk of vitamin B_{12} deficiency, such as lacto-ovo vegetarians or flexitarians. These groups may not abstain from animal-based foods completely, but the frequency may vary greatly and even beyond the point where the cobalamin intake from foods alone will be insufficient. Future research may therefore investigate frequency of the intake of animal-based foods and supplementation habits in these groups. Additionally, there is still a need for further research to establish optimal supplemental intake of cobalamin for vegans.

CONCLUSIONS

The first aim of our study was to identify how prevalent is the regular and irregular supplementation in Slovak and Czech vegans. We have found that most Czech and Slovak vegans used vitamin B_{12} supplements, with 55.27% supplementing regularly, 32.46% irregularly and only 12.27% not supplementing at all. Czech vegans were 5.04% more likely to use cobalamin supplements then Slovak vegans and also the rate of the regular supplementation was higher in Czechs. The rate of nonsupplementation was significantly higher among vegans following the diet short-term (17.99%) compared to medium-term (8.37%) and long-term vegans (7.50%). These results indicate that there is still a need for education about the importance of the cobalamin supplementation, particularly focused on new vegans.

Second aim of our study was to identify supplemental cobalamin intake of Czech and Slovak vegans. We have observed statistically significant differences in the supplemental frequency and total supplemental cobalamin intake between regularly and irregularly supplementing vegans. Irregularly supplementing vegans were supplementing on average 2.93 times a week, compared to 5.27 times a week in regularly supplementing vegans, resulting in 44.52% lower mean supplemental cobalamin intake. This result supports our initial hypothesis that the reason why some of the supplementing vegans in the study by Selinger et al. still developed cobalamin deficiency was an inadequate supplemental cobalamin intake. Despite the cobalamin supplementation rate being higher than in other countries, our results suggest that there is still

a need to raise awareness about the importance of regular and adequate cobalamin supplementation in the Czech and particularly Slovak vegan community. This is even more urgent due to the fact that the majority of vegans in our study were women in the reproductive age and the adequate cobalamin intake is the most important in pregnant and breastfeeding vegan women and their children.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

Annex. Questionnaire

Q No	Condition	Question	Note	Options	
1		How long have you been vegan (years)?	Please enter only the number, if you have been eating vegan for less than a year, enter 1	1	Number
2		How old are you?	Please enter a number only	2	Number
3		What is your highest level of education?		1	Secondary education or less
				2	Upper secondary education without maturity diploma
				3	Upper secondary education with maturity diploma
				4	Bachelor's degree or equivalent
				5	Master's degree
				6	Doctorate
4		Nationality		1	Slovak
				2	Czech
				3	Other
5		Sex		1	Female
				2	Male
6	Question 5 = 1	Are you currently pregnant or breastfeeding?		1	Pregnant
				2	Breastfeeding
				3	Pregnant and breastfeeding
				4	Neither
7		Do you use vitamin B_{12} supplements?	Tablets, drops and sprays with a defined vitamin B_{12} content are included, including multivitamins and B-complexes with a vitamin B_{12} content. Enriched foods and beverages, injectables, chewing gum, toothpaste, chlorella or other algae are not included.	1	Regularly
				2	Irregularly
				3	Not at all
8	Question 7 = 1, 2	What nutritional supplement do you use? *	Choose one or more answers (if you have more than one nutritional supplement)	1	Standalone vitamin B_{12} supplement
				2	Combination supplement (e.g. B-Complex, multivitamin)
9	Question 7 = 1, 2	How often do you take vitamin B_{12} supplements?	In case of irregular use, please estimate.	1	1x a week (weekly)
				2	2x a week
				3	3x a week
				4	4x a week
				5	5x a week
				6	6x a week
				7	7x a week (daily)
				8	Other frequency (describe)

10	Question 7 = 1, 2	What dose of vitamin B_{12} in micrograms (µg/mcg) are you taking?	Dose corresponds to the total amount of vit. B_{12} you take in one day. For example, if you take two tablets three times a week, this is the amount of vitamin B_{12} in two tablets. Please enter a number only. Decimal places are allowed.	1	Dose in µg:
				2	I do not know
11	Question 7 = 1, 2	What is the form of vitamin B_{12} in your nutritional supplement?	Select one or more answers (in case of a multiple forms)	1	Cyanocobalamin
				2	Methylcobalamin
				3	Adenosylcobalamin
				4	Hydroxocobalamin
				5	I do not know
12	Question 7 = 3	What are your reasons for not supplementing vitamin B_{12}?	Select one or more answers	1	I didn't know it was necessary on a vegan diet
				2	I haven't addressed it yet
				3	I get vit. B_{12} from other sources
				4	I have sufficient stores
				5	I'm worried about the effects of nutritional supplements
				6	Other (describe)
13	Question 12 = 3	What other sources do you get vit. B_{12} from?	Please list all your sources of vitamin B_{12}	1	text
14		Do you suffer or have suffered from any of the following diseases/ problems in the past?	Pernicious anemia, celiac disease, Crohn's disease, ulcerative colitis, atrophic gastritis, pancreatic insufficiency, gastrointestinal cancer, cobalamin (vitamin B_{12}) absorption disorder, decreased gastric acid production, gastric or small intestinal surgery. (affect the absorption of vitamin B_{12})	1	Yes
				2	No
15		Are you currently taking any of the following medicines?	Proton pump inhibitors (eg Pantoprazole, Omeprazole), H2 antagonists or other drugs to reduce stomach acid. (affect the absorption of vitamin B_{12})	1	Yes
				2	No
16		Have you informed your doctor about your vegan diet?		1	Yes
				2	No
17		Have you ever been tested for vitamin B_{12} status?	Not every blood test can detect vitamin B_{12} . Choose answer "yes" only if you know that test was focused specifically on the vitamin B_{12}.	1	Yes
				2	No
18	Question 17 = 1	Have you ever been diagnosed with vitamin B_{12} deficiency?		1	Yes
				2	No
19		Have you ever consulted your doctor or nutritionist about your vegan diet?		1	Yes
				2	No
20		From what sources do you get information on how to eat vegan properly?		1	Social media discussions
				2	friends and acquaintances
				3	Books
				4	Educational articles and videos
				5	Information materials for pro-vegan organizations
				6	Scientific articles and primary research
				7	Consultations with experts
				8	Other (describe)

21		Which of these sources is most important for you?	Which of these sources have you used the most to learn about vegan nutrition?	1	Social media discussions
				2	Friends and acquaintances
				3	Books
				4	Educational articles and videos
				5	Information materials for pro-vegan organizations
				6	Scientific articles and studies
				7	Consultations with experts
				8	Other (describe)
22		In what language do you get information about the vegan diet most often?		1	Slovak
				2	Czech
				3	English
				4	Other
23		How do you rate the availability of info on proper vegan nutrition in Slovak and Czech languages?		1	Sufficient
				2	Rather sufficient
				3	Rather insufficient
				4	Insufficient

REFERENCES

1. *Vou A*: Europe is going veg. Published online March 12, 2019. Accessed January 13, 2022. https://www.europeandatajournalism.eu/eng/News/Data-news/Europe-is-going-veg.
2. Ipsos: Vegan Trends & Demographics USA. Accessed January 13, 2022. https://www.ipsos-retailperformance.com/en/vegan-trends/.
3. Ipsos*:* Bezmasou Stravu Preferuje Desetina Mladých [A tenth of young people prefer a meat-free diet]; 2019. (Accessed January 13, 2022, in Czech). https://www.ipsos.com/cs-cz/bezmasou-stravu-preferuje-desetina-mladych.
4. *Vesanto M, Winston C, Levin S*: Position of the Academy of Nutrition and Dietetics: Vegetarian Diets. J Acad Nutr Diet. 2016;116(12):1970-1980. doi:10.1016/J.JAND.2016.09.025.
5. *Craig W.J. et al.:* The Safe and Effective Use of Plant-Based Diets with Guidelines for Health Professionals. Nutrients 2021;13(11):4144, doi: 10.3390/NU13114144.
6. *Madry E, Lisowska A, Grebowiec P, Walkowiak J*: The impact of vegan diet on B_{12} status in healthy omnivores: five-year prospective study. Acta Sci Pol Technol Aliment. 2012;11(2):209-212. Accessed January 13, 2022. https://pubmed.ncbi.nlm.nih.gov/22493163/.
7. *Selinger E, Kühn T, Procházková M, Anděl M, Gojda J*: Vitamin B_{12} Deficiency Is Prevalent Among Czech Vegans Who Do Not Use Vitamin B_{12} Supplements. Nutrients. 2019;11(12). doi:10.3390/NU11123019.
8. *Krajčovičová-Kudláčková M, Blažíček P, Kopčová J, Béderová A, Babinská K*: Homocysteine levels in vegetarians versus omnivores. Ann Nutr Metab. 2000;44(3):135-138. doi:10.1159/000012827.
9. *Agostoni C, Berni Canani R, Fairweather-Tait S, et al.*: Scientific Opinion on Dietary Reference Values for cobalamin (vitamin B_{12}). EFSA Journal. 2015;13(7):4150. doi:10.2903/J.EFSA.2015.4150.
10. *del Bo C, Riso P, Gardana C, Brusamolino A, Battezzati A, Ciappellano S*: Effect of two different sublingual dosages of vitamin B_{12} on cobalamin nutritional status in vegans and vegetarians with a marginal deficiency: A randomized controlled trial. Clin Nutr. 2019;38(2):575-583. doi:10.1016/J.CLNU.2018.02.008.
11. *Rajan S, Wallace JI, Brodkin KI, Beresford SA, Allen RH, Stabler SP*: Response of elevated methylmalonic acid to three dose levels of oral cobalamin in older adults. J Am Geriatr Soc. 2002;50(11):1789-1795. doi:10.1046/J.1532-5415.2002.50506.X.
12. *Koeder C., Perez-Cueto F. J. A.*: Vegan nutrition: a preliminary guide for health professionals. Crit Rev Food Sci Nutr. 2022: 1-38. doi: 10.1080/10408398.2022.2107997.
13. *Siebert AK, Obeid R, Weder S, et al*: Vitamin B_{12}–fortified toothpaste improves vitamin status in vegans: a 12-wk randomized placebo-controlled study. Am J Clin Nutr. 2017;105(3):618-625. doi:10.3945/AJCN.116.141978.
14. *Zant A, Awwad HM, Geisel J, Keller M, Obeid R*: Vitamin B_{12}-fortified toothpaste improves vitamin status in elderly people: a randomized, double-blind, placebo-controlled study. Aging Clin Exp Res. 2019;31(12):1817-1825. doi:10.1007/S40520-019-01125-6.
15. *Benham AJ, Gallegos D, Hanna KL, Hannan-Jones MT:* Vitamin B_{12} Supplementation Adequacy in Australian Vegan Study Participants. Nutrients. 2022;14(22):4781. doi:10.3390/NU14224781.
16. *Dawczynski C, Weidauer T, Richert C, Schlattmann P, Dawczynski K, Kiehntopf M:* Nutrient Intake and Nutrition Status in Vegetarians and Vegans in Comparison to Omnivores - the Nutritional Evaluation (NuEva) Study. Front Nutr. 2022;9:819106. doi:10.3389/FNUT.2022.819106/FULL.

17. *Gallego-Narbón A, Zapatera B, Barrios L, Vaquero MP:* Vitamin B_{12} and folate status in Spanish lacto-ovo vegetarians and vegans. J Nutr Sci. 2019;8:1-8. doi:10.1017/JNS.2019.2.
18. *Kristensen NB, Madsen ML, Hansen TH, et al.*: Intake of macro- and micronutrients in Danish vegans: Nutr J. 2015;14(1). doi:10.1186/S12937-015-0103-3.
19. *Světnička M, Sigal A, Selinger E, Heniková M, El-labababidi E, Gojda J*: Cross-Sectional Study of the Prevalence of Cobalamin Deficiency and Vitamin B_{12} Supplementation Habits among Vegetarian and Vegan Children in the Czech Republic. Nutrients. 2022;14(3). doi:10.3390/NU14030535.
20. *Chan CQH, Low LL, Lee KH:* Oral Vitamin B_{12} Replacement for the Treatment of Pernicious Anemia. Front Med (Lausanne). 2016;3(AUG). doi:10.3389/FMED.2016.00038.
21. *Obeid R.., Fedosov S.N., Nexo E.:* Cobalamin coenzyme forms are not likely to be superior to cyano- and hydroxyl-cobalamin in prevention or treatment of cobalamin deficiency. Mol Nutr Food Res, 2015;59(7):364, doi: 10.1002/MNFR.201500019.

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ORIGINAL ARTICLE

FOOD PURCHASING HABITS AT TRADITIONAL AND MODERN MARKETS AND CONSUMPTION OF NATURAL AND PROCESSED FOODS IN MOROCCAN HOUSEHOLDS

Imane Barakat[1,2], Sanaa El-Jamal[1], Houda Elfane[1], Mohammed Elayachi[1], Rekia Belahsen[1]

[1]Laboratory of Biotechnology, Biochemistry and Nutrition, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco
[2]Higher Institute of Professions Nurses and Health Techniques of Marrakesh, Morocco

ABSTRACT

Background. The locations of food purchase have an impact on the quality of food consumed.

Objective. To examine food purchasing habits at traditional and modern markets, their associated factors, and their effects on consuming natural and processed foods.

Material and methods. This work used a validated conceptual and methodological framework of a study conducted among 507 households in the Rabat-Salé-Kenitra region in Morocco. Data on sociodemographic and economic characteristics and the frequency of food purchasing were collected from household representatives through a population survey. The food frequency questionnaire was used to collect frequency of consumption of 20 foods, including 10 natural and 10 processed. The associations between the variables were studied by the *Chi*-square test with a level of significance of $p<0.05$.

Results. Among the households 70% were urban, 62% have nuclear families, 51.5% a size of 5 to 12 members, 41% middle standard of living, 87% frequented markets and souks (MS), and 19% frequented large and medium-sized stores (LMS) at least once a week. The households have in majority a frequency of natural foods consumption ≥ 3 times/week, including fresh vegetables (91%), olive oil (85%), and fresh fruit (84%); and processed foods, such as refined flours (68%), industrial cheese (65%) and industrial yoghurt (52%). MS and LMS frequentation were associated with environment ($p<0.001$), family type ($p=0.01$ and $p=0.002$, respectively), household size ($p=0.04$ and $p=0.002$ respectively) and standard of living ($p<0.001$). Foods whose consumption was associated with both MS and LMS frequentation included fresh vegetables ($p<0.001$) as natural foods and baked goods as processed foods (respectively, $p=0.01$ and $p=0.04$).

Conclusion. The results of this study argue for implementing a nutrition education strategy based on the choice of food purchase locations as well as the consumption of natural or processed foods as part of a sustainable Mediterranean diet.

Key words: *food stores, markets, souks, large and medium-sized supermarkets, natural foods, processed foods, Morocco*

INTRODUCTION

Food distribution systems in the emerging countries include traditional markets and modern stores such as hypermarkets, supermarkets, and minimarkets [1]. New modes of consumption and acquisition of foods [2] linked to the modern types of markets also emerged and are likely to convey new cultural values affecting tastes and eating habits [1], and favor the consumption of modern and industrial food products whose nutritional quality could be harmful to health [3]. This tends to abandon Mediterranean food model, which favors a healthy diet that is rich in biodiversity, respects the environment, and plays a beneficial role in the development of sustainable agriculture [4].

Although structured hypermarkets have acquired a good clientele, interest in local and farmers' markets is noted in both developed countries [5] and in developing countries in the southern Mediterranean area, where urban markets and daily or weekly rural souks persist in offering fresh and natural food products [6]. Indeed, food purchasing continues to be carried out from traditional markets [7] that represent spaces of exchange and communication that play a fundamental role both in sociability and in offering of various food choices. The traditional market favors the maintenance of personalized relations with the clientele [8] and preserves its social and cultural norms while granting payment facilities, unlike modern forms of markets [9, 10].

Corresponding author: Rekia Belahsen, Laboratory of Biotechnology, Biochemistry and Nutrition, Faculty of Sciences, Chouaib Doukkali University, El Jadida – 4000, Morocco, e-mail: b.rekia@gmail.com

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The factors that determine food purchase practices are related to the socioeconomic and demographic characteristics of households, the geographic and cultural origin of the population, and the characteristics of the market, specifically the availability of food products, and the guaranteed quality of both natural and processed products [8, 11]. In addition, food cost and socioeconomic levels are determining factors of food choice. However, increasing the amount of income available for food choices does not necessarily mean that individuals will have more balanced and healthy diets choices. Likewise, education and knowledge in terms of foods and nutrition are determinants of food choices. Thus, individuals who are educated and informed about healthy diets are more likely to make healthy food choices [12].

The purpose of the current work was to examine food purchasing habits in traditional and modern markets, their associated factors, and their effects on consuming natural and processed foods.

MATERIAL AND METHODS

This work is a part of a study based on a validated conceptual and methodological framework that was conducted on 507 households in the region of Rabat-Salé-Kenitra in Morocco [13].

Data collection

Data were collected from household representatives (HRs) who have a major role in food purchases through a survey on sociodemographic, socioeconomic, sociocultural, and dietary characteristics, as well as food shopping locations. The frequency of food consumption within households was collected by a food frequency questionnaire [14–16]. The latter was designed taking into account the methods of development of this type of instrument [17] and consisted of a closed list of 20 natural and processed foods. The natural foods studied were: whole wheat, fresh vegetables, pulses, fresh fruit, fish, olive oil, free-range chicken, free-range egg, fresh cheese and free-range butter. The processed foods studied were: refined flour, baked goods, corn flakes, potato chips, packaged milk, industrial cheese, industrial yogurt, industrial chicken, industrial eggs, and industrial butter.

Variables under study

1. Household characteristics: area of residence, family type, household size, and standard of living.
2. HRs characteristics: age, education, work status, marital status, ethnicity, and sources of food information.
3. Frequentation of markets and souks[1] (MS) and large and medium-sized stores (LMS) categorized into, 1) < once a week; 2) ≥ once a week.
4. Frequency of consumption of natural or processed foods, categorized as 1) < 3 times per week; 2) ≥ 3 times per week.

Statistical analysis

Statistical analysis was performed by SPSS for Windows (Statistical Package for the Social Sciences) version 21 and Microsoft Office Excel 2007. The *Chi*-square test was used to study associations between variables with a statistical significance level of $p<0.05$.

RESULTS

Characteristics of households and their representatives

The study results showed that 70% of the households are urban, 62% are nuclear families, 51.5% are composed of 5 to 12 members, 41% have an average standard of living, 87% go to the MS, and 19% go to the LMS, at least once a week. Moreover, 76% of the HRs are aged 35 to 65 years, 40% are illiterate, 13% have a higher education, 76% are inactive, 80% are married, 80% are of Arab ethnic origin, and all of them have as source of dietary information their parents and grandparents, 80% from the media, 59% from their entourage, 35% from health professionals, 26% from Internet websites, and 12% from scientific journals (Table 1).

Frequency of consumption of natural and processed foods within households

The majority of the surveyed households have a consumption frequency of ≥ 3 times per week of some natural foods, including fresh vegetables (91%), olive oil (85%), fresh fruits (84%), whole wheat (64%), and free-range butter (52%); and of some processed foods, namely, the packaged milk (90%), industrial butter (81%), refined flours (68%), industrial cheese (65%), and industrial yogurt (52%) (Figure1).

