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REVIEW ARTICLE

VITAMIN D IN SARS-COV-2 INFECTION

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ABSTRACT

Background. SARS-CoV-2 virus is one of the largest RNA viruses, included in the coronavirus group, showing tropism to airway epithelial cells. SARS-CoV-2 causes an acute respiratory infectious disease, Covid-19. According to WHO reports, mortality due to Covid-19 is higher in the elderly and in those burdened with comorbidities such as diabetes, obstructive pulmonary disease, coronary artery disease, cancer, hypertension, hepatitis B, obesity or chronic kidney disease.

Objective. The aim of the study was to review the current literature on the influence and importance of vitamin D levels on the course of SARS-CoV-2 infection.

Material and method. A systematic review of studies published from January 1, 2009 to June 31, 2021 has been performed. For this purpose, bibliographic databases such as PubMed and Scopus were searched. The following keywords and combinations were used: Covid-19, vitamin D, 25-hydroxy-vitamin D, vitamin D supplementation, SARS-CoV-2.

Results. It has been shown that vitamin D plays an important role in the mechanisms of the innate immunity in the course of the acute respiratory infections. The overlapping factors of the severity of COVID-19 disease, vitamin D deficiency, and the prevalence of obesity, age scare, ethnicity, has led some researchers to hypothesize that vitamin D supplementation may be promising as a preventive or therapeutic measure for COVID-19.

Conclusions. A very important factor that has an immunomodulatory character is vitamin D, the adequate supplementation of which can be a preventive or therapeutic measure in case of SARS-CoV-2 infection, especially in elderly people, with obesity and other chronic diseases.

Key words: *Covid-19, SARS-CoV-2, vitamin D3, supplementation*

STRESZCZENIE

Wstęp. Wirus SARS-CoV-2 to jeden z największych wirusów RNA, zaliczany do grupy koronawirusów, wykazujący tropizm do komórek nabłonka dróg oddechowych. SARS-CoV-2 powoduje ostrą chorobę zakaźną układu oddechowego, Covid-19. Według doniesień WHO śmiertelność z powodu Covid-19 jest wyższa u osób starszych i obciążonych chorobami współistniejącymi, takimi jak cukrzyca, obturacyjna choroba płuc, choroba wieńcowa, nowotwory, nadciśnienie, zapalenie wątroby typu B, otyłość czy przewlekła choroba nerek.

Cel. Celem pracy był przegląd aktualnej literatury dotyczącej wpływu i znaczenia poziomu witaminy D na przebieg zakażenia SARS-CoV-2

Materiał i metody. Przeprowadzono systematyczny przegląd badań opublikowanych od 1 stycznia 2009 r. do 31 czerwca 2021 r. W tym celu przeszukano bazy bibliograficzne, takie jak baza PubMed i baza Scopus firmy Elsevier. Zastosowano następujące słowa kluczowe i kombinacje: Covid-19, witamina D, 25-hydroksy-witamina D, suplementacja witaminą D, SARS-CoV-2.

Wyniki. Wykazano, że witamina D odgrywa ważną rolę w mechanizmach odporności wrodzonej w przebiegu ostrych infekcji dróg oddechowych. Nakładanie się czynników ciężkiego przebiegu choroby COVID-19, niedoboru witaminy D oraz występowania otyłości, straszy wiek, pochodzenie etniczne, doprowadził niektórych badaczy do hipotezy, że suplementacja witaminy D może być obiecująca jako środek zapobiegawczy lub terapeutyczny w przypadku COVID-19.

Wnioski. Bardzo ważnym czynnikiem o charakterze immunomodulującym jest witamina D, której odpowiednia suplementacja może być środkiem zapobiegawczym lub terapeutycznym w przypadku zakażenia SARS-CoV-2, zwłaszcza u osób starszych, z otyłością i innymi chorobami przewlekłymi.

Słowa kluczowe: *Covid-19, SARS-CoV-2, witamina D3, suplementacja*

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INTRODUCTION

SARS-CoV-2 virus is included in the coronavirus group, showing tropism to airway epithelial cells. SARS-CoV-2 causes the acute respiratory infectious disease - Covid-19. The first case of the disease was diagnosed in December 2019 in Wuhan, China. On 11 March 2020, the World Health Organization declared a Covid-19 pandemic, recognising that it is a seriously life-threatening disease to public health with international reach [26]. Penetration of the virus occurs through the receptor, which is located in the nasal mucosa, while the average incubation time is 5 days, but can reach up to 14 days. Most often, the course of the disease is asymptomatic or with slight fever, cough and dyspnoea [49]. In the advanced form, interstitial inflammatory lesions in the lungs appear, while severe forms develop acute respiratory failure, which progresses to multiple organ failure and eventually death of the patient. According to WHO reports, higher mortality rates have been reported in the elderly and those diagnosed with comorbidities such as diabetes, obstructive pulmonary disease, coronary artery disease, cancer, hypertension, hepatitis B, obesity, and chronic kidney disease [21]. Therefore, there is a need to implement a rapid, accessible and effective therapy to limit the spread of the virus and save lives. More and more studies and static studies are emerging that present the clinical course of this disease. In many countries around the world, preventive measures are being taken to limit the number and rapid growth of cases, so that the health care system remains viable. Great hopes are placed in the natural defence mechanisms of our organism, and their effectiveness depends on ourselves. Therefore, many scientists in the world are studying the influence of various factors that may significantly affect the body's response to the SARS-CoV-2 infection, with the aim of preventing or reducing its effects [22]. Vitamin D has been shown to play an important role in the mechanisms of innate immunity in the course of acute respiratory infections. Supplementation with this vitamin has also been shown to reduce the risk of respiratory infection [3].

The aim of this study was to review the current literature on the influencet and importance of vitamin D in the course of the SARS-CoV-2 infection.

VITAMIN D METABOLISM

Vitamin D, along with vitamins A, E, K is classified as a fat-soluble vitamin, now called a prohormone. One of the most important known functions of vitamin D is its role in calcium phosphate metabolism and thus in bone growth and mineralisation processes [15]. In the human body, provitamin D3, under the influence of solar UVB radiation is converted to previtamin D3, then isomerised to vitamin D3 under the influence of heat. The vitamin, which is formed in the deeper layers of the epidermis, which are close to blood and lymph vessels, is completely bound by DBP, a protein that is characterised by its high capacity and strength to bind vitamin D [13]. When the skin is overexposed to UV radiation, there is no intoxication with this vitamin, as the biologically inactive compounds lumisterol and tachysterol are formed. These biologically inactive photoproducts lack DBP's ability to bind them and are therefore systematically removed from the body with exfoliating epidermis [33]. In foods, vitamin D is found in two forms: vitamin D2 called ergocalciferol and D3 called cholecalciferol. Vitamin D2 is found in plants and mushrooms, while D3 is synthesised by animal organisms [47].

The exogenous and endogenous form of vitamin D is inactive in the human body and requires two steps of hydroxylation to convert it into a biologically active form. The first step takes place by 25-hydroxylase in a liver to produce 25-hydroxyvitamin D (calcidiol), which is a substance of moderate biological activity that is the main form of vitamin D in the bloodstream. The second step occurs among others in the kidney with the participation of 1-α-hycroxidase and leads to the formation of the active metabolite 1,25-dihydroxyvitamin D (calcitriol), which is widely considered as the most active form of vitamin D [8].

SOURCES OF VITAMIN D

Vitamin D occurs naturally in a small number of dietary products and it is estimated that 10-20% of the daily requirement comes from the diet. Vitamin D2 is supplied to the body only by eating products of plant origin and mushrooms (oyster mushroom, champignon, shitake mushroom). Sources of vitamin D3 in products of animal origin are fatty fish, eggs, milk and dairy products [5, 29]. A significant part of vitamin D3 found in the body (about 80%) comes from dermal synthesis. It has been shown that under Polish conditions, exposure on a sunny day, a minimum of 18% of the body surface from 10 AM to 15 PM for 15-20 minutes, results in adequate synthesis of vitamin D, without the use of sunscreens. Factors that reduce vitamin D synthesis up to 80% include: increased melatonin, use of UV filter creams, body weight, age, and skin pigmentation [25]. In Poland, there is a limited possibility to obtain the recommended serum 25OHD concentration through dermal synthesis due to its geographical location [24].

DETERMINATION OF VITAMIN D SERUM CONCENTRATION

Vitamin D deficiency is assessed by determination of plasma concentrations of the main circulating metabolite25-hydroxycholecalciferol,whichwillreflect the actual state of the body's supply of this vitamin. However, in order to provide the best assessment of the metabolism occurring in the body, it is also advisable to determine 1,25-dihydroxycholecalciferol, which will allow tracing the activity of 1-alpha-hydroxylase in the kidney and the production of active metabolites [36].

Many studies conducted in Poland and worldwide have shown that vitamin D deficiency is an increasingly common health problem for people living at higher latitudes, in all age groups. The main reasons for this condition are: staying most of the day indoors, diet poor in the aforementioned vitamin, not using supplementation and low active lifestyle [28]. The requirement for vitamin D should be considered in close relation to its concentration in the blood, by determining the blood metabolite 25-hydroxycholecalciferol. Despite the heterogeneous terminology and ongoing discussion about this concentration, currently most experts around the world adopt the following classification: optimal concentration 30-50 ng/ml, suboptimal 20-30 ng/ml, deficiency 10-20 ng/ml and severe deficiency 0-10 ng/ml [25].

According to the nutrition standards for the Polish population developed at the adequate intake (AI) level, the vitamin D intake for all population groups is 15 µg cholecalciferol/person/day, except for newborns for whom AI =10 µg [25].

Since 2013, Guidelines for vitamin D supplementation for Central Europe have been in force in Poland, where the indications for determination of vitamin D serum levels, the principles of supplementation and treatment of vitamin D deficiency are included [43].

BODY FUNCTIONS

Vitamin D plays an important role in maintaining calcium phosphate metabolism, but also has an important function in insulin secretion when conditions of increased demand for this hormone occur in the body, which is linked to the presence of the VDR (vitamin D receptor) also in the beta cells of the pancreas. Vitamin D deficiency may adversely affect insulin secretion and glucose tolerance in people with type 2 diabetes [49]. Vitamin D is also important for the normal development of nervous system tissue, as its metabolite 1,25(OH)2 D3 is produced locally in brain tissue and its receptors are found in the cerebellum, brainstem, hippocampus, forebrain, spinal cord, perivascular tissue, among others [46]. Vitamin D also exhibits anticancer effects by inhibiting excessive cell proliferation, stimulating apoptosis and cell differentiation, as well as regulating mRNA expression and modelling signalling pathways to inhibit inflammatory processes [1]. The anti-cancerogenic effect of vitamin D is also supported by the expression of 1α-hydroxylase in many extraskeletal tissues, as well as reduced synthesis of this enzyme in the course of various cancers, resulting in reduced calcitriol concentrations. The immunomodulatory properties of vitamin D in the prevention and treatment of autoimmune diseases, such as type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, vitiligo or multiple sclerosis, are increasingly being highlighted [29]. Vitamin D by regulating the expression of specific neurotrophins can stimulate neuronal growth. Another important function is to influence the maintenance of the normal epidermal barrier, by regulating calcium concentration, keratin gene expression, promoting their differentiation into corneocytes [23].

The result of calcium deficiency in the body is elevated parathormone levels, which will be an exponent of low vitamin D concentrations. Parathormone increases the production of calcitriol in the intestines and kidneys, which has a negative impact on the cardiovascular system and causes endothelial dysfunction, increased aortic stiffness, aortic valve calcification, the development of hypertension and dyslipidaemia. It has been shown that higher parathormone levels increase the risk of death from cardiovascular disease [20].

In a review by *Stefanowski* et al [45] it was shown that there is a lot of literature available showing the effect of vitamin D deficiency on the occurrence of depression and the severity of depressive symptoms.

INFECTIONS VERSUS VITAMIN D

In addition to the effects of vitamin D on bone metabolism and calcium balance, vitamin D has been shown to model the response of macrophages and monocytes against bacteria, viruses and microorganisms. In cases of visceral disease, inflammatory bowel disease, or pancreatic and liver diseases there are disorders of vitamin D absorption and metabolism leading to vitamin D deficiency in the body [29].

Several scientific publications have shown that vitamin D plays an important role in the body's innate response [44]. Through metabolism in the kidney and liver, the active Vitamin D metabolite - dihydroxycholecalciferol (1,25(OH)2D) interacts with numerous tissues through the VDR receptor.

It has been detected on monocytes, dendritic cells, macrophages, NK cells (natural killer cells – NK), T and B lymphocytes [39]. VDR activation results in the production of cytokines that inhibit the activation of the T helper cells (Th), while stimulating the activation of the regulatory T cells (Treg). The active metabolite of vitamin D also causes changes in dendritic cells, where an acceleration of their maturation, differentiation and migration is observed [15]. The role these cells play in stimulating lymphocytes and in the early detection of microorganisms indicates that an adequate supply of vitamin D may influence the speed of the immune response after contact with a foreign antigen. Vitamin D, by enhancing the phagocytic activity of macrophages and stimulating NK cells, has a direct effect on the body's ability to destroy microorganisms. In viral infections, lung epithelial cells convert inactive vitamin D into its active form, thus stimulating the production of the antimicrobial peptide, helping to fight the ongoing infection [10].

In a study conducted by *Ginde* [19], among 18,883 people aged over 12 years old, a correlation was found between vitamin D levels and the occurrence of upper respiratory tract infections. It was shown that serum vitamin D concentrations were inversely related to the occurrence of these infections. This relationship was even higher in patients with respiratory diseases (asthma and chronic obstructive pulmonary disease). It has been concluded that vitamin D supplementation can reduce the prevalence of upper respiratory tract infections and also reduce the severity of respiratory diseases. A study conducted in 2010, among 198 people, found that levels of this vitamin D≥38 ng/ml were associated with twice the frequency of the occurrence of the upper respiratory tract infections and also a shorter duration of infection [25]. Other researchers have assessed the effects of vitamin D on lung function in 10,000 Korean adults. The authors showed a positive correlation between lung function and 25 (OH) D concentration with regard to age, sex, height and season, and it was higher in patients with a history of tuberculosis [9].

VITAMIN D VERSUS COVID-19

The new disease entity Covid-19 is caused by SARS-CoV-2, which is responsible for severe acute respiratory distress syndrome. In the majority of cases infected with Covid-19, the disease is mild and there are no complications afterwards. In approximately 14% of all patients, the course of Covid-19 requires hospitalisation, oxygen therapy and has a severe nature, while 5% of patients require admission to an intensive care unit [48]. If the course of the disease is severe, complications may arise in the form of acute respiratory distress syndrome (ARDS), sepsis and septic shock, multi-organ failure including heart and kidney failure [52].

Given the dangerous course of Covid-19 infection, particularly among patients with coexisting respiratory, cardiovascular and diabetic diseases, there is a need for a vaccine to prevent infection, but also for therapeutic steps to help reduce the risk of contracting the disease and also to reduce the symptoms of an already existing infection [4, 32]. A very important factor that is immunomodulatory in nature is vitamin D, the adequate supplementation of which can be a preventive or therapeutic measure for SARS-CoV-2 infection, especially among the elderly, those with obesity and other chronic diseases [30]. Since the outbreak of the pandemic, many studies have been published that have investigated the association between vitamin D and Covid-19. These have been mostly observational, cross-sectional, retrospective and prospective cohort studies and randomised control trials [50].

A study by *Faniyi* et al [16] at the end of the first wave of the epidemic (n=392) among staff at NHS University Hospitals Birmingham showed that of the Covid -19 positive staff (55%), 15.6% (n=16) were vitamin D deficient. It was also shown that there were significantly lower levels of this compound among those from BAME groups (Black, Asian and Minority Ethnic), men and those with a higher BMI. Similar results were obtained in a study by *Maghboola* et al [31] conducted at Boston University School of Medicine, where vitamin D levels were measured in 235 patients hospitalised for coronavirus infection. They found that patients who had at least 30 ng/mL of 25-hydroxyvitamin D were significantly less likely to experience severe illness, including unconsciousness and hypoxia. A lower mortality rate was also achieved in this group. Patients with sufficient vitamin D levels had significantly lower blood levels of the inflammatory marker CRP and had a higher total blood lymphocyte number, suggesting that adequate vitamin D improved their immune function. Another small study of 107 patients in Switzerland also found that serum vitamin D levels were lower among patients with positive Covid-19 [11].

A review of recent findings by *Rhodes* et al [42] indicates that there is a lot of literature available demonstrating a beneficial effect of vitamin D on the course of Covid -19 infection. Vitamin D deficiency has been shown to be more prevalent in obese individuals, those with type 2 diabetes, hypertension, and most among ethnic minorities in Europe and North America - where darker skin pigmentation reduces skin synthesis, resulting in up to eight times higher prevalence of vitamin D deficiency. Greater vitamin D deficiency is observed, also among people placed in institutions, including prisoners and those in nursing homes. These results may suggest that UV radiation, and thus indirectly vitamin D, may be beneficial in reducing the prevalence of Covid-19. However, it should be noted that UV exposure also has a number of vitamin D-independent mechanisms such as a reduction in the number and activity of dendritic cells and macrophages which translates into impaired activation of T lymphocytes in the skin [6]. A survey of Italian patients (n=1486) diagnosed with *Parkinson's* disease showed that those taking vitamin D were less likely to have Covid-19 [17].

Another study conducted at Wuhan Hospital found that the prevalence of vitamin D deficiency was higher among Covid-19 patients (n=335), compared to a control group (n=560) studied in 2018-2019 (65.1% vs 40.7%; p <0.0001). In a linear regression analysis, age was directly associated with length of hospital stay, whereas serum 25(OH)D concentrations, gender, comorbidities, BMI or smoking were not associated with duration of hospitalisation. Serum 25(OH)D concentrations were significantly lower in the group of patients with severe Covid-19 symptoms than in the group with a mild course [30].

A study of 7807 patients in Israel found that suboptimal serum vitamin D levels may be a potential risk factor for Covid-19 infection, at high risk of hospitalisation, regardless of demographic characteristics and medical conditions [35]. A US observational study based on test data from Quest Diagnostics is the largest observational study to date, involving 191,779 patients aged 40-65 years found that higher incidence of Covid-19 was among patients with vitamin D deficiency (<20 ng/ml) than patients with optimal levels (30-34 ng / ml) and patients with values ≥ 55 ng / ml [27].

Low serum vitamin D concentrations are associated with hypocalcaemia. This condition has been frequently described in the course of other epidemics [51], as well as in 80% of Italians who were hospitalised for SARS-CoV-2 infection [14]. The causal relationship between serum vitamin D concentrations and the risk of SARS-CoV-2 infection and the severity of Covid-19 has not been definitively established. Age as well as comorbidities may play a greater role than vitamin D levels in fatal cases of Covid-19. In the long term, low vitamin D levels may be the determinant of the patient's comorbid condition and therefore a more or less direct determinant of Covid-19 severity [7].

Exposure to sunlight is one of the factors that is involved in endogenous biosynthesis of the vitamin D. The few published studies, which mainly refer to the first wave of the pandemic, describe a significant association between disease outbreak and latitude [42]. From an analysis, over 108 days, in 152 countries, *Moozhipurath* et al [37] found that a sustained unit increase in ultraviolet index was associated with a 1.2% decrease in the daily rate of increase in cumulative deaths from Covid-19 and a 1.0% decrease in the daily case fatality rate. A recent paper whose authors considered both the first and second waves of the pandemic (1 March to 30 April 2020 and 1 October to 30 November 2020, respectively), an analysis based on 40996 25(OH)D determinations, found no direct association between serum vitamin D levels, presumed UV dose and risk of SARS-CoV-2 infection [18].

During the pandemic, the recommendations regarding vitamin D supplementation in Poland have not changed. The key question is whether short-term vitamin D supplementation can be tested and also be a test for disease risk. In studies in people with a positive SARS-CoV-2 test result and vitamin D deficiency (25(OH)D ng/ml), a follow-up response to the result (60,000 biliary response or placebo for 7 days, no to the number of participants, including tests over 21 days [40].

Achieving adequate serum vitamin D concentrations in the population is a good clinical practice in preventing the adverse effects of vitamin D deficiency, which may also affect the course of Covid-19. Currently, there are no reliable intervention data available on vitamin D supplementation among patients hospitalized for COVID-19 [34]. There are also studies in which it has been shown that vitamin D supplementation did not improve the clinical outcomes of the studied patients [38].

CONCLUSIONS

The outbreak of the Covid-19 pandemic has created a global public health crisis and therefore preventive health measures are urgently needed that can reduce the risk of infection, progression and severity of this disease. Numerous studies have shown that vitamin D is an immunomodulatory hormone with proven efficacy in acute respiratory infections. Supplementation with this vitamin influences the immune system, and recent studies may suggest that a deficiency of this vitamin may have a significant impact on the course of Covid-19. In addition, attention is drawn to the fact that factors associated with higher mortality from Covid-19 (age, ethical background, obesity diabetes, hypertension) overlap with the risk of vitamin D deficiency. Vitamin D deficiency in the blood may impair immune function but, may also have a negative influence on Covid-19 treatment.

Although there is currently insufficient evidence to recommend vitamin D supplementation to reduce the risk of Covid-19, it is advisable to maintain concentrations of vitamin D in the blood at optimal levels. Further intervention studies are needed to confirm the hypothesis that vitamin D supplementation may be helpful in the prevention and treatment of Covid-19.

Author contributions

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. All authors provided substantial contribution to the design and implementation of this study and to the generation of the manuscript. The contributions of each author are as follows: *A. Decyk* conceived the design and purpose of the work. *A. Decyk, M. Kobylińska, K. Antosik, K. Kurowska* analyzed the data and interpreted the results based on the available literature. *M. Kobylińska and A. Decyk* drafted the manuscript. *A. Decyk, M. Kobylińska, K. Antosik and K. Kurowska* provided critical revisions and final approval of the version submitted.

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REVIEW ARTICLE

COVID-19 UPDATE: OMICRON VARIANT – A NEW EMERGING THREAT

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ABSTRACT

A new health threat in the form of a new variant of Covid-19 called Omicron has emerged globally in this holiday season. This new variant has been declared a variant of concern (VOC) by the World Health Organization and experts are expecting another wave of the pandemic. A lot still unknown about this variant and researchers worldwide are conducting studies to find the nature and characteristics of this mutated strain. Cases have begun to rise dramatically around the world and many countries have already imposed travel restrictions again to prevent disease transmission. It is being speculated that this particular variant has got tendency of immune escape and therefore can even infect fully vaccinated individuals. Vaccination and adhering to Covid-19 guidelines and protocols can curtail the spread of the virus. The present paper focuses on what is currently known about this variant, antigen diagnostic tests, importance of getting fully vaccinated and having booster dose etc. If we want to emerge from this pandemic, countries should practice vaccine equality and solidarity for the good of mankind.

Key words: *Covid-19, SARS-CoV-2, Omicron variant, vaccine, coronavirus pandemic*

INTRODUCTION

The COVID-19 pandemic caused by the SARS-CoV-2 virus has led to more than 270 million infections and 5.3 million of deaths worldwide [12]. Ever since the start of pandemic in December 2019, mankind has been trying to find out a cure that can put an end to this devastating disease. Things started to normalize slowly after the development and use of COVID-19 vaccines which have been given emergency use authorization by the US Food and Drug Administration (FDA) and the European Medicines Agency during this public health emergency [8]. However, emergence of different variants (alpha, delta, kappa etc.) of COVID-19 in the recent past has again threatened our existence. These are classified as Variants of Interest (VOI) and Variants of Concern (VOC) on the basis of transmissibility and mutability. These variants are thought to counter the immune response mediated by the vaccines and have the potential to cause COVID-19 even in fully vaccinated individuals [7]. New variants, genome sequencing, new travel advisories, partial lockdowns, and economic disruption, all sound familiar and seem to have become cyclic events.

The recent variant of COVID-19 known as 'Omicron' (B.1.1.529) first detected in samples from South Africa and Botswana on November 9, 2021, has been designated as VOC by World Health Organization (WHO) [15]. It is the fifth VOC and first since the emergence of the dominant 'Delta variant' (B.1.617) which resulted in millions of cases in several countries as it became the dominant variant globally. It has now spread into more than 100 countries worldwide and spreading rapidly into other nations as well. Till now more than 1700 cases of Omicron have been detected in India and a fresh wave of the epidemic is being anticipated [3]. An onrushing wave of the new variant can spell another onslaught on the health systems of various countries which have not yet fully recovered from the two previous waves of COVID-19 as it also needs time to recuperate.

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Omicron - current knowledge

Our knowledge regarding the new VOC-Omicron is still very limited. Researchers around the globe are conducting regular studies to better understand many aspects of Omicron and they continue to share their findings as they are becoming available. According to some researchers, this new variant Omicron has a 'very unusual constellation of mutations'. The available data suggests that Omicron has got 50 mutations, maximum for any COVID-19 variant so far, including 32 in spike proteins [4]. This spike protein is used by most of the viruses to enter body cells, and most vaccines are manufactured to target these spike proteins. The mutations on the Omicron variant are widely distributed on multiple proteins of SARS-CoV-2 such as NSP3, NSP4, NSP5, NSP6, NSP12, NSP14, S protein, envelope protein, membrane protein, and nucleocapsid protein.