Associations of household characteristics and their representatives with MS and LMS frequentation

Data analysis revealed that household characteristics that have a significant association with the frequentation of both MS and LMS are household size ($p=0.04$, $p=0.002$; respectively), environment ($p<0.001$, $p<0.001$; respectively), family type ($p=0.01$, $p=0.002$; respectively), and living standard ($p<0.001$, $p<0.001$; respectively). On the other hand, HRs char-

1 In the Arab world, souks are ephemeral fairs, usually held weekly. They are also places for commercial transactions. They're almost always outdoors. There are rural souks and urban souks. It contains everything that people in a traditional society need in terms of food.

Table1. Characteristics of households and their representatives (n=507)

Characteristics *			Values (%)	CI 95%
Household characteristics				
Area of residence	Urban		355 (70)	[67-73]
	Rural		152 (30)	[27-33]
Family type	Nuclear		316 (62)	[58-67]
	Composed		191 (38)	[33-42]
Household size	2-4 members		246 (48.5)	[43.8-53.1]
	5-12 members		261 (51.5)	[46.9-56.2]
Standard of living	Low		156 (31)	[27-35]
	Average		208 (41)	[37-45]
	High		143 (28)	[25-32]
Food purchase locations	MS	< Once a week	65 (13)	[10.1-16]
		≥ Once a week	442 (87)	[84-89.9]
	LMS	< Once a week	409 (81)	[76.9-84.2]
		≥ Once a week	98 (19)	[15.8-23.1]
Household representatives characteristics				
Age groups (years)	20-34		122 (24)	[20.1-27,8]
	35-65		385 (76)	[72.2-79.9]
Education level	Without		209 (40)	[37.3-45.4]
	Preschool and primary		100 (20)	[16.2-23.3]
	Secondary school		78 (16)	[12.2-18.5]
	Qualifying secondary		53 (11)	[7.7-13.2]
	Higher education		67 (13)	[10-16.4]
Professional occupation	Active		122 (24)	[20.3-27.6]
	Inactive		385 (76)	[72.4-79.7]
Marital status	Single		41 (8)	[5.9-10.3]
	Married		405 (80)	[76.5-83.4]
	Divorced		42 (8)	[5.9-10.7]
	Widower		19 (4)	[2.2-5.5]
Ethnic origin	Arabs		403 (80)	[76-83]
	Berber		104 (20)	[17-24]
Food information sources	Parents and grand parents		507 (100)	[100-100]
	Family and entourage		297 (59)	[54.2-62.7]
	Health Professionals		175 (35)	[30.4-38.9]
	Scientific journals		62 (12)	[9.7-15]
	Internet Websites		132 (26)	[22.1-29.8]
	Media		404 (80)	[76.1-83.2]

* = Expressed in size (%); CI = Confidence interval; MS = markets and souks; LMS = large and medium-sized stores.

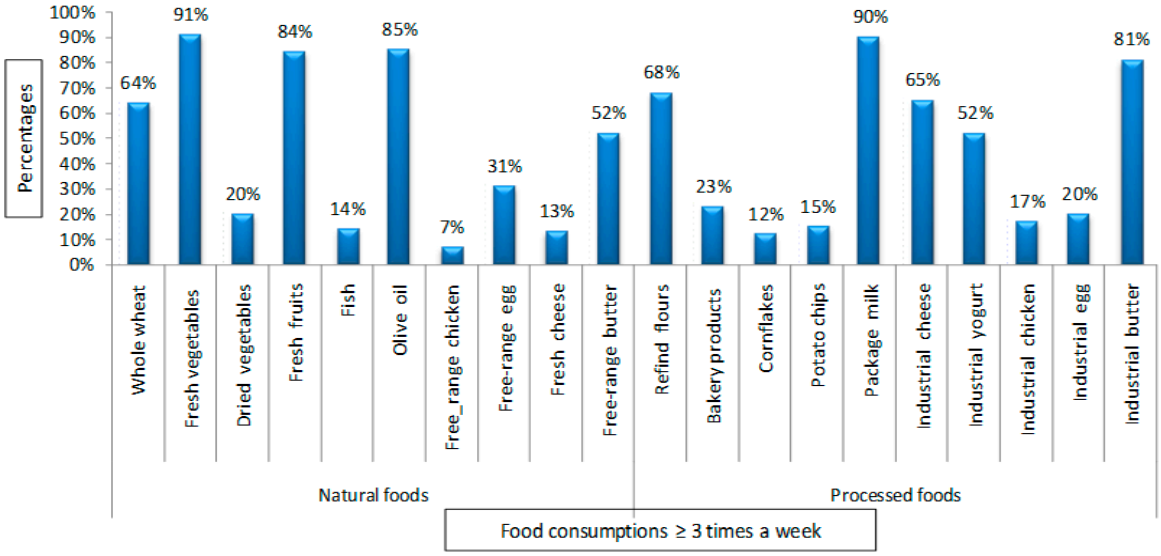

Figure 1. Distribution of households according to the frequency of consumption of natural and processed foods (n=507)

Table 2. Characteristics of households and their representatives associated with frequentation of MS and LMS (n=507)

Characteristics *		MS			LMS		
		<1/S (%)	**≥1/S (%)**	**P****	**<1/S (%)**	**≥1/S (%)**	**P****
Age groups (years)							
20-34		17 (14)	105 (86)	0.3 NS	103 (84)	19 (16)	0.1 NS
35-65		48 (12.5)	337 (87.5)		306 (79.5)	79 (20.5)	
Area of residence							
Urban		62 (17.5)	293 (82.5)	<0.001 ***	262 (74)	93 (26)	<0.001 ***
Rural		3 (2)	149 (98)		147 (97)	5 (3)	
Education level							
Without,		14 (6.7)	195 (93)	<0.001 ***	192 (92)	17 (8)	<0.001 ***
Preschool and primary		11 (11)	89 (89)		81 (81)	19 (19)	
Secondary school		6 (8)	72 (92)		58 (74)	20 (26)	
Qualifying secondary		17 (32)	36 (68)		37 (70)	16 (30)	
Higher education		17 (25)	50 (75)		41 (61)	26 (39)	
Professional occupation							
Active		23 (19)	99 (81)	0.02 ***	90 (74)	32 (26)	0.02 ***
Inactive		42 (11)	343 (89)		319 (83)	66 (17)	
Marital status							
Single		9 (22)	32 (78)	0.3 NS	24 (58.5)	17 (40.5)	0.001 ***
Married		49 (12)	356 (88)		330 (81.5)	75 (18.5)	
Divorced		5 (12)	37 (88)		39 (93)	3 (7)	
Widower		2 (10.5)	17 (89.5)		16 (84)	3 (16)	
Ethnic origin							
Arabic		57 (14)	346 (86)	0.04 ***	320 (79)	83 (21)	0.09 NS
Berber		8 (8)	96 (92)		89 (86)	15 (14)	
Food information sources							
Family and entourage	Yes	33 (11)	264 (89)	0.2 NS	241 (81)	56 (19)	0.7 NS
	No	32 (15)	178 (85)		168 (80)	42 (20)	
Health Professionals	Yes	32 (18)	143 (82)	0.01 ***	125 (71)	50 (29)	<0.001 ***
	No	33 (10)	299 (90)		284 (85.5)	48 (14.5)	
Scientific journals	Yes	14 (23)	48 (77)	0.02 ***	38 (61)	24 (39)	<0.001 ***
	No	51 (11.5)	394 (88.5)		371 (83)	74 (17)	
Internet Websites	Yes	32 (24)	100 (76)	<0.001 ***	88 (67)	44 (33)	<0.001 ***
	No	33 (9)	342 (91)		321 (86)	54 (14)	
Media	Yes	54 (13)	350 (87)	0.3 NS	320 (79)	84 (21)	0.09 NS
	No	11 (11)	92 (89)		89 (86)	14 (14)	
Family type							
Nuclear		49 (15.5)	267 (84.5)	0.01 ***	242 (77)	74 (23)	0.002 ***
Composed		16 (8)	175 (92)		167 (87)	24 (13)	
Household size							
2-4 members		42 (17)	204 (83)	0.04 ***	185 (75)	61 (25)	0.002 ***
5-12 members		23 (9)	238 (91)		224 (86)	37 (14)	
Standard of living							
Low		7 (4.5)	149 (95.5)	<0.001 ***	142 (91)	14 (9)	<0.001 ***
Average		29 (14)	179 (86)		169 (81)	39 (19)	
High		29 (20)	114 (80)		98 (68.5)	45 (31.5)	

* = Expressed in Size (%); ** = *Chi*-square test with a significance level of p<0.05; *** = Significant; NS = Not significant; MS: Markets and Souks; LMS: Large and Medium-sized Stores.

acteristics that had a significant association with MS frequentation were, ethnicity (p=0.04), education level (p<0.001), work status (p=0.02), consulting of health professionals (p=0.01), use of scientific journals (p=0.02), and internet websites (p<0.001). In addition, HRs characteristics that had a significant association with LMS frequentation, are age (p=0.003), education level (p<0.001), work status (p=0.02), marital status (p=0.001), consulting of health professionals (p<0.001), use of scientific journals (p<0.001) and internet websites (p<0.001) (Table 2).

Associations between frequentation of MS and LMS, and consumption frequency of natural and processed foods

The natural foods whose consumption was significantly associated with frequenting MS are whole wheat (p<0.001), fresh vegetables (p<0.001), fresh fruit (p<0.001), fish (p<0.001), and free-range chicken (p<0.001); and those whose consumption is significantly associated with the frequentation of LMS are whole wheat (p<0.001), fresh vegetables (p<0.001), dried vegetables (p<0.001), fish (p<0.001), and fresh cheese (p=0.02) (Table 3).

The processed foods whose consumption was significantly associated with MS frequentation were, cornflakes (p=0.01), potato chips (p=0.003), and industrial chicken (p=0.03); and those whose consumption is significantly associated with the frequentation of LMS are, bakery products (p=0.04), cornflakes (p=0.03), potato chips (p<0.001) and industrial yogurt (p=0.01) (Table 4).

DISCUSSION

The nutritional quality of foods introduced in recent decades has been reported to be incriminated in the etiology of many chronic diseases which are on the rise in both developed and developing countries, including Morocco. These diseases have been linked to the negative effects of the complex interplay of multiple nutritional factors directly linked to the excessive consumption of new foods and industrialized foods high in fats, refined sugars and salt. In addition, consumers' food choices are influenced by the global emerging trends. On the other hand, food preparation procedures even simple affect their initial nutritional quality. Around the world, the processed food sector is increasingly contributing to both the economy and people's eating habits [18]. Also, as in western countries worldwide, supermarkets and hypermarkets are expanding in developing countries including, in Morocco [19].

However, the present study shows that, markets and souks are more frequently visited by the studied population than large or medium-sized stores. This result proves that traditional trade is still resisting, despite the development of modern distribution networks. This can be explained by the fact that this type of trade fulfills, beyond its economic function, a mission of social link, personalized relationships, and payment facilities [1, 9, 20, 21]. All these advantages that are difficult to find in modern stores encourage the consumer to adopt a selective behavior towards traditional food shopping markets [22, 23].

The present study revealed also several statistically significant associations of household characteristics and their representatives with MS and LMS frequentation. Indeed, there are significant associations of sociodemographic, sociocultural, and socioeconomic characteristics with food practices as a whole and more specifically with food shopping [24–26]. It should be noted that purchasing power and area of residence are among the determining factors in the choice of places to buy food. Thus, frequenting supermarkets generally characterizes the wealthy socio-professional category living in urban areas and not those with low incomes, living in rural areas [27]. On the other hand, the size of the household would be proportional to the food expenses within the household and would direct the food purchase choices toward the most economically accessible sale points. Consistent with these data, it has been reported that the structure and quantity of foods and dishes consumed as well as in food preparation and consumption practices differ according to household size [11]. Monthly income and standard of living would also be proportional to food expenditure. In addition, income has been reported as a moderating factor of the relationships between involvement and intention to buy with food purchasing behavior [28]. Furthermore, low family income has been consistently associated with poorer food quality [11]. Another factor involved is ethnicity which is also associated with food purchasing practices. The cultural heritage specific to each ethnic group is indeed among others factors which can influence the choice of food purchases [29]. Also, the influence of the ethnic origin of the family in determining the eating behavior of individuals has been confirmed [30].

Educational level is also recognized as a determinant of food preferences. Indeed, food-buying behavior has been reported to be influenced by the education level [28]. Work status can also lead individuals to resort to local food stores due to lack of time and availability [29, 31].

Furthermore, the type of family influences the selection of food markets. Indeed, family members, depending on their nuclear or extended type, are involved in guiding food choices within the household. The extended family, which generally includes grandparents, aunts, uncles, etc., tries to ensure that good eating habits are adopted by family members and thus

Table 3. Associations between natural food consumption and frequentation of MS and LMS (n=507)

Food purchase locations \ Foods*		Whole wheat		Fresh vegetables		Dried vegetables		Fresh fruits		Fish		Olive oil		Free-range chicken		Free-range eggs		Fresh cheese		Free-range butter	
		<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w	<3/w	≥3/w
MS	<1/w	65 (36%)	-	46 (100%)	19 (4%)	54 (13%)	11 (11%)	39 (49%)	26 (6%)	46 (11%)	19 (28%)	12 (16%)	53 (12%)	45 (10%)	20 (53%)	48 (14%)	17 (11%)	54 (12%)	11 (16%)	32 (13%)	33 (12%)
	≥1/w	115 (64%)	327 (100%)	-	442 (96%)	353 (87%)	289 (89%)	40 (51%)	402 (94%)	392 (89%)	50 (12%)	64 (84%)	378 (88%)	424 (90%)	18 (47%)	300 (88%)	142 (89%)	386 (88%)	56 (84%)	209 (87%)	233 (88%)
	P	<0.001***		<0.001***		0.5 NS		<0.001***		<0.001***		0.4 NS		<0.001***		0.3 NS		0.3 NS		0.7 NS	
LMS	<1/w	128 (71%)	281 (86%)	23 (50%)	386 (84%)	344 (84%)	65 (65%)	59 (74%)	350 (82%)	394 (90%)	15 (22%)	65 (85%)	344 (80%)	378 (81%)	31 (82%)	284 (82%)	125 (79%)	348 (79%)	61 (91%)	201 (83%)	208 (78%)
	≥1/w	52 (29%)	46 (14%)	23 (50%)	75 (16%)	63 (16%)	35 (35%)	20 (25%)	78 (18%)	44 (10%)	54 (78%)	11 (15%)	87 (20%)	91 (19%)	7 (18%)	64 (18%)	34 (21%)	92 (21%)	6 (9%)	40 (17%)	58 (22%)
	P	<0.001***		<0.001***		<0.001***		0.1 NS		<0.001***		0.2 NS		0.8 NS		0.4 NS		0.02***		0.1 NS	

* = Expressed in size (%); **= *Chi*-square test with significance level p<0.05; *** = Significant; NS = Not Significant; MS = Markets and souks; LMS = Large and medium-sized stores; w= week.

Table 4. Associations between processed food consumption and frequentation of MS and LMS (n=507)

Food purchase locations \ Foods*		Refined flour		Bakery products		Cornflakes		Potato chips		Package milk		Industrial cheese		Industrial yogurt		Industrial chicken		Industrial egg		Industrial butter	
		<3/S	≥3/S	<3/S	≥3/S	<3/S	≥3/S	<3/S	≥3/S	<3/S	≥3/S	<3/S	≥3/S	<3/S	≥3/S	**<3/S**	**≥3/S**	**<3/S**	**≥3/S**	**<3/S**	**≥3/S**
MS	**<1/S**	18 (11%)	47 (13%)	42 (11%)	23 (19%)	56 (13%)	9 (15%)	47 (11%)	18 (23%)	5 (10%)	60 (13%)	22 (12%)	43 (13%)	37 (15%)	28 (11%)	60 (14%)	5 (6%)	51 (13%)	14 (14%)	15 (16%)	50 (12%)
	≥1/S	142 (89%)	300 (87%)	346 (89%)	96 (81%)	391 (87%)	51 (85%)	383 (89%)	59 (77%)	47 (90%)	395 (87%)	155 (88%)	287 (87%)	205 (85%)	237 (89%)	361 (86%)	81 (94%)	355 (87%)	87 (86%)	81 (84%)	361 (87%)
	P**	0.5 NS		0.01***		0.6 NS		0.003***		0.5 NS		0.8 NS		0.1 NS		0.03***		0.7 NS		0.4 NS	
LMS	**<1/S**	125 (78%)	284 (82%)	306 (79%)	103 (87%)	355 (79%)	54 (90%)	387 (90%)	22 (29%)	39 (75%)	370 (81%)	145 (82%)	264 (80%)	206 (85%)	203 (77%)	334 (79%)	75 (87%)	322 (79%)	87 (86%)	83 (87%)	326 (79%)
	≥1/S	35 (22%)	63 (18%)	82 (21%)	16 (17%)	92 (21%)	6 (10%)	43 (10%)	55 (71%)	13 (25%)	85 (19%)	32 (18%)	66 (20%)	36 (15%)	62 (23%)	87 (21%)	11 (13%)	84 (21%)	14 (14%)	13 (13%)	85 (21%)
	P**	0. 3 NS		0.04***		0.03***		<0.001***		0.3 NS		0.6 NS		0.01***		0.09 NS		0.1 NS		NS	

* = Expressed as size (%); ** = *Chi*-square test with significance level p<0.05; *** = Significant; NS = Not significant; MS = Markets and souks; LMS = Large and medium-sized stores.

gives their opinion on the food shopping for the household [32]. Also, the family represents the decision-making unit that is made by the couple [33]. In the same context, marital status is a factor associated with the choice of food shopping locations, as the opinions of both spouses are taken into consideration in any type of food purchase for the benefit of the family [11].

Finally, sources of food information are associated with choices for food purchase locations. However, vigilance and the ability to distinguish between good and bad information are required in this respect, to avoid places that offer foods that are potentially harmful to health and/or that are advised by people and/or authorities who are not qualified in the matter [34, 35, 36].

This study also showed that the majority of the surveyed households consume certain natural foods quite frequently (at least three times a week), including fresh vegetables and fruits, olive oil, whole wheat, and free-range butter. The positive effect of all these natural foods is reported in the literature. Indeed, the benefits of fresh vegetables and fruits are well known and include a diverse group of plant materials that vary considerably in energy and nutrient content. In addition, fruits and vegetables provide dietary fibers that are associated with a lower incidence of cardiovascular diseases and obesity, provide vitamins and minerals, and are sources of phytochemicals with antioxidants activities, phytoestrogens, and as anti-inflammatory agents [37, 38, 39]. Olive oil is also a foodstuff that has proven its virtues for good health, and well-being as well as its beneficial impact on lipid metabolism [40]. A study covering 28 years of follow-up showed indeed that higher consumption of olive oil was associated with a 19% lower risk of mortality from cardiovascular diseases, a 17% lower risk of mortality from cancer, a 29% lower risk of mortality from neurodegenerative diseases, and an 18% lower risk of mortality from respiratory diseases [41]. Regarding whole wheat consumption, whole grain intake has been shown to reduce the risk of several non-communicable diseases, including cardiovascular diseases, type 2 diabetes, and some types of cancer [42–44]. Unfortunately, despite its recognized nutritional and health benefits, whole grain consumption is below recommendations in almost all countries of the world [45]. Regarding the consumption of free-range butter, few studies have investigated its effect on health and particularly on the lipid profile compared with industrially produced butter. Nevertheless, there is generally considerable interest in free-range products, given their higher content of bioactive compounds [46].

On the other hand, the present study also showed that certain processed foods were quite frequently consumed (at least three times a week) by the majority of the investigated population. It is about, packaged milk, industrial butter, industrial cheese, industrial yogurt, and refined flour. Regarding the consumption of milk and other dairy products, recent evidence has shown controversial results regarding their role in deleterious processes such as inflammation [47]. The effect of dairy consumption on health has received considerable attention over the past decade. However, there is uncertainty about their health effects, as several prospective cohort studies have shown conflicting results. On the other hand, no association between the consumption of dairy products and the risk of all-cause mortality has been demonstrated in all the systematic reviews and meta-analyses on the risk of mortality linked to the consumption of dairy products [48]. Concerning refined flour, it has been reported that its consumption has adverse health effects, including an increased risk of cardiovascular diseases, type 2 diabetes, and obesity [49], which could be linked to a loss of both dietary fibers and mineral content by the refining of cereals as well as an increase in carbohydrate content [50].

The present study revealed that among the natural foods, the consumption of whole wheat, fresh vegetables, fresh fruits, fish, and free-range chicken is significantly associated with the frequentation of MS. Indeed, markets and souks represent a shopping area where various food products used daily in Moroccan cuisine are available. Moreover, MS offer healthy foods with nutritional virtues for health, given their richness in dietary fibers, minerals and vitamins, and proteins of high biological value. This study showed also that the natural foods whose consumption is associated with the frequentation of LMS are whole wheat, fresh vegetables, dried vegetables, fish, and fresh cheese. This justifies the diversity of foods offered by the LMS, which do not only offer processed foods as is generally assumed.

The data reported here also revealed that the processed foods whose consumption is significantly associated with the frequentation of MS are bakery products and industrial chicken. This diversity of available foods (natural and processed) is explained by the fact that MS provide everything that the populations require in terms of foodstuffs while also providing modern sales points such as bakeries and pastry shops, grocery stores, butcher shops, etc. Admittedly, LMS usually offer sophisticated or refined products while MS continue to be the priority recourse for the purchase of natural or traditional products [51, 52].

The present study did not, however, find an exclusive statistically significant association between the consumption of natural foods and the frequentation of traditional markets, or between the consumption of modern foods and the frequentation of LMS.

CONCLUSION

This work investigated factors associated with food purchasing habits at traditional and modern markets, as well as its implication on the consumption of natural and processed foods. These results will serve as the basis for the establishment of a nutrition education strategy on the choice of places for food shopping and on the consumption of natural and processed foods in the context of a sustainable Mediterranean diet.

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Conflicts of interest
The authors declare that they have no conflicts of interest.

REFERENCES

1. *Amine A., Lazzaoui N.*: Rôle de la distribution moderne dans l'évolution des pratiques de consommation dans les pays émergents: Cas de la distribution alimentaire au Maroc. Revue Marocaine de Recherche en Management et Marketing 2009; (1).
2. *Amine A.*: La grande distribution dans les pays émergents: caractéristiques, enjeux et perspectives. Marché et organisations 2012;15(1):117-41.
3. *Van Diepen S., Scholten AM., Korobili C., Kyrli D., Tsigga M., Van Dieijen T., et al.:* Greater Mediterranean diet adherence is observed in Dutch compared with Greek university students. Nutr Metab Cardiovasc Dis 2011;21(7):534-40.
4. *Belahsen R.*: Nutrition transition and food sustainability. Proc Nutr Soc 2014;73(3):385-8.
5. *Laisney C.*: L'évolution de l'alimentation en France: tendances émergentes et ruptures possible. Futuribles 2011;5-23.
6. *Mermier F., Peraldi M.*: Mondes et places du marché en Méditerranée; formes sociales et spatiales de l' échange. Paris 2011.
7. *Hannah C., Davies J., Green R., Zimmer A., Anderson P., Battersby J.,* et al.: Persistence of open-air markets in the food systems of Africa's secondary cities. Cities 2022;124:103608.
8. *Belloute R., Diour M.*: Comportement et critères d'achat des produits alimentaires au Maroc et les facteurs qui les influencent. Mersenne 2014;6 (141116 ISSN 2111-4706):1-14.
9. *Gahinet MC.*: The dimensions of proximity applied to food purchases. Revue d'économie régionale urbaine 2018;(5):1367-90.
10. *Goldman A., Ramaswami S., Krider RE.*: Barriers to the advancement of modern food retail formats: theory and measurement. Journal of Retailing 2002;78 (4):281-95.
11. *French SA., Tangney CC., Crane MM., Wang Y., Appelhans BM.*: Nutrition quality of food purchases varies by household income: the Shopper study. BMC Public Health 2019;19(1):231.
12. *Langley-Evans S.: M. J. Gibney, B. M. Margetts, J. M. Kearney* and *L. Arab* (editors). Public Health Nutrition. Oxford: Blackwell Science 2004. pp. 378. ISBN 0632056274**».** British Journal of Nutrition. Cambridge University Press 2006;95(1):214.
13. *Barakat I., Elayachi M., Belahsen R.:* Validation of conceptual and methodological framework for the study of dietary practices and nutritional status of an adult population. Rocz Panstw Zakl Hig 2022;73(2):199-207.
14. *Monteiro CA., Levy RB., Claro RM., Castro IRR de., Cannon G.*: A new classification of foods based on the extent and purpose of their processing. Cad Saude Publica 2010;26(11):2039-49.
15. *Moubarac JC., Martins APB., Claro RM., Levy RB., Cannon G., Monteiro CA.*: Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. Public Health Nutr 2013;16(12):2240-8.
16. *Moubarac JC., Batal M., Martins APB., Claro R., Levy RB., Cannon G., et al.*: Processed and ultra-processed food products: consumption trends in Canada from 1938 to 2011. Can J Diet Pract Res 2014;75(1):15-21.
17. *Rutishauser IH.*: Dietary intake measurements. Public Health Nutrition 2005; 8(7a):1100-7.
18. *Cordain L., Eaton SB., Sebastian A., Mann N., Lindeberg S., Watkins BA., O'Keefe JH., Brand-Miller J.*: Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 2005;81(2):341-54. doi: 10.1093/ajcn.81.2.341. PMID: 15699220.
19. *Reardon T., Timmer CP.*: The Economics of the Food System Revolution. Annual Review of Resource Economics 2012;4(1):225-64.
20. *Aladwani AM.*: A quality-facilitated socialization model of social commerce decisions. Int. J. Info Management 2018;40:1-7.
21. *Busalim AH., Hussin ARC.*: Understanding social commerce: A systematic literature review and directions for further research. Int J. Information Management 2016;36(6, Part A):1075-88.
22. *Skallerud K., Wien AH.*: Preference for local food as a matter of helping behaviour: Insights from Norway. Journal of Rural Studies 2019;67:79-88.
23. *Veeck A., Leingpibul T., Xie H., Veeck G.*: The role of personal factors in attitudes toward the adoption of new consumption behaviors in developing food systems. Appetite 2020; 149:104614.
24. *Aydın G.*: Do Personality Traits and Shopping Motivations Affect Social Commerce Adoption Intentions? Evidence from an Emerging Market. Journal of Internet Commerce 2019;18(4):428-67.
25. *Idoko EC., Ukenna SI., Obeta CE.*: Determinants of shopping mall patronage frequency in a developing economy: Evidence from Nigerian mall shoppers.

Journal of Retailing and Consumer Services 2019;48:186-201.
26. *Kusumawaty Y.*: Determinants of cross-shopping behaviour among modern and traditional food retail stores in Indonesia: An empirical analysis of Riau Province [Thesis]. Curtin University; 2016.
27. *El Mimouni D.*: Enquête grande distribution: où les Marocains font-ils leurs courses? Sunergia 2020.
28. *Gnoufougou D.*: Effet modérateur du niveau d'éducation et du niveau de revenu dans les relations entre implication du consommateur et intention d'achat et comportement d'achat biologique. Recherches et Pratiques Marketing 2021;(5).
29. *Reddy G., van Dam RM.*: Food, culture, and identity in multicultural societies: Insights from Singapore. Appetite 2020;149:104633.
30. *Labossiere W., Bouheni FB., Bellelah M.*: Effect of Ethnic Socialization Factors on Determination of Ethnicity and Consumption Behavior. La Revue des Sciences de Gestion 2018;293(5):61-70.
31. *Çalışkan C., Sabbağ Ç., Dedeoğlu B.*: The Importance of the Occupational Factors in Local Food Consumption Behavior. Anais Brasileiros de Estudos Turísticos - ABET. 2019; 9.
32. *Lindsay AC., Wallington SF., Lees FD., Greaney ML.*: Exploring How the Home Environment Influences Eating and Physical Activity Habits of Low-Income, Latino Children of Predominantly Immigrant Families: A Qualitative Study. International Journal of Environmental Research and Public Health 2018;15(5):978.
33. *Sdiri I., Bendhia B.*: Les déterminants de la décision d'achat du couple : Contexte Tunisien. Revue Internationale des Sciences de Gestion 2019;2(3).
34. *Clavier V.*: La place de l'information dans les pratiques professionnelles des diététiciennes : au croisement des missions d'éducation, de prévention et de soin. I2D - Information, données documents 2019;1(1):114-33.
35. Haut Commissariat au Plan (Maroc).: Indicateurs Sociaux. HCP 2016. https://www.hcp.ma/region-drda/attachment/834622/.
36. *Quaidoo EY., Ohemeng A., Amankwah-Poku M.*: Sources of nutrition information and level of nutrition knowledge among young adults in the Accra metropolis. BMC Public Health 2018;18:1323.
37. *Morris AL., Mohiuddin SS.*: Biochemistry, Nutrients. In: StatPearls. Treasure Island (FL): Stat Pearls Publishing 2022.
38. Organisation Mondiale de la Santé (OMS): Alimentation Saine. 2018. https://www.who.int/fr/news-room/fact-sheets/detail/healthy-diet.
39. *Slavin JL., Lloyd B.*: Health benefits of fruits and vegetables. Adv Nutr 2012;3(4):506-16.
40. *Tomé-Carneiro J., Crespo MC., López de Las Hazas MC., Visioli F., Dávalos A.*: Olive oil consumption and its repercussions on lipid metabolism. Nutr Rev 2020;78(11):952-68.
41. *Guasch-Ferré M., Li Y., Willett WC., Sun Q., Sampson L., Salas-Salvadó J., et al.* : Consumption of Olive Oil and Risk of Total and Cause-Specific Mortality Among U.S. Adults. Journal of the American College of Cardiology 2022;79(2):101-12.
42. *Aune D., Keum N., Giovannucci E., Fadnes LT., Boffetta P., Greenwood DC., et al.*: Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. BMJ 2016;353:i2716.
43. *Chanson-Rolle A., Meynier A., Aubin F., Lappi J., Poutanen K., Vinoy S., et al.*: Systematic Review and Meta-Analysis of Human Studies to Support a Quantitative Recommendation for Whole Grain Intake in Relation to Type 2 Diabetes. PLoS One 2015;10(6):e0131377.
44. *Johnson J., Wallace TC.*: Introduction to Whole Grains and Human Health. In: Whole Grains and their Bioactives. John Wiley & Sons, Ltd 2019. p. 1-17.
45. *Meynier A, Chanson-Rollé A, Riou E.* Main Factors Influencing Whole Grain Consumption in Children and Adults-A Narrative Review. Nutrients. 2020;12(8):2217. doi:10.3390/nu12082217.
46. *Werner LB., Hellgren LI., Raff M., Jensen SK., Petersen RA., Drachmann T., et al.*: Effects of butter from mountain-pasture grazing cows on risk markers of the metabolic syndrome compared with conventional Danish butter: a randomized controlled study. Lipids Health Dis 2013;12:99.
47. *Ulven SM., Holven KB., Gil A., Rangel-Huerta OD.*: Milk and Dairy Product Consumption and Inflammatory Biomarkers: An Updated Systematic Review of Randomized Clinical Trials. Adv Nutr 2019;10(suppl_2):S239-50.
48. *Cavero-Redondo I., Alvarez-Bueno C., Sotos-Prieto M., Gil A., Martinez-Vizcaino V., Ruiz JR.*: Milk and Dairy Product Consumption and Risk of Mortality: An Overview of Systematic Reviews and Meta-Analyses. Adv Nutr 2019;10(suppl_2):S97-104.
49. *Gaesser GA.*: Perspective: Refined Grains and Health: Genuine Risk or Guilt by Association? Adv Nutr 2019;10(3):361-71.
50. *Jeantet R., Croguennec T., Schuck P., Brule G.*: Handbook of Food Science and Technology 2: Food Process Engineering and Packaging. John Wiley & Sons 2016. 346 p.
51. *Potvin L.*: Les aliments ultra-transformés, un concept utile pour la santé publique. Can J Public Health 2019;110(1):1-3.
52. *Sproesser G., Ruby MB., Arbit N., Akotia CS., Alvarenga M dos S., Bhangaokar R.,* et al.: Understanding traditional and modern eating: the TEP10 framework. BMC Public Health 2019;19(1):1606.

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ORIGINAL ARTICLE

PREVALENCE AND PREDICTORS OF DEPRESSION, ANXIETY, AND STRESS AMONG RECOVERED COVID-19 PATIENTS IN VIETNAM

Le Thanh Thao Trang[1,2], Cua Ngoc Le[1,3], Nirachon Chutipatana[1,3], Shamarina Shohaimi[4], Charuai Suwanbamrung[1,3]

[1]Master of Public Health Program in Public Health Research, School of Public Health, Walailak University, Nakhon Si Thammarat, Thailand
[2]Faculty of Basic Science and Public Health, Dong Thap Medical College, Dong Thap, Vietnam
[3]Excellent Center for Dengue and Community Public Health (EC for DACH), School of Public Health, Walailak University, Nakhon Si Thammarat, Thailand
[4]Department of Biology, Faculty of Science, University Putra Malaysia, Malaysia

ABSTRACT

Background. During the COVID-19 pandemic, a high prevalence of mental health distress has been reported among people who have recovered from the disease.

Objectives. To assess the prevalence of depression, anxiety, and stress as well as identify predictors among recovered COVID-19 patients after more than six months of being discharged in Dong Thap Province, Vietnam.

Material and methods. The cross-sectional study was conducted among 549 eligible participants recruited by stratified sampling. Data was collected using the depression, anxiety and stress scale – 21 items had Content Validity Index = 0.9, and Cronbach's alpha for depression, anxiety and stress sub-scales were 0.95, 0.81, and 0.86, respectively. Descriptive statistics were used to measure the prevalence levels and distribution of characteristics of the participant, while factors influencing depression, anxiety, and stress were predicted using binary logistic regression.

Results. The overall prevalence of depression, anxiety, and stress were 24.8% (95% CI: 21.2-28.6), 41.5% (95% CI: 37.4-45.8), and 25.3% (95% CI: 21.7-29.2), respectively. The predictors of depression were living in urban area (OR = 1.97; 95% CI: 1.27-3.08), holding a bachelor's degree (OR:3.51; 95% CI: 1.13-10.8), having a high monthly income (OR: 2.57; 95% CI: 1.03-6.38), diabetes (OR: 2.21; 95% CI: 1.04-4.68), heart disease (OR: 3.83; 95% CI: 1.79-8.17), respiratory disease (OR: 3.49; 95% CI: 1.24-9.84), and diarrhea (OR: 4.07; 95% CI: 1.06-15.6). Living in the urban area (OR: 1.57; 95% CI: 1.07-2.29), having sleep disturbance (OR: 2.32; 95% CI: 1.56-3.46), and fatigue (OR: 1.57; 95% CI: 1.03-2.39) were predictors for anxiety. Having respiratory disease (OR: 3.75; 95% CI: 1.47-9.60) or diarrhea (OR: 4.34; 95% CI: 1.18-15.9) were predictors of stress.

Conclusion. People who have recovered from COVID-19 should be assessed for symptoms of depression, anxiety, and stress. Primary healthcare providers should develop interventions to support their recovery.

Key words: *prevalence, predictor, depression, anxiety, stress, COVID-19*

Abbreviations: DASS-21: The 21-item depression anxiety and stress scale; DAS: Depression, anxiety, and stress; CSS: Current signs and symptoms; UD: underlying disease; COVID-19: Coronavirus disease. VND: Vietnam Dong

INTRODUCTION

The coronavirus disease (COVID-19) has had a devastating impact on global health, with over 663 million confirmed cases and 6.7 million deaths as of December 31st, 2022 [50]. Recent studies have shown that COVID-19 has physical and long-term psychological effects on recovered patients [23, 25, 38]. Studies have shown that COVID-19 patients often experience severe psychological stress during hospitalization, which can continue even after they are discharged [36]. Depression is a common mental illness marked by persistent feelings of sadness and disinterest in previously enjoyable activities, as well as a lack of energy and the inability to carry out everyday activities [2, 33]. Anxiety is an emotional state characterized by a feeling of fear, dread and

Corresponding author: Charuai Suwanbamrung, Master of Public Health Program in Public Health Research, Excellent Center for Dengue and Community Public Health (EC for DACH), School of Public Health, Walailak University, Thasala District, Nakhon Si Thammarat Province +6680160, Thailand, Tel: +66 81 479 3034, E-mail: scharuai@wu.ac.th or yincharuai@gmail.com.

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uneasiness [1, 29, 34], while stress is a feeling of emotional or physical tension [3, 30, 34].

As the number of COVID-19 cases continues to increase, the number of discharged COVID-19 patients is also rising. The COVID-19 pandemic has caused to an increase in depression and anxiety by 27.6% and 25.6% globally, respectively [48]. Hospital-discharged patients, in particular, may experience mental health symptoms due to risk factors such as infection, isolation, lockdown, social factors and job-related factors [6]. Within three months of being discharged, more than half of recovered patients have reported experiencing symptoms that were not present prior to their COVID-19 infection, such as fatigue, cognitive symptoms, and dyspnea [8, 32]. Furthermore, survivors posted a high prevalence of mental health issues after three months up to one year following diagnosis [15, 31, 52], with depression, anxiety, and stress (DAS) scores appearing to be significantly higher in patients infected with COVID-19 than in healthy control groups [16, 27]. Individuals with severe mental illness have a shortened life expectancy, a 10-20 years shorter lifespan than the general population [49], and are at a higher risk for suicide [7]. Studies have shown that at least 90% of suicide cases are related to mental disorders, particularly depression and anxiety [4, 7, 40]. The mental health has been severely impacted due to this crisis [49].