Transmissibility: It is still unclear whether the new variant is more transmissible as compared to other variants like Delta. Currently, new infections are being reported from various countries and with each passing day new information is becoming available. Experts are of the opinion that high number of mutations alone is not enough to predict the behaviour of the virus [5].

Disease severity: Recent reports suggest that there have been increase in the rates of hospitalization due to COVID-19 in South Africa, US and Europe and it is attributed primarily due to the new variant Omicron which is fuelling new infections. Lot of information will become available in the coming days and weeks that will show how serious the threat of Omicron is [5].

Omicron variant: impact on antigen diagnostic tests (As of 28/12/2021)

Ever since the start of the COVID-19, the FDA has been continuously monitoring and evaluating the potential impact of genetic variants on antigen tests. The FDA has collaborated with the National Institutes of Health's (NIH) RADx program to study the performance of various antigen tests with samples of patients containing live virus having Omicron variant [14]. Early data indicates that antigen tests are able to detect the omicron variant but may have reduced sensitivity. It should be noted that these laboratory data are not a replacement for ongoing clinical study evaluations which are done using patient samples with live virus.

The performance of these antigen tests using patient samples with live virus are being continuously evaluated by FDA and RADx. The FDA has authorized the use of these tests as directed in the authorized labelling and people should continue to use them in accordance with the instructions included in the test kit. Antigen tests are generally less sensitive and less likely to pick up very early infections as compared to molecular tests. The FDA's analysis to date has identified certain EUA-authorized molecular tests that have the ability to detect the Omicron variant with a specific gene drop out detection pattern.

Role of monoclonal antibodies against Omicron

Corticosteroids and IL6 Receptor Blockers will still be effective for managing patients with severe COVID-19. The U.S. FDA has issued emergency use authorizations (EUAs) for monoclonal antibodies (Bamlanivimab plus etesevimab & Casirivimab plus imdevimab) for non-hospitalized patients with mild or moderate COVID-19 disease and for individuals exposed to COVID-19 as post-exposure prophylaxis. Unfortunately, it is now evident that these are much less effective against Omicron variant [11]. These two drugs played an important role in early treatment of COVID-19 in people who were unvaccinated or vaccinated but high risk. Sotrovimab is the only monoclonal antibody which is effective against this new variant but it is not widely available right now because of limited purchase and distribution by the governments.

Effectiveness of vaccines against Omicron

Omicron variant has raised serious concerns about reducing the efficacy of vaccines and neutralization antibodies due to its vast mutations [6]. Despite it short comings, vaccination still remains the most potent weapon against this new and emerging threat. While we have made tremendous strides in this respect in the recent past, vaccination coverage in poor and developing countries is still abysmal. Governments in several countries have started rolling out precautionary or 'booster' doses for those people who are fully vaccinated especially frontline health care workers and elderly people [9].

Recent research indicates that Omicron has the potential to infect even those who are fully vaccinated; vaccination is supposed to protect against severe disease and it will continue to be the mainstay of our fight against new variants of COVID-19. It has been observed by experts that even a small number of unvaccinated people in counties having high vaccination coverage, can start a new wave of the pandemic. This is being called as 'pandemic of the unvaccinated.' Therefore, it is crucial to extend the vaccination coverage in unvaccinated population groups.

Following COVID-19 appropriate behaviour

Another important way to prevent Omicron is to follow covid-appropriate behaviour by not lowering our guards [1, 16]. Individuals should continue to wear a well-fitting mask; open windows to improve ventilation; avoid poorly ventilated or crowded spaces; keep hands clean; cough or sneeze into a bent elbow or tissue; and get vaccinated when it's their turn (Figure 1). One of the reasons for occurrence of second wave (due to the Delta variant) was due to non-adherence to Covid protocols and guidelines which lead to loss of millions of lives across the world. Positive heath behaviours inculcated during the first and second wave have diminished in intensity with time owing to pandemic fatigue. For example, a disproportionate chunk of Omicron cases is among the people who are fully vaccinated. This indicates that the fully vaccinated for obvious reasons, have lowered their guards and are indulging more freely in risky behaviour like not wearing face masks and flouting social distancing norms. While they may be less prove to severe disease, they can perpetuate and spread infection. Similarly, health communication is also vital in our prevention efforts. Severity of Omicron should be backed by hard evidence as it could have adverse impact on collective health behaviour. Only a robust and thoughtful communication strategy can prove effective `in developing appropriate health behaviour.

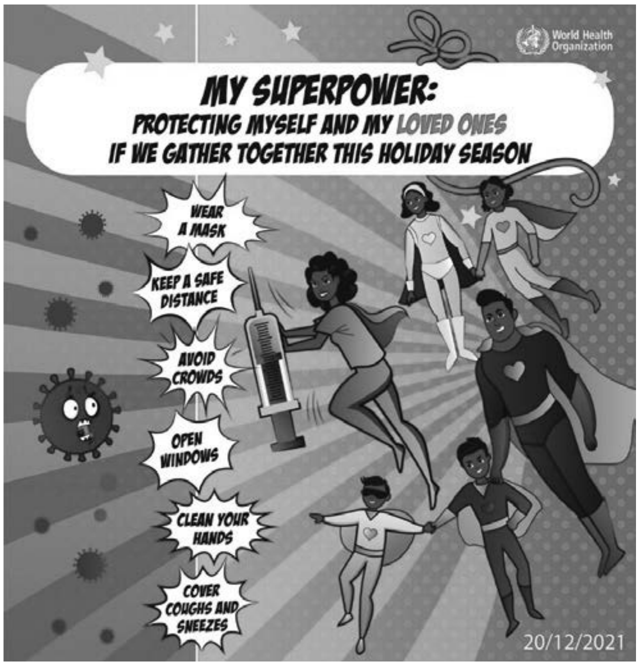

Figure 1. Practising Covid appropriate behaviour by the WHO

Omicron and Delta driving tsunami of cases – WHO

According to latest reports by WHO, the combination of Delta and Omicron is driving a dangerous tsunami of COVID-19 cases [2]. Record breaking figures are being reported from US, France, Italy, Denmark, Portugal, UK and Australia till now. This will continue to put pressure on exhausted health workers and health systems on the brink of collapse. According to estimates given by America's top infectious expert, Omicron infections are likely to peak at the end of January in the US, given its population size and vaccination rate. According to recent studies conducted in the UK (31st December 2021), a third dose of the vaccine can boost vaccine effectiveness against Omicron to 88% [13]. Therefore, several wealthy nations have launched booster drives to give third doses of Covid vaccines especially to health workers and elderly population.

Omicron in India

India is looking at an imminent Omicron wave as a good chunk of Indian population is yet to be fully vaccinated with a likely under prepared vaccine supply chain [10]. More than 1700 cases of Omicron have been detected in India as per recent reports. The government has made a recent announcement to this effect, allowing booster or precautionary doses for frontline health workers and elderly co-morbid. Moreover, elections to five states- Uttar Pradesh, Punjab, Uttarakhand, Goa and Manipur are on the cards. Political parties are gearing up for elections and drawing large crowds in public rallies. Complacent behaviour has been noticed in fully vaccinated individuals during recent festivals. Health experts and epidemiologists are being consulted by the Election Commission to give advice on pandemic situation in order to take call on the poll schedule. Indian policy makers need to learn from the past and trusted information should be shared and communicated timely by the Union Health Ministry.

CONCLUSION

The new variant Omicron has created panic worldwide as epidemiologists are expecting another wave of the pandemic in the days to come. As a result, many countries have already implemented travel restrictions to prevent virus transmission. Still lot of things about this variant are unclear but many studies are underway that will help us to understand this variant in a better way soon. However, as the number of cases has risen sharply within few days around the world, countries should continue to implement the effective public health measures to combat the spread of the new variant. Moreover, individuals who are not fully vaccinated yet should get themselves vaccinated. It has been reported that many countries are hoarding their vaccines allowing them to expire, offering booster shots while a large proportion of population in low and middle income countries have not received their first dose of the vaccine. There should be vaccine equity and solidarity among all countries in order to end this pandemic. Lastly, in the race to protect the global population against novel SARS-CoV-2 VOCs and VOIs, there is an urgent need to create much more effective one-dose vaccines that can protect people over their entire lifetime.

Conflict of interest

*The authors declare no conflict of interest***.**

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ORIGINAL ARTICLE

COVID-19 KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG HEALTHCARE WORKERS IN URBAN COMMUNITY BANGKOK, THAILAND

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ABSTRACT

Objective. This study examined associations between socio-demographic characteristics and knowledge, attitudes, and practices (KAP) related to COVID-19 among healthcare workers in Bangkok, Thailand.

Materials and methods. A cross-sectional online survey was distributed among healthcare workers in Bangkok, Thailand from July - August 2021.

Results. A total of 637 responses were received. Most participants were clinical workers (68.4%), and nearly half (47.3%) had been at risk of infection with COVID-19 (ever screening test). Binary logistic regression analysis found associations between high knowledge scores and the 26–35-year age group (OR=1.776-1.562, 95%CI 1.021–2.853), having a bachelor's degree or higher (OR=1.672, 95%CI 1.058–2.644), and clinical workers (OR=1.784, 95CI% 1.188–2.678). The 36 year and above age group was associated with higher attitude scores (OR=2.406, 95%CI 1.567–3.695). Higher practice scores were associated with females (OR=1.913, 95%CI 1.057–3.464), and clinical workers (OR=1.903, 95CI% 1.170–3.095). Correlation analysis found a positive correlation between practice scores and knowledge ($r=0.322$, $p<0.001$) and attitudes ($r=0.263$, $p<0.001$).

Conclusion. Although healthcare workers demonstrated overall high knowledge, attitude, and practice scores, this study identified several factors that influence KAP. This study can guide public health strategies regarding healthcare workers during the third wave of the COVID-19 pandemic in Thailand.

Key words: *COVID-19, knowledge, attitudes, practices, healthcare workers, Thailand*

Abbreviations

COVID-19 – Coronavirus disease; KAP – Knowledge, Attitudes, Practices; HCWs – Healthcare workers; ICU – Intensive Care Unit; OR – Odds Ratio; CI – Confidence Interval; r – correlation coefficient.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has emerged as a serious threat to human societies worldwide. An increasing proportion of the populations most at risk of serious illness or death are becoming infected [1]. Healthcare workers (HCWs) are at the forefront in the containment of COVID-19, and they are at increased risk of exposure to the virus [2]. Understanding the factors contributing to the increased rates of infection among HCWs can mitigate virus transmission among HCWs and patients alike [3, 4].

Although direct transmission in hospitals cannot be ruled out as a cause of infection among HCWs, the data do not indicate widespread nosocomial transmission among either patients or HCWs [5]. Nonetheless, COVID-19 represents an occupational health risk among HCWs due to their frequent exposure to

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infected individuals [6]. However, studies on factors influencing infection rates among HCWs are scarce and have methodological limitations that include poor control of the amount and intensity of exposure as well a lack of control of confounding factors [7].

People's adherence to infection control measures will largely be affected by their knowledge, attitude, and practices (KAP). Among existing studies, hospital environmental contamination and lack of isolation room facilities that lead to transmission between HCWs have been associated with overcrowding [8]. Another study reported that compared with non-HCWs, HCWs with COVID-19 were younger, had less severe illness, and were less likely to be admitted to hospitals [9]. These findings suggest the possibility of socio-demographic and facility-based factors in COVID-19 transmission among HCWs, which could indicate that some HCWs have inadequate knowledge and practices related to infection prevention. Although several studies have found adequate and good knowledge among HCWs during the COVID-19 pandemic [2, 3, 6, 10, 11, 12, 13], others have reported poor knowledge and improper practices [14, 15, 16]. Insufficient knowledge related to COVID-19 could lead HCWs to engage in inappropriate practices, increase their stress and anxiety, and undermine the adequacy of their medical judgments [6]. The public must routinely practice precautionary behaviors to control the spread of COVID-19, and requiring people to adhere to social distancing and appropriate preventative practices can help prevent or contain outbreaks [17, 18]; however, effective pandemic management requires an adequate understanding of the factors that influence behavioral changes [19], including the ways that KAP affect individuals' adherence to government measures [20].

Multiple waves of COVID-19 outbreaks in Thailand have impacted many provinces, with the largest outbreaks being associated with sites that attracted crowding, extended interactions, and high turnover [21, 22, 23]. It is important that HCWs adhere to the recommended COVID-19 prevention measures in order to support efforts to mitigate the impacts of this disease. Findings of a few previous studies on gaps in KAP regarding COVID-19 transmission in Thailand have been used to produce targeted educational videos that have contributed to with subsequent improvements on retesting [24]; however, such research has largely been focused on the general public, and few have specifically focused on HCWs.

This study aimed to help address the above-mentioned research gap by conducting a cross-sectional survey study among HCWs in Bangkok, Thailand. Specifically, this study aimed to: 1) collect information on HCWs' baseline knowledge, attitudes, and practices related to COVID-19; 2) examine potential associations between socio-demographic characteristics and COVID-19-related knowledge, attitudes, and practices; 3) compare clinical and non-clinical KAP; and 4) investigate possible correlations between knowledge, attitude, and practice dimensions. In so doing, this study can help identify various characteristics of HCWs who are more likely to be vulnerable to the effects of COVID-19. In addition, understanding how the virus spreads reinforces the importance of prevention measures. Knowing how COVID-19 has impacted people of all ages may reinforce the need for everyone to adopt health-promoting behaviors.

MATERIAL AND METHODS

Study design

This study conducted a cross-sectional survey study of HCWs working at the Faculty of Medicine Vajira Hospital, Bangkok, Thailand. Data collection occurred from July – August 2021. This study was approved by the Ethics Committee of Faculty of Medicine, Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand (Approval no. COA 116/2564).

Participants

HCWs aged 18 years and above agreed to participate in the study. All participants were of Thai nationality and living in the urban community of Bangkok, Thailand. The sample size was calculated using G*Power based on the estimated population of HCWs in the city.

Data collection

Questionnaires were completed using an online survey (Google forms). Participants were recruited on social media using a snowball technique based on social distancing during the COVID-19 pandemic. The invitation requested voluntary participation of HCWs and provided instructions for filling in the questionnaire.

Questionnaire

The questionnaire was designed based on a previous KAP study on COVID-19 [25] and adapted to the situation in Thailand by a team of experts. The questionnaire takes about 10 minutes to complete and is divided into four sections. The first part collected socio-demographic information, including gender, age, education, occupation, income, marital status, residence type, and risk of contracting COVID-19 (Table 1). The remaining three sections were dedicated to KAP items (Table 2), including: 1) ten knowledge items (K1-K10) rated No=0 and Yes=1 for a total score ranging from 0 to 10 and a cut-off point of 60% (≥8 points) indicating adequate knowledge; 2) nine attitudes items (A1-A9) rated on scale comprised of Agree=2; Not Sure=1; Disagree=0 for a total score ranging 0 to 18 and a cut-off point of 13 points denoting favorable attitudes; and 3) seven practice items (P1-P7) rated as Practice=2; Not sure=1; Not practice=0 for a total score ranging from 0 to 14 and a cut-off point of 13 points demonstrating proper practices. Several attitude, and practice items were based on participants' perspectives of whether the country could win the fight against the COVID-19 pandemic (see Table 2 for details).

Statistical analysis

We summarized the descriptive statistics and compared sociodemographic characteristics using binary logistic regression to test the association between socio-demographic characteristics with COVID-19 related knowledge, attitudes, and practices and investigate differences between clinical and non-clinical workers. We used *Spearman's* correlation analysis to examine relationships between knowledge, attitudes, and practices scores. The level of significance was set at $p<0.05$. The statistical analysis was performed using the Statistical Package for the Social Sciences Program (SPSS), version 22.

RESULTS

The original number of participants was 758; however, applying inclusion and exclusion criteria resulted in 715 responses. Among these, we obtained a total of 637 valid responses. Sociodemographic characteristics are presented in Table 1. Among the included HCWs, the majority (88.5%) were female, and the mean age was 37.86±11.70. Most participants had attained bachelor's degree or above (79.4%), were clinical workers (68.4%), earned an income of at least 25,000 Thai Baht (54.0%), and were single (61.5%) The largest group were living in homes (37.7%). Nearly half reported having been at risk of contracting COVID-19 (ever had a screening test; 47.3%). These characteristics are representative of the social demographics of the HCWs living in Bangkok.

Responses on knowledge, attitudes, and practices

The mean knowledge score was 8.53±1.49 (Table 2). Nearly 60% of participants showed good knowledge (n=370) and just over 40% demonstrated poor knowledge (n=267). Most participants understood that wearing two masks can prevent infection better than one layer (68.9%), recognized frequent handwashing as a preventative measure (95.3%), and knew that vaccines prevent severe symptoms (78.6%).

The mean attitudes score was 13.52±2.49 (Table 2). Participants were more or less evenly divided between favorable (50.9%) and unfavorable (49.1%) attitudes. Approximately 70% expressed a favorable attitude toward the idea that wearing two masks is better than one mask, and over 90% agreed that avoiding crowds and public places can protect COVID-19 (n=587). However, fewer than 40% believed that COVID-19 can be controlled (n=236), and most agreed that the pandemic has affected daily life (97.8%) and family (96.7%).

The mean practices score was 13.62±0.89 (Table 2). Approximately 80% of participants reported maintaining proper practices (n=512). The majority reported frequently washing their hands or cleaning them with alcohol (99.1%) and covering their mouths and noses with an elbow or tissue when coughing or sneezing (89.8%). Most reported that they strictly complied with government guidelines (96.9%), and followed news updates about the COVID-19 situation (98.4%).

Table 1. Sociodemographic characteristics of participants (n = 637)

Socio-demographics	n (%)
Gender	
Male	73 (11.5%)
Female	564 (88.5%)
Age (years)	
≤25	126 (20.3%)
26-35	176 (27.6%)
≥36	332 (52.1%)
Education	
Less than a bachelor's degree	131 (20.6%)
bachelor's degree or above	506 (79.4%)
Occupation	
Non-clinical worker	201 (31.6%)
Clinical worker	436 (68.4%)
Income	
<15,000	45 (7.1%)
15,001-20,000	138 (21.7%)
20,001-25,000	110 (17.3%)
>25,000	344 (54.0%)
Status	
Single	392 (61.5%)
Married	223 (35.0%)
Divorced	22 (3.5%)
Residence type	
House	240 (37.7%)
Condominium or apartment	180 (28.3%)
Hospital service	217 (34.1%)
Risk of contracting COVID-19	
Have been infected	13 (2.0%)
Experienced risk (ever screening test)	301 (47.3%)
Never at risk	194 (30.5%)
Not sure	129 (20.3%)

Table 2. Knowledge, attitude, and practice scores

	Knowledge	n (%)	
		True	False
K1	COVID-19 is spread through human-to-human transmission.	627 (98.4%)	10 (1.6%)
K2	COVID-19 can be spread by droplets and aerosols.	626 (98.3%)	11 (1.7%)
K3	COVID-19 symptoms include mild fever, tiredness, dry cough, and muscle pain.	604 (94.8%)	33 (5.2%)
K4	Everyone has the same risk of infection COVID-19.	560 (87.90%)	77 (12.1%)
K5	There currently is no treatment for COVID-19.	556 (87.3%)	81 (12.7%)
K6	Wearing two layers of masks can prevent infection from COVID-19 better than one layer.	439 (68.9%)	198 (31.1%)
K7	Washing hands frequently reduces the risk of COVID-19 infection	607 (95.3%)	30 (4.7%)
K8	Anyone who comes in contact with someone infected with COVID-19 should be immediately isolated for 14 days.	583 (91.5%)	54 (8.5%)
K9	Vaccines prevent severe COVID-19 symptoms.	501 (78.6%)	136 (21.4%)
K10	There currently is no information on the efficacy and safety of vaccination against COVID-19.	334 (52.4%)	303 (47.6%)

Knowledge mean±SD = 8.53±1.49; Max score=10
Good knowledge: 370 (58.1%), Poor knowledge: 267 (41.9%)
True=1, Note sure and False=0

	Attitudes	n (%)		
		Agree	Not Sure	Disagree
A1	COVID-19 can be controlled	236(37.0%)	297(46.6%)	104(16.3%)
A2	COVID-19 has affected my daily life.	623 (97.8%)	10 (1.6%)	4 (0.6%)
A3	COVID-19 has affected my family.	616 (96.7%)	15 (2.4%)	6 (0.9%)
A4	Cleaning inside households or offices daily can protect against COVID-19	424 (66.6%)	180(28.3%)	33 (5.2%)
A5	Wearing two masks is more effective than one mask	445 (69.9%)	160(25.1%)	32(5.0%)
A6	Avoiding crowds and public places can protect from COVID-19	587 (92.2%)	40 (6.3%)	10 (1.6%)
A7	Vaccination is the best protection against COVID-19.	347 (54.5%)	187(29.4%)	103(16.2%)
A8	The government's clear and accurate information and media will be able to control the spread of COVID-19.	260 (40.8%)	230(36.1%)	147(23.1%)
A9	I have confidence that governmental measures will be able to control the spread of COVID-19.	98 (15.4%)	224(35.2%)	315(49.5%)

Attitudes mean±SD = 13.52±2.49, Max score=18
Favorable attitudes: 324 (50.9%), Unfavorable attitudes: 313 (49.1%)
Agree=2; Not Sure=1; Disagree=0

	Practices	n (%)		
		Practice	Not sure	Not practice
P1	I wear a mask every time I leave the house.	633 (99.4%)	2 (0.3%)	2 (0.3%)
P2	I wash my hands or clean them with alcohol often.	631 (99.1%)	5 (0.8%)	1 (0.2%)
P3	I cover my mouth and nose with my elbow or a cloth or tissue. when I cough or sneeze	572 (89.8%)	22 (3.5%)	43 (6.8%)
P4	I maintain at least 1 meter distance from others in public places.	593 (93.1%)	31 (4.9%)	13 (2.0%)
P5	I avoid crowds and public places.	609 (95.6%)	25 (3.9%)	3 (0.5%)
P6	I follow news updates about the COVID-19 situation.	627 (98.4%)	9 (1.4%)	1 (0.2%)
A7	I have strictly complied with government guidelines.	617 (96.9%)	20 (3.1%)	0

Practice mean±SD = 13.62±0.89, Max score=14,
Proper Practices: 512(80.4%), Improper Practices: 125(19.6%)
Practice=2; Not sure=1; Not practice=0.

Association between sociodemographic characteristics and knowledge, attitudes, and practices (KAP)

Table 3 presents the results of the multivariate analysis. The multivariate analysis of knowledge showed that being at least 26 years of age was associated with greater knowledge about COVID-19 (*p* < 0.05; OR=1.776-1.562, 95%CI 1.021–2.853). In addition, having a bachelor's degree or higher (*p* = 0.028; OR=1.672, 95%CI 1.058–2.644) and being a clinical worker (*p* = 0.005; OR = 1.784, 95CI% 1.188–2.678) were associated with greater knowledge about COVID-19.

Being aged 36 or older was associated with more positive attitudes about COVID-19 (p < 0.001; OR = 2.406, 95%CI 1.567–3.695). Notably, being a clinical worker was not associated with positive attitudes about COVID-19.

Table 3. Results of binary logistic regression analysis

Association between sociodemographic characteristics, knowledge, attitudes, and practices (KAP)						
Multivariate						
Socio-demographics	Knowledge		Attitudes		Practice	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Gender						
Male	Ref.		Ref.		Ref.	
Female	1.030 (0.601-1.766)	>0.05	0.860 (0.501–1.474)	>0.05	1.913 (1.057–3.464)	**0.032**
Age (years)						
≤25	Ref.		Ref.		Ref.	
26-35	1.776 (1.109–2.853)	**0.017**	0.942 (0.588–1.509)	>0.05	1.400 (0.789–2.485)	0.250
≥36	1.562 (1.021–2.388)	**0.040**	2.406 (1.567–3.695)	**<0.001**	1.673 (0.989–2.830)	**0.055**
Education (degree)						
< bachelor's degree	Ref.		Ref.		Ref.	
≥bachelor's degree	1.672 (1.058–2.644)	**0.028**	1.099 (0.700–1.725)	>0.05	1.847 (0.855–2.586)	0.160
Occupation						
Non-clinical worker	Ref.		Ref.		Ref.	
Clinical worker	1.784 (1.188–2.678)	**0.005**	1.202 (0.800–1.806)	>0.05	1.903 (1.170–3.095)	**0.009**
Knowledge scores (1 = < 8 scores, 2 = ≥ 8 scores). Attitudes scores (1 = < 13 scores, 2 = ≥ 13 scores). Practice scores (1 = < 13 scores, 2 = ≥ 13 scores). OR, odds ratio; CI, confidence interval. Significant at *p*-value < 0.05.						
Association between KAP question items with occupational variable						
Occupation	KAP-questions items					
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Knowledge(K)	K6		K7		K9	
Non-clinical worker	Ref.		Ref.		Ref.	
Clinical worker	0.640 (0.416–0.984)	**0.042**	2.749 (1.234–6.126)	**0.013**	1.745 (1.132–2.689)	**0.012**
Attitudes(A)	A1		A5		A6	
Non-clinical worker	Ref.		Ref.		Ref.	
Clinical worker	1.458 (0.993–2.141)	0.054	0.675 (0.449–1.013)	0.058	2.133 (1.115–3.940)	**0.015**
Practice(P)	P2		P3		P7	
Non-clinical worker	Ref.		Ref.		Ref.	
Clinical worker	2.139 (0.264–17.376)	0.477	1.629 (0.933–2.844)	0.086	0.628 (0.188–2.095)	0.449
Health care worker is non-clinical worker =0, clinical worker =1. Knowledge(K) is good knowledge = 0, poor knowledge = 1. Attitudes(A) is favorable attitudes = 0, unfavorable attitudes = 1. Practice(P) is proper practices = 0, improper practices = 1. OR, odds ratio; CI, confidence interval. Significant at p-value < 0.05.						