By the end of December 2022, over 11.5 million cases and over 43,000 deaths have been recorded in Vietnam [14]. While the impacts of COVID-19 on psychological have been well-documented [48], recent studies on mental health in Vietnam during the pandemic have primarily focused on the general population [21, 43] and healthcare workers [12, 45]. However, the psychological consequences of COVID-19 on patients discharged from the hospital have not been fully explored. The objectives of this study were to assess the prevalence of DAS and identify factors influencing DAS among recovered COVID-19 patients after more than six months of being discharged in Vietnam. The result is essential to develop effective interventions to support their recovery and improve their overall well-being.

MATERIAL AND METHODS

Study design and population

A cross-sectional study was conducted among people who have recovered from COVID-19 in Dong Thap Province, Vietnam, from August to October 2022. The participants must meet the following criteria: (1) aged 18 or older, (2) have been infected with COVID-19, and (3) have been discharged from the hospital for treatment of COVID-19 for more than six months.

The sample size was calculated based on a standard formula of prevalence [20] where n is the sample size of the study, Z = 1.96 (95% confidence level), p is the estimated proportion of the target population (p = 0.31) [28], and d is desired precision (d = 0.04). In order to obtain reliable data, the researcher has increased the sample size by 10%, accounting for 565 participants.

According to authorities of the Dong Thap Department of Health, participants were recruited by stratified sampling from the database of COVID-19 patients in Dong Thap Province. With a total of 28,638 people discharged from hospitals around the province from January 2022 to June 2022, eligible participants were divided into 12 subgroups based on location (12 districts). The sample size of each subgroup was proportionate to the patient population. In each subgroup, research subjects were selected based on simple random sampling. The data was collected from 1st October to 15th November 2022.

Research instrument

Participants were instructed by well-trained research assistants to complete a structured questionnaire consisting of three parts. Part 1 included 8 items about the sociodemographic characteristics of the respondent, such as gender, age, living area, marital status, education level, monthly income (calculated in Vietnam Dong (VND)), occupation, and having family members who had COVID-19 infection. Part 2 (6 items) gathered disease information. Participants were asked 'yes/no' questions to indicate whether they had any underlying diseases (hypertension, heart disease, cancer, respiratory disease, diabetes, kidney disease) and experienced any current signs and symptoms after the COVID-19 stage (cough, chest pain, headache, shortness of breath, dizziness, fatigue, joint pain, diarrhea, decreased appetite, change in smell, forgetfulness). Types of COVID-19 diagnosis were categorized into 5 levels based on medical record (asymptomatic, mild, moderate, severe, critical) and types of COVID-19 treatment were classified into 2 types, whether required oxygen/ventilator. The length of hospital stay was calculated as the number of days in the hospital for COVID-19 treatment. Sleep disturbance was categorized by the average number of sleeping hours per day, with more than 9 hours or less than 7 hours per day classified as sleep disturbance [18].

In part 3, the 21-item depression anxiety and stress scale (DASS-21) was used to assess DAS symptoms [26], which was validated, reliable, and widely used during the COVID-19 pandemic [9, 17, 46, 51] and also in Vietnam [22, 44]. This self-reported instrument was developed by *Lovibond* and *Lovibond* [26] and consisted of 3 sub-scales, with a total of 21-item questions on negative emotion over the past week, with a 4-point

Likert scale (0 = Did not apply to me at all; 1 = applied to me some degree or some of the time; 2 = applied to me to a considerable degree or a good part of the time; 3 = applied to me very much, or most of the time) [26]. With 7 items for each sub-scale, the total score of each scale was multiplied by 2 to calculate the final score based on the scale manual [26]. The total depression subscale score was categorized as normal (0-9), mild (10-13), moderate (14-20), severe (21-27), and extremely severe (≥28). The total anxiety subscale score was categorized as normal (0-7), mild (8-9), moderate (10-14), severe (15-19), and extremely severe (≥20). The total stress subscale score was classified as normal (0-14), mild (15-18), moderate (19-25), severe (26-33), and extremely severe (≥34) [26]. The content validity of DASS-21 was assessed by three experts through the Content Validity Index to be 0.9. Regarding internal consistency reliability, a pilot study was conducted on a sample of 30 participants to measure Cronbach's alpha. In the present study, Cronbach alpha for DAS sub-scales were 0.95, 0.81, and 0.86, respectively.

Data analysis

Descriptive statistics were used to characterize the study population. Categorical variables were displayed as frequency and percentages. The prevalence of DAS were calculated using percentages. Before running statistical analyses, the *Kolmogorov-Smirnov* test was performed to test for normality of distribution, and the Variance Inflation Factors greater than 10 were applied to detect multicollinearity. The preliminary analysis was assessed using *Chi*-Square or *Fisher* exact test to detect associated factors, as appropriate. Factors with $p<0.05$ were entered into the multivariable analysis. A binary logistic regression model was carried out to identify predictor variables associated with DAS. A statistical significance level of $p<0.05$ was considered for all tests.

Ethical consideration

All processes were carried out in accordance with the principle of the Helsinki Declaration. The study was approved by the Human Research Ethics Committee, Walailak University (Ref: WU-EC-PU-0-214-65). Prior to participating in the survey, all respondents were given a full explanation and agreed to participate by written informed consent. All personal identification information of the participants remained confidential.

RESULTS

Sociodemographic characteristic

There was a total of 549 participants in this study. The age of the respondents ranged from 18 to 79 years old, with a mean and standard deviation of 42.9 and 16.4, respectively. The majority of the respondents were over 40 years old (54.1%), female (56.5%), and married (74%). 56.3% of the participants lived in urban areas, 27.5% had obtained up to primary school education, and a small proportion had achieved a postgraduate degree (2.4%). Most of the respondents (71.4%) had an income of up to 5,000,000 VND, and 39.5% were freelancers. Almost all the study participants had a family member infected with COVID-19 (92.3%). (Table 1).

Disease condition

A vast majority of participants (65.6%) reported no underlying diseases. Additionally, hypertension (20.4%) and diabetes (9.1%) were the most prevalent underlying disease, while cancer was the least common (0.5%). In terms of types of COVID-19 diagnosis, 64.7% and 21.9% of participants experienced mild and moderate levels. Most respondents (91.1%) had medical treatment in the hospital, while 8.9% had oxygen/ventilator support.

Concerning current signs and symptoms, forgetfulness (49.2%), fatigue (34.8%), cough (26.2%), and joint pain (20%) were the most common symptoms of the respondents after discharge. On the other hand, 25.1% of respondents reported not having any signs and symptoms after being infected with COVID-19 (Table 2).

Prevalence of depression, anxiety, and stress among recovered COVID-19 patients

With 549 participants included in this study, the overall prevalence of DAS were 24.8% (95% CI: 21.2-28.6), 41.5% (95% CI: 37.4-45.8) and 25.3% (95% CI: 21.7-29.2), respectively. We found mild depression occurred in 10.9% (95% CI: 8.4-13.8) of participants, followed by moderate depression (8.9%, 95% CI: 6.7-11.6), severe depression (2.9%, 95% CI: 1.7-4.7), and extreme depression (2.0%, 95% CI: 1-3.6). With regard to the level of anxiety, extremely severe anxiety was the least to be experienced (4.7%, 95% CI: 3.1-6.9). By contrast, mild, moderate, and severe levels accounted for 14.8% (95% CI: 11.9-18), 16% (95% CI: 13.1-19.4) and 6% (95% CI: 4.2-8.3), respectively. Last but not least, mild and moderate levels of stress accounted for the majority, 12.2% (95% CI: 9.6-15.2) and 8.2% (95% CI: 6-10.8), respectively. Meanwhile, participants who had severe and extremely severe levels were 4.2% (95% CI: 2.7-6.2) and 0.7% (95% CI: 0.2-1.9) (Figure 1).

Association of characteristic factors with depression, anxiety, and stress among recovered COVID-19 patients

A significant association was observed between types of COVID-19 treatment and sleep disturbance with DAS ($p<0.01$). The living area, monthly income,

Table 1. Sociodemographic characteristics of study participants

Sociodemographic characteristic		Frequency (n=549)	Percentage (%)
Gender	Male	239	43.5
	Female	310	56.5
Age (years)	18- ≤43	282	51.4
	>43	267	48.6
Living area	Urban	309	56.3
	Rural	240	43.7
Education	No schooling completed	35	6.4
	Up to Primary	151	27.5
	Up to Middle school	146	26.6
	Up to High school	126	23
	Bachelor's degrees	78	14.2
	Postgraduate and higher	13	2.4
Marital status	Single	124	22.6
	Married	406	74.0
	Separated/divorced	5	0.9
	Widow/widower	14	2.6
Monthly income (VND)	<50000,000	392	71.4
	5,000,000 – <10,000,000	127	23.1
	10,000,000 and above	30	5.5
Occupation	Employee	119	21.7
	Freelancer	217	39.5
	Housewife	98	17.9
	Unemployed	22	4.0
	Student	53	9.7
	Retired	40	7.3
Family members infected with COVID-19	Yes	507	92.3
	No	42	7.7

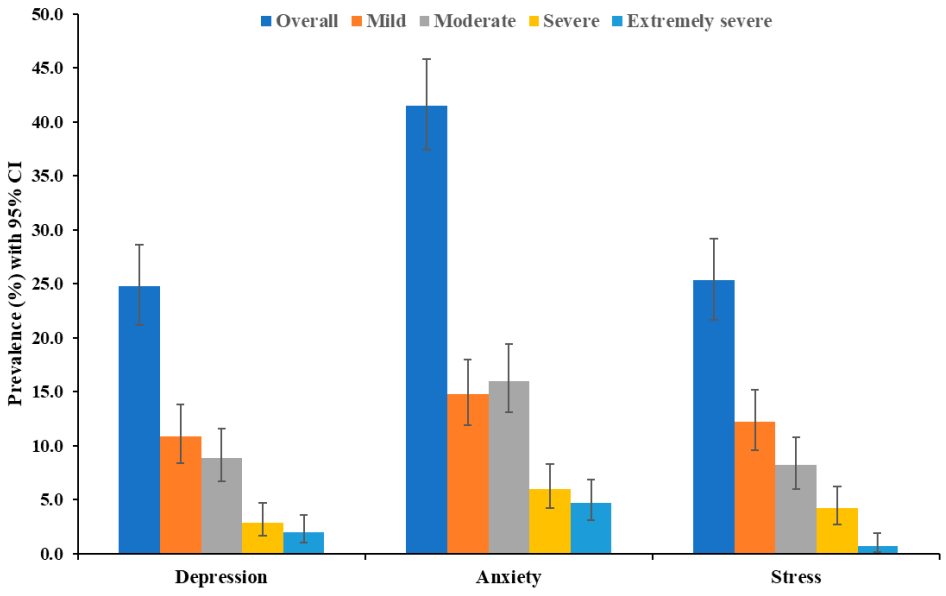

Figure 1. Prevalence of depression, anxiety, and stress among recovered COVID-19 patients

Table 2. Descriptive statistics for underlying disease condition

Characteristic		Frequency (n=549)	Percentage (%)
Underlying diseases	Hypertension	112	20.4
	Heart disease	48	8.7
	Cancer	3	0.5
	Respiratory disease	22	4.0
	Diabetes	50	9.1
	Kidney disease	13	2.4
	Others	30	5.5
Types of COVID-19 diagnosis from medical record	Asymptomatic	36	6.6
	Mild	355	64.7
	Moderate	120	21.9
	Severe	27	4.9
	Critical	11	2.0
Length of hospital stay (days)	≤14	415	75.6
	>14	134	24.4
Types of COVID-19 treatment	Hospital + medicine	500	91.1
	Hospital + medicine + oxygen/ ventilator	49	8.9
Sleep disturbance	Yes	199	36.2
	No	350	63.8
Current signs and symptoms	Cough	144	26.2
	Chest pain	46	8.4
	Headache	103	18.8
	Shortness of breath	72	13.1
	Dizziness	76	13.8
	Fatigue	191	34.8
	Joint pain	110	20.0
	Diarrhea	11	2.0
	Decreased appetite	9	1.6
	Change in smell	11	2.0
	Forgetfulness	270	49.2
	Other symptoms	12	2.2
	No symptoms	138	25.1

and types of COVID-19 diagnosis were associated with depression and anxiety ($p<0.05$). Depression and stress were associated with having family members infected with COVID-19. Education was associated with depression, while age was significantly associated with anxiety (Table 3).

Association of the underlying disease with depression, anxiety, and stress among recovered COVID-19 patients

A significant association were observed between heart disease and cancer with DAS ($p<0.05$). The association between diabetes, respiratory disease, and kidney disease with stress were significant, as well as depression ($p<0.05$). Hypertension was associated with depression and anxiety ($p<0.05$) (Table 4).

Association of current signs and symptoms factors with depression, anxiety, stress among recovered COVID-19 patients

A significant association were observed between fatigue, diarrhea, decreased appetite, and change in smell with DAS ($p<0.05$). Otherwise, chest pain and shortness of breath were associated with anxiety ($p<0.01$) (Table 5).

Table 3. Preliminary analysis of the association between characteristic factors with depression, anxiety, and stress among recovered COVID-19 patients (n=549)

Characteristic factors	Depression			Anxiety			Stress		
	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2
Gender									
Male	64 (26.8)	175 (73.2)	0.91	98 (41.0)	141 (59.0)	0.05	63 (26.4)	176 (73.6)	0.24
Female	72 (23.2)	238 (76.8)		130 (41.9)	180 (58.1)		76 (24.5)	234 (75.5)	
Age									
> 43 years old	72 (27.0)	195 (73.0)	1.34	131 (49.1)	136 (50.9)	12.15***	71 (26.6)	196 (73.4)	0.45
≤ 43 years old	64 (22.7)	218 (77.3)		97 (34.4)	185 (65.6)		68 (24.1)	214 (75.9)	
Living area									
Urban	94 (30.4)	215 (69.6)	12.1**	142 (46.0)	167 (54.0)	5.69*	83 (26.9)	226 (73.1)	0.89
Rural	42 (17.5)	198 (82.5)		86 (35.8)	154 (64.2)		56 (23.3)	184 (76.7)	
Education									
No schooling completed	8 (22.9)	27 (77.1)	23.96***	15 (42.9)	20 (57.1)	3.71	9 (25.7)	26 (74.3)	6.13
Primary school	38 (25.2)	113 (74.8)		70 (46.4)	81 (53.6)		34 (22.5)	117 (77.5)	
Middle school	20 (13.7)	126 (86.3)		55 (37.7)	91 (62.3)		34 (23.3)	112 (76.7)	
High school	32 (25.4)	94 (74.6)		49 (38.9)	77 (61.1)		35 (27.8)	91 (72.2)	
Bachelor's degrees	32 (41.0)	46 (60.0)		35 (44.9)	43 (55.1)		26 (33.3)	52 (66.7)	
Postgraduate and higher	6 (46.2)	7 (53.8)		4 (30.8)	9 (69.2)		1 (7.7)	12 (92.3)	
Marital status									
Single	32 (25.8)	92 (74.2)	3.41	46 (37.1)	78 (62.9)	2.33	30 (24.2)	94 (75.8)	0.47
Married	96 (23.6)	310 (76.4)		176 (43.3)	230 (56.7)		103 (25.4)	303 (74.6)	
Separated/ divorced/ Widow	8 (42.1)	11 (57.9)		6 (31.6)	13 (68.4)		6 (31.6)	13 (68.4)	
Monthly income (VND)									
<5,0000,000	89 (22.7)	303 (77.3)	14.04**	173 (44.1)	219 (55.9)	7.24*	97 (24.7)	295 (75.3)	0.24
5,000,000 – <10,000,000	31 (24.4)	96 (75.6)		40 (31.5)	87 (68.5)		34 (26.8)	93 (73.2)	
10,000,000 and above	16 (53.3)	14 (46.7)		15 (50)	15 (50)		8 (26.7)	22 (73.3)	
Occupation									
Employee	34 (28.6)	85 (71.4)	3.98	52 (43.7)	67 (56.3)	5.53	30 (25.2)	89 (74.8)	3.48
Freelancer	51 (23.5)	166 (76.5)		83 (38.2)	134 (61.8)		57 (26.3)	160 (73.7)	
Housewife	20 (20.4)	78 (79.6)		47 (48.0)	51 (52.0)		21 (21.4)	77 (78.6)	
Unemployed	4 (18.2)	18 (81.8)		8 (36.4)	14 (63.6)		4 (18.2)	18 (81.8)	
Student	14 (26.4)	39 (73.6)		18 (34.0)	35 (66.0)		13 (24.5)	40 (75.5)	
Retired	13 (32.5)	27 (67.5)		20 (50)	20 (50)		14 (35)	26 (65)	
Family members infected with COVID-19									
Yes	119 (23.5)	388 (76.5)	6.01*	207 (40.8)	300 (59.2)	1.34	123 (24.3)	384 (75.7)	3.92*
No	17 (40.5)	25 (59.5)		21 (50)	21 (50)		16 (38.1)	26 (61.9)	

Table 3 cont.