Being female was associated with more proper practices (p = 0.032; OR=1.913, 95%CI 1.057–3.464), as was being 36 years of age or older (p = 0.055; OR=1.673, 95%CI 0.989–2.830) and being a clinical worker (p = 0.009; OR=1.903, 95CI% 1.170–3.095).

Association between KAP items and sociodemographic variables

When examining associations between sociodemographic characteristics and individual items, being a clinical worker was associated with higher scores than non-clinical workers on several knowledge items (Table 3), including the benefits of frequent handwashing and the effectiveness of vaccines for preventing severe symptoms (p < 0.05; OR=1.745-2.749, 95CI% 1.234–6.126), that wearing two mask layers can prevent infection better than one layer (p < 0.05; OR=0.640, 95CI% 0.416–0.984).

Being a clinical worker was also associated with more favorable attitude item scores related to the effectiveness of avoiding crowds and public places to protect against COVID-19 (p = 0.015; OR= 2.133, 95CI% 1.115–3.940). Differences between clinical and non-clinical workers optimism regarding whether COVID-19 can be controlled and wearing two masks is more effective than only one mask approached the level of significance (p >0.05). Being a clinical worker was not associated with any difference in practices.

Correlations between knowledge, attitude, and practice scores

Table 4 presents the results of the *Spearman's* correlation analysis. There were weak-to-moderate positive correlations between practices and both knowledge and attitudes.

Table 4. Spearman correlation analysis between knowledge, attitudes, and practices scores on healthcare workers

Correlation coefficient (*r*)		
Variables	Practice	*p*-value
Knowledge	0.322	<0.001
Attitudes	0.263	<0.001
Practice	1.000	-

Correlation coefficient significant at p-value < 0.05.

DISCUSSION

This study was conducted among HCWs working in an urban community during the third wave of the COVID-19 pandemic in Thailand. The findings show that sociodemographic factors such as gender, age, and education can influence health care workers' knowledge, attitudes, and practices, and practices are positively and significantly correlated with knowledge and attitudes. The findings reinforce the need for COVID-19-related training and education among HCWs as well as adequate supports for front-line workers in order to prevent or mitigate further outbreaks [26].

The finding that nearly half of the participants had experienced sufficient risk of infection that they had obtained a COVID-19 screening highlights HCWs' heightened occupational health risk due to their frequent exposure to infected individuals [6, 27]. One study argued that more robust national surveillance testing methods are needed to accurately monitor HCWs' COVID-19 infections and deaths as a means to improve safety [28]. The risk factors driving high infection rates among both patient-facing and non-patient-facing front-line HCWs require the implementation of special public health interventions and occupational health policies [29].

Association between sociodemographic characteristics and knowledge, attitudes, and practices

The finding that HCWs aged 26 years and above demonstrated a 1.77-fold increase in knowledge scores over those aged 25 below is in agreement with previous studies [30, 31], as is the result that participants with higher education levels showed a 1.67-fold increase in knowledge scores over those with less than a bachelor's degree [10, 32]. In addition, being a clinical worker was associated with a 1.78-fold increase in knowledge scores over non-clinical workers reinforces the results of previous research [30, 32, 33]. However, the high level of knowledge might be due to the advanced information networks of the current modern world and higher education level of the people [34].

The finding that HCWs aged 36 above showed a 2.40-fold increase in favorable attitudes than younger age groups is agreement with a previous study [35]. Notably, although being a clinical worker was not significantly associated with more favorable attitudes about COVID-19, the fact that hi group showed a 1.20-fold increase in attitude scores than non-clinical workers is similar to the results of a previous study [35]. Studies have suggested that HCWs were anxious about their families becoming infected with COVID-19 due to their occupation [36]. Positive attitudes are an important factor in implementing effective healthcare management [37].

The finding that being female was associated with a 1.91-fold increase in proper practices over males could be attributable to the tendency for women to be more directly involved with family responsibilities [38, 39]; however, some researchers have suggested that the reasons for gender and specialty differences require further exploration [40]. The result that clinical workers showed 1.90-fold increase in proper practices over non-clinical workers reinforces the recommendation of other studies that occupational factors must be addressed [38]. One study found limited prevention practices during the outbreak [33]; however, other research has demonstrated that COVID-19-related practices were significantly related to education [35].

Association between KAP question items and occupation

The finding that clinical workers showed 1.74-fold increase over than non-clinical workers in the knowledge items related to handwashing and the effectiveness of vaccines is in agreement with previous studies that have indicated that clinical workers have greater self-defense awareness than other groups [12, 13]. Like other studies [2, 3, 6, 10–13], we found that HCWs demonstrated overall adequate knowledge of COVID-19 transmission and preventative practices. There is potential utility for HCWs and government authorities to work together to further build vaccine awareness [41]. The current study also found that clinical workers showed a .64-fold reduction than non-clinical workers concerning the utility of wearing two masks rather than one. This result could be interpreted as an indication that clinical workers do not perceive that mask are sufficient protection; rather they may be aware that wearing personal protective equipment (PPE) is more effective at preventing transmission than masks [11]. Studies have reported that N95 face masks offer superior protection than other types [32]. Other studies have indicated that the use of PPE and infection control training are associated with decreased infection risk [7].

Our finding that clinical workers showed 2.13-fold increase over than non-clinical workers concerning the attitude that avoiding crowds and public places could help prevent COVID-19 infection aligns with previous research [13]. Studies have shown that transmission cases have been dominated by superspreading events or contexts, crowded spaces, indoor venues, and unventilated places [42]. We also found that clinical workers had more favorable attitudes than non-clinical workers that COVID-19 can be controlled, which is in agreement with a previous study that showed that this disease can be prevented [43]. Clinical workers' favorable attitudes may be based on a greater awareness of the real situation of COVID-19. Casual attitudes regarding personal safety have been identified as an important barrier to preventative practices [44]. Directing more rigid mask policies toward high-risk rather than low-risk setting or activities is expected to foster mask adherence and acceptance and decrease mask-related discomfort and fatigue [42]. Scientific evidence indicates that mask wearing reduces transmissibility per contact by reducing the transmission of infected respiratory particles in both laboratory and clinical contexts [45]. The current study found that most participants felt that the pandemic had affected their daily lives and families, and a substantial proportion had limited confidence that government measures would be able to control the spread of COVID-19. Hence, we recommend that hospitals and government agencies implement policies to specifically protect the safety of HCWs.

There were no significant differences in COVID-19-related practices between clinical and non-clinical HCWs. Both groups engaged in practices such as frequent handwashing and covering the mouth or nose while sneezing, as experts have recommended [46]. In addition, like HCWs in other studies [13, 47], most participants reported that they had strictly complied with government guidelines are similar to those of other published study and followed news update about the COVID-19 situation. However, other studies have reported poor knowledge and improper practices among HCWs during COVID-19 pandemic [14, 15, 16]. Education and consistent risk communication with the public are critical for an effective pandemic response [42]. One study suggested that close following of such information may reflect the impact and role of mass media and social media marketing on the way we perceive our world and our everyday lives on individual, social and societal levels during these critical times [47]. Health authorities should further increase publicity to raise public awareness of the disease.

Correlations between knowledge, attitudes, and practices scores

We found modest positive correlations between practices and both knowledge and attitudes, which is in agreement with a previous study [11]. However, another study found that although participants practiced preventive measures, some lacked optimistic attitudes [10]. Like other studies, we found that participants were concerned about dealing with the pandemic long-term [48]. One study suggested that differences between attitudes and practices are likely due to the perception of HCWs of the magnitude of the pandemic [47]. Research has demonstrated that a strong perception of the seriousness of a pandemic and its health consequences is an independent predictor of protective behaviors [49]. Hence, knowledge can play a crucial role in enhancing preventative practices [18].

Limitations

This study was conducted online using self-reported data, which may introduce selection bias. Secondly, this was a cross-sectional study conducted among HCWs in a single hospital, and the responses may not reflect the situation in other regions or at other points in time. Finally, we did not evaluate the questionnaire's reliability because it was designed based on a previous COVID-19-related KAP study [25] and adapted by a team of experts to reflect the local situation and incorporate participants' perceptions of whether the country could win the fight against the COVID-19 pandemic through implementing social distancing and other restrictive measures for the prevention and control of the COVID-19.

CONCLUSIONS

During the third waves of the COVID-19 pandemic in Thailand, HCWs demonstrated overall good knowledge, favorable attitudes and proper practices. However, this study identified several influencing factors that can guide public health strategies for HCWs. We recommend that health education campaigns target less educated HCWs. It is critical to ensure the continuous provision of PPE and training of all HCWs on proper infection prevention measures. Strategies should also focus on reducing fear and improving attitudes toward the care of COVID-19 patients as well as the promotion of preventive practices.

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Authors' contributions

JK., contributed to study design, data collection, data analysis, interpretation, writing and revision of the manuscript. PY., contributed to data analysis, interpretation, and writing. BS., contributed to study design, data analysis, interpretation. BW., contributed to study design, data analysis, interpretation. CS., contributed to data collection, design, data analysis, interpretation, and writing. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

WEIGHT STATUS AND ITS DETERMINANTS AMONG MOROCCAN ADOLESCENTS IN THE PROVINCE OF EL JADIDA

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ABSTRACT

Background. Despite the efforts of health systems, the prevalence of malnutrition reflected by weight status still poses challenges for many countries around the world. In fact, with the decrease in undernutrition, excess weight and obesity are gradually increasing in parallel with that of chronic diseases. Among school-going adolescents, however, weight status is less studied, particularly in developing countries.

Objective. The objective of this research is to examine the situation and the determinants of the weight status of adolescents in the province of El Jadida in Morocco.

Material and methods. The study was carried out on a sample of 463 students from the province of El Jadida of which 58.1% are boys and 41.9% girls, ages 9 and 17. Nutritional status was assessed by anthropometric measurements including height, weight and waist and hip circumference. Body mass index (BMI) was calculated to determine underweight, overweight and general obesity and waist circumference and the waist circumference to hip circumference ratio were used to determine abdominal obesity.

Results. The results indicate that only 59.6% of students had normal weight status, 40.4% had abnormal BMI values corresponding to thinness in 18.8% and overweight (overweight and obesity) in 21.6%. A significant difference in the prevalence of abnormal weight status by sex and level of physical activity was found. The results of the present study reveal an abnormal weight status in a large proportion of adolescents linked in addition to the peculiarities of the puberty phase to the sedentary lifestyle in the study population.

Conclusions. The results obtained revealed the coexistence of over-nutrition and undernutrition which require a prevention policy based on regular monitoring of weight status as well as on nutritional education and the promotion of physical activity for children and parents.

Key words: *underweight, overweight, obesity, weight status, socioeconomic status, adolescents, Morocco*

INTRODUCTION

Malnutrition is a global problem that affects both developed and developing countries, despite the efforts of health systems. It is manifested as undernutrition, a problem of poor countries, which includes among other problems, underweight, micronutrient deficiency or stunted growth in children. On the other hand, over-nutrition, which is a characteristic of rich countries, includes overweight, obesity and associated non-communicable diseases [35]. Like other emerging countries, Morocco is undergoing in parallel with economic, demographic and epidemiological transitions, a nutritional transition associated with changes in eating habits and changes in lifestyles [3, 30] leading to an increase in obesity and its comorbidities [30].

In children and young people obesity has become a major public health problem in the world and is on the rise in Mediterranean countries. In the countries of the southern Mediterranean, including Morocco, the problems of overweight and obesity are more present in the adult population. In these countries, few studies exist on the extent of this problem and its various factors among young people. Adolescence is considered as the period of transition between childhood and adulthood [36]. Numerous studies in countries in the northern rim of the Mediterranean

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report a gradual and steady decrease of the quality of diet of adolescents over time [16, 23], with unhealthy eating practices and behaviors harmful for current and future Public Health. This age category that coincides also with the period of life when young people make their own food choices without the direct control of their families is also associated with a decrease in the level of physical activity or a sedentary lifestyle leading to a rapid rise in childhood obesity that become increasingly worrisome in adolescents [15]. Indeed, the WHO estimated 42 million overweight children in 2010, of which almost 35 million (83%) are in developing countries [33]. In Tunisia, the prevalence of overweight and childhood obesity has been estimated at 8.7% [26] and 23.1% in Algeria [5, 35] . In Morocco, the WHO reports that a child and an adolescent Moroccans in ten are affected by the scourge of obesity, corresponding to 10.3% of boys and 9.9% of girls aged 5 to 19 [35]. The adolescents and the very young adults age category (15 to 20 years old) constituting future adults, represent 20% of the Mediterranean population [34]. In Morocco, it was estimated in 2017 at 6,035 million constituting 17.3% of the total population [36]. This figure reflects the magnitude of the problems associated with overweight and obesity among young people and which persist into adulthood. The prevention and management of this problem involves regular monitoring of weight status and the evaluation of overweight and malnutrition in general, in children and adolescents, with a preventive and curative goal [7, 32].

The problems of overweight and obesity in the countries of the southern shore compared to those of the northern rim of the Mediterranean's, are more present in adults. Few studies exist on the extent of these problems and on the weight status of adolescents, particularly in developing countries including Morocco.

Given the evolution of lifestyles and that the young population of today is the adult population of tomorrow, the objective of this research was to determine the prevalence and the determining factors of the weight status and the nutritional status of schoolchildren in the Moroccan province of El Jadida.

MATERIAL AND METHODS

Survey

This cross-sectional study involved a sample of 463 children and adolescents schoolers recruited from three schools, one primary and two secondary, in the urban area of the province of El Jadida (Casa-Settat region). The survey was carried out during the 2016/2017 and 2017/2018 school years, over a period from September 2016 to the end of June 2018. A structured questionnaire was used to collect information on demographic and socioeconomic characteristics of the participants and their parents, and anthropometric parameters were measured. The participants are of both sexes (58.1% girls and 41.9%% boys) aged 9 to 17 years old.

The information collected on demographic and socioeconomic characteristics are age, sex, the schoolers educational level with 3 level categories : Ppimary, middle and high school, parental education level determined by the highest level of one of the schoolers parents divided in 3 categories according to the number of years of education attained: Low (<7years), Medium (7 to <11years), High (>11years), household size, the housing type categorized in owner or renters status and the socio-economic status (SES) determined by the type of the parents professional situation in addition to the monthly household income. The SES level was classified into four categories: 1 - very low (<2500DH), 2 - low (2500 -7000DH), 3 - Medium (> 7000DH- 15000DH) and 4 - High (>15000DH).

Anthropometric measurements

The anthropometric parameters measured on the children studied were weight, height, circumference of the abdomen and circumference of the hips. These measurements were recorded by the protocol and standard instruments of the World Health Organization (WHO, 1995). The students' weight to the nearest 0.1 kg was measured using a standard mechanical beam scale, with light clothing. Standing height was measured without shoes, nearest 0.1cm, using a measuring rod, with the legs straight, heels together, arms dangling and shoulders relaxed. Body mass index (BMI) is calculated by dividing weight (in kg) by height in meters squared (kg/m^2). The distribution of BMI values is used to determine BMI Z-scores to obtain a numerical indication of the standard deviation (SD) from the median for sex and age. Depending on the value of the Z-score obtained, the adolescent's weight status is considered in the category indicating un<+ 1DS", overweight if "Z Score ≥ + 1DS" and obesity if "Z Score ≥ + 2DS" [35].

The waist circumference (or the circumference the weight if "Z Score ≤-2DS", normal weight if "-2DS <Z Score of the abdomen) is measured in the children/adolescents participating in this study using a tape measure halfway between the costal edge (bottom of the lower ribs and the antero-superior iliac spine/crest on the mid-axillary line, at the narrowest level of the torso at the end of a normal exhalation and without exerting pressure on the skin. Waist circumference (WC) is linked to the risk of cardiovascular and other chronic diseases [2, 21] . Waist circumference (WC) to height ratio (H) WHTR) is also calculated and the cutoff of 0.5 is used to define presence or absence of abdominal obesity in boys and girls [19]. A WHTR ≥

0.5 means the presence of abdominal fat and a WHTR <0.5 means no abdominal fat [19].

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) software for Windows version 23.0. Quantitative variables are expressed as means +/- standard deviations, and qualitative variables as frequencies and percentages. The children were classified into 4 categories based on the reference standards established by WHO for sex and age and using the WHO Anthro software for SPSS.

Analysis of variance (ANOVA) was used for the comparison of several means. Student's test was used for independent samples and the chi-square test to compare categorical variables.

Univariate and multivariate binary regression analyzes were performed. P values less than 0.05 are used to consider the differences statistically significant.

Ethical considerations

The study is carried out after obtaining authorization from the Regional Academy of Education, officials of the selected schools and after obtaining the oral consent of the schoolers and their parents. All the participants were informed of the objectives, the progress of the investigation as well as on their willingness to withdraw from the study at any time, if they so wish.

RESULTS

Demographic, socioeconomic and anthropometric characteristics

Table 1 presents the anthropometric parameters of the school-going adolescents studied. The mean age of the sample was 14.16 ± 1.93 years, mean weight was 50.35 ± 13.43 kg and the mean height was 1.57 ± 0.12 m. The mean BMI was 20.03 ± 3.83. The comparison of the means of these biodemographic parameters by sex using *Student's* t test, shows that weight, height, waist circumference and ratio WHTR were significantly higher ($p \leq 0.05$) in boys compared to girls while the mean BMI were comparable in both sexes.

The socio-demographic characteristics of the study population are shown in the Table 2. The overall sample (n=463) is composed of 58.1% of girls and 41.9% of boys. The majority of the youngsters participants were of 15 to 17 years old (58.74%), followed by the 12-14 years age group (29.8%) and that of 9 to 11 years old (11.44%). According to their school levels, 21.59% of participants were at primary level, 26.99% at intermediate level while 51.4% were at the high school level.

Concerning the children' parents education levels, the results show that about 33% have a low while 43.6% have high education level with about 43% of the pupils' fathers and 19.9% of their mothers of university level. Concerning the professional occupation of the parents, (results not shown), 72.4% of the students mothers were housewives without any remunerated employment, 14.5% were public employees, 8.9% were craftsmenand4.3%were workers. For the pupils' fathers, the majority (44.5%) were craftsmen, 28.5% public employees, 21. 8% were workers and 3.7% were businessmen. The Table 2 shows also that for the housing status, the parents of the majority of students (74.7%) were owners and only 25.3% were rent of their house.

THE NUTRITIONAL STATUS OF THE STUDIED CHILDREN

Weight status classes and the demographic and socioeconomic characteristics in the studied children

Table 3 shows the prevalence of the different BMI categories according to different demographic and socioeconomic characteristics of the study objects. The results show that overweight (including obesity) was more prevalent in girls (67%) than boys (33%) and in the 15 to 17 years age category (61%). The prevalence

Table 1. Biodemographic characteristics of all schoolchildren of both sexes

	Total Mean ± SD	Girls (n=269) Mean ± SD	Boys (n=194) Mean ± SD	P-value
Age (years)	14.16±1.93	14±1.93	14.37±1.92	0.039*
Height (m)	1.57±0.12	1.55±0.1	1.60±0.14	<0.001***
Weight (Kg)	50.35±13.43	49.28±12.07	51.84±15.02	0.043*
BMI (kg/m^2)	20.03±3.83	20.25±3.88	19.71±3.76	NS
Waist circumference (cm)	48.51±23.43	45.18±22.97	53.11±23.34	<0.001***
WHTR	0.31±0.15	0.29±0.15	0.33±0.14	0.007**

BMI: Body Mass Index; WHTR: waist-to-height ratio; values are Mean ± Standard deviation; *: Significant; NS: Not significant; test performed is t-test.

Table 2. Socio-demographic and economic characteristics of the schoolers by sex

	Total 463	Females 269(58.1)	Males 194 (41.90)
	N (%)	N (%)	N (%)
Age (years)			
9-11	53(11.44)	32(11.9)	21(10.82)
12-14	138(29.80)	94(34.94)	44(22.68)
15-17	272(58.74)	143(53.16)	129(66.49)
Education level			
Primary	100(21.6)	53(19.70)	47(24.22)
Middle school	125(27)	95(35.31)	30(15.46)
High school	238(51.4)	121(44.98)	117(60.30)
Housing type			
Owners	346(74.7)	160(59.47)	112(57.73)
renters	117(25.3)	109(40.52)	82(42.26)
Socioeconomic status (SES)			
SES 1	163(35.2)	93(34.2)	70(35.6)
SES 2	85(18.4)	50(18.6)	35(18)
SES 3	112(24.2)	66(24.5)	46(23.7)
SES 4	103(22.2)	60(22.3)	43(22.2)
Education levels of the parents			
Low	155(33.47)	49(18.2)	35(18)
Medium	105(22.67)	98(36.4)	57(29.4)
High	202(43.62)	122(45.4)	102(52.6)
Household size			
3-4	203(43.8)	115(42.8)	88(45.4)
5-6	212(45.8)	121(45)	91(46.9)
7-8	48(10.4)	33(12.3)	15(7.7)

N (%): number (percentage); SES 1-4: Socioeconomic status categories (Very low, Low, Medium, High)

of overweight/obesity was also more marked (90%) in households with a size between 3 to 6 people and in the schoolers with parents of high education level (52%) while it was present at comparable percentages whatever the socioeconomic status.

Table 3 shows also that underweight was more present in boys (56.3%) and in adolescents of 12to 17years old (79.3%). These malnourished youngsters were more present in households of a size between 3 to 6 people (87.3%), in poor socio economic status (39.1%) and with parents with medium (32.2%) or high (37.9%) education level.

Nutritional status of children according to age and socioeconomic characteristics

Table 4 shows that in the overall sample the prevalence of underweight was higher in boys (25.25%) compared to girls (14.13%) and increases with age in both sexes affecting more than half (51.0%) of boys and (31.6%) of girls aged between 15 and 17 years.

On the other hand, overweight including obesity was prevalent in about a quarter (24.90%) of girls and 17.01% of boys and more markedly for students in the 15 to 17 age group of both sexes, where this prevalence was 69.7% in boys and 56.7% in girls.

Abdominal fat evaluated by the waist to height ration (WHTR) was prevalent in around 11% of the schoolers sample comparable in girls and boys with a high prevalence in the 15-17years age group in both sexes.

Table 5 shows that overweight including obesity was more prevalent among boys of high socioeconomic level (30.3%), whose parents are with high education level (54.5%) and of households' size of 3 to 6 people (96.9%). On the contrary, being underweight was more present in boys of poor economic status (42.9%). In girls, overweight (including obesity) was more prevalent among those of poor economic status (32.8%) and those whose parents had a high education level (50.7%).

Table 6 shows that in general, more than 56% of girls practiced sport for at least one hour per day with 10% of them exerted it for more than 3 hours/day; 22%

Table 3. Weight status of the study adolescents according to demographic and socioeconomic characteristics

		BMI categories			
		Normal weight	Overweight & obesity	Underweight	P-value
		N (%)	N (%)	N (%)	
Sex	Males	112 (40.6)	33(33)	49(56.3)	0.04*
	Females	164(59.4)	67(67)	38(43.7)	
Age categories	9-11	27(9.8)	8(8)	18(20.7)	0.004**
	12-14	75(27.2)	31(31)	32(36.8)	
	15-17	174(63)	61(61)	37(42.5)	
School level	Primary School	55(19.9)	16(16)	29(33.3)	0.003**
	Middle School	67(24.3)	29(29)	29(33.3)	
	High School	154(55.8)	55(55)	29(33.3)	
Housing type	Owners	152(55.1)	68(68)	52(59.8)	NS
	Renters	124(44.9)	32(32)	35(40.2)	
Household size	3-4	123(44.6)	43(43)	37(42.5)	NS
	5-6	126(45.7)	47(47)	39(44.8)	
	7-8	27(9.8)	10(10)	11(12.6)	
Parents education level	Low	48(17.4)	10(10)	26(29.9)	0.009**
	Medium	89(32.2)	38(38)	28(32.2)	
	High	139(50.4)	52(52)	33(37.9)	
Socioeconomic status (SES)	SES 1	98(35.5)	31(31)	34(39.1)	NS
	SES 2	51(18.5)	17(17)	17(19.5)	
	SES 3	72(26.1)	24(24)	16(18.4)	
	SES 4	55(19.9)	28(28)	20(23)	

BMI : body mass Index ; N (%): number(percentage); SES 1-4: Socioeconomic status categories (Very low, Low, Medium, High).; *: Significant; NS: Not significant; test performed is *Chi*^{2}.