Characteristic factors	Depression			Anxiety			Stress		
	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2
Types of COVID-19 diagnosis from medical record									
Asymptomatic	14 (38.9)	22 (61.1)		15 (41.7)	21 (58.3)		13 (36.1)	23 (63.9)	
Mild	70 (19.7)	285 (80.3)		118 (33.2)	237 (66.8)		81 (22.8)	274 (77.2)	
Moderate	36 (30)	84 (70)	20.45***	70 (58.3)	50 (41.7)	33.52***	30 (25)	90 (75)	9.28
Severe	9 (33.3)	18 (66.7)		17 (63.0)	10 (37.0)		9 (33.3)	18 (66.7)	
Critical	7 (63.6)	4 (36.4)		8 (72.7)	3 (27.3)		6 (54.5)	5 (45.5)	
Length of hospital stay (days)									
>14	32 (23.9)	102 (76.1)	0.08	60 (44.8)	74 (55.2)	0.77	32 (23.9)	102 (76.1)	0.19
≤14	104 (25.1)	311 (74.9)		168 (40.5)	247 (59.5)		107 (25.8)	308 (74.2)	
Types of COVID-19 treatment									
Hospital + medicine + oxygen/ ventilator	21 (42.9)	28 (57.1)	9.44**	32 (65.3)	17 (34.7)	12.5***	20 (40.8)	29 (59.2)	6.83**
Hospital + medicine	115 (23)	385 (77)		196 (39.2)	304 (60.8)		119 (23.8)	381 (76.2)	
Sleep disturbance									
Yes	69 (34.7)	130 (65.3)	16.42***	116 (58.3)	83 (41.7)	36.11***	67 (33.7)	132 (66.3)	11.51**
No	67 (19.1)	283 (80.9)		112 (32)	238 (68)		72 (20.6)	278 (79.4)	

χ2: Chi-square test; * p<0.05; ** p<0.01; ***p<0.001; [a]: Fisher's exact test

Table 4. Preliminary analysis of the association between underlying disease factors and depression, anxiety, and stress among recovered COVID-19 patients (n=549)

UD Factors	Depression			Anxiety			Stress		
	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2
Hypertension									
Yes	36 (32.1)	76 (67.9)	4.10*	56 (50)	56 (50)	4.15*	33 (29.5)	79 (70.5)	1.27
No	100 (22.9)	337 (77.1)		172 (39.4)	265 (60.6)		106 (24.3)	331 (75.7)	
Diabetes									
Yes	21 (42)	29 (58)	8.76**	25 (50)	25 (50)	1.62	20 (40)	30 (60)	6.27*
No	115 (23.0)	384 (77.0)		203 (40.7)	296 (59.3)		119 (23.8)	380 (76.2)	
Heart disease									
Yes	25 (52.1)	23 (47.9)	21.05***	30 (62.5)	18 (37.5)	9.52**	20 (41.7)	28 (58.3)	7.43**
No	111 (22.2)	390 (77.8)		198 (39.5)	303 (60.5)		119 (23.8)	382 (76.2)	
Cancer									
Yes	3 (100)	0 (0)	9.16*[a]	3 (100)	0 (0)	4.24[a]	3 (100)	0 (0)	8.89*[a]
No	133 (24.4)	413 (75.6)		225 (41.2)	321 (58.8)		136 (24.9)	410 (75.1)	
Respiratory disease									
Yes	13 (59.1)	9 (40.9)	14.48***	11 (50)	11 (50)	0.68	13 (59.1)	9 (40.9)	13.82***
No	123 (23.3)	404 (76.7)		217 (41.2)	310 (58.8)		126 (23.9)	401 (76.1)	
Kidney disease									
Yes	4 (30.8)	9 (69.2)	0.26[a]	7 (53.8)	6 (46.2)	0.83	4 (30.8)	9 (69.2)	0.21[a]
No	132 (24.6)	404 (75.4)		221 (41.2)	315 (58.8)		135 (25.2)	401 (74.8)	

UD: Underlying disease; χ2: *Chi*-square test; * p<0.05; ** p<0.01; ***p<0.001; [a] = Fisher's exact test

Table 5. Preliminary analysis of the association between current signs and symptoms factors with depression, anxiety, and stress among recovered COVID-19 patients (n=549)

CSS Factors	Depression			Anxiety			Stress		
	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2	Yes n (%)	No n (%)	χ^2
Fatigue									
Yes	58 (30.4)	133 (69.6)	4.91*	101 (52.9)	90 (47.1)	15.54***	59 (30.9)	132 (69.1)	4.80*
No	78 (21.8)	280 (78.2)		127 (35.5)	231 (64.5)		80 (22.3)	278 (77.7)	
Cough									
Yes	36 (25)	108 (75)	0.005	66 (45.8)	78 (54.2)	1.49	38 (26.4)	106 (73.6)	0.12
No	100 (24.7)	305 (75.3)		162 (40)	243 (60)		101 (24.9)	304 (75.1)	
Chest pain									
Yes	14 (30.4)	32 (69.6)	0.87	29 (63)	17 (37)	9.57**	16 (34.8)	30 (65.2)	2.37
No	122 (24.2)	381 (75.8)		199 (39.6)	304 (60.4)		123 (24.5)	380 (75.5)	
Headache									
Yes	28 (27.2)	75 (72.8)	0.40	48 (46.6)	55 (53.4)	1.35	20 (19.4)	83 (80.6)	2.33
No	108 (24.2)	338 (75.8)		180 (40.4)	266 (59.6)		119 (26.7)	327 (73.3)	
Shortness of breath									
Yes	21 (29.2)	51 (70.8)	0.86	40 (55.6)	32 (44.4)	6.71**	23 (31.9)	49 (68.1)	1.92
No	115 (24.1)	362 (75.9)		188 (39.4)	289 (60.6)		116 (24.3)	361 (75.7)	
Dizziness									
Yes	20 (26.3)	56 (73.7)	0,11	35 (46.1)	41 (53.9)	0.74	18 (23.7)	58 (76.3)	0.13
No	116 (24.5)	357 (75.5)		193 (40.8)	280 (59.2)		121 (25.6)	352 (74.4)	
Joint pain									
Yes	32 (29.1)	78 (70.9)	1.37	54 (49.1)	56 (50.9)	3.29	30 (27.3)	80 (72.7)	0.28
No	104 (23.7)	335 (76.3)		104 (23.7)	335 (76.3)		109 (24.8)	330 (75.2)	
Diarrhea									
Yes	6 (54.5)	5 (45.5)	5.33*[a]	11 (100)	0 (0)	18.80***[a]	7 (63.6)	4 (36.4)	8.71**[a]
No	130 (24.2)	408 (75.8)		217 (40.3)	321 (59.7)		132 (24.5)	406 (75.5)	
Decreased appetite									
Yes	4 (44.4)	5 (55.6)	1.90[a]	6 (66.7)	3 (33.3)	2.38[a]	3 (33.3)	6 (66.7)	0.31[a]
No	132 (24.4)	408 (75.6)		222 (41.1)	318 (58.9)		136 (25.2)	404 (74.8)	
Change in smell									
Yes	5 (45.5)	6 (54.5)	2.57[a]	8 (72.7)	3 (27.3)	4.50[a]	5 (45.5)	6 (54.5)	2.40[a]
No	131 (24.3)	407 (75.7)		220 (40.9)	318 (59.1)		134 (24.9)	404 (75.1)	
Forgetfulness									
Yes	65 (24.1)	205 (75.9)	0.14	108 (40)	162 (60)	0.51	73 (27.0)	197 (73.0)	0.830
No	71 (25.4)	208 (74.6)		120 (43)	159 (57)		66 (23.7)	213 (76.3)	

CSS: Current signs and symptoms; $\chi 2$: *Chi*-square test; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; [a]: Fisher's exact test

Predictors of depression, anxiety, and stress among recovered COVID-19 patients

The binary logistic model analyses showed that living areas were significantly associated with depression and anxiety. Living in urban areas was 1.97 (95% CI: 1.27-3.08) and 1.57 (95% CI: 1.07-2.29) times the risk of depression and anxiety than in rural areas, respectively. People with a bachelor's degree were likely to experience depression 3.51 times more than those who have not completed school (OR = 3.51; 95% CI: 1.13-10.8). Moreover, people were 2.57 times more likely to suffer from depression in the group with an income above 10,000,000 VND compared to the group with an income less than 5,000,000 VND (OR = 2.57; 95% CI: 1.03-6.38). Occurrence of depression increased for people with diabetes (OR = 2.21; 95% CI: 1.04-1.72), heart disease (OR = 3.83; 95% CI: 1.79-8.17), respiratory disease (OR = 3.49; 95% CI: 1.24-9.84) or diarrhea (OR = 1.07; 95% CI: 1.06-15.6), compared to those without. Individuals who sleep

Table 6. Predictors of depression, anxiety, and stress in recovered COVID-19 patients

Predictors	Depression	Anxiety	Stress
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age			
>43 years old		1.41 (0.93-2.13)	
≤43 years old		1	
Living area			
Urban	1.97 (1.27-3.08)*	1.57 (1.07-2.29)*	
Rural	1	1	
Education			
No schooling completed	1		
Primary school	1.37 (0.49-3.79)		
Middle school	0.77 (0.26-2.31)		
High school	1.99 (0.67-5.91)		
Bachelor's degrees	3.51 (1.13-10.8)*		
Postgraduate and higher	2.89 (0.55-14.9)		
Monthly income (VND)			
<5,0000,000	1	1	
5,000,000 – <10,000,000	1.14 (0.65-1.99)	0.68 (0.43-1.08)	
10,000,000 and above	2.57 (1.03-6.38)*	1.29 (0.57-2.91)	
Family members infected with COVID-19			
Yes	0.64 (0.29-1.43)		0.59 (0.29-1.20)
No	1		1
Types of COVID-19 diagnosis from medical record			
Asymptomatic	1	1	
Mild	0.53 (0.22-1.28)	0.81 (0.38-1.71)	
Moderate	0.75 (0.29-1.93)	1.79 (0.80-4.00)	
Severe	0.38 (0.07-1.88)	1.14 (0.28-4.52)	
Critical	1.39 (0.18-10.2)	1.74 (0.26-11.4)	
Types of COVID-19 treatment			
Hospital + medicine + oxygen/ventilator	1.30 (0.39-4.32)	1.19 (0.42-3.31)	1.15 (0.57-2.31)
Hospital + medicine	1	1	1
Sleep disturbance			
Yes	1.56 (0.97-2.52)	2.32 (1.56-3.46)*	1.48 (0.96-2.27)
No	1	1	1
Hypertension (UD)			
Yes	0.91 (0.48-1.72)	0.78 (0.46-1.32)	
No	1	1	
Diabetes (UD)			
Yes	2.21 (1.04-4.68)*		1.69 (0.88-3.24)
No	1		1
Heart disease (UD)			
Yes	3.83 (1.79-8.17)*	1.28 (0.63-2.60)	1.63 (0.84-3.17)
No	1	1	1
Respiratory disease (UD)			
Yes	3.49 (1.24-9.84)*		3.75 (1.47-9.60)*

Table 6 cont,

Predictors	Depression	Anxiety	Stress
	OR (95% CI)	OR (95% CI)	OR (95% CI)
No	1		1
Fatigue (CSS)			
Yes	1.40 (0.87-2.25)	1.57 (1.03-2.39)*	1.32 (0.86-2.02)
No	1	1	1
Chest pain (CSS)			
Yes		1.62 (0.79-3.30)	
No		1	
Shortness of breath (CSS)			
Yes		1.08 (0.59-1.96)	
No		1	
Diarrhea (CSS)			
Yes	4.07 (1.06-15.6)*		4.34 (1.18-15.9)*
No	1		1

Binary logistic regression statistics; * p<0.05; OR: Odd Ratio; 95% CI: 95% Confidence Interval; UD: Underlying disease; CSS: Current sign and symptoms

more than 9 hours or less than 7 hours were 2.32 times more likely to have anxiety than those who sleep 7-9 hours a day (OR = 2.32; 95% CI: 1.56-3.46). Fatigue after being discharged from the hospital can escalate the risk of anxiety 1.57 times (95% CI: 1.03-2.39). Stress was 3.75 and 4.34 times more likely to occur in individuals with respiratory disease and diarrhea, respectively (Table 6).

DISCUSSION

Our study aims to assess the prevalence of DAS as well as identify predictors of DAS among recovered COVID-19 patients following six months of discharge from the hospital. In the present study, the prevalence of DAS were 24.8%, 41.5%, and 25.3%, respectively. These findings are higher than those from a cohort study conducted in France among COVID-19 patients four months after discharge, which established the prevalence of depression and anxiety were 20.6% and 31.5%, respectively [32]. Another follow-up study in China found 37.5% and 41.7% of COVID-19 patients suffered depression and anxiety one year after discharge, which is higher than our finding [52]. The results showed the prevalence of DAS symptoms were higher compared to earlier studies carried out in Vietnam on different populations during the initial stage of the pandemic, which was notable [11, 13]. For instance, among healthcare workers, the prevalence of DAS were 13.11%, 14.75%, and 4.92%, respectively [11]. Additionally, among the general population, the prevalence of DAS were 23.5%, 14.1%, and 22.3%, respectively [13]. These results indicated that symptoms of DAS occurred more severely among recovered COVID-19 patients and did not end with hospital discharge, highlighting the need for intervention and prevention strategies to support their recovery.

Our study found that certain characteristic factors, such as living area, education level, monthly income, and sleep disturbance, were identified as predictors of DAS. Living in an urban area was a predictor of depression and anxiety, which is in line with previous studies [35, 42]. Individuals who reside in urban areas and have recovered from COVID-19 are more susceptible to depression and anxiety. Urban areas with higher population density result in a greater chance of exposure to the virus and being subjected to quarantine. The sudden halt in work and other professional activities, combined with the possibility of financial losses, can contribute to psychological distress [45]. Furthermore, a previous study has demonstrated that individuals exposed to COVID-19-positive patients or patients with other illnesses were associated with increased DAS symptoms [41]. Our findings concurred with previous studies [24, 42] that individuals holding bachelor's degrees were associated with higher rates of depression. This can be attributed to the fact that higher-educated individuals may have greater access to information about the COVID-19 pandemic and may be more concerned about its long-term health consequences [36]. In addition, people with high education may have higher expectations for their future careers, which may be negatively impacted by the economic downturn caused by the pandemic and the resulting job market prospects [5]. These findings imply that the government should take measures to mitigate the effects of the economic fallout and provide financial support for those affected by the pandemic.

However, our result revealed conflicting patterns between higher monthly income with increased levels of depression, which is in contrast to other studies [18]. Additionally, having a higher income may bring about higher societal expectations and an increased sense of responsibility to perform at a high level. Furthermore, the economic fallout caused by the COVID-19 pandemic may have led to companies reducing the number of employees, causing job pressure among those still employed. A study in Vietnam showed that 37.9% of employees had to work overtime due to the outbreak [21]. This highlights the pressure that individuals may face to maintain their workplace position. Furthermore, our study confirmed the previous finding [18, 19], which indicated that experiencing sleep disturbance increases the risk of anxiety. As per the literature, it has been established that individuals who slept less than 7 hours or more than 9 hours had significantly higher scores on the DASS-21 scale [19]. The results suggest that healthcare providers should pay attention to sleep disturbance and provide education and interventions on the importance of healthy sleep habits.

In terms of disease conditions, the relationship between depression and stress with underlying diseases was also found in our results, which included diabetes, heart disease and respiratory disease. This finding supports previous research that has shown chronic diseases were known as risk factors for increased severity of DAS symptoms [15, 18, 37, 42]. This could be attributed to individuals with poor self-rated health status or a history of chronic disease, which was more vulnerable to the psychological impact of the outbreak and displayed higher levels of DAS [47]. These findings suggested that such people, particularly those with underlying diseases, should receive mental health support after hospital discharge.

Surprisingly, there were no associations between DAS and clinical features during hospital stays, including types of COVID-19 treatment and types of COVID-19 diagnosis. Previous literature on this topic had shown mixed evidence, with a study found an association between mental health outcomes and the severity level of COVID-19 within the first month after hospitalization [24], while others did not find this relationship in follow-up studies conducted three months after hospitalization [15, 38]. This discrepancy could be attributed to the difference in post-discharge assessment time. The existing literature implied no correlation between the frequency of depressive symptoms and the severity of acute COVID-19 for more than 12 weeks following infection [39]. As a result, it is reasonable to expect that the severity of COVID-19 will not result in DAS after patients are discharged for an extended period.

On the other hand, researchers have stated that the greater the number of symptoms after discharge, the higher the level of depression and anxiety [24], as seen in our study, where individuals who experienced fatigue and diarrhea had an increased risk of DAS. Having persistent symptoms such as fatigue and diarrhea can impact mental health as it can limit a person's ability to engage in activities they enjoy, leading to feelings of sadness and loneliness [10]. Studies have shown that individuals who feel they can participate in daily life and regain certain functions experience a reduction in symptoms of depression [10]. Therefore, it is important to address these symptoms in order to improve mental health outcomes for individuals who have recovered from COVID-19.

Limitations

There are some limitations of this study. First, self-reported information about DAS as well as current signs and symptoms, should not be considered a diagnosis. Second, recall bias may be a limitation. Third, a causal association could not be demonstrated since the cross-sectional study design restricted the temporal relationship between exposure and result. The longitudinal design could be conducted to demonstrate a causal relationship between DAS symptoms and predictors as well as minimizing recall bias.

CONCLUSIONS

The prevalence of DAS symptoms were high among COVID-19 patients who recovered for more than six months after hospitalization. Predictors of DAS, including living in an urban area, higher educational level, higher monthly income, diabetes, heart disease, respiratory disease, sleep disturbance, fatigue, and diarrhea, can be utilized to identify populations that are vulnerable to developing DAS following hospital discharge. Our finding provides valuable insight into the mental health needs of recovered COVID-19 patients and highlights the importance of monitoring and addressing potential DAS symptoms of COVID-19 patients after hospitalization. Further interventions are needed to minimize the long-term mental health impact of the pandemic.

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Conflict of interest

There are no conflicts of interest in regard to this study.

REFERENCES

1. American Psychological Association: Anxiety (2017). Available https://www.apa.org/topics/anxiety (Accessed 15 May 2022)
2. American Psychological Association: Depression (2019). Available https://www.apa.org/topics/depression (Accessed 15 May 2022)
3. American Psychological Association: What's the difference between stress and anxiety? (2022). Available https://www.apa.org/topics/stress/anxiety-difference#:~:text=People%20under%20stress%20experience%20mental,the%20absence%20of%20a%20stressor. (Accessed 02 June 2022)
4. *Arsenault-Lapierre G., Kim C., and Turecki G.:* Psychiatric diagnoses in 3275 suicides: a meta-analysis. BMC Psychiatry 2004;4(1). 1-11. doi: 10.1186/1471-244X-4-37.
5. *Bareket-Bojmel L., Shahar G., and Margalit M.:* COVID-19-related economic anxiety is as high as health anxiety: findings from the USA, the UK, and Israel. International Journal of Cognitive Therapy 2021;14(3):566-574. doi: 10.1007/s41811-020-00078-3.
6. *Bourmistrova N.W., Solomon T., Braude P., Strawbridge R., and Carter B.:* Long-term effects of COVID-19 on mental health: A systematic review. J Affect Disord 2022;299:118-125. doi: 10.1016/j.jad.2021.11.031.
7. *Brådvik L.:* Suicide Risk and Mental Disorders. Int J Environ Res Public Health 2018; 15(9). doi: 10.3390/ijerph15092028.
8. *Ceban F., Ling S., Lui L.M.W., Lee Y., Gill H., Teopiz K.M., Rodrigues N.B., Subramaniapillai M., Di Vincenzo J.D., Cao B., Lin K., Mansur R.B., Ho R.C., Rosenblat J.D., Miskowiak K.W., Vinberg M., Maletic V., and McIntyre R.S.:* Fatigue and cognitive impairment in Post-COVID-19 Syndrome: A systematic review and meta-analysis. Brain Behav Immun 2022;101:93-135. doi: 10.1016/j.bbi.2021.12.020.
9. *Crawford J.R. and Henry J.D.:* The Depression Anxiety Stress Scales (DASS): normative data and latent structure in a large non-clinical sample. Br J Clin Psychol 2003;42(Pt 2):111-31. doi: 10.1348/014466503321903544.
10. *DeJean D., Giacomini M., Vanstone M., and Brundisini F.:* Patient experiences of depression and anxiety with chronic disease: a systematic review and qualitative meta-synthesis. Ont Health Technol Assess Ser 2013;13(16):1-33.
11. *Do Duy C., Nong V.M., Van A.N., Thu T.D., Do Thu N., and Quang T.N.:* COVID-19-related stigma and its association with the mental health of health-care workers after quarantine in Vietnam. Psychiatry and Clinical Neurosciences 2020;74(10):566. doi: 10.1111/pcn.13120.
12. *Doan Q.H., Tran N.N., Than M.H., Nguyen H.T., Bui V.S., Nguyen D.H., Vo H.L., Do T.T., Pham N.T., Nguyen T.K., Cao D.C., Nguyen V.T., Tran T.T., Pham B.H., Tran A.L., Nguyen V.T., Nguyen V.T., Tran X.T., Lai D.T., Vu Q.H., and Otsu S.:* Depression, Anxiety and Associated Factors among Frontline Hospital Healthcare Workers in the Fourth Wave of COVID-19: Empirical Findings from Vietnam. Trop Med Infect Dis 2021;7(1). doi: 10.3390/tropicalmed7010003.
13. *Duong K.N.C., Le Bao T.N., Nguyen P.T.L., Van T.V., Lam T.P., Gia A.P., Anuratpanich L., and Van B.V.:* Psychological Impacts of COVID-19 During the First Nationwide Lockdown in Vietnam: a Web-Based, Cross-Sectional Survey Study. JMIR formative research 2020;4(12). e24776. doi: 10.2196/24776.
14. *Edouard Mathieu H.R., Lucas Rodés-Guirao, Cameron Appel, Charlie Giattino, Joe Hasell, Bobbie Macdonald, Saloni Dattani, Diana Beltekian, Esteban Ortiz-Ospina and Max Roser*: Coronavirus Pandemic (COVID-19) (2020). Available https://ourworldindata.org/coronavirus (Accessed 31 December 2022).
15. *Gramaglia C., Gambaro E., Bellan M., Balbo P.E., Baricich A., Sainaghi P.P., Pirisi M., Baldon G., Battistini S., Binda V., Feggi A., Gai M., Gattoni E., Jona A., Lorenzini L., Marangon D., Martelli M., Prosperini P., and Zeppegno P.:* Mid-term Psychiatric Outcomes of Patients Recovered From COVID-19 From an Italian Cohort of Hospitalized Patients. Front Psychiatry 2021;12.667385. doi: 10.3389/fpsyt.2021.667385.
16. *Hao F., Tam W., Hu X., Tan W., Jiang L., Jiang X., Zhang L., Zhao X., Zou Y., Hu Y., Luo X., McIntyre R.S., Quek T., Tran B.X., Zhang Z., Pham H.Q., Ho C.S.H., and Ho R.C.M.:* A quantitative and qualitative study on the neuropsychiatric sequelae of acutely ill COVID-19 inpatients in isolation facilities. Transl Psychiatry 2020;10(1):355. doi: 10.1038/s41398-020-01039-2.
17. *Henry J.D. and Crawford J.R.:* The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative data in a large non-clinical sample. Br J Clin Psychol 2005;44(Pt 2):227-39. doi: 10.1348/014466505x29657.
18. *Islam M.S., Ferdous M.Z., Islam U.S., Mosaddek A.S.M., Potenza M.N., and Pardhan S.:* Treatment, Persistent Symptoms, and Depression in People Infected with COVID-19 in Bangladesh. International Journal of Environmental Research and Public Health 2021;18(4):1453. doi: 10.3390/ijerph18041453.
19. *Islam M.S., Sujan M.S.H., Tasnim R., Sikder M.T., Potenza M.N., and van Os J.:* Psychological responses during the COVID-19 outbreak among university students in Bangladesh. PLoS One 2020;15(12). e0245083. doi: 10.1371/journal.pone.0245083.
20. *Kotrlik J. and Higgins C.:* Organizational research: Determining the appropriate sample size in survey research. Information technology, learning, and performance journal 2001;19(1):43.

21. *Le H.T., Lai A.J.X., Sun J., Hoang M.T., Vu L.G., Pham H.Q., Nguyen T.H., Tran B.X., Latkin C.A., Le X.T.T., Nguyen T.T., Pham Q.T., Ta N.T.K., Nguyen Q.T., Ho R.C.M., and Ho C.S.H.:* Anxiety and Depression Among People Under the Nationwide Partial Lockdown in Vietnam. Front Public Health 2020;8:589359. doi: 10.3389/fpubh.2020.589359.
22. *Le M.T.H., Tran T.D., Holton S., Nguyen H.T., Wolfe R., and Fisher J.:* Reliability, convergent validity and factor structure of the DASS-21 in a sample of Vietnamese adolescents. PLoS One 2017;12(7):e0180557. doi: 10.1371/journal.pone.0180557.
23. *Li W., Zhang H., Zhang C., Luo J., Wang H., Wu H., Zhu Y., Cui H., Wang J., Li H., Zhu Z., Xu Y., and Li C.:* The Prevalence of Psychological Status During the COVID-19 Epidemic in China: A Systemic Review and Meta-Analysis. Frontiers in Psychology 2021;12. doi: 10.3389/fpsyg.2021.614964.
24. *Liu D., Baumeister R.F., Veilleux J.C., Chen C., Liu W., Yue Y., and Zhang S.:* Risk factors associated with mental illness in hospital discharged patients infected with COVID-19 in Wuhan, China. Psychiatry Research 2020; 292. 113297. doi: 10.1016/j.psychres.2020.113297.
25. *Lopez-Leon S., Wegman-Ostrosky T., Perelman C., Sepulveda R., Rebolledo P.A., Cuapio A., and Villapol S.:* More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Scientific reports 2021;11(1):1-12. doi: 10.1038/s41598-021-95565-8.
26. *Lovibond S.H. and Lovibond P.F.*, Manual for the Depression Anxiety Stress Scales. 1996: Psychology Foundation of Australia.
27. *Mattioli F., Stampatori C., Righetti F., Sala E., Tomasi C., and De Palma G.:* Neurological and cognitive sequelae of Covid-19: a four-month follow-up. J Neurology 2021;268(12). 4422-4428. doi: 10.1007/s00415-021-10579-6.
28. *Mazza M.G., De Lorenzo R., Conte C., Poletti S., Vai B., Bollettini I., Melloni E.M.T., Furlan R., Ciceri F., and Rovere-Querini P.:* Anxiety and depression in COVID-19 survivors: Role of inflammatory and clinical predictors. Brain, Behavior, and Immunity 2020;89:594-600. doi: 10.1016/j.bbi.2020.07.037.
29. MedlinePlus: Anxiety (2020). Available https://medlineplus.gov/anxiety.html (Accessed 22 Feb 2022)
30. MedlinePlus: Stress and your health (2020). Available https://medlineplus.gov/ency/article/003211.htm#:~:text=Stress%20is%20a%20feeling%20of,danger%20or%20meet%20a%20deadline (Accessed 22 Feb 2022)
31. *Méndez R., Balanzá-Martínez V., Luperdi S.C., Estrada I., Latorre A., González-Jiménez P., Bouzas L., Yépez K., Ferrando A., and Reyes S.:* Long-term neuropsychiatric outcomes in COVID-19 survivors: A 1-year longitudinal study. J Int. Medicine 2022; 291(2). 247-251.
32. *Morin L., Savale L., Pham T., Colle R., Figueiredo S., Harrois A., Gasnier M., Lecoq A.-L., Meyrignac O., and Noel N.:* Four-month clinical status of a cohort of patients after hospitalization for COVID-19. Jama 2021; 325(15). 1525-1534. doi: 10.1001/jama.2021.3331.
33. National Institutes of Mental Health: Depression (2021). Available https://www.nimh.nih.gov/health/publications/depression (Accessed 2022 22 Feb)
34. National Institutes of Mental Health: I'm So Stressed Out! Fact Sheet (2021). Available https://www.nimh.nih.gov/health/publications/so-stressed-out-fact-sheet#part_6631 (Accessed 22 Feb 2022)
35. *Özdin S.* and *Bayrak Özdin Ş.*: Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: The importance of gender. Int J Soc Psychiatry 2020; 66(5). 504-511. doi: 10.1177/0020764020927051.
36. *Pei H., Wu Q., Xie Y., Deng J., Jiang L., and Gan X.:* A Qualitative Investigation of the Psychological Experiences of COVID-19 Patients Receiving Inpatient Care in Isolation. Clinical Nursing Research 2021; 30(7). 1113-1120. doi: 10.1177/10547738211024807.
37. *Prakash J., Dangi A., Chaterjee K., Yadav P., Srivastava K., and Chauhan V.S.:* Assessment of depression, anxiety and stress in COVID-19 infected individuals and their families. Med J Armed Forces India 2021; 77. S424-S429. doi: 10.1016/j.mjafi.2021.06.013.
38. *Rass V., Beer R., Schiefecker A.J., Kofler M., Lindner A., Mahlknecht P., Heim B., Limmert V., Sahanic S., and Pizzini A.:* Neurological outcome and quality of life 3 months after COVID-19: A prospective observational cohort study. European Journal ofNneurology 2021; 28(10). 3348-3359. doi: 10.1111/ene.14803.
39. *Renaud-Charest O., Lui L.M.W., Eskander S., Ceban F., Ho R., Di Vincenzo J.D., Rosenblat J.D., Lee Y., Subramaniapillai M., and McIntyre R.S.:* Onset and frequency of depression in post-COVID-19 syndrome: A systematic review. J Psychiatr Res 2021;144:129-137. doi: 10.1016/j.jpsychires.2021.09.054.
40. *Robins E., Murphy G.E., Wilkinson Jr R.H., Gassner S., and Kayes J.:* Some clinical considerations in the prevention of suicide based on a study of 134 successful suicides. American Journal of Public Health and the Nations Health 1959;49(7):888-899.
41. *Shi L., Lu Z.-A., Que J.-Y., Huang X.-L., Liu L., Ran M.-S., Gong Y.-M., Yuan K., Yan W., Sun Y.-K., Shi J., Bao Y.-P., and Lu L.:* Prevalence of and Risk Factors Associated With Mental Health Symptoms Among the General Population in China During the Coronavirus Disease 2019 Pandemic. JAMA Network Open 2020;3(7).e2014053-e2014053. doi: 10.1001/jamanetworkopen.2020.14053.
42. *Srivastava A., Bala R., Devi T.P., and Anal L.:* Psychological trauma and depression in recovered COVID-19 patients: A telecommunication-based observational study. Trends Psychiatry Psychother 2021. doi: 10.47626/2237-6089-2021-0381.
43. *Tran B.X., Nguyen H.T., Le H.T., Latkin C.A., Pham H.Q., Vu L.G., Le X.T.T., Nguyen T.T., Pham Q.T., Ta N.T.K., Nguyen Q.T., Ho C.S.H., and Ho R.C.M.:* Impact of COVID-19 on Economic Well-Being and Quality of Life of the Vietnamese During the National Social Distancing. Frontiers in Psychology 2020; 11. doi: 10.3389/fpsyg.2020.565153.

44. *Tran T.D., Tran T., and Fisher J.:* Validation of the depression anxiety stress scales (DASS) 21 as a screening instrument for depression and anxiety in a rural community-based cohort of northern Vietnamese women. BMC Psychiatry 2013;13:24. doi: 10.1186/1471-244x-13-24.
45. *Tuan N.Q., Phuong N.D., Co D.X., Son D.N., Chinh L.Q., Dung N.H., Thach P.T., Thai N.Q., Thu T.A., and Tuan N.A.:* Prevalence and factors associated with psychological problems of the healthcare workforce in Vietnam: findings from COVID-19 hotspots in the national second wave. Healthcare 2021;9(6):718. doi: 10.3390/healthcare9060718.
46. *Wang C., Chudzicka-Czupała A., Tee M.L., Núñez María I.L., Tripp C., Fardin M.A., Habib H.A., Tran B.X., Adamus K., Anlacan J., García M.E.A., Grabowski D., Hussain S., Hoang M.T., Hetnał M., Le X.T., Ma W., Pham H.Q., Reyes P.W.C., Shirazi M., Tan Y., Tee C.A., Xu L., Xu Z., Vu G.T., Zhou D., Chan N.A., Kuruchittham V., McIntyre R.S., Ho C.S.H., Ho R., and Sears S.F.:* A chain mediation model on COVID-19 symptoms and mental health outcomes in Americans, Asians and Europeans. Scientific Reports 2021;11(1):6481. doi: 10.1038/s41598-021-85943-7.
47. *Wang C., Pan R., Wan X., Tan Y., Xu L., Ho C.S., and Ho R.C.:* Immediate Psychological Responses and Associated Factors during the Initial Stage of the 2019 Coronavirus Disease (COVID-19) Epidemic among the General Population in China. Int J Environ Res Public Health 2020;17(5). doi: 10.3390/ijerph17051729.
48. World Health Organization: Mental Health and COVID-19: Early evidence of the pandemic's impact: Scientific brief, 2 March 2022 (2022). Available https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci_Brief-Mental_health-2022.1 (Accessed 15 March 2022)
49. World Health Organization: Policy Brief: COVID-19 and the Need for Action on Mental Health (2020). Available (Accessed 13 May 2022)
50. World Health Organization: WHO Coronavirus (COVID-19) Dashboard (2022). Available https://covid19.who.int/ (Accessed 31 December 2022)
51. *Zanon C., Brenner R.E., Baptista M.N., Vogel D.L., Rubin M., Al-Darmaki F.R., Goncalves M., Heath P.J., Liao H.Y., Mackenzie C.S., Topkaya N., Wade N.G., and Zlati A.:* Examining the Dimensionality, Reliability, and Invariance of the Depression, Anxiety, and Stress Scale-21 (DASS-21) Across Eight Countries. Assessment 2021;28(6):1531-1544. doi: 10.1177/1073191119887449.
52. *Zhou F., Tao M., Shang L., Liu Y., Pan G., Jin Y., Wang L., Hu S., Li J., Zhang M., Fu Y., and Yang S.:* Assessment of Sequelae of COVID-19 Nearly 1 Year After Diagnosis. Front Med (Lausanne) 2021;8:717194. doi: 10.3389/fmed.2021.717194.

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ORIGINAL ARTICLE

BENZENE HEALTH RISK ASSESSMENT FOR NEUROLOGICAL DISORDERS OF GAS STATION EMPLOYEES IN RAYONG PROVINCE, THAILAND

Anamai Thetkathuek[1], *Chan Pattama Polyong*[2], *Wanlop Jaidee*[3]

[1]Department of Industrial Hygiene and Safety, Faculty of Public Health, Burapha University, Chonburi, Thailand
[2]Occupational Health and Safety Program, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok, Thailand
[3]Department of Public Health Foundations, Faculty of Public Health, Burapha University, Chonburi, Thailand

ABSTRACT

Background. The main job of employees working in the area of fuel service stations is to provide refueling services to customers. Therefore, operators at petrol stations may be exposed to chemicals for long periods, potentially affecting their health in nervous system.

Objectives. This study aims to assess the risk of benzene exposure to the nervous system in gas station operators. Data were collected from 100 fuel service personnel working at fuel dispensers and 100 employees working outside fuel dispensers, accounting to 200 cases.

Material and methods. Data were collected using interview questionnaires. Urine samples were used for the analysis of t,t-muconic acid.

Results. The results showed that t,t-muconic acid concentration is 431.23 ± 233.69 µg/g.cr (449.28 ± 213.32 µg/g.cr at fuel dispensers vs 413.18 ± 252.20 µg/g.cr outside fuel dispensers). The risk characterization results showed that most of the risks were at level 1 (low risk), as observed in 108 people (54.0%). The results of the analysis of the relationship between t,t-muconic acid concentrations classified by 3 levels of percentile and neurological disorders of the study group, the results showed that there was a statistically significant relationship (p-value <0.05).

Conclusion. Therefore, the benzene neurotoxic risk assessment model could be utilized in field practice.

Key words: *health risk assessment, benzene, petrol station, worker, Rayong province, Thailand*

INTRODUCTION

Employees working in gas stations are one of the personnel that contributes to the development of a country. However, employees working at a gas station do not only work as refueling service employees but also perform various services, which include working in grocery stores, restaurants, coffee shops, sanitary facilities, engine repairs, or car washes [1, 2]. Rayong province is one of the provinces with investment in transportation expansion of the industry, agriculture, and marine tourism. As a result, there are more businesses to support the economic growth of a country [3].

The main job of employees working in the area of fuel service stations is to provide refueling services to customers. However, there are also other occupations operating in these areas, with employees normally working for more than 8 h per day [1]. Therefore, these employees are more likely to be exposed to latent occupational hazards [4, 5]. Operators at petrol stations may be exposed to chemicals for long periods, potentially affecting their health in many systems [6]. The main effect is in the nervous system, which is now attracting concern [7, 8]. If employees are exposed to excessive amounts of benzene, this can damage the central nervous system (CNS), until they abnormal symptoms such as depression [8], resulting in cognitive and behavioral disorders [9]. It is very important that benzene is classified as a carcinogen and can be harmful to health, causing leukemia [9, 10] even after long-term exposure to low benzene concentrations [11].

Health surveillance for high-risk groups exposed to benzene while working in the gas station was achieved by assessing the biomarker of exposure in

Corresponding author: Anamai Thetkathuek, Department of Industrial Hygiene and Safety, Faculty of Public Health, Burapha Universit, Longhad Bangsaen Road, Chonburi 20131, Thailand, Tel: 66-81-6921013; Fax 66-38-393-253, E-mail: anamai@buu.ac.th, anamai@go.buu.ac.th

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the urinary benzene in the form of t,t-muconic acid, or S-phenyl mercapturic acid [12]. In addition, health impact assessments, including changes in biochemical indicators [13] are also gaining interest. In addition, symptoms of various system disorders can be assessed, especially assessment of neurological disorders by using a neurological disorder questionnaire such as the modified EURO QUEST questionnaire [14] and using neurobehavioral system tests [15] to assess the neurological health of at-risk groups, etc.

Therefore, health risk assessment of benzene in gas stations for health is very important. In this case, health risk assessment for toxic pollutants including the low dose of benzene must be carried out to determine the possible adverse effects of exposure to this substance [16]. Particularly, benzene is a carcinogen and is highly toxic to the body [9]. In Thailand, there are health risk assessment guidelines according to the TIS 2012 standard, which is used for the assessment of chemical health risks. It will be helpful to figure out how to manage to avoid exposure to benzene at the source [17].

Previous studies have examined the risks of exposure to low concentrations of benzene, to the health of workers in China [7]. Health risk assessments of various chemicals, including benzene, take many models, such as the model of the United States Environmental Protection Agency [16, 18] and the biomatrix of health risk assessment model, which recommends the use of t,t-MA [1]. No previous studies have been conducted to assess the risk of benzene on neurological symptoms which will be useful as a guideline for health surveillance among employees working at fuel stations that currently do not have annual health checks based on risk factors like in the industry which appears only in research studies [19, 20]. Therefore, the researcher is interested in studying to assess the risk of benzene in the nervous system of employees working at a fuel service station in Rayong province. This study is expected to be useful as a tool for screening health risks to prevent the risk of benzene on the nervous system in the at-risk group.

MATERIAL AND MEDTHODS

Study site and population

The study population consists of employees of gas stations in Rayong province. Initially, the researcher coordinated with the municipality for public relations and chose a fuel service station adjacent to the main road of Rayong. A simple random sampling was then performed for the selection of employees. The researchers randomly selected a group (cluster random sampling), i.e., refueling service workers, according to the inclusion criteria by selecting everyone voluntarily at each gas station. If the number of samples was insufficient, employees in the next gas station were chosen.

Sample size calculation

In a sample of 200 cases, calculated from a previous study by *Chaiklieng* and *Nantanuch* [21], 83.7% of workers who had worked at gas stations experienced adverse reactions (P = 0.837), with a 5% error (e = 0.05) 95% CI (Z = 1.96). The sample size was 200 cases, which are classified as the exposure group of 100 people who work in a gas station with a duty to provide fuel services and the control group (non-exposure group) of 100 employees who work in a fuel service station without having to directly service the fuel. The inclusion criteria included fuel service station employees aged between 18 and 60 years, able to read, listen, and write Thai consent to participate in the research, and able to work on the data collection day for 8 h. The exclusion criterion included employees who cannot complete 8 h of work and are unable to participate in research activities.