Table 4. Nutritional status of girls and boys by age groups

	Total N (%)	Boys N= 194			p-value	Total N (%)	Girls N= 269			p-value
		[9-11]	[12-14]	[15-17]			[9-11]	[12-14]	[15-17]	
		N (%)	N (%)	N (%)			N (%)	N (%)	N (%)	
Normal weight	112 (57.73)	9 (8.0)	22 (19.60)	81 (72.30)	NS	164 (60.96)	18 (11)	53 (32.3)	93 (56.7)	0.02*
Overweight & obesity	33 (17.01)	3 (9.1)	7 (21.20)	23 (69.70)		67 (24.90)	5 (7.5)	24 (35.8)	38 (56.7)	
Underweight	49 (25.25)	9 (18.4)	15 (30.60)	25 (51.00)		38 (14.12)	9 (23.7)	17 (44.7)	12 (31.6)	
WHTR≥0.5	22 (11.34)	31 (3.6)	5 (22.70)	14 (63.60)	NS	28 (10.40)	8 (28.6)	4 (14.3)	16 (57.1)	0.004**
WHTR <0.5	172 (88.65)	18 (10.5)	39 (22.70)	115 (66.90)		241 (89.59)	24 (10)	90 (37.3)	127 (52.7)	

N (%): number (percentage); WHTR: waist-to-height ratio; *significant, NS: Not significant; test performed is *Chi*^{2}.

used to take a siesta, 27% occasionally consume fast food snacks and 63% use food delivery from outside.

Among overweight girls, 82.1% do not take a siesta and 55.2% exert sport activity for less than an hour per day. In addition, 67.2% of these girls never take fast food snacks while 64.2% occasionally have home food deliveries.

Table 5. Nutritional status of boys and girls according to socioeconomic status

			Normal weight N (%)	Overweight & obesity N (%)	Underweight N (%)	p-value	WHTR≥0.5 N (%)	p-value
Boys	Socioeconomic status (SES)	SES 1	39 (35.7)	9 (27.3)	21(42.9)	0.48 NS	8(36.4)	0.55NS
		SES 2	19 (17.0)	6 (18.2)	10(20.4)		4(18.2)	
		SES 3	31 (27.7)	8 (24.2)	7(14.3)		3(13.6)	
		SES 4	22 (19.6)	10 (30.3)	11(22.4)		7(31.8)	
	Household size	3-4	54 (48.2)	14 (42.4)	20(40.8)	0.62 NS	9(40.9)	0.24NS
		5-6	48 (42.9)	18 (54.5)	25(51.0)		13(59.1)	
		7-8	10 (8.9)	1 (3.0)	4(8.2)		0(0.0)	
	Parents education level	Low	16 (14.3)	3 (9.1)	16(32.7)	0.02*	3(13.6)	0.77NS
		Medium	31 (27.7)	12 (36.4)	14(28.6)		6(27.3)	
		High	65 (58.0)	18 (54.5)	19(38.8)		13(59.1)	
Girls	Socioeconomic status (SES)	SES 1	57 (34.8)	22 (32.8)	13(34.2)	0.96 NS	12(42.9)	0.55NS
		SES 2	32 (19.5)	11 (16.4)	7(18.4)		6(21.4)	
		SES 3	41 (25.0)	16 (23.9)	9(23.7)		4(14.3)	
		SES 4	33 (20.1)	18 (26.9)	9(23.7)		6(21.4)	
	Household size	3-4	69 (42.1)	29 (43.3)	17(44.7)	0.62 NS	15(53.6)	0.41NS
		5-6	78 (47.6)	29 (43.3)	14(36.8)		11(39.3)	
		7-8	17 (10.4)	9 (13.4)	7(18.4)		2(7.1)	
	Parents education level	Low	32 (19.5)	7 (10.4)	10(26.3)	0.94 NS	4(14.3)	0.81NS
		Medium	58 (35.4)	26 (38.8)	14(36.8)		10(35.7)	
		High	74 (45.1)	34 (50.7)	14(36.8)		14(50.0)	

N (%): number (percentage); WHTR: waist-to-height ratio; SES 1-4: Socioeconomic status categories (Very low, Low, Medium, High); * significant, NS: Not significant; test performed is Chi^2.

Generally, in boys a quarter have siesta every day or sometimes, and about 73% practice a sport at least 1 hour/day with 11% that exercise that more than 3 hours/day, around 41% take fast snacks and 70% use food delivered from outside. Among the overweight boys, the results show that 75.8% do not take a siesta and that 57.6% have a sporting activity for 1 to 3 hours per day. In addition, 39.4% of these boys occasionally have fast food snacks and 72.7% occasionally use home food deliveries. Likewise, among boys with abdominal obesity, 59.1% participate in an average sport activity of 1 to 3 hours/day and 36.4% less than an hour per day.

Relationship between BMI classes (overweight including obesity and underweight), lifestyle and socio-demographic of school children

The results of the univariate and multivariate analyses performed to assess the interrelation among the variables and abnormal weight status (underweight and overweight including obesity), using logistic regression model are presented in Table 7. The table indicates that except for WHTR and fast food all variables were not significantly associated to overweight (including obesity). The results of the univariate logistic regression analysis showed that underweight is associated with gender, age, school level, parents education level, fast food and food delivery uses. After adjusting for sex and age, multiple regression analysis showed that sex ([ORa]: 2.22, 95% CI: 1.33-3.70), age ([ORa]: 3.53, 95% CI: 1.73-7.21), Food delivery ([ORa]: 0.44, 95% CI: 0.26-0.75) significantly influence the prevalence of underweight, whereas the school level of the pupils, the parents education level and the fast food were no longer significant after the adjustment for sex and age.

On the other hand, the WHTR and fast foods remain associated to overweight (including obesity) after adjusting for sex and age ([ORa]: 12.31, 95% CI: 5.92-25.59 and ORa: 0.10, 95% CI: 0.01-0.90, respectively). As shown in the Table 7, elementary school students were more likely to be underweight than high school students ([OR]: 2.80, 95% CI: 1.54 - 5.10, and OR: 2.30, 95% CI: 1.28 - 4.14, respectively).

Children of 9 to 11 age group were approximately 3.14 times (95% CI: 1.57–6.28) more likely to be underweight than those aged 12 to 14yrs. This finding suggests that high school students are more likely to be overweight (including obesity) than middle and elementary school students. Thus, age can be a critical

Table 6. Nutritional status according to boys and girls lifestyles

		Total	Normal weight	Overweight & obesity	Underweight	p-value	WHTR≥0.5	p-value
Girls (N=269)								
		N(%)	N (%)	N (%)	N (%)		N (%)	
Siesta	Yes	55 (18.58)	40 (24.4)	8 (11.9)	7 (18.4)	0.21NS	3(10.7)	0.35NS
	Sometimes	13(4.83)	6 (3.7)	4 (6.0)	3 (7.9)		1(3.6)	
	No	201(74.72)	118 (72.0)	55 (82.1)	28 (73.7)		24(85.7)	
Sport activity	<1 hour/d	117(43.49)	66 (40.2)	37 (55.2)	14 (36.8)	0.22NS	12(42.9)	0.14NS
	1- 3 hours /d	125(46.46)	79(48.2)	26 (38.8)	20 (52.6)		16(57.1)	
	> 3 hours/d	27(10.04)	19 (11.6)	4 (6.0)	4 (10.5)		0(0.0)	
Fastfood snacks	Never	196(72.86)	124 (75.6)	45 (67.2)	27 (71.1)	**0.04***	16(57.1)	**0.002****
	Occasionally	70(26.02)	40 (24.4)	19 (28.4)	11 (28.9)		10(35.7)	
	Everyday	3(1.12)	0 (0.00)	3 (4.5)	0 (0.0)		2(7.10)	
Food delivery	Never	96(35.69)	55 (33.5)	22 (32.8)	19 (50.0)	0.15NS	5(17.9)	0.05NS
	Occasionally	170(63.2)	108 (65.9)	43 (64.2)	19 (50.0)		22(78.6)	
	Everyday	3(1.12)	1 (0.6)	2 (3.00)	0 (0.0)		1(3.60)	
Boys (N=194)								
		Total	Normal weight	Overweight & obesity	Underweight	p-value	WHTR≥0.5	p-value
		N(%)	N (%)	N (%)	N (%)		N (%)	
Siesta	Yes	38 (19.59)	23 (20.5)	4 (12.1)	11 (22.4)	0.25NS	1 (4.5)	0.13NS
	No	146 (75.26)	84 (75.0)	25 (75.8)	37 (75.5)		19 (86.4)	
	Sometimes	10 (5.15)	5 (4.5)	4 (12.1)	1 (2.0)		2 (9.1)	
Sport activity	<1hour/d	52 (26.80)	27 (24.1)	11 (33.3)	14 (28.6)	0.29NS	8 (36.4)	0.38NS
	1- 3hours /d	120 (61.86)	75 (67.0)	19 (57.6)	26 (53.1)		13 (59.1)	
	> 3hours/d	22 (11.34)	10 (8.9)	3 (9.1)	9 (18.4)		1 (4.5)	
Fastfood snacks	Never	112 (57.73)	69 (61.6)	19 (57.6)	24 (49.0)	0.57NS	10 (45.5)	0.27NS
	Occasionally	79 (40.72)	42 (37.5)	13 (39.4)	24 (49.0)		11 (50.0)	
	Every day	3 (1.54)	1 (0.9)	1 (3.0)	1 (2.0)		1 (4.5)	
Food delivery	Never	59 (30.41)	29 (25.9)	8 (24.2)	22 (44.9)	0.10NS	5 (22.7)	0.61NS
	Occasionally	130 (67.0)	79 (70.5)	24 (72.7)	27 (55.1)		16 (72.7)	
	Everyday	5 (2.58)	4 (3.60)	1 (3.0)	0 (0.0)		1 (4.5)	

N (%): number (percentage); WHTR: waist-to-height ratio; * significant, NS: Not significant; test performed is *Chi*^{2}.

factor in predicting overweight (including obesity) and underweight in children and adolescents.

Table 7 also that the OR for sex with overweight (including obesity) was 1.39 (95% CI: 0.86-2.24) with girls more likely to be overweight than boys and the OR for sex and underweight was (OR = 0.53, 95% CI: 0.33 -0.86) with girls less likely to be underweight than boys. In addition, children whose parents had low education level were about 2.28 times (95% CI 1.24 -4.20) more likely to be underweight. This result suggests that children whose parents have a high level of education are more likely to be overweight (including obesity).

DISCUSSION

The present study revealed a coexistence of overweight and underweight problems in the Moroccan adolescent studied, testifying the existence of the double burden of malnutrition in this young population. Indeed, data from this study reports that about fifth of children were underweight, one fifth were overweight and 1.1% were obese. The reported prevalence of overweight in this work is higher than that found in children of other Moroccan cities such as Marrakech city (8%) [18], Rabat city (8.7%) [6] and than that obtained in a study carried out in a primary school of Marrakech which reported a prevalence of overweight of 12.9% in girls and 9.1% in boys [6].

Table 7. Association of weight status with lifestyle, demographic and socioeconomic characteristics of the study school children

		Overweight & obesity				Underweight			
		Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis	
		OR (IC 95%)	p-value	OR_a (IC 95%)	p-value	OR (IC 95%)	p-value	OR_a (IC 95%)	p-value
Sex	Females	1.39 (0.86-2.24)	0.183	0.73 (0.45-1.19)	0.20	0.53 (0.33-0.86)	**0.01***	2.22 (1.33-3.70)	0.001**
Age categories	9-11	0.85 (0.36-1.96)	0.695	0.82 (0.35-1.90)	0.64	3.14 (1.57-6.28)	**0.001****	3.53 (1.73-7.21)	**0.001****
	12-14	1.18 (0.71-1.96)	0.527	1.12 (0.67-1.88)	0.67	2.01 (1.16-3.46)	**0.01***	2.33 (1.33-4.09)	**0.001****
	15-17	reference				reference		reference	
WHTR	WHTR≥0.5	9.64 (4.89-19.00)	0.001**	12.31 (5.92-25.59)	0.001**	0.44 (0.10-1.98)	0.28	0.35 (0.08-1.65)	0.19
School level	Primary School	0.81 (0.43-1.54)	0.527	0.52 (0.13-1.98)	0.33	2.80 (1.54-5.10)	0.001	1.61 (0.44-5.80)	0.47
	Middle School	1.21 (0.71-2.07)	0.48	0.80 (0.31-2.12)	0.66	2.30 (1.28-4.14)	**0.01***	2.55 (1.00-6.51)	0.05
	High School	reference				reference		reference	
Household size	5-6	reference				reference			
	7-8	0.94 (0.42-2.11)	0.888	0.98 (0.43-2.24)	0.97	0.74 (0.33-1.63)	0.45	0.92 (0.54-1.58)	0.77
	3-4	1.01 (0.45-2.24)	0.986	1.03 (0.46-2.31)	0.95	0.76 (0.35-1.67)	0.49	1.15 (0.50-2.64)	0.75
Parents education level	Low	0.56 (0.26-1.18)	0.127	0.49 (0.21-1.12)	0.09	2.28 (1.24-4.20)	**0.01***	1.32 (0.63-2.79)	0.46
	Medium	1.14 (0.70-1.87)	0.601	1.03 (0.60-1.78)	0.91	1.33 (0.75-2.34)	0.33	0.98 (0.51-1.87)	0.94
	High	reference				reference		reference	
Socio-economic status (SES)	SES 1	reference				reference			
	SES 2	0.62 (0.34-1.14)	0.125	0.61 (0.32-1.13)	0.12	0.96 (0.49-1.88)	0.91	1.03 (0.51-2.07)	0.94
	SES 3	0.65 (0.32-1.34)	0.244	0.62 (0.30-1.28)	0.19	0.64 (0.33-1.25)	0.19	0.76 (0.38-1.52)	0.43
	SES 4	0.65 (0.34-1.25)	0.201	0.64 (0.33-1.22)	0.18	1.05 (0.55-1.99)	0.89	1.38 (0.70-2.74)	0.35
Sport activity	1- 3 hours /d	reference				reference			
	> 3 hours/d	2.14 (0.87-5.24)	0.096	2.09 (0.85-5.13)	0.11	0.99 (0.58-1.70)	0.98	0.82 (0.46-1.45)	0.50
	<1 hour/d	1.21 (0.50-2.95)	0.674	1.26 (0.51-3.07)	0.62	1.49 (0.68-3.24)	0.32	1.22 (0.54-2.75)	0.63
Fastfood snacks	Never	reference				reference		reference	
	Everyday	1.18 (0.72-1.93)	0.521	0.07 (0.01-0.68)	0.02*	1.62 (0.98-2.67)	**0.06***	0.20 (0.01-3.29)	0.26
	Occasionally	12.0 (61.32-109.90)	0.027*	0.10 (0.01-0.90)	0.04*	3.78 (0.23-61.55)	0.35	0.41 (0.02-6.87)	0.54
Food delivery	Never	reference				reference		reference	
	Everyday	1.00 (0.61-1.66)	0.99	0.51 (0.11-2.32)	0.38	0.50 (0.31-0.83)	**0.01***	0.44 (0.26-0.75)	**0.001****
	Occasionally	1.68 (0.38-7.46)	0.495	0.55 (0.12-2.39)	0.42	0.04 (0.02-0.01)	0.999	0.03 (0.02-0.01)	0.999

OR: l'odds ratio ORa; l'odds ratio after adjusting for sex and age; WHTR: waist-to-height ratio ;* significant

The prevalence of overweight including obesity observed in the survey sample is somewhat comparable or lower than that reported in some Mediterranean countries. Indeed, this prevalence was estimated as 36.3% in Algeria in 2014 [8], 31.1% in Lebanon [37], 37.9% in Syria and 32.4% in Greece [4]. In Tunisia, the prevalence of overweight including obesity was found to be 13.2% in 2011 [26]. Overweight and obesity are highly prevalent among adolescents in the European countries of the northern shore of the Mediterranean basin. It is the case of Italy where a study has reported that 50.2% of adolescents aged 15 are overweight [20], in France where 28% of adolescents aged 10 to 18 are overweight with 12.8 % were girls and 15.2% boys [13] and in Spain where a study also revealed a prevalence of 42.8% of overweight or obesity in young people with a higher prevalence among boys (25.3%) than girls (17.5%) [10] .

While the difference in excess weight between the sexes of adolescents is variable in industrialized countries [14], in the present work girls are more affected by the scourge of overweight than boys. It is known that the prevalence of overweight is greater in the puberty phase, and that the difference between boys and girls can be explained by the specificities of growth linked to gender and the different evolution of the distribution of fat mass according to sex [25]. However, this may be associated with the societal constraints encountered by girls, unlike boys, in the population studied as they have less freedom to exercise physical activities outside the home.

Childhood obesity is reported to be 16.9% in the United States [24] and around already 15% in 2002 in France [24]. In Africa, although low prevalence is recorded for some countries such as Togo, where a percentage of 2.86% for obesity and 1.72% for overweight is reported [11], a prevalence of 8.5 % was recorded in 2010 among African children and, this percentage was expected to reach 12.7% by 2020. The same is true in other parts of the world such as Asia where a relatively low prevalence of overweight and obesity estimated at 4.9% in 2010 is reported in Indian children [9]. In these developing and underdeveloped countries, where undernutrition was the rule reflected by underweight which is decreasing thanks to the efforts of governments, the problem of overweight and obesity is emerging and on continuous rise. This observation is linked to several factors including dietary changes and lifestyle characterized by a growing sedentary lifestyle linked to urbanization and globalization [1, 27].

In this study, the data confirms the nutritional transition in place in Morocco, as in other countries of the Mediterranean region and elsewhere in the world. Indeed, although in Morocco where the diet is of Mediterranean-type known as healthy, a gradual decrease in adherence to this diet especially among young people has already been reported in Greece, Spain, Italy and Cyprus with unhealthy eating practices and behaviors more marked in the 12-17 year age group likely to pose health problems in the future in this population once in adulthood [23]. Hence the need to promote a healthy diet among young people to avoid the development of weight excess and its consequences on health.

The present data also reports the presence of underweight among the participants in this study indicating the existence of the double burden of malnutrition, expressed by the coexistence of both over-nutrition and undernutrition characterized by being overweight and underweight respectively. Indeed, in many emerging countries the increase in obesity often coexists in the same population with chronic undernutrition [17].

Despite that the difficulty to compare prevalence of overweight, obesity and underweight are difficult to compare with those mentioned in the literature, sociodemographic and socioeconomic characteristics have been reported as determinant factors of weight status [12, 28, 29]. Accordingly age, sex, parents education level, the SES examined in the current study adolescents appeared to be associated with both under and overweight. In addition to these factors, a lifestyle characterized by a low level of physical activity or a sedentary lifestyle also contributes to childhood obesity [22, 31]. Among the life-style factors examined in this study fast food consumption and physical activity level are found as characteristics of the both underweight and overweight in the present young study population. Indeed, the fact that girls this study practiced less physical activities than boys (52.3% and 37.1% respectively) could explain the higher percentage of girls with the overweight (66.6%) compared to boys (33.7%).

The present results confirm the complex association of changes in diet and in eating behavior, industrialization, urbanization and increasingly sedentary lifestyles with overweight and obesity. The data reported here also bear witness to the unsustainability of diet and the need for policies to combat malnutrition by promoting adequate food in quantity and quality in addition to physical activity targeting school-aged children.

CONCLUSIONS

The results from this survey identified a number of the risk factors for malnutrition (overweight, obesity and underweight) that would provide information for the implementation of a prevention policy based on regular monitoring of body weight status as well as nutrition education as well as the promotion of physical activity for children and parents.

Declaration of interest

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

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ORIGINAL ARTICLE

EFFECT OF SELECTED LIFESTYLE FACTORS ON THE NUTRITION STATE OF ELDERLY PEOPLE WITH METABOLIC SYNDROME

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ABSTRACT

Background. The formation and development of the metabolic syndrome (MetS) is largely caused by lifestyle factors. Many studies have shown that excessive consumption of simple carbohydrates, alcohol, salt, physical inactivity and smoking increase the risk of MetS.

Objective. The aim of the study was to assess the nutritional status and its relationship with selected lifestyle factors in elderly people with MetS.

Material and methods. 81 people aged 51-75 participated in the study. According to the guidelines, all tests were performed on an empty stomach. The comparison of individual quantitative variables between the groups was performed using the *Student's* t-test for independent measurements or using the *Mann-Whitney* U test. The relationship between quantitative variables was verified with the *Spearman's* correlation coefficient. All statistical tests were based on a significance level of $p<0.05$.

Results. The BMI, the percentage of body fat and the waist circumference significantly exceed the norm for the population in the examined persons. The responses of respondents aged 51-65 show that 36% of people sweetened their drinks, 65.6% were salted their food, 51.6% consumed alcohol, and at the age of 66-75, respectively: 47.1%, 52.9%, 41.2%. There was no correlation between sweetening beverages and salting food and the concentration of glucose and lipids. However, a positive correlation was found between the amount of salt consumed and the heart rate ($r=0.28$, $p<0.05$). In both age groups, statistically significant differences in the concentration of triglycerides depending on alcohol consumption or non-consumption were found. Due to the lack of precise data on the amount of alcohol consumed, the correlation between alcohol consumption and the concentration of glucose and lipids was not analyzed. Physical activity was not undertaken by 39.1% of patients aged 51-65 years and 41.2% of patients aged 66-75 years. In the group of elderly people without physical activity, a statistically significantly higher glucose concentration was found in relation to those who were physically active (130 mg/dl vs. 105 mg/dl; $p=0.031$). Patients aged 51-65 who engaged physical activity had statistically significantly lower body weight, BMI, waist circumference and lean body mass, which requires further studies.

Conclusions. The anthropometric indices and parameters of MetS patients indicated disturbances in the nutritional status. Unhealthy lifestyle was shown mainly in the younger group of patients 51-65 years old (they sweetened drinks more often, salted dishes, consumed alcohol). Patients with metabolic syndrome did not undertake physical activity as often as recommended.

Key words: *metabolic syndrome, lipid profile, body composition, anthropometric measurements, lifestyle*

STRESZCZENIE

Wprowadzenie. Za powstanie i rozwój zespołu metabolicznego (MetS) w dużej mierze odpowiadają czynniki związane ze stylem życia. Wyniki wielu badań wskazują, że spożycie w nadmiarze węglowodanów prostych, alkoholu, soli, brak aktywności fizycznej oraz palenie papierosów zwiększa ryzyko MetS.

Cel. Celem badania była ocena stanu odżywienia i jego związki z wybranymi czynnikami stylu życia u osób starszych z MetS.

Materiał i metody. W badaniu udział wzięło 81 osób w wieku 51-75 lat. Zgodnie z wytycznymi wszystkie badania wykonane były na czczo. Porównanie poszczególnych zmiennych ilościowych pomiędzy grupami wykonano za pomocą testu *t-Studenta* dla pomiarów niezależnych lub za pomocą testu U *Manna-Whitneya*. Zależność pomiędzy zmiennymi ilościowymi była weryfikowana współczynnikiem korelacji *Spearmana*. Wszystkie testy statystyczne oparto na poziomie istotności $p<0,05$.

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Wyniki. Wskaźnik BMI, procent tkanki tłuszczowej oraz obwód talii znacząco przewyższają u badanych osób normę dla populacji. Z odpowiedzi ankietowanych w wieku 51-65 lat wynika, że 36% osób słodziło napoje, 65,6 % dosalało potrawy, 51,6% spożywało alkohol, a w wieku 66-75 lat odpowiednio: 47,1%, 52,9%, 41,2%. Nie wykazano związku pomiędzy słodzeniem napojów i soleniem potraw a stężeniem glukozy i lipidów. Stwierdzono natomiast dodatnią korelację pomiędzy ilością spożywanej soli a tętnem (r=0,28, p<0,05). Wykazano w obu grupach wiekowych istotne statystycznie różnice w stężeniu triglicerydów w zależności od spożycia alkoholu lub jego niespożywania. Z powodu braku precyzyjnych danych dotyczących ilości spożywanego alkoholu w ciągu 1 tygodnia lub 1 miesiąca nie wykonano analizy korelacji pomiędzy spożyciem alkoholu a stężeniem glukozy i lipidów.
Aktywności fizycznej nie podejmowało 39,1% pacjentów w wieku 51-65 lat i 41,2% w wieku 66-75 lat. W grupie osób starszych bez aktywności fizycznej stwierdzono istotnie statystycznie większe stężenie glukozy w odniesieniu do wykazujących aktywność fizyczną (130 mg/dl vs. 105 mg/dl; p=0,031). Pacjenci w wieku 51-65 lat wykazujący aktywność fizyczną mieli istotnie statystycznie mniejszą masę ciała, wskaźnik BMI, obwód talii oraz beztłuszczową masę ciała co wymaga dalszych badań.
Wnioski. Wskaźniki i parametry antropometryczne pacjentów z MetS wskazywały na zaburzenia stanu odżywienia. Niezdrowy styl życia wykazywała głównie młodsza grupa pacjentów 51-65 lat (częściej słodzili napoje, solili potrawy, spożywali alkohol). Pacjenci z zespołem metabolicznym nie podejmowali aktywności fizycznej tak często jak jest to rekomendowane.

Słowa kluczowe: *zespół metaboliczny, lipidogram, skład ciała, pomiary antropometryczne, styl życia*

INTRODUCTION

Ongoing civilization changes related to technical progress and the widespread availability of mobile multifunctional devices are the cause of the increase in the frequency of metabolic disorders, especially in the area of carbohydrate-lipid metabolism, leading, inter alia, to the metabolic syndrome (MetS), which includes several risk factors for cardiovascular disease and type 2 diabetes. The 2009 integrated MetS definition assigns equal levels of importance to all its components; abdominal obesity as measured by waist circumference (WC), elevated triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL), elevated blood pressure (BP) and elevated fasting glucose [1].

For the formation and development of the metabolic syndrome environmental factors related to lifestyle correspond to: improper diet (excessive consumption of simple carbohydrates, salt), insufficient physical activity, smoking. In contrast, several studies have shown a significant reduction in the risk of cardiovascular events and all-cause mortality from the consumption of light / moderate alcoholic beverages [2, 3].

Alcohol consumption is positively correlated with plasma HDL concentration [4]. However, high alcohol consumption adversely affects abdominal obesity, TG levels, blood pressure and possibly insulin sensitivity [5, 6, 7, 8].

A meta-analysis of observational studies found that the beneficial metabolic effect appeared to be limited to moderate alcohol consumption (<20 g/d for women and <40 g/d for men) [9]. The consequence of improper diet is excessive body weight, and the main method of treating people with metabolic syndrome is weight reduction and increasing physical activity, regardless of age. In contrast, inactivity can cause loneliness, social isolation, progressive disability, and even premature mortality, especially among the elderly [10].

This is all the more worrying because we may soon be observers of the growing percentage of older people using online social networks.

The aim of the study was to assess the nutritional status and its relationship with selected lifestyle factors such as: physical activity, sweetening drinks, adding salt to food, alcohol consumption in elderly people with metabolic syndrome, and to compare the lifestyle of pre-retirement and retired people.

MATERIAL AND METHODS

The study involved 81 people diagnosed with the metabolic syndrome based on the 2009 consensus, including 64 aged 51-65 years and 17 people aged 66-75 years. All persons gave their written consent to participate in the research.

In accordance with WHO guidelines, anthropometric measurements and body composition tests were performed using the bioelectric impedance method with the Akern BIA 101 analyzer. Measurements of height and weight were used in the study, on the basis of which the body mass index (BMI) was calculated, which allows to estimate the frequency of underweight, overweight and obesity. BMI was assessed according to the criteria indicated by WHO. Biochemical determinations (lipogram, glucose) were made by enzymatic methods with the use of Johnson & Johnson reagents. According to the guidelines, all tests were performed on an empty stomach. Statistical calculations were performed using the statistical program Statistica 6.0. The comparison of individual quantitative variables between the groups was performed using the *Student's* t-test for independent measurements or using the *Mann-Whitney* U test.

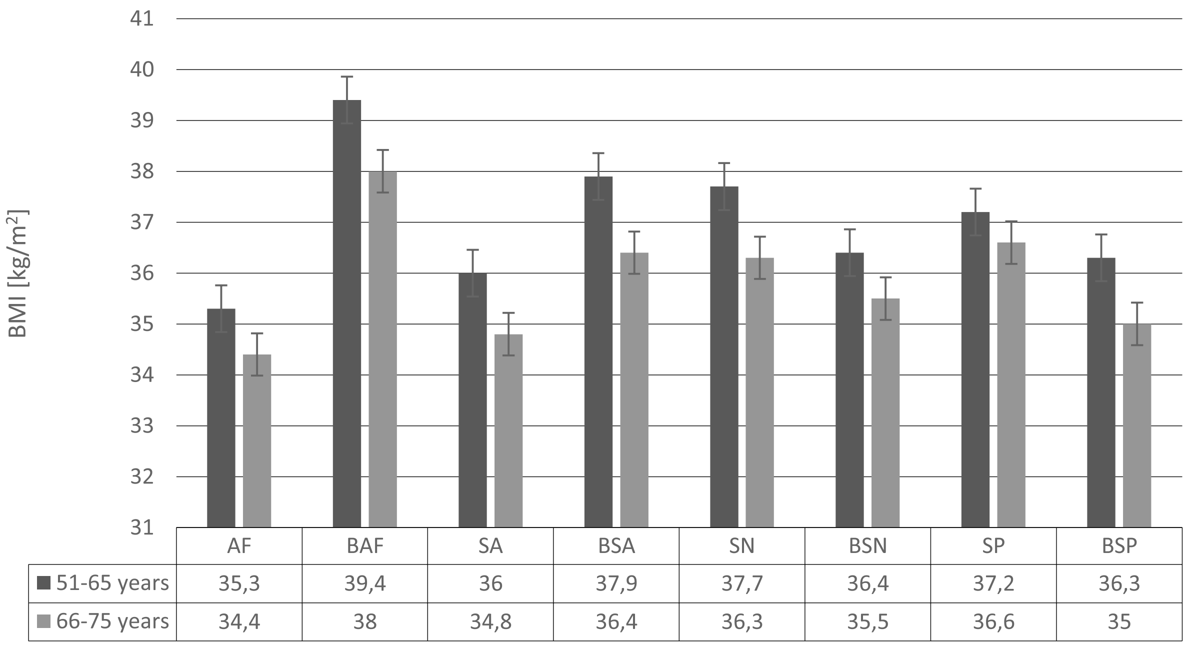
The relationship between quantitative variables was verified with the *Spearman* correlation coefficient. All statistical tests were based on a significance level of p <0.05.

RESULTS

Table 1 presents the characteristics of people with metabolic syndrome, taking into account selected lifestyle factors. Lack of physical activity was found in 39.1% of people aged 51-65 years and 41.2% of people aged 66-75 years. In the group of younger patients, there were more people consuming alcohol (51.6%) and adding salt to their dishes (65.6%). On the other hand, more elderly people (66-75 years old) sweetened beverages (47.1%) and did not drink alcohol (52.9%) (Table 1). Our study examined the effect of selected lifestyle factors on body mass index (BMI) (Figure 1). Statistically significantly higher BMI was found in physically inactive people compared to active people (aged 51-65 by 4.1 kg/m^2 ($p<0.05$), in the group 66-75 by 3.6 kg/m^2 ($p<0.05$). People who consumed alcohol had a lower BMI than those who declared not drinking alcohol (in the 51-65 age group by 1.9 kg/m^2, and in the 66-75 age group by 1.6 kg/m^2). BMI values were found in both age groups in people who sweeten drinks and add extra salt (Figure 1). In the group of physically inactive elderly people, a statistically significantly higher glucose concentration was found in relation to physically active people (130 mg/dl vs 105 mg/dl; p = 0.031) (Table 2). People aged 51-65 years old demonstrating physical activity had statistically significantly lower body weight, BMI, waist circumference and lean body mass (Table 2).

Table 1. Characteristics of the studied patients with metabolic syndrome

	Patients aged 51-75 years (total)	Patients aged 51-65 years	Patients aged 66-75 years
n	81	64	17
Physically active person, n (%)	49 (60.5)	39 (60.9)	10 (58.8)
Physically inactive person, n (%)	32 (39.5)	25 (39.1)	7 (41.2)
Alcohol drinkers, n (%)	40 (50)	33 (51.6)	7 (41.2)
Non-alcohol drinkers, n (%)	40 (50)	31 (48.4)	9 (52.9)
Persons who sweeten drinks, n (%)	31 (38.3)	23 (35.9)	8 (47.1)
Persons who not sweeten drinks, n (%)	50 (61.7)	41 (64.1)	9 (52.9)
Persons who salt food, n (%)	51 (63)	42 (65.6)	9 (52.9)
Persons who do not add salt to food, n (%)	30 (37)	22 (34.4)	8 (47.1)



AF - persons physically active, BAF - persons physically inactive, SA - alcohol drinkers, BSA - non alcohol drinkersconsumption, SN - persons who sweeten drinks, BSN - persons who non-sweeten drinks, SP - persons who salt food products and meals, BSP - persons who do not add salt to food products and meals, BMI - body mass index

Figure 1. Influence of lifestyle factors on BMI in patients aged 51-75 years with metabolic syndrome

The following tables examined the effect of alcohol consumption (Table 3), sweetening drinks (Table 4) and salting meals on a plate (Table 5) for blood pressure, heart rate, lipids and glucose serum level, body weight, waist circumference, total water content (TBW), lean mass (FFM), and body fat mass (FM). BMI, percentage of body fat and waist circumference are well above the norm for the study population. The responses of respondents aged 51-65 show that 36% of people sweetened their drinks, 65.6% added salt to their meals, 51.6% consumed alcohol, and at the age of 66-75, respectively: 47.1%, 52.9%, 41.2%. There was no correlation between the sweetening of beverages and the salting of dishes and the concentration of glucose and lipids. However, a positive correlation was found between the amount of consumed salt and heart rate ($r = 0.28$, $p < 0.05$). In both age groups, statistically significant differences in the concentration of triglycerides were found depending on the consumption or non-consumption of alcohol. Due to the lack of precise data on the amount of alcohol consumed, the correlation between alcohol consumption and the concentration of glucose and lipids was not analyzed. In the group of elderly people, a statistically significantly higher blood glucose concentration was found in relation to physically active people (130 mg/dL vs 105 mg/dL; $p = 0.031$). Patients aged 51-65 years who showed physical activity had statistically significantly lower body weight, BMI, waist circumference and weight of adipose tissue.

DISCUSSION

Lifestyle is undoubtedly a very important factor influencing human health, regardless of age. Dietary preferences, physical activity and maintaining a healthy body weight play an important role in counteracting civilization diseases, such as diabetes, cardiovascular diseases, cancer, especially of the large intestine, osteoporosis and depression [11, 12, 13]. Meta-analyzes of long-term prospective cohort studies and short-term randomized controlled trials indicate that total sugars or sugar-sweetened beverages are associated with a higher risk of metabolic syndrome and type 2 diabetes [14, 15, 16]. Sugar-sweetened beverages (SBB) are not as filling as solid foods, which can lead to excessive energy consumption. In our study, a significant proportion of patients in both age groups sweetened their drinks, with more people consuming sugar in the elderly (47.1%). However, no correlation was found between the sweetening of the beverages and the concentration of glucose or TG in the blood serum. However, meta-analyzes of long-term prospective cohort studies and short-term RCTs have shown that total consumption of sugar or sugar-sweetened beverages (SSB) is associated with weight gain or a higher BMI [11], a greater risk of diabetes [14, 16], dyslipidemia [17] and hypertension [18, 19].

However, it was found that lowering blood pressure was significantly influenced by salt restriction, while diets rich in sodium chloride increased blood pressure in people with metabolic syndrome compared to people without this syndrome [20]. In our study, 63% of people salted their meals on a plate, despite the fact that they had previously been diagnosed with metabolic syndrome, mainly in the age group 51-65, who had a statistically significantly higher heart rate compared to people who did not salt their dishes. A risk factor for metabolic syndrome and mortality is lack of physical activity ($r=0,28$, $p<0,05$). Regular physical activity is associated with a reduced risk of MetS, type 2 diabetes, ischemic heart disease, cognitive impairment, depression and osteoporosis [21]. Doctors recommend regular aerobic training to prevent MetS: walking, cycling, and swimming [22, 23]. People with obesity are recommended 60-90 minutes of moderate-intensity training in combination with a properly balanced diet. Part of this decline in performance with age can be prevented by regular exercise. Exercise is a planned and conscious attempt, at least in part, to improve fitness and health and maintain mental well-being. Part of the decline in insulin sensitivity in the elderly is due to lack of exercise. Experts recommend that older people engage in moderate-intensity physical activity, such as brisk walking for at least 30 minutes 5 days a week (150 minutes/week) or high-intensity physical activity for at least 20 minutes three days a week [24].

Lima and the team showed that the three-month training program consisting of aerobic exercise had a positive effect on the reduction of abdominal fat, blood glucose concentration and an increase in the maximum VO_2 max in people exercising with metabolic syndrome. However, this did not affect the blood pressure values [25]. The Navigator trial studies showed that for every additional 2,000 steps per day, the risk of cardiovascular events was reduced by 10% [26], diabetes by 5.5% [27], and the risk index of the metabolic syndrome was 0.29 z-score [28]. In a Finnish 3-month intervention study involving 78 subjects with impaired oral glucose tolerance, an improvement in some cardiometabolic markers was observed in those taking an average of 5,576 steps per day compared to 4,434 steps per day in the control group [29]. In a 5-year study of approximately 500 Australians, there was a 13% reduction in the risk of dysglycemia for every 1,000 steps per day (as measured by a pedometer) and an increase in the number of steps over 5 years, which is favorably associated with obesity and insulin sensitivity [30].

Regarding mortality, research in Australia (n = 2576) [31], Great Britain (n = 1,655) [32] and

Table 2. Effect of physical activity on blood pressure, heart rate, lipid levels, glucose levels, body weight and body composition in people with MetS in two age groups

Patients 51-65 years of age	Physical activity (n=39) mean± SD	Physical inactivity (n=25) mean± SD	95% CI	p	Patients 66-75 years of age	Physical activity (n=10) mean± SD	Physical inactivity (n=7) mean± SD	95% CI	p
Systolic blood pressure (mmHg)	137.58±15.26	138.04±22.9	-11.19 to 10.26	0.931	Systolic blood pressure (mmHg)	135.00±10.05	128.14±15.29	-8.48 to 22.19	0.344
Diastolic blood pressure (mmHg)	83.45±9.97	79.04±14.47	-2.55 to 11.36	0.207	Diastolic blood pressure (mmHg)	74.86±7.03	76.80±10.28	-14.65 to 10.76	0.726
Pulse	73.70±13.26	73.30±10.25	-6.14 to 6.94	0.903	Pulse	67.00±9.82	66.50±7.82	-12.13 to 13.13	0.929
TCH (mg/dl)	189.18±44.83	178.76±49.7	-14.25 to 35.09	0.399	TCH (mg/dl)	183.44±36.36	192.14±64.52	-70.38 to 52.98	0.757
TG (mmol/L)	2.10±0.89	2.53±2.07	-1.32 to 0.46	0.332	TG (mmol/L)	1.94±0.72	2.22±1.06	-1.33 to 0.75	0.553
HDL (mg/dl)	46.97±10.49	42.96±10.42	-1.36 to 9.39	0.140	HDL (mg/dl)	55.11±11.75	49.43±12.09	-7.34 to 18.70	0.363
LDL (mg/dl)	106.97±40.06	89.88±35.70	-2.48 to 36.68	0.086	LDL (mg/dl)	94.89±30.48	103.43±54.39	-60.51 to 43.43	0.718
Glucose (mg/dl)	120.2±39.9	111.6±16.2	-5.72 to 22.98	0.233	Glucose (mg/dl)	105±17.0	130±15.4	-37 to -2.0	0.031*
Body weight (kg)	99.27±20.51	114.17±23.0	-26.28 to -3.52	0.011*	Body weight (kg)	94.44±15.56	108.27±27.47	-39.96 to 12.29	0.260
WC (cm)	112.71±15.67	122.48±19.0	122.48 to19.0	0.028*	WC (cm)	108.33±12.13	119.43±19.46	-29.92 to 7.73	0.217
TBW(kg)	43.62±9.42	50.22±12.25	-12.42 to 0.77	0.027*	TBW(kg)	41.10±6.90	46.33±10.71	-15.76 to 5.30	0.295
TBW (%)	44.81±6.29	44.29±5.34	-2.52 to 3.57	0.732	TBW (%)	44.45±5.70	43.64±4.55	-4.92 to 6.53	0.766
FM (kg)	40.23±13.59	45.73±11.76	-11.96 to 0.95	0.093	FM (kg)	40.03±10.29	49.10±15.75	-24.60 to 6.45	0.222
FM (%)	40.51±8.92	40.56±8.00	-4.38 to 4.27	0.979	FM (%)	43.05±8.45	45.46±5.67	-10.40 to 5.58	0.525
FFM (kg)	57.39±14.99	67.54±17.47	-18.73 to -1.59	0.021*	FFM (kg)	52.54±9.50	57.19±13.53	-18.17 to 8.88	0.464
FFM (%)	59.59±9.02	59.31±8.28	-4.29 to 4.86	0.901	FFM (%)	56.71±9.09	54.54±5.67	-6.85 to 11.85	0.604

* statistically significant differences

TCH (total cholesterol), LDL (low density cholesterol), HDL (high density cholesterol), TG (triacylglycerols), TBW (total body water), FM (fat mass), FFM (fat free mass), WC (waist circumference)

Table 3. Effect of alcohol consumption on blood pressure, heart rate, lipid levels, glucose levels, body weight and body composition in people with MetS in two age groups

Patients 51-65 years of age	Drinking alcohol (n=33) mean± SD	No drinking alcohol (n=31) mean± SD	95% CI	p	Patients 66-75 years of age	Drinking alcohol (n=7) mean± SD	No drinking alcohol (n=9) mean± SD	95% CI	p
Systolic blood pressure (mmHg)	138.88±20.25	136.57±16.49	-7.05 to 11.67	0.623	Systolic blood pressure (mmHg)	134.00±11.20	129.89±14.66	-13.16 to 21.38	0.596
Diastolic blood pressure (mmHg)	83.23±12.75	80.30±11.07	-3.19 to 9.04	0.342	Diastolic blood pressure (mmHg)	82.67±13.65	73.38±4.81	-22.68 to 41.26	0.359
Pulse	73.85±11.22	73.23±13.19	-6.15 to 7.39	0.855	Pulse	72.33±10.69	63.29±6.24	-13.89 to 31.99	0.277
TCH (mg/dl)	191.48±53.41	178.32±38.01	-9.23 to 36.25	0.259	TCH (mg/dl)	208.67±44.13	180.44±47.98	-24.52 to 80.96	0.265
TG (mmol/L)	2.76±1.84	1.74±0.59	0.33 to 1.70	0.005*	TG (mmol/L)	2.66±0.75	1.68±0.79	0.09 to 1.86	0.034
HDL (mg/dl)	46.39±10.85	44.35±10.33	-3.25 to 7.33	0.444	HDL (mg/dl)	50.83±12.22	55.67±11.09	-18.65 to 8.99	0.454
LDL (mg/dl)	97.52±43.06	103.19±35.05	-25.64 to 14.29	0.571	LDL (mg/dl)	110.67±37.70	95.89±43.53	-31.33 to 60.89	0.498
Glucose (mg/dl)	120.67±33.11	112.81±32.55	-8.55 to 24.27	0.342	Glucose (mg/dl)	113.33±17.53	127.22±42.82	-48.89 to 21.11	0.402
Body weight (kg)	105.68±26.71	104.45±17.55	-10.02 to 12.49	0.827	Body weight (kg)	105.80±28.98	93.63±13.67	-15.14 to 39.48	0.335
WC (cm)	117.32±20.55	115.68±14.07	-7.13 to 10.41	0.709	WC (cm)	112.17±22.40	111.44±10.70	-22.82 to 24.27	0.944
TBW(kg)	48.05±12.20	44.37±9.51	-1.83 to 9.19	0.186	TBW(kg)	51.06±9.09	38.17±4.11	1.82 to 23.97	0.030*
TBW (%)	46.65±5.76	42.57±5.38	-1.20 to 6.96	0.006*	TBW (%)	48.70±5.65	41.33±2.49	0.48 to 14.25	0.040*
FM (kg)	39.76±14.56	45.15±10.93	-11.87 to 1.09	0.101	FM (kg)	42.02±20.80	44.74±9.57	-28.10 to 22.61	0.793
FM (%)	37.78±8.52	43.36±7.60	-9.65 to 1.52	0.008*	FM (%)	37.94±8.41	47.78±3.86	-18.30 to -0.40	0.042*
FFM (kg)	64.74±17.43	57.98±15.33	-1.51 to 15.02	0.107	FFM (kg)	64.04±12.83	48.06±4.25	0.27 to 31.70	0.048*
FFM (%)	62.59±8.68	56.38±7.60	1.99 to 10.43	0.005*	FFM (%)	62.05±8.40	51.43±3.23	0.37 to 20.89	0.045*

* statistically significant differences

TCH (total cholesterol), LDL (low density cholesterol), HDL (high density cholesterol), TG (triacylglycerols), TBW (total body water), FM (fat mass), FFM (fat free mass), WC (waist circumference)

Table 4. Effect of sweetening beverages on blood pressure, heart rate, lipid levels, glucose levels, body weight and body composition in people with MetS in two age groups

Patients 51-65 years of age	Sweetening the drinks (n=23) mean± SD	No sweetening of the drinks (n=41) mean± SD	95% CI	p	Patients 66-75 years of age	Sweetening the drinks (n=8) mean± SD	No sweetening of the drinks (n=9) mean± SD	95% CI	p
Systolic blood pressure (mmHg)	141.87±16.05	135.33±19.46	-2.63 to 15.71	0.159	Systolic blood pressure (mmHg)	133.83±9.50	129.88±15.44	-10.67 to 18.59	0.566
Diastolic blood pressure (mmHg)	82.52±8.39	81.34±13.75	-4.49 to 6.85	0.679	Diastolic blood pressure (mmHg)	76.17±9.95	75.17±6.82	-10.17 to 12.17	0.844
Pulse	74.90±12.49	72.66±11.97	-4.72 to 9.21	0.518	Pulse	63.60±6.77	69.33±9.16	-16.63 to 5.16	0.264
TCH (mg/dl)	184.00±42.67	185.73±49.32	-25.35 to 21.89	0.884	TCH (mg/dl)	167.88±39.78	206.63±51.78	-88.57 to 11.07	0.117
TG (mmol/L)	2.26±0.81	2.27±1.74	-0.65 to 0.63	0.975	TG (mmol/L)	1.95±0.65	2.18±1.07	-1.20 to 0.74	0.610
HDL (mg/dl)	43.65±10.04	46.39±10.85	-8.15 to 2.67	0.314	HDL (mg/dl)	51.00±13.06	54.25±11.17	-16.31 to 9.81	0.601
LDL (mg/dl)	102.77±38.03	99.03±40.00	-16.96 to 24.45	0.717	LDL (mg/dl)	82.25±31.40	115.00±45.16	-74.94 to 9.44	0.117
Glucose (mg/dl)	120.26±36.23	114.95±31.05	-12.83 to 23.45	0.558	Glucose (mg/dl)	113.13±15.92	131.50±44.52	-56.34 to 19.59	0.301
Body weight (kg)	106.42±24.51	104.34±21.68	-10.30 to 14.46	0.736	Body weight (kg)	103.59±26.50	97.07±17.31	-17.49 to 30.53	0.565
WC (cm)	116.87±15.22	116.33±18.97	-8.16 to 9.24	0.901	WC (cm)	115.88±19.31	110.50±13.14	-12.56 to 23.31	0.527
TBW(kg)	47.17±10.45	45.71±11.44	-4.22 to 7.16	0.610	TBW(kg)	46.54±10.45	40.91±7.08	-4.74 to 15.99	0.255
TBW (%)	45.23±6.74	44.23±5.37	-2.36 to 4.35	0.553	TBW (%)	44.17±5.01	43.99±5.39	-5.62 to 5.99	0.946
FM (kg)	42.20±15.59	42.53±11.61	-7.90 to 7.24	0.930	FM (kg)	48.00±15.23	40.99±11.73	-8.56 to 22.58	0.344
FM (%)	39.52±9.59	41.11±7.87	-6.34 to 3.18	0.506	FM (%)	44.79±6.28	43.64±8.22	-6.97 to 9.27	0.765
FFM (kg)	63.23±14.94	60.37±17.66	-5.53 to 11.26	0.497	FFM (kg)	58.17±13.09	51.68±9.44	-6.67 to 19.67	0.300
FFM (%)	60.48±9.59	58.87±8.12	-3.25 to 6.47	0.507	FFM (%)	55.21±6.28	56.04±8.82	-9.84 to 8.19	0.844

TCH (total cholesterol), LDL (low density cholesterol), HDL (high density cholesterol), TG (triacylglycerols), TBW (total body water), FM (fat mass), FFM (fat free mass), WC (waist circumference)

Table 5. Effect of adding salt in dishes on blood pressure, heart rate, lipid levels, glucose levels, body weight and body composition in people with MetS in two age groups