Research ethics

This study is conducted in consideration of human research ethics from the Human Research Committee of Burapha University (Research Project Code No. HS 031/2020, certified on July 22, 2020, completed before data collection).

Data collection tools

Step 1. **Data collection: demographic information, work history, symptoms of nervous system disorders, and t,t-muconic acid in urine.**

The instruments and data collection in this study is divided into two parts, i.e., the interview form and the urine sample collection device.

Interview questionnaires: The research tool and data collection were interview questionnaires, divided into 3 parts, totaling 30 items, scored by selecting answers and filling in the words.

Part 1 - includes ten personal data, such as gender, age, body mass index, marital status, education level, and history of smoking and consumption of alcohol.

Part 2 - includes the work history of five items such as work experience (years), the number of hours worked in a day (hours), the number of days worked per week (days), overtime (hours/week), and sleep time per night (hours).

Part 3 - includes 15 neurological symptoms, i.e., (1) Vertigo, (2) headache, (3) dizziness, (4) nausea and vomiting, (5) fatigue/easy fatigue, (6) more drowsy than usual, (7) lack of concentration and poor memory, (8) stressed and easily irritated, (9) lack of smell, (10) taste changes, (11) numbness of the hands and feet, (12) facial numbness, (13) limb weakness, (14) hand tremor, and (15) decreased sexual sensation. These

neurological symptoms are classified into five levels: (1) 0–2 symptoms with a score of 1, (2) 3–5 symptoms with a score of 2, (3) 6–8 symptoms with a score of 3, and (4) 9–11 symptoms with a score of 4 points, and (5) 12–15 or more symptoms with a score of 5.

Quality test of the questionnaires: A quality test of the interview questionnaire by considering the content validity, structure, and objectives of the research including the appropriateness of the language. The reliability was checked by finding *Cronbach's* alpha coefficient equal to 0.88.

Urine sampling: The equipment used for the biological samples is a delivery slip, plastic jar, and polyethylene tubes intended for urine collection. The levels of t,t-muconic acid are assessed based on the interpretation criteria recommended by the American Conference of Governmental Industrial Hygienists [22].

Data collection

Collecting data from interview questionnaires: Data collection by interview questionnaires starts with the researcher explaining to the research assistants to understand the question line accordingly. Later, the researchers met with managers again at each gas station's appointment and started collecting research data by interviewing each individual at each gas station. The interview time was 10–15 min at the office area of each gas station.

Urine sample collection: The employees were informed to collect a mid-stream urine sample in the plastic cup provided. At least 50 ml of this sample was placed into cold storage immediately. The urine specimen collections were sent to the laboratory each day and stored at -20 C to analyze the t,t-MA concentration for benzene. The urine samples were analyzed using HPLC following the method described in these studies [23, 24].

Step 2. Assessing the risk of benzene on the nervous system

Hazard identification: In this first step, the researcher uses the information collected from personal data, work history (i.e., exposure frequency (EF), exposure duration, the amount of substance, t,t-muconic acid in the urine of the sample consisted of the refueling service department, cashier, loading petrol, car washing, car repair, selling in convenience stores, raw materials to identify the hazards of benzene in the work area. The researchers search, collect and study the chemical information (Safety Data Sheet, SDS) of benzene, benzene exposure, and health effect information.

*Health effect assessment***:** Assessment of the effects of benzene on the nervous system as health effect rating (HER) is classified using the results collected from the first step of the assessment of 15 neurological disorders classified into 5 levels: 1) 0–2 symptoms = 1 point, 2) 3–5 symptoms = 2 points, 3) 6–8 symptoms = 3 points, 4) 9–11 symptoms = 4 points, 5) 12–15 or more symptoms = 5 points

Exposure assessment: Exposure assessment of benzene was calculated using data from exposure frequency and urinary t,t-muconic acid levels as follows:

(a) Exposure frequency (EF) consisted of five levels: (1) the frequency of exposure once a year, (2) exposure 2–3 times a year, (3) exposure 2–3 times a month, (4) exposure for 2–4 h consecutively in one shift, and (5) exposure throughout the shift, respectively.

(b) Concentration rating (CR): Urinary t,t-muconic acid levels are calculated by comparing t,t-muconic acid levels with the biological exposure index (BEI) [22] resulting in the chemical concentrations as (Concentration rating, CR) which are classified into 5 levels: Level 1 (Below BEI 10%), level 2 (Below 50%), level 3 (Below 75%), level 4 (75–100), and level 5 (Above 100%), respectively [17].

(c) Exposure assessment score: EAS is calculated by multiplying EF (level 1–5) and CR (level 1–5) as (EAS) = [EF × CR]. EAS was classified into five exposure rating (ER) groups as follows: Level 1: Acceptable (1–3 points), Level 2: Low (4–9 points), Level 3: Moderate (10–16 points), Level 4: High (17–20 points), Level 5: Very high (21–25 points) [17].

Risk characterization

Risk characterization is calculated by multiplying the ER and the HER as [hazard characterization = ER × HER]. The multiplied scores of ER and HER were then used to classify the 5 levels of risk characteristics as follows: Level 1: 1–3 points = acceptable or no significance, Level 2: 4–9 points = low, Level 3: 10–16 points = medium, Level 4: 17–20 points = high, Level 5: 21–25 points = very high [17].

Statistics analysis

The statistics used in the study were descriptive statistics, including frequency, percentage, mean, standard deviation, and range (minimum/maximum). *Chi*-square statistics were used to analyze the data to determine the relationship between t,t-muconic acid of BEI levels and neurologic disorder symptoms.

RESULTS

Part 1. Demographic information, work history, and neurological disorders

This study found the number of males and females is similar amount of which 68.5% were females more than males. The mean age was 30.25 ± 11.105 years, and the average body mass index is 23.63 ± 5.26 kg/sqm classified as at the fuel dispenser at 32.0% and

outside the fuel dispenser at 10%, drinking alcoholic beverages at 49.0% at the fuel dispenser 49% and outside the fuel dispenser 42.0%.

Work history: The results of this study found that working experience is 2.44 ± 4.06 years (1.61 ± 2.85 years at the fuel dispensers vs 3.81 ± 5.27 years outside the fuel dispensers), hours worked are 9.05 ± 1.57 h/day (9.09 ± 1.54 h/day at the fuel dispensers vs 8.98 ± 1.63 h/day outside the fuel dispensers), the number of days worked is 6.31 ± 2 day/week (6.32 ± 0.49 years at the fuel dispensers vs 6.30 ± 0.46 years outside the fuel dispensers), operation overtime is 6.42 ± 4.87 h/week (4.94 ± 2.61 h/week at the fuel dispensers vs 5.44 ± 2.60 h/week outside the fuel dispensers). Table 1 presents the details.

Table 1. Work history at fuel dispenser and outside the fuel dispenser

Work history	Fuel dispenser area	Outside fuel dispenser area	Total
Work experience (years), Mean±SD	1.61±2.848	3.81±5.266	2.44±4.063
Working hours per day, Mean±SD	9.09±1.535	8.98±1.628	9.05±1.568
Workdays per week, Mean±SD	6.32±0.489	6.30±0.460	6.31±0.477
Overtime work hours/week, Mean±SD	6.53±4.777	6.23±5.145	6.42±4.871

Table 2. Number (percentage) of neurological disorders among workers in gas station workers

Neurological disorders	n (%) of neurological disorders								
	Fuel dispenser area			Outside fuel dispenser area			Total		
	No	Sometimes	Frequent	No	Sometimes	Frequent	No	Sometimes	Frequent
1 Vertigo	58 (58.0)	40 (40.0)	2 (2.0)	57 (57.0)	38 (38.0)	5 (5.0)	115 (57.5)	78 (39.0)	7 (3.5)
2 Headache	53 (53.0)	44 (44.0)	5 (5.0)	49 (49.0)	41 (41.0)	10 (10.0)	102 (51)	83 (41.5)	15 (41.5)
3 Dizziness	64 (64.0)	34 (34.0)	2 (2.0)	62 (62.0)	33 (33.0)	5 (5.0)	126 (63.0)	67 (33.5)	7 (3.5)
4. Nausea/vomiting	82 (82.0)	18 (18.0)	0 (0.0)	85 (85.0)	14 (14.0)	1 (1.0)	167 (83.5)	32 (16.0)	1 (5.0)
5. Tired, easily tired	70 (70)	29 (29)	1 (1)	71 (71.0)	25 (25.0)	4 (4.0)	141 (70.5)	54 (27.0)	5 (2.5)
6. More drowsy than usual	78 (78.0)	20 (20.0)	2 (2.0)	77 (77.0)	21 (21.0)	2 (2.0)	155 (77.5)	41 (20.5)	4 (2.0)
7.Lack of concentration and poor memory	79 (79.0)	21 (21.0)	0 (0.0)	74 (74.0)	24 (24.0)	2 (2.0)	153 (76.5)	45 (22.5)	2 (1.0)
8. Stressed, easily irritated	68 (68.0)	29 (29.0)	3 (3.0)	55 (55.0)	37 (37.0)	8 (8.0)	123 (61.5)	66 (33.0)	11 (5.5)
9. lack of smell	91 (91)	8 (8.0)	1 (1.0)	92 (92.0)	7 (7.0)	1 (1.0)	183 (91.5)	15 (7.5)	2 (1.0)
10. Taste changes	93 (93.0)	7 (7.0)	0 (0.0)	95 (95.0)	5 (5.0)	0 (0.0)	188 (94.0)	12 (6.0)	0 (0.0)
11. Numbness of the hands and feet	81 (81.0)	16 (16.0)	3 (3.0)	74 (74.0)	20 (20.0)	6 (6.0)	155 (77.5)	36 (18.0)	9 (95.5)
12. Facial numbness	97 (97.0)	3 (3.0)	0 (0.0)	98 (98.0)	2 (2.0)	0 (0.0)	195 (97.5)	5 (2.5)	0 (0)
13. Weak limbs	86 (86.0)	12 (12.0)	2 (2.0)	87 (87.0)	10 (10.0)	3 (3.0)	173 (86.5)	22 (11.0)	5
14. Hand tremor	89 (89.0)	11 (11.0)	0 (0.0)	81 (81.0)	14 (14.0)	5 (5.0)	170 (85.0)	25 (12.5)	5 (2.5)
15. Decreased sexual sensation	94 (94.0)	4 (4.0)	2 (2.0)	88 (88.0)	11 (11.0)	1 (1.0)	182 (91.0)	15 (7.5)	3 (1.5)

Neurological disorders in employees working at and outside the fuel dispensers were observed during the past 3 months to the present, which sometimes include headaches (44.0% at the fuel dispensers vs 41.0% outside the fuel dispensers) and dizziness (40.0% at the fuel dispensers vs 38.0% outside the fuel dispensers), as presented in Table 2.

Part 2. Assessment of neurological risk from benzene exposure

Exposure assessment

The results of the analysis of t,t-muconic acid levels showed the mean ± SD of the t,t-muconic acid concentration was 431.23 ± 233.69 µg/g.cr (449.28 ± 213.32 µg/g.cr at fuel dispensers vs 413.18 ± 252.20 µg/g.cr outside the fuel dispensers), and the range(min, max) is 393.40 (59.71–1482.46 µg /g.cr) classified as at fuel dispensers area 428.23 (95.58 - .1202.56 µg /g.cr and outside the fuel dispensers 375.57 (59.71–1,482.46), as presented in Table 3.

The results showed that urinary t,t-muconic acid levels compared to BEI as exposure concentration (EC) were mostly EC levels >100% of BEI (500 µg/g.cr) in 59 cases (29.5%) and EF level of benzene exposure continuously throughout the shift of 200 cases (100%), the details of which are shown in Table 4.

The result showed that the majority of ER were moderate or level 3 as observed in 92 cases (46.0%), followed by very high or level 5 as observed in 59 cases (29.5%), as presented in Table 5.

Table 3. Concentration of t,t-muconic acid in the urine of workers working at the fuel dispenser and outside the fuel

Benzene exposure indicators	Fuel dispenser area n=100		Outside fuel dispenser area n=100		Total (n=200)	
	n	%	n	%	n	%
t,t-Muconic acid in the urine (µg/g.cr)						
<500	67	67.0	74	74.0	141	70.5
≥500	33	33.0	26	26.0	59	29.5
Mean±SD	449.284±213.323		413.179±252.200		431.232±233.686	
Median Min-max	428.23 95.58-1202.56		375.57 59.71-1482.46		393.40 59.71-1482.46	
Percentile of t,t-muconic acid in urine (µg/g.cr)						
25					274.59	
50					393.62	
75					519.17	

Table 4. Number and percentage of urinary t,t-muconic acid levels classify groups by comparing with BEI values, EC, and BEF

Level of exposure concentration (µg/g.cr)	Urinary t,t-muconic acid levels classify groups by comparing with BEI values	n (%)
1 (<25)	<10% of BEI (500 µg/g.cr)	0 (0.0)
2 (<250)	<50% of BEI (500 µg/g.cr)	40 (20.0)
3 (<375)	<75% of BEI (500 µg/g.cr)	52 (26.0)
4 (375-500)	<75-100% of BEI (500 ug/dl)	49 (24.5)
5(>500)	>100% of BEI (500 µg/g.cr)	59 (29.5)
Level of the frequency of exposure to benzene (1–5)	The frequency of exposure to benzene	
1	Infrequently	0 (0.0)
2	2-3 times a year	0 (0.0)
3	2-3 times a month	0 (0.0)
4	2-4 h per shift	0 (0.0)
5	Continuous exposure throughout the shift	200 (100.0)

BE – biological exposure index, EC – exposure concentration, BEF – benzene exposure frequency

Classification of effects on the nervous system (HER)

The results of the study on the classification of effects on the nervous system (HER) showed that most of the severity belonged to level 1 (no impact), as observed in 101 cases (40%), followed by level 2 (low impact) in 48 cases (24%), respectively, as presented in Table 6.

Risk characterization rating

Regarding risk characterization, the results found that most of them are at level 1 (low level), as observed in 108 cases (54.0%), followed by level 0 (acceptable) in 45 cases (22.5%), as presented in Table 7.

Table 5. Number and percentage of urinary benzene exposure rating (ER)

EF	t,t-muconic acid of BEI					Exposure rating (ER)		n	%
	1	2	3	4	5	EAS	Classification		
1	1	2	3	4	5	1-3	1=Acceptable	0	0.0
2	2	4	6	8	10	4-6	2=Low	0	0.0
3	3	6	9	13	15	10-16	3=Moderate	92	46.0
4	4	8	12	16	20	17-20	4=High	49	24.5
5	5	10	15	20	25	21-25	5=Very high	59	29.5

EF – Exposure frequency, EAS - Exposure assessment score

Table 6. Number and percentage of the severity of neurological symptoms

Level	Adverse symptom level	Nervous system disorders (symptoms)	n	%
1	Non-symptomatic	1 (0-2)	101	50.5
2	Low	2 (3-5)	48	24.0
3	Moderate	3 (6-8)	27	13.5
4	High	4 (9-11)	14	7.0
5	Very high	5 (12-15)	10	5.0

Table 7. Number and percentage of risk level calculated from exposure rating (ER) × severe health effect rating (HER)

Risk level			n	%
Score range (1-3/4-9)	Level	Level		
1-3	Acceptable	0	45	22.5
4-9	Low	1	108	54.0
10-16	Moderate	2	34	17.0
17-20	High	3	10	5.0
21-25	Very high	4	3	1.5
Total			200	100

Table 8. Relationship between urinary benzene concentrations and neurological disorders among employees in the fuel dispenser worker group and those outside the fuel dispenser worker group

Neurological disorder (Symptoms)	t,t-muconic acid in the urine (µg/g.cr)			Total, n (%)	P-value
	Percentile 25 (274.59 µg/g.cr), n (%)	Percentile 50 (293.62 µg/g.cr), n (%)	Percentile 75 (519.17 µg/g.cr), n (%)		
Classification of symptoms					
1 (0-2)	27 (54.0)	22 (44.0)	52 (52)	101 (50.5)	0.030*
2 (3-5)	11 (22.0)	12 (24.0)	25 (25.0)	48 (24.0)	
3 (6-8)	6 (12.0)	14 (28.0)	7 (7.0)	27 (13.5)	
4 (9-11)	4 (8.0)	2 (4.0)	8 (8.0)	14 (7.0)	
5 (12-15)	2 (4.0)	0 (0)	100 (100)	10 (5.0.03)	

Part 3. Examining the relationship between urinary benzene concentrations and neurological disorders

Assessment of the relationship between t,t-muconic acid, was classified according to 3 levels of percentile, 25, 50, and 75, with neurological disorders of the workers in the fuel dispenser area and the workers outside the fuel dispenser. The results showed that there was a statistically significant relationship (p-value <.05), as presented in Table 8.

Zones of gasoline stations and areas of work about the likelihood of exposure levels, adverse effects, and risk characterization are shown in Table 9

DISCUSSION

This study aims to assess the risk of benzene exposure to the nervous system among fuel service providers according to a health risk assessment model applied from the notification of the Ministry of Industry, Chemical Risk Assessment B.E. 2012 [17]. The results found that the mean age was 30.25 (±11.015) years, considered to be the working age consistent with the results of studies in Indonesia, indicating that the average age of employees working at gas stations was 29.90 years [25] as well as in Brazil with an average age of 30-year-old employees [26]. This study reported more female (68.5%) than male (36.5%) subjects, consistent with the 2021 National Statistical Report, indicating that the population is classified by labor status and sex. Throughout the kingdom, there are more female workers than males [27]. However, this study was not consistent with the study of *Tunsaringkasm* et al. [13] who studied the characteristics of employees at a gas station, in which most subjects were male.

Regarding the evidence of studies in humans, gender-specific differences appear to be variables of interest when considering benzene exposure and health effects. Previous research suggests the likelihood of a particular risk of health consequences for women [28]. Epidemiological evidence shows that women are involved greater in the effects of benzene exposure on health than males [29]. Finally, evidence that emerged about a different rate of metabolization of benzene in women rather than in men calls for further biochemical surveys to understand how this difference could lead to toxicologically relevant effects [30].

Assessing the risk of benzene on neurological disorders

The results of this study showed that the researcher applied a model of chemical health risk assessment according to the guidelines of the Ministry of Industry [17] with the following criteria: frequency of exposure, chemical concentration, and the severity of health symptoms. In this research, the focus was on

Table 9. Zones of gasoline stations and areas of working with the likelihood of exposure levels, adverse effects, and risk characterization (n=200)

Parameters	Likelihood of exposure levels					Adverse health effects					Risk characterization				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Zone															
Control zone 1	0(0.0)	0(0.0)	61(66.3)	33(67.3)	42(72.9)	75(74.3)	29(60.4)	17(63.0)	12(85.7)	4(40.0)	30(66.7)	77(71.3)	23(67.6)	5(50.0)	2(66.7)
Control zone 2	0(0.0)	0(0.0)	31(33.7)	16(32.7)	16(27.1)	26(25.7)	19(39.6)	10(37.0)	2(14.3)	6(60.0)	15(33.3)	31(28.7)	11(32.4)	5(50.0)	1(33.3)
Work area															
Oil distribution	0(0.0)	0(0.0)	42(45.7)	26(53.1)	32(54.2)	52(51.5)	27(56.3)	13(48.1)	5(37.5)	3(30.0)	18(40.0)	62(57.4)	16(47.1)	3(30.0)	1(33.3)
Outside oil distribution	0(0.0)	0(0.0)	50(54.3)	23(46.9)	27(45.8)	49(48.5)	21(43.8)	14(51.9)	9(64.3)	7(70.0)	27(60.0)	46(42.6)	18(52.9)	7(70.0)	2(66.7)

Control zone 1 – Control zone inside the pollution; Control zone 2 – control zone outside the pollution

neurological symptoms, which were found to be the main effect of benzene exposure on health. Especially, employees working at gas stations exposed to a wide range of low levels of chemicals should be a concern as well. This requires modern strategies for risk assessment and management of chemical health risks [31].