Patients 51-65 years of age	Salting food (n=42) mean± SD	No salting of dishes (n=22) mean± SD	95% CI	p	Patients 66-75 years of age	Salting food (n=9) mean± SD	No salting of dishes (n=8) mean± SD	95% CI	p
Systolic blood pressure (mmHg)	140.15±18.66	133.10±17.40	-2.60 to 16.70	0.148	Systolic blood pressure (mmHg)	137.00±12.06	126.14±12.17	-3.25 to 24.96	0.119
Diastolic blood pressure (mmHg)	83.51±11.29	78.25±12.76	-1.55 to 12.08	0.126	Diastolic blood pressure (mmHg)	76.14±9.08	75.00±7.62	-9.69 to 11.97	0.818
Pulse	75.46±12.83	69.13±9.14	0.05 to 12.62	0.048	Pulse	64.00±6.13	70.00±10.07	-18.43 to 6.44	0.286
TCH (mg/dl)	181.36±39.92	192.27±57.87	-39.01 to 17.18	0.435	TCH (mg/dl)	168.89±34.12	210.86±57.16	-96.97 to 13.02	0.119
TG (mmol/L)	2.12±0.82	2.55±2.24	-1.46 to 0.58	0.384	TG (mmol/L)	2.00±0.66	2.14±1.13	-1.22 to 0.95	0.781
HDL (mg/dl)	45.71±10.16	44.82±11.54	-5.01 to 6.80	0.760	HDL (mg/dl)	51.89±12.50	53.57±11.89	-14.89 to 11.52	0.788
LDL (mg/dl)	98.62±37.33	104.00±43.20	-28.26 to 17.50	0.636	LDL (mg/dl)	82.33±26.65	119.57±48.87	-83.81 to 9.33	0.104
Glucose (mg/dl)	114.69±31.13	121.00±36.21	-24.72 to 12.11	0.492	Glucose (mg/dl)	114.00±13.44	133.00±48.53	-64.02 to 26.03	0.349
Body weight (kg)	106.21±23.49	102.95±21.05	-8.34 to 14.87	0.574	Body weight (kg)	103.62±24.54	96.21±18.69	-15.04 to 29.86	0.492
WC (cm)	116.70±14.70	116.18±22.50	-10.31 to 11.35	0.923	WC (cm)	116.38±18.95	110.00±13.41	-11.41 to 24.16	0.456
BW(kg)	46.54±11.73	45.70±9.83	-4.75 to 6.43	0.764	TBW(kg)	46.19±10.52	40.51±6.18	-3.94 to 15.29	0.222
TBW (%)	44.53±6.16	44.75±5.55	-3.33 to 2.90	0.890	TBW (%)	43.69±4.19	44.51±6.17	-3.96 to 5.30	0.770
FM (kg)	42.62±13.24	42.02±13.07	-6.39 to 7.59	0.863	FM (kg)	47.40±14.05	40.67±12.82	-8.27 to 21.72	0.350
FM (%)	40.40±8.87	40.75±7.96	-4.75 to 4.06	0.874	FM (%)	44.64±7.12	43.64±7.70	-7.36 to 9.35	0.800
FFM (kg)	62.60±16.81	59.22±16.51	-5.47 to 12.23	0.446	FFM (kg)	58.49±13.85	50.39±6.09	-3.98 to 20.18	0.166
FFM (%)	59.62±9.16	59.25±7.96	-4.15 to 4.91	0.868	FFM (%)	55.36±7.11	55.98±8.37	-10.10 to 8.85	0.886

TCH (total cholesterol), LDL (low density cholesterol), HDL (high density cholesterol), TG (triacylglycerols), TBW (total body water), FM (fat mass), FFM (fat free mass), WC (waist circumference)

Japan (n = 419) [33] have shown that a higher number of steps (as assessed by a pedometer or accelerometer) is associated with lower mortality rates during follow-up ranging from 5 to 10 years. For Australians, every 1,000 steps per day increase was associated with a 6% risk reduction, and for British participants, for a 14% risk reduction. Among Japanese participants, only the most active quartile, with an average of 10,241 steps / day, experienced a significant reduction in risk; however, the statistical power was limited. A study by Min Lee and his team published in 2019 in the journal JAMA found that in women aged 72, around 4,400 steps/day was significantly associated with lower mortality compared to around 2,700 steps/day. The recommended 10,000 steps per day has also been found to be of limited scientific basis, having shown that around 7,500 steps per day are associated with lower mortality [34]. On the other hand, the Statistics Poland (GUS) research showed that 73.4% of Poles aged 50–59 were physically inactive [35]. The report of the Ministry of Sport and Tourism shows that only 12–15% of the elderly were active in line with WHO recommendations [36]. Low physical activity and age entail changes in body composition, which result in increasing body fat, loss of muscle mass and strength. The total body hydration as well as the proportions between the extracellular and intracellular water content are also changing in an unfavorable direction. The changes in body composition that occur during the aging process are inherently unfavorable. They also include disproportions in body composition, i.e. changes in the direction of a decrease in lean body mass (including muscle mass), with a simultaneous increase in adipose tissue (both subcutaneous and deposited between internal organs). Therefore, a natural consequence is the appearance of visceral obesity, which is responsible for a number of cardiovascular, internal secretion and immune diseases. This obesity may contribute to the cascade of metabolic changes typical of the metabolic syndrome, which is one of the most serious risk factors for cardiovascular diseases [37].

In the inhabitants of Biała Podlaska (Poland), abdominal obesity based on the waist circumference was found in 72.23% of women (WC>88 cm) and 41.35% of men (WC>102 cm) [38].

In our study physical activity was not undertaken by 39.1% of patients aged 51-65 years and 41.2% of patients aged 66-75 years. In the group of elderly people without physical activity, a statistically significantly higher glucose concentration was found in relation to those who were physically active (130 mg/dl vs. 105 mg/dl; p=0.031). Patients aged 51-65 who showed physical activity had statistically significantly lower body weight, BMI, waist circumference and lean body mass, which requires further studies.

CONCLUSIONS

The anthropometric indices and parameters of MetS patients indicated disturbances in the nutritional status. In both age groups, patients consuming alcohol were found to have statistically higher concentration of triglycerides. In the group of younger patients without physical activity, body weight and BMI were statistically significantly higher. In the group of older patients without physical activity, statistically significantly higher glucose concentration was found. A statistically significantly higher heart rate was also found in people aged 51-65 years using food.

Unhealthy lifestyle was shown mainly by the younger group of patients 51-65 years old (they sweetened drinks more often, salt dishes, consumed alcohol).

Patients with metabolic syndrome did not undertake physical activity as often as recommended.

Modification of eating habits and reduction of alcohol consumption in combination with increased physical activity may reverse this unfavorable trend.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

EFFECTS OF DIETARY COMPONENTS ON INTESTINAL SHORT-CHAIN FATTY ACIDS (SCFAs) SYNTHESIS IN HEALTHY ADULT PERSONS FOLLOWING A KETOGENIC DIET

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ABSTRACT

Background. The ketogenic diet (KD) has been used for almost 100 years in the treatment of drug-resistant epilepsy in children - and adults. The intestinal microbiome has a climax character, and the main factor changing its composition and functions is the diet. Both increased biodiversity and the production of short-chain fatty acids (SCFAs) are important indicators of gut barrier function. SCFAs are synthesized by microorganisms through the fermentation of dietary fibre provided with the diet. They are an important element in signal transduction from the digestive system to other tissues. To date, there is little research to determine how the use of KD alters the SCFAs profile of the human stool.

Objective. To assess the SCFAs profile in the stool of healthy and active KD users.

Material and methods. Study group: amateur athletes following KD. Control group: amateur athletes following a regular diet (carbohydrates min. 50%); gender: men and women aged 18-60. Material: stool sample (1x10 g). SCFAs content was determined in stool samples using gas chromtography method. Participants completed a Food Frequency Questionnaire (FFQ) and a 72-hour food diary.

Results. There research has shown differences in the amount of SCFAs, as far as the results obtained from the two groups are concerned. The discrepancies referred to the levels of acetic, butyric, iso-butyric, valeric, and isovaleric acids. *Spearman's* rank correlation analysis showed a strong relationship between the consumption of selected dietary components (vegetables, fruits, red meat, poultry, fish, nuts and seeds, sugar, sugar substitutes, fats) and the SCFAs content in the stool of the study group.

Conclusions. High consumption of cruciferous and leaf vegetables, berries and nuts on a ketogenic diet may have a positive effect on the profile of short-chain fatty acids produced by the gut microbiome. Changing the diet towards a greater supply of plant products may prevent proteolytic fermentation and reduce the negative effects of microbiome changes caused by an oversupply of protein and fat in the ketogenic diet.

Key words: *ketogenic diet, intestinal microbiome, short-chain fatty acids, proteolytic fermentation*

STRESZCZENIE

Wprowadzenie. Dieta ketogeniczna (DK) jest stosowana już niemal 100 lat w leczeniu padaczki lekoopornej u dzieci oraz dorosłych. Dieta jest głównym czynnikiem zmieniającym skład i funkcje mikrobioty. Bioróżnorodność mikrobiomu i produkcja krótkołańcuchowych kwasów tłuszczowych (SCFAs) to ważne wskaźniki funkcji bariery jelitowej. SCFAs są produkowane przez mikrobiom podczas fermentacji włókna pokarmowego z diety. Stanowią ważny element w transdukcji sygnału z jelit do tkanek. Do tej pory istnieje niewiele badań określających zmiany profilu SCFAs w stolcu u ludzi na DK.

Cel. Ocena profilu SCFAs w stolcu zdrowych i aktywnych osób stosujących dietę ketogeniczną.

Materiał i metody. Grupa badana: zdrowe osoby stosujące DK jako swój wybór od minimum miesiąca. Grupa kontrolna: zdrowe osoby stosujące dietę zwyczajową (węglowodany min. 50%); płeć: mężczyźni i kobiety w wieku 18-60 lat.
Materiał: kał (1x 10 g) od osób z grupy badanej i kontrolnej. Zawartości SCFAs w próbkach kału oznaczano metodą chromatografii gazowej. Zastosowano kwestionariusz częstotliwości spożycia (FFQ; ang. Food Frequency Questionaire) oraz 72-godzinny pamiętnik żywieniowy. Analiza statystyczna: Med. Calc 19.2 (Ostend, Belgia).

Wyniki. Wykazano różnice w zawartości SCFAs w stolcu pomiędzy grupami. Różnice dotyczą zawartości kwasów: octowego, masłowego, izomasłowego, walerianowego i izowalerianowego. Analiza korelacji rang *Spearman'a* wykazała silne zależności pomiędzy spożyciem wybranych składników diety (warzywa, owoce, czerwone mięso, drób, ryby, orzechy i nasiona, cukier, substytuty cukru, tłuszcze) a zawartością SCFAs w stolcu grupy badanej.

Wnioski. Duże spożycie warzyw krzyżowych, warzyw liściastych, owoców jagodowych oraz orzechów na diecie ketogenicznej wydaje się korzystnie wpływać na profil SCFAs w stolcu. Zmiana diety w kierunku większej podaży

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produktów roślinnych może zapobiegać fermentacji proteolitycznej i niwelować negatywne skutki zmian mikrobiomu wywołane nadmierną podażą białka i tłuszczu na diecie ketogennej.

Słowa kluczowe: *dieta ketogeniczna, mikrobiom jelitowy, krótkołańcuchowe kwasy tłuszczowe, SCFAs, fermentacja proteolityczna*

INTRODUCTION

The ketogenic diet (KD) has been used for almost 100 years in the treatment of drug-resistant epilepsy in children [1, 2, 3] and adults [4, 5, 6]. Currently, KD due to the progressive popularization, most often due to social media, is gaining more and more recognition among people who want to reduce fat mass [15, 16]. The benefits of the ketogenic diet seem to be based not only on weight reduction but according to the latest research, it can be part of the dietary prevention of lifestyle diseases [30].

Low-carbohydrate diets have long been used by athletes to reduce body weight [15]. It turns out, however, that a certain percentage of amateur athletes, as well as a slightly smaller percentage of professional athletes, decide to use KD not only to try to increase their sports achievements but rather for the broadly understood potential health benefits. However, it's worth taking a closer look at the interactions between the ketogenic diet and lifestyle and overall health. The ketogenic diet is based on a significant reduction in carbohydrates in the diet (<50 g per day) and increasing the consumption of high-fat products and total fats [6]. Diet is one of the major regulators of the epigenome [17]. Dietary fibre provided by the diet is a substrate for bacterial synthesis of butyrate and propionate, which exhibit epigenetic effects, and *beta*-hydroxybutyric acid itself is one of the histone deacetylase inhibitors (HDAC) [17, 18]. As a result of bacterial fermentation in the intestinal lumen, various bioactive compounds, both beneficial and unfavourable to the host's health, may be formed [19]. The qualitative composition of the diet seems to have the greatest impact on the composition and functions of the gut microbiota [7, 19]. The intestinal microbiome has a climax character, and the main factor changing its composition and functions is the diet. Both increased biodiversity and the production of short-chain fatty acids (SCFAs) are important indicators of gut barrier function.

SCFAs are synthesized by microorganisms through the fermentation of dietary fibre provided with the diet [7]. They are an important element in signal transduction from the digestive system to other tissues [8, 9]. Short-chain fatty acids are the most common product of the metabolism of microorganisms. SCFAs consist of three most abundant acids: acetic acid (approx. 60%), propionic acid (approx. 25%) and butyric acid (approx. 15%) [19]. The highest concentration of SCFAs is observed in the ascending colon, with slightly lower levels in the transverse and descending colon. One of the more important features of short chain fatty acids appears to be their ability to induce specific metabolic effects along with acting on the host as signalling molecules [8]. SCFAs have the ability to cross the blood-brain barrier, which is an important aspect in the functioning of the gut-brain axis. The metabolic functions of SCFAs include: elimination of lipopolysaccharide induced inflammation, direct modulation of systemic immunity, improvement of insulin sensitivity, regulation of appetite, induction of adipose tissue differentiation (e.g. browning), increase of energy expenditure by enhancing thermogenesis, increasing lipolysis. It should be noted that most studies assessing SCFAs production are carried out on stool samples, which may not be fully reliable due to limitations related to intestinal transit time, intestinal permeability, metabolite transport rate, or the method of collecting and storing the sample itself [27]. To date, there is little research to determine how the use of a ketogenic diet alters the SCFAs profile of the human stool. Therefore, the aim of this study was to assess the SCFAs profile in the stool of healthy and active persons following a ketogenic diet.

MATERIAL AND METHODS

Group characteristics

Healthy and physically active adults participated in the study. The study participants were selected into two groups: the study group (n = 14) and the control group (n = 16).

S*tudy group*: healthy active people voluntarily following a ketogenic diet. *Control group:* healthy active people following a habitual diet, characterized by the consumption of carbohydrates corresponding to a minimum of 50% of energy. The estimated group size was at least 10 people in each group (for the assumed test power of 0.95; $p < 0.05$, G Power test); gender: men and women aged 18-60.

I*nclusion criteria*: individuals who voluntarily wish to submit a stool sample and complete surveys; healthy active people (men and women) who have been on a ketogenic diet for at least a month (study group) and healthy active people (men and women) using habitual nutrition.

E*xclusion criteria:* people> 60 years of age; people with irregular physical activity; people with metabolic disorders (coronary artery disease, hypertension, diabetes); people with liver failure; people diagnosed with inflammatory bowel disease (Crohn's disease, *Colitis ulcerosa*); people with biliary disorders; obese people; people taking antibiotic therapy for at least 3 months from the start of the study; people who supplemented probiotics, antibiotics and butyric acid in the period preceding the study by less than 3 months.

Surveys

The first step was to conduct a short metric survey and to determine the duration of the ketogenic diet. The subjects of the study gave their formal consent to participate in the study and collect samples. The Food Frequency Questionnaire (FFQ) was conducted and the 72-day food diary was recorded. All data was collected from participants using online survey tools. The consumption of certain groups of food products was recorded with the use of the FFQ questionnaire. The participants completed it considering the period: a) for the test group after switching to the ketogenic diet (up to 12 months back); b) for the control group, the period of the last 12 months. All products and subgroups of food products were divided into the following groups: total fats, coconut oil, animal fat, butter (all types), berries, vegetables (all types), meat and fish products, artificial sweeteners, sugar and honey, alcoholic beverages, sweetened and energy drinks, poultry and rabbit meat, red and game meat, milk, eggs, and natural dairy products, natural unsweetened plant drinks, nuts, seeds and seeds, dried fruit and sweet fruit preserves, polyols and stevia, sweetened dairy products, products cereal (total), snacks (salty, sweet) and fast-food, processed meat products, total fish, fruit and vegetable juices, cream and mayonnaise and dressings, medium-chain fatty acids, vegetable fats (including olive oil and avocado), other vegetables, legumes, green leafy vegetables and cruciferous vegetables, potatoes and sweet potatoes and their derivatives. The ranks from the FFQ questionnaire were converted into the frequency of consumption according to the formula presented in Table 1. Microsoft Excel (Version 16.37 (20051002)) was used to calculate the ranks.

Sampling and analysis

We collected stool samples from participants. The participants were informed about the critical points necessary for the proper storage and transport of the samples. The content of short-chain fatty acids (acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid, valeric acid, caproic acid, isocaproic acid, heptanoic acid) was determined in the stool samples. Any data obtained by analysis in an Agilent technology 1260 A GD gas chromatograph with a flame ionization detector (FID) using a column (DB-FFAP, 30m × 0.53mm × 0.5um). Injection parameters: hydrogen carrier gas with a flow rate of 14.4 ml/min, separation conditions: initial temperature (100°C) / 0.5 minutes, then increase to 180°C and 200°C (5 min). The results were transferred into a Microsoft Excel version 16.37 (20051002) database and presented in both percentages (%) and concentrations (mg/g).

Bioethical Committee

This research received the consent of the Bioethical Committee of the Pomeranian Medical University in Szczecin.

Statistical analysis

The statistical analysis covered the responses from the FFQ questionnaires and the database with the SCFAs analysis results. Statistical analysis was performed using the Med Calc program (19.2.6; Ostend, Belgium). The performed statistical calculations were based on the normal distribution *Shapiro-Wilk* test, the *Spearman's* rank correlation test, and the *Chi*-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Statistical analysis showed significant differences in the content of individual fatty acids between the study group and the control group. Significant diversities were also noticed in the consumption of certain groups of products and certain eating habits. Anthropometric data for both groups are presented

Table 1. The diagram of converting FFQ ranks into frequency of consumption. Based on the FFQ-6 Food Consumption Frequency Questionnaire prepared by *Wądołowska L.* and *Niedźwiedzka E.* [31]

Consumption frequency categories	Ranks assigned to frequency categories	Daily frequency (times / day)
Never or hardly ever	1	0.0
Once a month or less	2	0.025
Several times a month	3	0.100
Several times a week	4	0.571
Everyday	5	1.000
Several times a day	6	2.000

Based on the FFQ-6 Food Consumption Frequency Questionnaire [31].

in the tables below. The individual groups had an approximate gender distribution, and there were more men in the control group (9 vs.7).

The analysis of the auxiliary questions included in the FFQ questionnaire showed that people following the ketogenic diet consumed significantly fewer meals during the day than people eating habitually. The approximate number of meals consumed per day ranged from 1 to 3 in the test group and from 3 to 4 meals in the control group. Additionally, there was a trend (p = 0.068) in which people following the ketogenic diet had a fixed mealtime as opposed to the control group, where half of the participants did not have a fixed eating time. Intermittent fasting consists of eating meals for 8 hours and fasting for 16 hours until the next day. People following the ketogenic diet also used intermittent fasting significantly more often (p = 0.003). Moreover, in the study group, no person reported taking iron supplements, as opposed to the control group, where over 35% of participants took iron supplements. Descriptive statistics for the entire study group broken down by gender showed a strong trend in the percentage (%) of isovaleric acid and the concentration (mg/g) of caproic acid.

The analysis of SCFAs concentrations between the control and intervention groups showed significant differences in the percentage of acetic acid as well as the concentration of isovaleric acid and valeric acid. There was also a strong trend for butyric acid (p = 0.0552) and isobutyric acid (p = 0.0513) between the groups.

Table 2. Anthropometric parameters

	Intervention group			Control group		
	n	Median	25 - 75 P	n	Median	25 - 75 P
Body weight	14	71.0000	61.000 to 82.000	16	74.0000	60.500 to 85.000
Hip circumference	14	93.5000	91.000 to 100.000	16	93.2500	89.500 to 100.000
Waist circumference	14	84.0000	74.000 to 90.000	16	85.0000	76.000 to 90.000
Age	14	28.5000	23.000 to 32.000	16	24.0000	23.000 to 26.000
Height	14	175.5000	168.000 to 184.000	16	176.0000	169.000 to 181.000
BMI	14	23.0500	21.610 to 24.220	16	23.890	21.180 to 25.950
WHR	14	0.9	0.81 to 0.9	16	0.91	0.85 to 0.9

BMI – Body Mass Index; WHR – waist–hip ratio

Table 3. Comparison of SCFAs content between gender

	Women			Men			
	n	Median	25 - 75 P	n	Median	25 - 75 P	P [a]
Acetic acid [%]	14	40.0335	31.838 - 44.748	16	41.0388	33.204 to 46.974	0.5889
Acetic acid [mg/g]	14	0.08778	0.0650 - 0.127	16	0.116	0.0896 - 0.181	0.1637
Propionic acid [%]	14	17.0049	13.694 - 19.943	16	14.9617	13.219 - 23.939	1
Propionic acid [mg/g]	14	0.04311	0.0270 - 0.0656	16	0.04728	0.0331 - 0.0720	0.5746
Butyric acid [%]	14	24.151	18.136 - 27.591	16	24.2946	17.232 - 33.573	1
Butyric acid [mg/g]	14	0.06295	0.0275 - 0.0830	16	0.07562	0.0369 - 0.163	0.2891
Isobutyric acid [%]	14	4.7979	3.675 - 5.870	16	4.8069	3.738 - 5.593	0.8679
Isobutyric acid [mg/g]	14	0.00949	0.00719 - 0.0170	16	0.01253	0.00737 - 0.0184	0.4669
Isovaleric acid [%]	14	7.9259	5.865 - 10.626	16	4.6226	2.536 - 10.052	0.0558
Izovaleric acid [mg/g]	14	0.01621	0.0132 - 0.0267	16	0.01539	0.00860 - 0.0215	0.5466
Valeric acid [%]	14	5.2814	4.463 - 6.142	16	4.4803	3.959 - 6.071	0.2444
Valeric acid [mg/g]	14	0.01092	0.00820 - 0.0209	16	0.01427	0.00882 - 0.0192	0.5746
Caproic acid [%]	14	1.64561	0.238 - 2.802	16	0.2854	0.176 - 0.548	0.0674
Caproic acid [mg/g]	14	0.00225	0.000671 - 0.00501	16	0.0006801	0.000503 - 0.00152	0.0585
Izocaproic acid [%]	14	0.20481	0.106 - 0.284	16	0.1229	0.0695 - 0.230	0.1975
Isocaproic acid [mg/g]	14	0.000469	0.000265 - 0.000910	16	0.0003587	0.000271 - 0.000607	0.5193
Heptanoic acid [%]	14	0.2104	0.0102 - 0.299	16	0.02977	0.0183 - 0.179	0.3184
Heptanoic acid [mg/g]	14	0.000338	0.0000481 - 0.000567	16	0.0001184	0.0000643 - 0.000304	0.5746

Table 4. Comparison of SCFAs content between the intervention group and control group

	Intervention group			Control group			
	n	Median	25 - 75 P	n	Median	25 - 75 P	P [a]
Acetic acid [%]	14	33.6201	29.783 - 41.353	16	43.3003	40.140 - 46.555	0.0034
Acetic acid [mg/g]	14	0.09889	0.0650 - 0.153	16	0.09136	0.0802 - 0.119	0.9369
Propionic acid [%]	14	17.5325	13.648 - 22.118	16	15.1032	13.049 - 20.355	0.3768
Propionic acid [mg/g]	14	0.06016	0.0300 - 0.0776	16	0.03820	0.0267 - 0.0514	0.1493
Butyric acid [%]	14	25.3448	20.150 - 33.131	16	19.0485	15.538 - 25.957	0.1702
Butyric acid [mg/g]	14	0.08301	0.0648 - 0.154	16	0.03719	0.0272 - 0.0775	0.0552
Isobutyric acid [%]	14	5.3615	4.198 - 6.474	16	4.9286	3.758 - 5.808	0.3294
Isobutyric acid [mg/g]	14	0.01732	0.00843 - 0.0241	16	0.01003	0.00714 - 0.0134	0.0513
Isovaleric acid [%]	14	7.8807	4.350 - 9.492	16	6.3459	4.960 - 11.719	0.6920
Isovaleric acid [mg/g]	14	0.02259	0.0142 - 0.0296	16	0.01440	0.0129 - 0.0180	0.0324
Valeric acid [%]	14	5.5977	4.460 - 6.397	16	4.8846	4.108 - 5.667	0.1609
Valeric acid [mg/g]	14	0.01897	0.00898 - 0.0250	16	0.01153	0.00822 - 0.0142	0.0235
Caproic acid [%]	14	0.4627	0.216 - 2.857	16	0.4318	0.238 - 2.060	0.7839
Caproic acid [mg/g]	14	0.001287	0.000618 - 0.00581	16	0.001087	0.000507 - 0.00311	0.3664
Isocaproic acid [%]	14	0.1704	0.0793 - 0.294	16	0.1797	0.107 - 0.249	0.9514
Isocaproic acid [mg/g]	14	0.0005387	0.00025 - 0.000992	16	0.0003417	0.000276 - 0.000585	0.4190
Heptanoic acid [%]	14	0.1072	0.0116 - 0.356	16	0.07301	0.0219 - 0.290	0.9757
Heptanoic acid [mg/g]	14	0.0001552	0.0000525- 0.00059	16	0.0002231	0.0000580 - 0.000526	0.8368

This table shows differences in stool SCFAs content between intervention and control group

There were statistically significant differences ($p < 0.05$) in the consumption of fatty foods (coconut oil, animal fat, butter (all types), medium-chain fatty acids, vegetable fats (including olive and avocado)), berries, sugar and honey, nuts, seeds and seeds, non-berry fruit, dried fruit, and fruit preserves, sweetened dairy products, total cereal products, salty and sweet snacks and fast-food, total fish, vegetable and fruit juices, cream and mayonnaise and dressings, vegetable legumes, green leafy and cruciferous vegetables, and potatoes, sweet potatoes, and their derivatives.

In the study group, a statistically significant negative correlation was observed between the consumption of animal fats and the percentage content of acetic acid, caproic acid, and heptanoic acid. Additionally, a significant positive correlation was found between the consumption of animal fats and the increased percentage of propionic acid and a mean positive correlation for the concentration of isovaleric acid. The consumption of nuts, seeds, and seeds negatively correlated with the percentage of butyric acid. There was a tendency where the consumption of vegetable fats and avocados correlated negatively with the content of iso-butyric acid and positively with the content of isocaproic acid. At the same time, there was a strong positive correlation between the consumption of medium-chain fatty acids and the concentration of butyric acid, and a mean positive correlation for the percentage concentration. There was also an average positive correlation between the consumption of these fats and the concentration and percentage of propionic acid. The declared consumption of medium-chain fatty acids negatively correlated with the percentage of acetic acid and caproic acid, and there was a strong negative correlation with the percentage of heptanoic acid.

In the control group, there was an average negative correlation between the intake of nuts, seeds, and seeds and the percentage of acetic acid. The consumption of coconut oil negatively correlated with the concentration of propionic acid, and the consumption of animal fats negatively correlated with the percentage of caproic acid. There was a weak trend towards increased amounts of valeric acid with increased consumption of animal fats and medium-chain fatty acids.

In the study group, the consumption of red meat, venison, and processed meat products correlated negatively with the percentage of acetic acid. Additionally, the consumption of red meat and venison positively correlated with the content of propionic acid. The consumption of fish positively correlated with the concentration of heptanoic acid.

In the control group, a negative correlation was observed between the consumption of poultry and rabbit meat and the percentage of propionic acid ($RHO = -0.517$; $p = 0.0403$), and a positive correlation ($RHO = 0.562$; $p = 0.0235$) between the consumption of red meat and venison and the concentration of valeric acid. There was also a strong correlation between the consumption of fish and a higher percentage of acetic

Table 5. Consumption of fatty products and SCFAs content in the intervention group

Intervention group								
		Total fats	Coconut oil	Animal fat	Butter	Vegetable fats (incl. avocado and olive oil)	Nuts and seeds	MCT oil
Acetic acid [%]	RHO	-0.281	-0.007	**-0.688**	-0.253	0.467	0.228	**-0.653**
	p	0.331	0.9809	**0.0066**	0.4036	0.0922	0.4328	**0.0113**
	n	14	14	14	13	14	14	14
Acetic acid [mg/g]	RHO	0.108	-0.106	0.333	0.319	-0.213	-0.299	**0.495**
	p	0.713	0.719	0.245	0.2876	0.4655	0.2985	**0.0719**
	n	14	14	14	13	14	14	14
Propionic acid [%]	RHO	0.194	-0.045	**0.823**	0.48	-0.239	-0.091	**0.593**
	p	0.5056	0.8797	**0.0003**	0.0965	0.4098	0.7575	**0.0256**
	n	14	14	14	13	14	14	14
Propionic acid [mg/g]	RHO	0.065	-0.127	0.401	0.211	-0.255	-0.395	**0.545**
	p	0.8257	0.6656	0.1548	0.4881	0.3791	0.1625	**0.044**
	n	14	14	14	13	14	14	14
Butyric acid [%]	RHO	-0.022	-0.028	0.011	0.015	-0.364	**-0.574**	**0.611**
	p	0.9416	0.9239	0.9691	0.9623	0.201	**0.032**	**0.0204**
	n	14	14	14	13	14	14	14
Butyric acid [mg/g]	RHO	0.151	-0.07	0.315	0.334	-0.154	-0.328	**0.788**
	p	0.6056	0.8108	0.2734	0.2649	0.5994	0.252	**0.0008**
	n	14	14	14	13	14	14	14
Isobutyric acid [mg/g]	RHO	0.065	0.052	0.408	0.095	**-0.49**	-0.333	0.421
	p	0.8257	0.8607	0.1472	0.7581	**0.0754**	0.2453	0.1342
	n	14	14	14	13	14	14	14
Isovaleric acid [mg/g]	RHO	**0.497**	0.023	**0.566**	0.118	-0.368	-0.166	0.299
	p	0.0705	0.9365	**0.0348**	0.7008	0.1959	0.5699	0.2993
	n	14	14	14	13	14	14	14
Valeric acid [%]	RHO	0.108	0.04	**0.496**	0.131	-0.249	-0.002	-0.088
	p	0.7134	0.8923	**0.0714**	0.6696	0.3911	0.994	0.7652
	n	14	14	14	13	14	14	14
Caproic acid [%]	RHO	-0.238	0.157	**-0.585**	-0.274	0.04	-0.027	**-0.687**
	p	0.4136	0.5914	**0.028**	0.3655	0.8923	0.9281	**0.0066**
	n	14	14	14	13	14	14	14
Isocaproic acid [%]	RHO	0.022	-0.35	-0.334	0.058	**0.479**	0.419	-0.306
	p	0.9416	0.2203	0.2438	0.8501	**0.0833**	0.1363	0.2867
	n	14	14	14	13	14	14	14
Isocaproic acid [mg/g]	RHO	0.195	**-0.475**	0.234	**0.497**	0.18	0.062	0.45
	p	0.5051	**0.0864**	0.4198	**0.0839**	0.5386	0.833	0.1064
	n	14	14	14	13	14	14	14
Heptanoic acid [%]	RHO	-0.324	-0.162	**-0.628**	-0.376	0.387	0.237	**-0.807**
	p	0.2586	0.5802	**0.0161**	0.2059	0.1713	0.4146	**0.0005**
	n	14	14	14	13	14	14	14

This table shows the correlation between consumption of the fatty products and the stool SCFAs content in the intervention group. RHO – *Spearman's* rank correlation coefficient.

Table 6. Consumption of fatty products and SCFAs content in the control group

Control group								
		Total fats	Coconut oil	Animal fat	Butter	Vegetable fats (incl. avocado and olive oil)	Nuts and seeds	MCT oil
Acetic acid [%]	RHO	0.272	0.186	0.313	-0.102	-0.183	**-0.509**	0.108
	p	0.3085	0.4895	0.2371	0.7068	0.4965	**0.0443**	0.6909
	n	16	16	16	16	16	16	16
Propionic acid [mg/g]	RHO	-0.103	**-0.57**	-0.132	-0.312	0.282	0.107	-0.349
	p	0.7034	**0.0212**	0.625	0.2389	0.2901	0.694	0.1846
	n	16	16	16	16	16	16	16
Valeric acid [%]	RHO	-0.008	0.22	-0.125	0.043	-0.003	-0.079	**0.444**
	p	0.9767	0.4124	0.6452	0.8735	0.9911	0.7724	**0.0846**
	n	16	16	16	16	16	16	16
Valeric acid [mg/g]	RHO	-0.436	-0.037	**0.443**	-0.158	-0.141	-0.366	0.267
	p	0.0917	0.8919	**0.0858**	0.5596	0.6025	0.163	0.3165
	n	16	16	16	16	16	16	16
Caproic acid [%]	RHO	-0.084	0.12	**-0.522**	-0.326	0.061	-0.01	-0.371
	p	0.7564	0.6577	**0.0381**	0.2174	0.8235	0.9696	0.1571
	n	16	16	16	16	16	16	16
Caproic acid [mg/g]	RHO	-0.292	0.018	-0.481	-0.418	0.161	-0.083	**-0.449**
	p	0.2716	0.9458	0.0593	0.1076	0.5522	0.7598	**0.0813**
	n	16	16	16	16	16	16	16

This table shows the correlation between consumption of the fatty products and the stool SCFAs content in the control group. RHO – *Spearman's* rank correlation coefficient.

Table 7. Consumption of meat and fish products and SCFAs content in the intervention group

Intervention group					
		Poultry and rabbit meat	Red meat and game	Processed meat products	Fish
Acetic acid [%]	RHO	0	**-0.571**	**-0.55**	-0.009
	p	1	**0.033**	**0.0416**	0.9757
	n	14	14	14	14
Propionic acid [%]	RHO	-0.27	**0.587**	0.372	0.004
	p	0.3513	**0.0273**	0.1905	0.9878
	n	14	14	14	14
Propionic acid [mg/g]	RHO	-0.13	**0.491**	0.17	0.067
	p	0.6569	**0.0744**	0.5621	0.8188
	n	14	14	14	14
Isovaleric acid [%]	RHO	0.096	0.305	0.251	**0.48**
	p	0.7442	0.289	0.3871	0.082
	n	14	14	14	14
Valeric acid [mg/g]	RHO	0.05	**0.48**	0.3	0.027
	p	0.8644	**0.0825**	0.2981	0.9271
	n	14	14	14	14
Heptanoic acid [mg/g]	RHO	**0.508**	0.099	-0.282	**0.582**
	p	**0.0638**	0.7371	0.3288	**0.0289**
	n	14	14	14	14

This table shows the correlation between consumption of the following animal products and the stool SCFAs content in the intervention group. RHO – *Spearman's* rank correlation coefficient.

acid (RHO = 0.732; p = 0.0013). In the ketogenic group, a correlation was observed between the consumption of natural unsweetened plant drinks (RHO = 0.657; p = 0.0108) and cream, mayonnaise, and dressings (RHO = 0.558; p = 0.0379) and the percentage of isovaleric acid. Much more correlations were found in the control group, including correlation between consumption of sweetened dairy products and acetic, propionic, butyric, iso-butyric, isovaleric, caproic, and heptanoic acids. The consumption of natural dairy products positively correlated with the percentage of isocaproic acid. The declared consumption of cream, mayonnaise, and dressings correlated positively with the content of butyric acid and negatively with the content of isovaleric acid.

The consumption of berries in the ketogenic group was significantly higher than in the control group. Additionally, significant positive correlations were observed between their consumption and higher amounts of acetic, propionic, iso-butyric, and isocaproic

Table 8. Vegetable and fruit consumption and SCFAs content in the intervention group

Intervention group								
		Non-berry fruit	Berries	Vegetables (all kinds)	Other vegetables	Legumes	Green leafy and cruciferous vegetables
Acetic acid [%]	RHO	-0.281	-0.007	**-0.688**	-0.253	0.467	0.228
	p	0.331	0.9809	**0.0066**	0.4036	0.0922	0.4328
	n	14	14	14	13	14	14
Propionic acid [%]	RHO	0.194	-0.045	**0.823**	0.48	-0.239	-0.091
	p	0.5056	0.8797	**0.0003**	0.0965	0.4098	0.7575
	n	14	14	14	13	14	14
Butyric acid [%]	RHO	-0.022	-0.028	0.011	0.015	-0.364	**-0.574**
	p	0.9416	0.9239	0.9691	0.9623	0.201	**0.032**
	n	14	14	14	13	14	14
Isobutyric acid [mg/g]	RHO	0.065	0.052	0.408	0.095	**-0.49**	-0.333
	p	0.8257	0.8607	0.1472	0.7581	**0.0754**	0.2453
	n	14	14	14	13	14	14
Isovaleric acid [mg/g]	RHO	**0.497**	0.023	**0.566**	0.118	-0.368	-0.166
	p	**0.0705**	0.9365	**0.0348**	0.7008	0.1959	0.5699
	n	14	14	14	13	14	14
Valeric acid [%]	RHO	0.108	0.04	**0.496**	0.131	-0.249	-0.002
	p	0.7134	0.8923	**0.0714**	0.6696	0.3911	0.994
	n	14	14	14	13	14	14
Valeric acid [mg/g]	RHO	0.238	0.021	**0.468**	0.144	-0.403	-0.335
	p	0.413	0.9428	**0.0917**	0.638	0.1531	0.2419
	n	14	14	14	13	14	14
Caproic acid [%]	RHO	-0.238	0.157	**-0.585**	-0.274	0.04	-0.027
	p	0.4136	0.5914	**0.028**	0.3655	0.8923	0.9281
	n	14	14	14	13	14	14
Isocaproic acid [%]	RHO	0.022	-0.35	-0.334	0.058	**0.479**	0.419
	p	0.9416	0.2203	0.2438	0.8501	**0.0833**	0.1363
	n	14	14	14	13	14	14
Isocaproic acid [mg/g]	RHO	0.195	**-0.475**	0.234	**0.497**	0.18	0.062
	p	0.5051	**0.0864**	0.4198	**0.0839**	0.5386	0.833
	n	14	14	14	13	14	14
Heptanoic acid [%]	RHO	-0.324	-0.162	**-0.628**	-0.376	0.387	0.237
	p	0.2586	0.5802	**0.0161**	0.2059	0.1713	0.4146
	n	14	14	14	13	14	14

This table shows the correlation between consumption of the following vegetable and fruit products and the stool SCFAs content in the intervention group. RHO – *Spearman's* rank correlation coefficient.

acid. The consumption of non-berry fruit positively correlated with the amount of butyric acid. A negative correlation was noted between the consumption of legumes and the content of acetic and caproic acid, as well as a positive correlation for propionic acid, poultry and rabbit meat and the percentage of propionic acid (RHO = -0.517; p = 0.0403) and a positive correlation (RHO = 0.562; p = 0.0235) between the consumption of red meat and venison and the concentration of valeric acid. There was also a strong correlation between the consumption of fish and a higher percentage of acetic acid (RHO = 0.732; p = 0.0013). In the ketogenic group, a correlation was observed between the consumption of natural unsweetened plant drinks (RHO = 0.657; p = 0.0108) and cream, mayonnaise, and dressings (RHO = 0.558; p = 0.0379) and the percentage of isovaleric acid. Much more correlations were found in the control group, including correlation between consumption of sweetened dairy products and acetic, propionic, butyric, iso-butyric, isovaleric, caproic, and heptanoic acids. The consumption of natural dairy products positively correlated with the percentage of isocaproic acid. The declared consumption of cream, mayonnaise, and dressings correlated positively with the content of butyric acid and negatively with the content of isovaleric acid.

The consumption of berries in the ketogenic group was significantly higher than in the control group. Additionally, significant positive correlations were observed between their consumption and higher amounts of acetic, propionic, iso-butyric, and isocaproic acid. The consumption of non-berry fruit positively correlated with the amount of butyric acid. Moreover, a negative correlation was noted between the consumption of legumes and the content of acetic and caproic acid, as well as a positive correlation for propionic acid.

The control group was characterized by significant negative correlations between the consumption of fruit, total vegetables, vegetables other than leafy greens and cruciferous vegetables, and legumes, and the content of valeric acid. The consumption of total fruit and berry fruit positively correlated with the content of caproic acid. The consumption of vegetables other than leafy and cruciferous vegetables negatively correlated with the content of isocaproic acid.

In the control group, there were no statistically significant correlations between the consumption of cereal products, potatoes, sweet potatoes, and derivatives and the content of SCFAs. In contrast, in the ketogenic group, statistically significant correlations between the consumption of potatoes, sweet potatoes, and similar products and the content of SCFAs were observed. There were no such correlations for cereal products. In the study group, between these variables there was a negative correlation for acetic acid in percent (RHO = -0.532; p = 0.0501) and positive correlations for acetic acid expressed in mg/g (RHO = 0.577; p = 0.0307), for propionic acid as a percentage (RHO = 0.667); p = 0.0091) and concentration (RHO = 0.765;

Tab. 9. Vegetable and fruit consumption and SCFAs content in the control group

Control group							
		Non-berry fruit	Berries	Vegetables (all kinds)	Other vegetables	Legumes	Green leafy and cruciferous vegetables
Valeric acid [mg/g]	RHO	**-0.578**	0.188	**-0.55**	**-0.553**	**-0.558**	-0.416
	p	**0.019**	0.486	**0.0273**	**0.0262**	**0.0248**	0.1087
	n	16	16	16	16	16	16
Caproic acid [%]	RHO	**0.571**	0.42	0.39	0.085	0.17	0.056
	p	**0.0209**	0.1057	0.1353	0.753	0.5295	0.8362
	n	16	16	16	16	16	16
Caproic acid [mg/g]	RHO	**0.463**	0.504	0.23	-0.111	0.041	-0.112
	p	**0.0708**	0.0465	0.3915	0.6824	0.8814	0.6784
	n	16	16	16	16	16	16
Isocaproic acid [mg/g]	RHO	-0.229	-0.103	0.057	**-0.501**	-0.012	-0.28
	p	0.3944	0.7033	0.8325	**0.0481**	0.9647	0.2943
	n	16	16	16	16	16	16
Heptanoic acid [mg/g]	RHO	0.282	**0.46**	0.092	-0.187	-0.074	-0.296
	p	0.2905	**0.0728**	0.7356	0.487	0.7863	0.2652
	n	16	16	16	16	16	16

This table shows the correlation between consumption of the following vegetable and fruit products and the stool SCFAs content in the control group. RHO – *Spearman's* rank correlation coefficient.

p = 0.0014), for the concentration of iso-butyric acid (RHO = 0.616; p = 0.0191) and for the concentration of valeric acid (RHO = 0.547; p = 0.0431).

DISCUSSION

The stool samples obtained from the test group (ketogenic diet) showed a higher content of isovaleric acid, iso-butyric acid, butyric acid, and a lower amount of acetic acid (compared to the control group - people on a standard diet). The higher fat intake of the ketogenic diet and the drastic elimination of carbohydrates seems to have a big impact on the bacterial metabolome. Isovaleric acid and iso-butyric acid are branched short-chain fatty acids which are the product of proteolytic (putrefactive) fermentation in the large intestine (with the participation of proteolytic microbiota from the *Proteobacteria* group) [20]. Higher content of *Proteobacteria* (in the large intestine) combined with a decrease in the number of microbiota that metabolizes non-digestible carbohydrates (complex glycans, polysaccharides (cellulose, hemicellulose, lignin, resistant starch pectin, and oligosaccharides), monosaccharides, mucins, mucopolysaccharides may be associated with colon cancer progression), inflammation, and the phenomenon of insulin resistance [20, 21]. The large differences in the content of isovaleric acid and iso-butyric acid (BCFAs) obtained in these studies between the groups may indicate differences in proteolytic fermentation, which takes place in a different way depending on the availability of dietary fibre in the diet. The amount of BCFAs increases significantly within 24 hours of consuming more protein. Higher protein intake increases the overall pool of isovaleric acid, which is present in trace amounts in a norm-protein diet [8, 22, 23]. Still little is known about the role of BCFAs in our bodies. It is known from individual reports that some of them can be used as an energy source in the event of an insufficient amount of butyrate (SCFAs are also produced because of proteolytic fermentation (mainly from glutamine and asparagine), but their total pool is much smaller if this fermentation does not come from carbohydrate substrates) [8]. Due to the lack of scientific data regarding the influence of BCFAs, including isovaleric acid on the host's health, the results obtained in this study can only be speculated.

The process of isovaleric acid formation is associated with an increased synthesis of ammonia, which is a toxic product whose content in blood and tissues must be strictly controlled. Ammonia from proteolytic fermentation (along with BCFAs) can be lowered by adding dietary fibree to the diet. Increasing the fermentation of dietary fibree will result in an influx of ammonia to bacterial cells because of the demand for nitrogen, as well as preferential fermentation of carbohydrate substrates. In addition, an acidic environment in the distal colon alone can influence the metabolism of gut bacteria to such an extent that BCFAs synthesis is reduced [8, 22]. However, there are such fractions of dietary fibree that can increase the production of iso-butyric and isocaproic acid. Low pH in the large intestine and the availability of dietary fibree is associated with an increase in bacterial differentiation and a reduction in putrid fermentation. It seems that starch may be of great importance in reducing the intensity of proteolysis, but the process still depends on the pH in the intestinal lumen. BCFAs production is 60% lower at pH=5.5 compared to pH=6.88.20.22. As proved by *Pieper R. et al.*, Increasing the amount of dietary fibre from 10 g to 30 g per day resulted in a preferential utilization of fibree over protein, and increased the overall amount of SCFAs, and shortened intestinal transit [23]. It is very important from the point of view of the carcinogenic activity of the metabolites of putrefactive microflora contained in the faeces, as well as from the point of view of the putrid fermentation itself, which takes place in the final sections of the gastrointestinal tract. The shorter the intestinal transit, the less putrid metabolites are likely to be produced. The lower amount of acetic acid in the ketogenic group compared to the test group may be related to the diet (providing a smaller pool of carbohydrates including fibree) and the likely greater absorption of acetate into the circulation. Acetate is trapped in the brain (it crosses the blood-brain barrier) and accumulates in the hypothalamus, changing the expression of the regulatory neuropeptide profile, and thus the regulation of appetite, which could explain the lower number of meals consumed during the day in the study group [8, 9]. Acetic acid produced by bacteria min. *Bacteroidetes* can be used for the synthesis of propionic and butyric acid by *Firmicutes* [24]. It seems that this could to some extent explain the ratio of acetic acid to butyric acid. The greater amount of butyric acid in the stool of the test group may also be due to the much greater consumption of vegetables (mainly leafy and cruciferous) by the ketogenic group compared to the control group, which may mitigate to some extent the effects caused by the greater amount of potentially harmful branched fatty acids. A greater amount of butter acid could also result from the growth of *Akkermansia mucinifila*, which was observed after the implementation of the ketogenic diet in the studies conducted by *Olson et al.* [26] and *Ma et al.* [25]. It should be noted that the very estimation of the degree of short-chain fatty acids production is methodologically problematic because we observe their rapid and continuous uptake by colonocytes, as well as their use for metabolism not only in the host but also in the microbiome. Currently, SCFAs can be performed in faeces and serum, but both measurements have some limitations. We used stool samples in our study, so it should be remembered that only 5% of the total pool of SCFAs produced is likely to be found in the sample [8, 9].

In the study group, a positive correlation was observed between the consumption of total fat and animal fat and the increased amount of isovaleric acid (compared to the control group). The results are consistent with the high consumption of protein in the diet of athletes. The result confirms previous studies in which the protective role of carbohydrate fermentation (saccharolytic fermentation) was observed. It has been found that the consumption of carbohydrates limits putrefaction, and the fermentation of carbohydrates itself, even though more protein is present in the lumen of the gut, may reduce the production of putrefactive metabolites. The consumption of animal fats in the study group also strongly positively correlated with the content of propionic acid (RHO = 0.823). A similar observation was made for medium-chain fatty acids (MCTs). Propionic acid induces some metabolic functions, such as increasing energy expenditure, leptin expression, reducing energy consumption, increasing PYY (YY peptide) expression, increasing intestinal gluconeogenesis, and hepatic gluconeogenesis. Increased propionate may also be associated with suppression of appetite and its function of glucose production in the process of gluconeogenesis, which is more active during the ketogenic diet [23, 27, 28, 29]. The consumption of medium-chain fatty acids in the ketogenic group was significantly higher than in the control group. MCTs showed correlations with a large amount of short-chain fatty acids, at the same time these correlations were different than those associated with the consumption of long-chain animal fats. The strongest positive correlation was observed between the consumption of MCTs and the increase in the amount of butyric acid. This may coincide with the results of the study by *Rial S. et al* discussing the beneficial effects of MCTs fats in obesity [28]. Microbiome changes induced by MCTs consumption included, among others, the protective effect of MCTs on toxicity and endotoxemia induced by bacterial lipopolysaccharide, improvement of intestinal barrier integrity, increase of sIgA secretion, and decrease of faecal pH. The consumption of medium-chain fatty acids did not correlate with the increase in the amount of isovaleric and iso-butyric acids, which proves their different metabolism compared to animal fats, which showed this correlation. MCTs fats are characterized by fast digestion and absorption and show a different cellular metabolism than long-chain triglycerides [23, 27, 30]. The consumption of vegetables in the ketogenic group was positively correlated with the amount of valeric acid, while in the control group the correlation was negative. This may indicate a significant difference in the composition and function of the microbiome in the face of the drastic elimination of one nutrient (carbohydrates) and a large percentage of another nutrient (fats). Potting fermentation and its products appear to vary with the amount of carbohydrate and fat in the habitual diet. The consumption of berries in the study group negatively correlated with the content of isovaleric acid, which may indicate the potential protective and prebiotic effect of polyphenols contained in colourful and tart berries [8, 27, 30]. The differences in the frequency of consumption of given product groups showed that the ketogenic group consumed vegetables and berries, nuts, seeds and seeds, vegetable fats, and fish significantly more often, which is not insignificant for health. The Mediterranean variant of KD seems to be the most pro-health, but it should still be approached with scepticism if a person wants to use this model of nutrition as usual, without specific health indications [29, 30]. A significant difference in minus was the greater consumption of cream, mayonnaise, and dressings, which may have an adverse effect on the bacterial metabolome due to the high degree of processing of these products, as well as the unfavourable composition (trans fatty acids, disturbed *omega*-3 to *omega*-6 ratio), added sugar, salt, and preservatives). The opposition is significantly lower consumption of salty snacks, sweet snacks, and fast-food products in the ketogenic group, which may indicate greater health and consumer awareness of people implementing this model of nutrition.

CONCLUSIONS

High consumption of cruciferous and leaf vegetables, berries and nuts on a ketogenic diet may have a positive effect on the profile of short-chain fatty acids produced by the gut microbiome. Changing the diet towards a greater supply of plant products may prevent proteolytic fermentation and reduce the negative effects of microbiome changes caused by an oversupply of protein and fat in the ketogenic diet.