Exposure to low concentrations of benzene in the workplace can pose a health risk. Therefore, benzene exposure should be assessed even at substandard concentrations [7]. In addition, the level of risk varies by job type and exposure to specific chemicals [32] as well as the type of health risk assessment [16, 18, 19]. The benefits of chemical risk assessment are for health monitoring of employees exposed to benzene [1] and for formulating control measures to reduce workers' exposure to chemicals [7, 33]. Health risk assessment is an intuitive and attractive method because it can be used even where information is limited and does not require specialization. It also shows a quick way to recognize the problem of risk, the severity of danger, and frequency/probability [34].

Exposure assessment

There is a way to start the framework of chemical risk assessment for human health, which is to consider exposure to external chemicals or infer endogenous chemical exposure from exogenous concentration measurements by modeling. However, some uncertainties may be characteristic of this exposure assessment method because it is possible that an overestimation may occur or may be too low [7]. Bioassays have been used to assess benzene exposure [19]. The biomarker should be as specific as possible to the chemical and highly sensitive to detect chemicals even at low levels [7]. The results of this study assessed biological exposure by evaluating urinary t,t-muconic acid in fuel service workers at the end of shift, as recommended by ACGIH [22] as BEI.

Analysis results showed that the concentration at the fuel dispenser was higher than outside the fuel dispenser area. In this study, the concentration of t,t-muconic acid was found to be higher than the level seen in the study of *Geraldino* [4], which is consistent with the study of *Fakhrinnur* et al. [25]. The urinary t,t-MA level of the exposure group was 480.74. (219.65) µg/g creatinine, which was significantly higher compared to 229.96 (127.80) µg/g creatinine in office workers.

It is important to keep in mind that benzene is a carcinogen [9], so airborne benzene levels must not exceed the chemical limit. Especially, for BEI, t,t-MA levels should be below 500 µg/g of creatinine. However, levels of benzene below the standard may present a health risk [7]. In operations at a gas station, employees working at and outside the fuel dispensers are both likely exposed to benzene because it is a vapor and can easily spread from the source to different areas [4]. Therefore, *in vivo* exposure to benzene should be assessed to predict benzene internal exposure. Examination of benzene in the blood is a reliable biomarker of exposure; however, many researchers have used urinary benzene to quantify exposure [35]. In this study, the urinary exposure of benzene in the form of t,t-muconic acid was assessed, comparing the t,t-muconic acid concentration with the BEI. It was found that most of the EC were >100% of the BEI (500 µg/g.cr) [22] of 59 cases (29.5%), inconsistent with the study by *Chaiklieng* [1], who found that the t,t-MA metabolite was detected in 77 workers (32.8%) and 16 workers (6.8%) with the highest level of more than 500 µg/g.cr (13 fueling workers and 3 cashiers).

In terms of operating frequency, most of the employees who work in gas stations have several working days per week were 6.31 ± 2.48 days. It can be seen that most employees have more than 40 h of work per week, which exceeds the law of the Labor Protection Act B.E. 1998 set in section [36]. Therefore, employees should focus on adequate bedtime to reduce the risk of benzene, therefore, employees have the opportunity to be exposed to benzene in the body throughout the working period due to 100% of employees with continuous operating frequency throughout the shift, this allows being exposed to benzene throughout the working period. This study found that the majority of ER results were moderate, as observed in 92 cases (46%). More attention is still needed to occupational health risks exposure to benzene, toluene, and xylenes lower than OEL [7].

Classification of adverse health effects

Regarding adverse health effects or health effect rating (HER) results of this study, most were at level 1 (no impact), as observed in 101 (40%) subjects. It can be seen that this model for categorizing the severity of benzene from neurodegenerative disorders is convenient for use in assessing neurologic risk. However, the benzene severity classification scheme differs from the Dick FD [37] categorization of neurologic symptoms, categorized into four stages: Type (1), symptoms only (symptoms); Type (2A), sustained personality or mood change; Type (2B), impairment in intellectual function; and Type (3), dementia: cognitive impairment.

In addition, the severity of symptoms in this study differed from the study by *Chaiklieng* et al. [1], which classified the severity of benzene as having an impact on health classified according to the severity of the symptoms, including all systems of the whole body. Therefore, those with related duties must be aware that gas station operators are exposed to low levels of benzene. Employees also have the opportunity to be exposed to benzene, which can affect the nervous system and other systems [7, 38].

Benzene is known to be one of the major constituents of BTEX as a neurotoxic constituent of fuel. Gasoline-induced neurotoxicity increased mood disorders, as reported in past studies that describe a link between exposure to benzene in fuel and risk of neurological symptoms after benzene exposure [8, 9]. A good understanding of the mode of action for benzene for the benefit of deciding the severity of neurotoxic effects can influence health risk assessments for benzene [38].

The case of benzene toxicity on the nervous system as neurotoxins may damage the CNS and cause depression, facial flushing, ataxia, vertigo, mental confusion, dizziness, giddiness, nausea, weakness, and headache if exposed to low benzene concentrations. It can also cause abnormal symptoms [7]. Therefore, continuous surveillance for neurological symptoms should be undertaken.

Risk characterization

The results of this study were able to identify the risk characterization. It was found that the majority were at level 1 (low risk. Risk characterization by pollution control area and outside pollution control area and the operating area around and outside the fuel dispenser showed that the level of risk characteristics was similar. However, although the majority of employees are at a low level of neurotoxicity with benzene, they should not be ignored even in the very high-risk group of only 10 cases (5%). Therefore, the health of employees' nervous systems should be monitored on an ongoing basis [7].

The neurotoxicity risk assessment of benzene, although using the same health risk assessment model as the other studies, is conducted (likelihood on exposure × severity) [1]. This has different educational contexts such as study methods, sample groups, and study areas, among others. Importantly, the area studied in this study is in Rayong province which is an area developed in industry, transportation, and tourism and has a very high economic growth [39]. In addition, this study examined the group of workers in the fuel service station that covers operators outside the fuel dispenser, who can also be exposed to benzene, such as the food and beverage trade group, convenience store groups, and others.

Urinary benzene concentrations and neurological symptoms

The urinary t,t-muconic acid levels were classified according to three levels of percentile and neurological abnormalities of the subjects. The results showed that there was a statistically significant relationship (p-value <.05). The association results of this study support the results of assessing the risk of benzene exposure to neurological disorders. However, the effects of benzene on the nervous system may be influenced by several factors. Therefore, it is advisable to take proactive measures to measure chemicals in the working environment. Measures to enable labor to reduce benzene exposure should be instituted together with biochemical blood assessments for high-risk individuals [7, 9]. The study found that the risk of benzene level 5 was in 10 (5%) people, benzene may have a disturbing effect on the nervous system. Therefore, surveillance for nervous system disorders by assessing acetylcholinesterase enzyme, which may be an appropriate parameter [13].

The highlight of this study was that it assessed the risk of benzene on the nervous system covering a sample of people working at gas stations, both around and outside fuel dispensers. Both of these groups have the potential to be exposed to and be harmed by benzene. In addition, the health risks of employees operating at gas stations within and outside the pollution control area were also described. The weak point in this study was that severity in the nervous system is not assessed through a diagnosis by a specialist, and the severity was not ranked according to the severity of the symptoms but was rather assessed according to the number of neurological symptoms. However, the neurological symptom assessment passed the quality assessment from three qualified persons who are occupational medicine physicians. Therefore, this assessment tool is considered a quality tool and is convenient for use in screening neurological disorders in field areas. The present study also assessed the relationship between urinary benzene concentrations, t,t-muconic acid, and neurological disorders. The results of the study found that a statistically significant correlation would support and confirm the appropriateness of the risk assessment model to continue to assess the risk of benzene on the nervous system in the field.

CONCLUSION AND RECOMMENDATIONS

The results of identifying the risk characterization of benzene on the nervous system showed that most of the risks were at level 1 (low risk), as observed in 108 subjects (54.0%), followed by level 0 (acceptable) in 45 subjects (22.5%). The correlation between t,t-muconic acid concentrations classified by 3 Percentile levels and neurological symptoms of the study subjects were found to be statistically correlated (p-value <0.05. Therefore, the benzene neurotoxic risk assessment model was useful for implementation. However, the researchers suggest that more studies be conducted in larger samples and a manual be created for assessing the risk of benzene on the nervous system, which includes creating an application and evaluating the effectiveness of the application to assess the risk of benzene on the nervous system in the real area.

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Authors' contributions
Anamai Thetkathuek and Chan Pattama Polyong initiated the idea to conduct the study and collect data. Anamai Thetkathuek wrote the first draft of the manuscript and planned the design of the study. Wanlop Jaidee helped with the research methodology. All authors read and approved the final manuscript.

Disclosure statement
All authors declare no conflicts of interest.

Abbreviations
ACGIH: American Conference of Governmental Industrial Hygienists, BEF: Benzene exposure frequency, BEI: Biological Exposure Index, CNS: Central nervous system, CR: Concentration rating, EAS: Exposure assessment score, EEC: Eastern Economic Corridor, ER: Exposure ratings, HER: Health effect rating, IARC: The International Agency for Research on Cancer, OEL: Occupational exposure limits, t,t-MA: t,t-muconic acid, SDS: Safety Data Sheet.

REFERENCES

1. *Chaiklieng S., Suggaravetsiri P., Astrup H.* Biomatrix of health risk assessment of benzene-exposed workers at Thai gasoline stations. J Occup Health 2021 Jan;63(1):e12307. DOI: 10.1002/1348-9585.1230.
2. *Šafranić P., Petljak K., Naletina, D.* The growing importance of petrol stations as channels for expanding the retail services. 2022. [cited 2022] Available from https://www.academia.edu/38481044/Growing_importance_of_petrol_stations.
3. Department of Land Transport, Planning Division. Transportation statistics report for the year 2020. 2021. [cited 2021] Available from https://web.dlt.go.th/statistics/.
4. *Geraldino B.R., Nunes R.F.N., Gomes J.B., Giardini I, da Silva P.V.B., Campos É., da Poça K.S., Hassan R., Otero U.B., Sarpa M.* Analysis of Benzene Exposure in Gas Station Workers Using Trans, Trans-Muconic Acid. Int J Environ Res. Public Health 2020;17(15):5295, DOI: 10.3390/ijerph17155295.
5. *Tunsaringkarn T., Siriwong W., Rungsiyothin A., Nopparatbundit S.* Occupational exposure of gasoline station workers to BTEX compounds in Bangkok, Thailand. Int J Occup Environ Med 2012;3(3):1174–25.
6. *Smith M.T.* Advances in understanding benzene health effects and susceptibility. Annu Rev Publ Health 2010;31:133-48 2 p following 148. doi: 10.1146/annurev.publhealth.012809.103646.
7. *Wang A.H., Leng P.B., Li X.H., Mao G.C., Xu G.Z.* Occupational Health Risk Assessment of Low Concentrations Benzene Toluene and Xylenes]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2019;7(8):627-632. DOI: 10.3760/CMA.j.issn.1001-9391.2019.08.018.
8. *Sabbath E.L., Gutierrez L.A, Okechukwu C.A., Singh-Manoux A., Amieva H., Goldberg M., Zins M., Berr C.* Time may not fully attenuate solvent-associated cognitive deficits in highly exposed workers. Neurology 2014;82(19):1716-23. DOI: 10.1212/WNL.0000000000000413.
9. International Agency for Research on Cancer (IARC). Benzene. 2022. [cited 2022] Available http://monographs.iarc.fr/ENG/Classification/latest classif.php.
10. *Azari M.R., Konjin Z.N., Zayeri F., Salehpour S., Seyedi M.D.* Occupational exposure of petroleum depot workers to BTEX compounds. Int. J. Occup. Environ. Med 2012;3:39–44.
11. WHO. Preventing disease through healthy environments exposure to benzene: A major health concern. 2022. [cited 2022] Available from https://www.who.int/ipcs/features/benzene.pdf.
12. *Scheepers P., Wendt D., Dael M., Anzion R., Vanoirbeek J., Corneliu DR., Creta M, Godderis L., Warnakulasuriya D., Devanarayana NM.* Assessment of exposure of gas station attendants in Sri Lanka to benzene, toluene, and xylenes. Environ Res 2019;178:108670.
13. *Tunsaringkarn T., Zapuang K., Rungsiyothin A.* The Correlative Study of Serum Pseudo cholinesterase, Biological Parameters, and Symptoms Among Occupational Workers. Indian J Clin Biochem 2013;28(4):396–402. DOI: 10.1007/s12291-013-0322-3. Epub 2013 Apr 3.
14. *Mandiracioglu A., Akgur S., Kocabiyik N., Sener U.* Evaluation of neuropsychological symptoms and exposure to benzene, toluene, and xylene among two different furniture worker groups in Izmir. Toxicol Ind Health 2011;27(9):802–9. DOI: 10.1177/0748233711399309. Epub 2011 Mar 18.
15. *Anger WK.* Neurobehavioural tests and systems to assess neurotoxic exposures in the workplace and community. Occup Environ Med 2003;60:531–538.
16. *Edokpolo B., Yu QJ., Connell S.* Health Risk Assessment of Ambient Air Concentrations of Benzene, Toluene and Xylene (BTX) in Service Station Environments. Int J Environ Res Public Health 2014;11(6):6354–6374, doi: 10.3390/ijerph110606354.
17. Ministry of Industry. Chemical health risk assessment for industrial workers 2012. [cited 2022] Available from

http://www.fio.co.th/web/tisi_fio/fulltext/TIS2535-2012.pdf.
18. *Soltanpour Z., Mohammadian Y., Fakhri Y.* The concentration of benzene, toluene, ethylbenzene, and xylene in ambient air of the gas stations in Iran: A systematic review and probabilistic health risk assessment. Toxicol Ind Health 2021;37(3):134–141.
19. *Chaiklieng S., Suggaravetsiri P., Astrup H.* Risk Assessment on Benzene Exposure among Gasoline Station Workers. Int J Environ Res Public Health 2019;16(14):2545.
20. *Tongsantia U., Chaiklieng S., Suggaravetsiri P., Andajani S., Astrup H.* Factors Affecting Adverse Health Effects of Gasoline Station Workers. Int J Environ Res Public Health 2021;18(19):10014.
21. *Chaiklieng S., Nantanuch R.* Factors Associated with Adverse Symptoms related to Benzene Toxicity among Workers at Gasoline Stations. SRIMEDJ;30(5):458-66.
22. ACGIH. TLVs and BEIs: Threshold limits values for chemical substances and physical agents: biological exposure indices. American Conference of Governmental Industrial Hygienists, 1330 Kemper Meadow Drive, Cincinnati, OH. 2021.
23. *Onchoi C., Kongtip P., Yoosook W., Chantanakul S.* High-performance liquid chromatography for determination of urinary metabolites of toluene, xylene, and styrene, and its application. Southeast Asian J Trop Med Public Health 2008;39(6):1164–1171.
24. Perkin Elmer, Inc. Environmental: BTEX on Brownlee analytical C 18, Aromatics HPLC. 2013, United States of America.
25. *Fakhrinnur MT.; Dewanti L.* Factor associated with urine Trans, TransMuconic Acid (tt-MA) levels of gas station workers. Int J Adv Eng, Manag, and Science (IJAMS) 2016;2(6):640–4.
26. *Laurelize PRL, Cezar-Vaz M.R, de Almeida MCV., Bonow CA., da Silva MS., da Costa VZ.* Use of personal protective equipment by gas station workers: a nursing contribution. Texto Contexto Enfermagen 2014;23(1):193–202.
27. National Statistical Office. Labor Statistics. Available from http://statbbi.nso.go.th/staticreport/page/sector/th/02.aspx. Accessed 9 Feb 2022.
28. *Li X., Guo Y., Song X., He Y., Zhang H., Bao H., Li X., Liu Y., Zhai Y., Wamg J., et al.* A cross-sectional survey based on blood VOCs, hematological parameters and urine indicators in a population in Jilin, Northeast China. Environ. Geochem. Health 2019;41:1599–1615. DOI: 10.1007/s10653-019-00241-6.
29. *Poli D., Mozzoni P., Pinelli S., Cavallo D., Papaleo B., Caporossi L.* Sex Difference and Benzene Exposure: Does It Matter? Int J Environ Res Public Health 2022;19(4):2339.
30. *Brown E.A., Shelley M.L., Fisher J. W.* A pharmacokinetic study of occupational and environmental benzene exposure about gender. Risk Anal 1998;18:205–213. DOI: 10.1111/j.1539-6924.1998.tbj00932.x.
31. *Bonzini M., Leso V., Iavicoli I.* Towards a toxic-free environment: perspectives for chemical risk assessment approaches. Med Lav 2022;113(1):e2022004. DOI: 10.23749/mdl.v113i1.12748.
32. *Chalak M.H., Bahramiazar G., Rasaee J., Fahimi R., Anbardan A.N., Jafari H., Nasab F.R.* Occupational health risk assessment at healthcare institutions: Developing a semi-quantitative risk method. Int J Risk Saf Med 2021;32(4):265–278. DOI: 10.3233/JRS-200048.
33. *Gul M., Ak F.M.* A comparative outline for quantifying risk ratings in occupational health and safety risk assessment. J Clean Prod 2018;196:653–664. DOI: 10.1016/j.jclepro.2018.06.106.
34. *Li J., Bao C., Wu D.* How to design rating schemes of risk matrices: a sequential updating approach. Risk Analysis 2018;38:99–117. doi:10.1111/risa.12810.
35. *Arnold S.M., Angerer J., Boogaard P.J., Hughes M.F., O'Lone R.B., Robison S.H., Schnatter A.R.* The use of biomonitoring data in exposure and human health risk assessment: benzene case study. Crit Rev Toxicol 2013;43(2):119–53. DOI: 10.3109/10408444.2012.756455.
36. Ministry of Labor labor protection. 2021. [cited 2021] Available from https://lb.mol.go.th/.
37. *Dick F.D.* Solvent neurotoxicity. Occup Environ Med 2006;63(3):221–226. DOI: 10.1136/oem.2005.022400.
38. *North C.M, Rooseboom M, Kocabas NA, Schnatter A.R., Faulhammer F., Williams S.D.* Modes of action considerations in threshold expectations for health effects of benzene. Toxicol Lett 2020;1;334:78-86. DOI: 10.1016/j.toxlet.2020.09.005. Epub 2020 Sep 14.
39. EEC. Launched the 'Public Health Development' plan EEC' supporting emerging diseases and population expansion. 2022. [cited 2022] Available from https://www.eeco.or.th/en.

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