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Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

CHEMICAL COMPOSITION, ANTIOXIDANTS AND ANTIMICROBIAL ACTIVITIES OF MOROCCAN SPECIES OF *PSIDIUM GUAJAVA* EXTRACTS

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ABSTRACT

Background. During the recent years, appropriate attention has been paid to the oxidative stress which damages the body's cells, proteins, and DNA. Therefore, the need of antioxidants becomes a therapeutic and preventive priority. In addition, microbial infections also constitute a public health problem.

Objective. To find efficient, reliable and safe alternatives sources to synthetic antioxidants, antibiotics and antifungals drugs.

Materials and methods. Extract and essential oil of *Psidium guajava* were screened for their antioxidant and antimicrobial activities against gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), gram negative bacteria (*Citrobacter freundii, Escherichia coli* and *Pseudomonas sp*) and fungi (*Candida albicans,Candida tropicalis* and *Cryptococcus neoformans*), as well as to determine the functional groups of phytochemicals present in the essential oil by Fourier transform infrared spectroscopy (FTIR).

Results. The results indicate that *P. guajava* leaves extract demonstrated very high antioxidant activity and *P.guajava* essential oil showed the highest polyphenols content. The antioxidant capacity showed a significant negative linear correlation to total polyphenolic content (TPC) with *Pearson's* correlation coefficients. P. guajava essential oil shows high antibacterial and antifungal activity against all the studied bacteria and fungi.The FTIR analysis of *P. guajava* essential oil showed the presence of several functional groups (ethers, esters, ketones, terpenes, alkanes, aldehydes, aromatic hydrocarbons, alcohols, and phenols). The relationship between the chemical composition and antimicrobial activity of *P.guajava* essential oil suggests that the attribution of its antimicrobial activity to a particular compound or a synergistic effects between its different constituents remains difficult.

Conclusions. The present study demonstrated that *Psidium guajava* is a valuable source of active compounds with anti-oxidant and antimicrobial activities. This finding suggests the new use of the fruits and the leaves extracts of this plant in the treatment of bacterial and fungal infections, as well as for the extraction of new antioxidants. Therefore, it is necessary to be carried out in another study to identify the active(s) compound(s) in *P.guajava* essential oil with respect to their mechanisms and synergistic actions.

Key words: *antibacterial activity, antifungal activity, polyphenols, medicinal plants, essential oil*

INTRODUCTION

In recent years, there has been an increased interest in the exploitation of medicinal plants in the pharmaceutical, medicinal and agri-food industries for the search of new antibiotics and new antioxidant, this is mainly due to the fact that the medicinal products derived from these plants have been found to be safe for human health and have no side effects compared to chemical synthetic drugs [1].

Psidium guajava, commonly known as guava and belonging to the *Myrtaceae* family,native to Mexico and extends throughout the South America, European, Asia and Africa, has been reported to have several chemical and biological activities. An aqueous extract of guava leaves demonstrated antibacterial activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* [2] and antifungal activity against *Candida albicans* [3] and effects of the guajaverine from guava leaves on growth inhibition

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of *Streptococcus mutans*, a pathogen for dental caries, has been described. It's verified that Flavonoids such as quercetin have expressed significant antioxidant and antibacterial activity [2, 4]. In addition, the lycopene has been found to reduce the risk of cancer through these antioxidant effects.

The leaves of guava contain an essential oil rich in flavonoids, cineol, tannins, resin, eugenol, chlorophyll, malic acid, cellulose and a number of other active compounds [5]. Guava fruits have been reported to have antioxidant activity, contain vitamin C, iron calcium and phosphorus, β-caryophyllene, limonene, antioxidant compound (polyphenols, flavonoids, proanthocyanidins, triterpenes and other constituents), antioxidant dietary fiber [6, 7, 8, 9].

In the present study, the extracts and the essential oil from leaves and fruits of *Psidium guajava* were screened for antioxidant, antibacterial and antifungal activities against gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), gram negative bacteria (*Citrobacter freundii, Escherichia coli*and *Pseudomonas sp*) and fungi (*Candida albicans, Candida tropicalis* and *Cryptococcus neoformans*) as well as to determine the functional groups of the phytochemicals present in *Psidium guajava* essential oil.

MATERIAL AND METHODS

Plant material

The studied *Psidium guajava* (local name Guava) was collected in the region of El Jadida. This cultivated plant was not treated by pesticides. The leaves and the fruits were collected from the surrounding areas, identified, and authenticated by a taxonomist. The leaves and fruits were thoroughly rinsed using water treated and shade-dried over during 2-4 weeks at room temperature. The leaves oriented to the extraction of the essential oil are preserved in the whole state, the leaves and the dried fruits oriented to the preparation of the dichloromethane/ethanol extract have been crushed separately to obtain fine powder.

Plant extraction

The dried and powdered leaves and fruits (100 g) was macerated separately for 48 hours at room temperature in a mixture of two solvents, a polar solvent (Ethanol) and a non-polar solvent (Dichloromethane) with a proportion of 50%:50%. The mixture was filterd using Whatman filter. The filtrat was concentrated under low pressure at 40°C using a Rotary evaporator until the total elimination of the solvent and the dried crude extract is obtainedstored in a freezer at 4°C until further tests.Essential oils has been extracted by hydrodistillation technique using Clevenger apparatus. The dried aerial parts of *P.Guajava* (300 g) were hydrodistilled using a Clevenger-type apparatus to extract the essential oils during 4 h. The distilled essential oils has been recovered, filtered and stored at +4°C.

Test microorganisms

Five bacteria species and three fungi from Collection of the Pasteur Institute in Paris (CIP) and from American Type Culture Collection (ATCC) were used (Table 1).

Antimicrobial efficacy testing

The antimicrobial activity of different *P.guajava* extracts was studied using the disc diffusion method. The inoculums of bacteria and fungi were prepared from colonies in phase of exponential growth from the culture from 18 to 24 hours old on *Mueller-Hinton* agar for bacteria and *Sabouraud* agar for fungi.

The evaluation of the antibacterial and antifungal activity of all extracts were validated by the measure of the diameters of the zones of inhibition appearing around the disks in comparison with the standard antibiotics (Ampicillin 30 µg) or the standard antifungal (Econazole 30µg). Every test was realized in triplicate mean inhibition zone was computed.

Antioxidant activity testing

The antioxidant activity of *P.guajava* extracts was determined by a DPPH (diphenyl-1-picrylhydrazyl) assay. The percentage of DPPH inhibition was calculated using the following formula:

Table 1.Bacteria and yeasts used for antimicrobial activity testing

	Microorganisms	Gram	Reference	Origin
Bacteria	*Citrobacter freundii*	Gram-	ATCC8090	American Type Culture Collection
	Escherichia coli		CIP54127	Collection of the Pasteur Institute, Paris
	Pseudomonas sp		ATCC10145	American Type Culture Collection
	Enterococcus faecalis	Gram+	ATCC19433	American Type Culture Collection
	Staphylococcus aureus		CIP 209	Collection of the Pasteur Institute, Paris
Yeasts	Candida albicans		CIP 48.72	Collection of the Pasteur Institute, Paris
	Candida tropicalis R2		CIP1275.81	Collection of the Pasteur Institute, Paris
	Cryptococcus neoformans		CIP960	Collection of the Pasteur Institute, Paris

I%=((Ac-As)/Ac) x100

Where:
I%: percentage of DPPH inhibition; Ac: the negative control's absorbance; As: the sample's absorbance tested. The standard of the reaction is the butylatedhydroxytoluene (BHT).

All the tests were made in triplicates and the results were expressed as a mean of the three assays. Ethanolic solution of extract was prepared at concentrations from 0 to 5000 µg/ml. DPPH (0.04 g/l) was added to 0.5 ml of each solution. The negative control was prepared by adding 0.5 ml of methanol to 1.5 ml of the DPPH methanolic solution. Discolorations were measured by the spectrophotometer at 517 nm after incubation of the mixture for 30 min at room temperature in the dark. The absorbance of the positive control (BHT) was measured in the same conditions as well as the extracts. The percentage of DPPH inhibition (I%) was calculated and the IC_{50} values for all the samples were determined using «Origin®Pro8» software.

Polyphenols' content

The method is adapted by *Singleton* and *Rossi* (in 1965) with the reactive of *Folin-Ciocalteu* [10]. Briefly, 2.5 mL of*Folin-Ciocalteu* reagent (diluted10 times) was added to 0.5 mL of aqueous extract (diluted 200times). Sodium carbonate (Na_2CO_3) (75g/L) was added (what favours an alkaline environment to activate the redox reaction). The mixture was incubated in a water bath at a temperature of 50°C during 5 min. Then, the absorbance was measured at 760 nm by a spectrophotometer UV-3100 PC VWR.

The total polyphenols content was calculated from the calibration curve established with a solution of gallic acid (calibration range 0 - 80 µg/ml). The negative control of the reaction was a polyphenol content free. The determination was done in triplicates. The results were expressed by milligram of gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g dw).

FTIR analysis of P. guajava essential oil

To study the chemical composition of *P.guajava* essential oil, the essential oil was scanned in the wavelength range of 4000 - 400 cm^{-1} with a resolution of 2 cm^{-1} using an FTIR spectrometer of type JASCO 4000, equipped with a detector (TGS) and a ceramic source, separated by an optical system using a *Michelson* interferometer. The room was kept at a controlled ambient temperature (25 °C) and relative humidity (30%).

Precisely weighed, essential oil (2 µL) were coated on the KBr tablets to form thin liquid films for infrared spectrometry analysis.The background air spectrum, water vapor and CO_2 interference were subtracted from these spectra. After baseline correction and smoothing were performed using the OMNIC8.0 software, the spectrum data were imported in Unscrambler 9.7 software to standardize the normal variations. the characteristic peaks and their functional groups were detected. FTIR peak values were recorded. Each analysis was repeated three times for spectrum confirmation.

Statistical analysis

All the assays were performed in triplicate and the *Pearson's* correlation coefficient (r) statistics was used. The coefficient of determination (R2) between antioxidant activity and total polyphenolic content (TPC) was carried out using the regression analysis by Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Antioxidant activity

The results obtained are represented in Figure 1.

The antioxidant activity of the extracts and the essential oils of *P. guajava* expressed by IC_{50} (concentration of the extract necessary to reduce 50% of the radical DPPH) is for: *P. guajava* leaves 102 µg/ml, *P. guajava* fruits 966.77 µg/ml, *P. guajava* essential oil 2366.29 µg/ml, and standard antioxidant (BHT) 79.81 µg/ml. The results of the antioxidant characteristics of the different extracts of *P. guajava*, estimated by the DPPH scavenging activity gave the following classification: *P. guajava* leaves >*P. guajava* fruits > *P. guajava* essential oil.

In recent years, appropriate attention has been directed to natural antioxidants. Antioxidant-based drug formulations are used as therapeutic or preventive against several infections and diseases; they synthesize a wide range of secondary metabolic molecules that have antioxidant activities with therapeutic power. Phenolic compounds such as flavonoids, phenolic acids, coumarins, stilbenes and tannins are considered to be the most abundant plant antioxidants [11]. Polyphenols and any reducing compounds, even non-electroactive species, will contribute for the overall antioxidant power. Therefore, reducing sugars, polysaccharides, vitamin C may influence the results of antioxidant activity in plant material [12].

The reduction power is generally due to the existence of one or more hydroxyl functions carried by the benzene ring that exert an antioxidant action by donating a hydrogen atom to break the free radicals chain reaction or to prevent the formation of peroxide [13].

The analytical principle of DPPH radical scavenging assays is based on the conversion of former radical (DPPH°) to the reduced form(DPPH-H), which is observed by the discoloration effect (transition

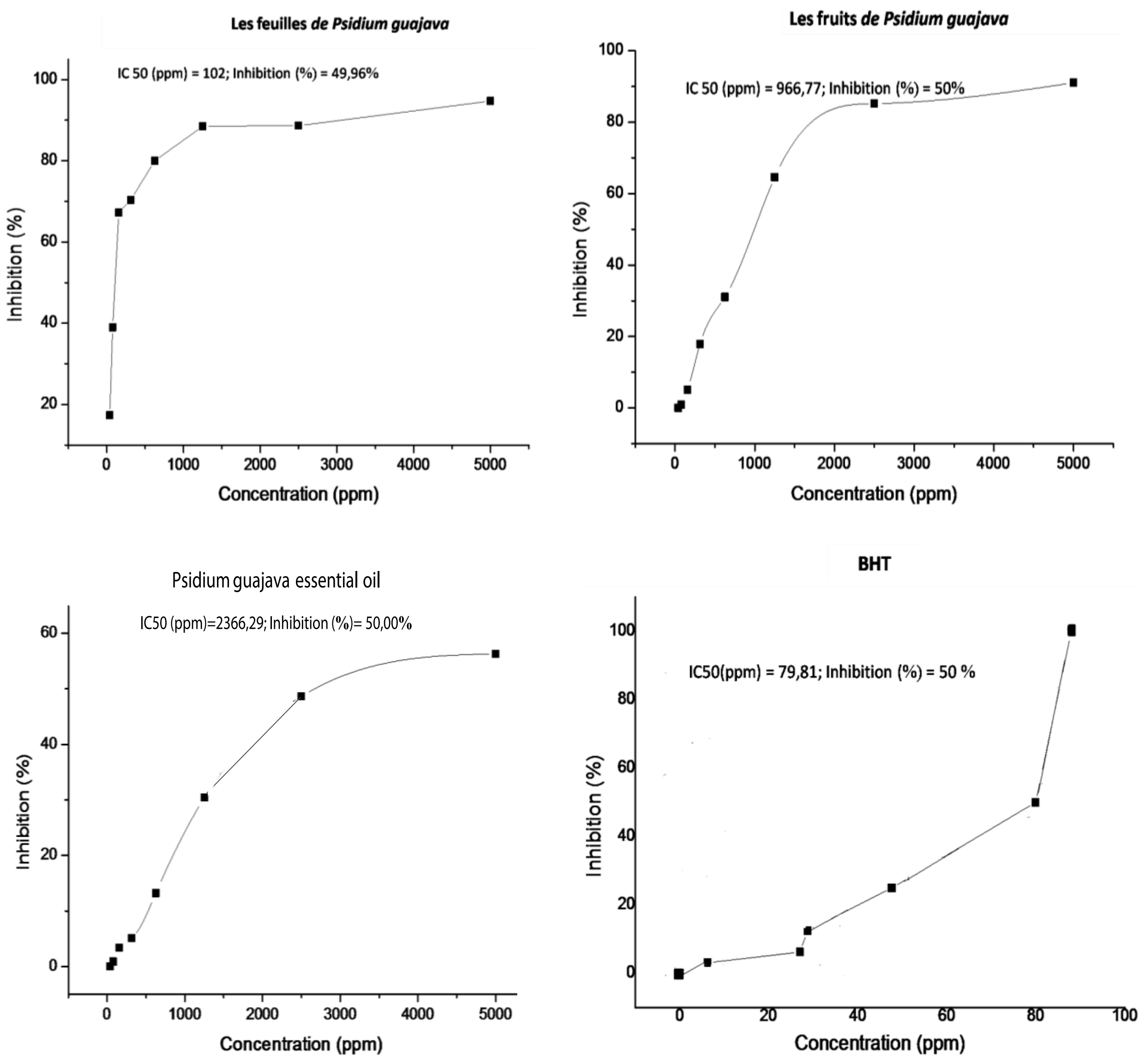

Figure 1. Inhibition percentage of different plant extracts based on extract concentration

from violet to yellow) measured spectrometrically at wavelength of 517 nm [14]. Thus, the lower is the IC_{50} value, stronger is the antioxidant activity.

P. *guajava* extracts, evaluated in this study, demonstrated variability in antioxidant characteristics. This is the first report for antioxidant capacity of *P. guajava* in Morocco, since is recently introduced in Morocco, it's native to the Caribbean and Central America. Thee extract of *P. guajava* leaves extract demonstrated very high antioxidant activity (IC_{50} =102 µg/ml) very close to that of BHT (IC_{50} =79.81 µg/ml), despite that this extract contains a low content of polyphenol (2.52±1.12 mg GAE/g dw). These results are in accord with high antioxidant activity (IC_{50} =100 µg/ml) reported previously for the aqueous extract of *P. guajava* leaves in South Korea [15]. This high antioxidant activity may be due to blockage of the chain reaction of linoleic acid [16] or free radical scavenging activity by quercetin, quercetin-3-o-glucopyranoside murine [4] and ferulic acid [17] or other antioxidants such as phenolic compounds like flavonoids, phenolic acids or carotenoids. However, the effectiveness of flavonoids as effective antioxidants depends on several factors such as environmental factors, which can even alter their effectiveness as antioxidants.

The dichloromethane/ethanolic extract of *P. guajava* fruits exhibited low to moderate activity by the DPPH, (IC_{50}=966.77 µg/ml), compared to that of BHT (IC50= 79.81 µg/ml) [18]. Several previous studies have obtained similar IC_{50} values[19,20]. In other study, *Ademiluyi* et al.[19] have shown that even if the IC_{50} value obtained was 92 0µg/ml, but this value has been interpreted as ted as signifying a high antioxidant activity due to the richness of this fruit in polyphenols [21], as shown by the results obtained with the total content of polyphenols of 18.09 ± 3.4 mg GAE/g dw, which is confirmed by the presence of Kaempferol, Quercetin, Schottenol ferulate and Esculin in *P.guajava* fruits extract [22].

Concerning *P.guajava* essential oil, the results obtained in our study indicate that it showed low antioxidant activity with a IC50 value of 2366.29 µg/ml, even if the polyphenols content is 45.67±2.88 mg GAE/g dw. Indeed, this result agrees favorably with previous reports suggests weak antioxidant activity of *P.guajava* essential oil (IC50 values between 18.52 - 33.72 mg/ml), this result can be explained by the absence of compounds as flavonoids, one of the main responsible compounds for the antioxidant activity of medicinal plants [23,24,25].

Polyphenols content

Phenolic compounds, such as catechins, quercetin, caffeic acid, chlorogenic acid, rutin, naringin and gallic acid, are the most important in the plant constituents known for their antioxidant power [26]. The total phenolic content (PC) data is presented in Table 2. Among all the tested extracts, the highest PC was observed in *P.guajava* essential oil 195.67±2.88 mg GAE/g dw and was the least in dichloromethane/ ethanol leaf extract 2.52±1.12 mg GAE/g dw.

Table 2. Total polyphenols contents in extracts from *P.guajava* (mg GAE/g dw)

Extracts	Total phenolic mg GAE/g dw
P. guajava leaves	2.52 ± 1.12
P.guajava fruits	18.09 ± 3.41
P.guajava essential oil	195.67±2.88

The comparison contents of total phenolic compounds in the three extracts of *P.guajava*, indicates the following order: *P.guajava* essential oil > *P.guajava* fruits extract > *P.guajava* leaves extract. This finding is in agreement with reported data from studies carried out by *Mahomoodally* et al., which showed similar result (209.16 ± 6.15 mg GAE/g) [27]. As regarding to *P.guajava* fruits extract its previously confirmed that its polyphenol content remains significant compared to other studies[18]. For *P.guajava* leaves extract with low polyphenolic content not exceeding 2.52 ± 1.12 mg GAE/g dw, even if this value is higher than that found in other study that is interpreted to be very rich in polyphenols [28]. Therefore, we can conclude that this extract is being rich in phenolic compounds as gallic acid, quercetin, protocatechuic acid, chlorogenic acid, caffeic acid, kaempferol and ferulic acid [28].

Correlation between phenolic compounds and antioxidant activity

The phenolic compounds were supposed to play an important role in the antioxidant activity. To reveal the correlation between total polyphenols content (TPC) and antioxidant activity (estimated by $1/IC_{50}$) is in Figure 2. This correlation showed a low determination coefficient ($R^2 = 0.371$, ($Y= -0.029x + 5.901$)). *Pearson's* correlation coefficient was applied to evaluate the relationship between antioxidant activity and total polyphenolic contents.

The antioxidant capacity showed a significant negative linear correlation to TPC with *Pearson's* correlation coefficients of r= -0.59. For example, the leaves extract of *P.guajava*, which had the lowest polyphenols content (2.52±1.12 mg GAE/g dw), showed the highest antioxidant activity (102 ppm). Indeed, several studies have demonstrated that there is no correlation between antioxidant activity and total polyphenols content [29]. This suggests that the relationship between polyphenols and antioxidant requires an explanation. Firstly, the free radical scavenging activity is not only affected by polyphenols concentrations, but also by the structure of the polyphenol compounds in the extract. Indeed, for

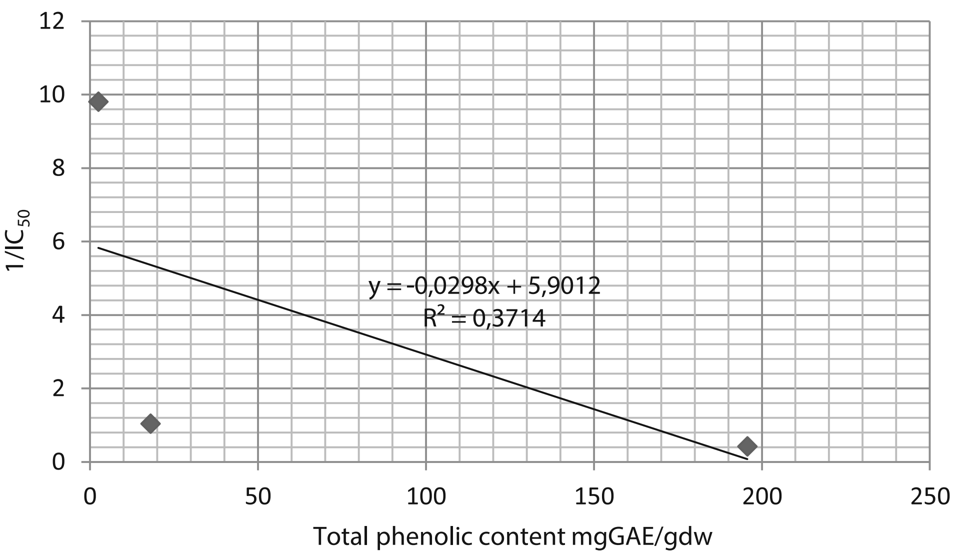

Figure 2. Correlation between total phenolic compounds and antioxidant activity ($1/IC_{50}$)

polyphenols that act *via* reactive species scavenger's pathway, their activity is affected by the positions and the numbers of phenolic hydroxyl groups in the structure of aromatic ring in phenols [30].

Also, the degree of stability conferred on the flavonoid phenoxyl radicals is the most effective radical scavengers, participant in electron delocalization [31]. The glycosylation of phenolic compounds can also decrease antioxidant activity. Moreover, the DPPH using in this study does not consider the effect of polyphenols others than free radical scavenging activity, via pathway of lipoxygenase inhibition or via reducing agents for metmyoglobin which requires others analysis method [32]. Others finding indicate that no correlations confirm that phenolic compounds are not the only contributor to the antioxidant activities of the medicinal plant extracts, several others non-phenolic antioxidants as nitrogen compounds, alkaloids, carotenoids, ascorbic acid, vitamin E and β-carotene may be responsible for the antioxidant activity [33, 34, 35]. Moreover, the antioxidant activity is the result of a synergetic effect between phenolic antioxidants and non-phenolic antioxidants [36].

Antibacterial activity

The antibacterial activity screening results presented in Table 3 show that *P.guajava* essential oil (PgEO) shows high antibacterial activity against all the studied bacteria, with maximum activity against *Enterococcus faecalis* and minimum activity against *Escherichia coli*. *P.guajava* leaves extract also shows antibacterial activity against all bacteria tested, but with an inhibition zone of average diameters, ranging from 8 to 11 mm. *P.guajava* fruit extract exhibit moderate antibacterial activity against *E. coli, Pseudomonas sp, E. faecalis* and *S. aureus*, with an inhibition zone of medium diameters between 8 mm and 12 mm.

The sensitivity of the bacteria according to their Gram to the *P.guajava* extracts studied shows that the antibacterial action of the three studied extracts of *P.guajava* is more pronounced on Gram positive bacteria compared to Gram negative bacteria, which are the most resistant. Indeed, Gam negative bacteria recorded lower inhibition diameters (between 8 mm and 20 mm) compared to Gram-positive bacteria, which showed higher inhibition zones reaching 24 mm (Figure 3).

The essential oil of *P. guajava* (PgEO), indicated strong antibacterial activity against *E.coli, C. freudii, S.aureus* and *E. faecalis* with an inhibition zone ranging from 11.67±2.08 mm to 24±3.61mm. *Hanif* et al. concluded that PgEO has moderate antibacterial potential against *E.coli* (15.0±0.8 mm), *S. aureus*

Table 3. Antibacterial activity of *P.guajava* extracts from leaves, fruits and essential oil (EO)

Extracts	Inhibition zone diameter (mm)				
	Gram negative bacteria			Gram positive bacteria	
	Escherichia coli	Pseudomonas sp	Citrobacter freundii	Enterococcus faecalis	Staphylococcus aureus
P. guajava (leaves)	8±1.00	11±2.00	8±1.00	11±1.73	11±2.65
P.guajava (fruits)	9±1.73	12±0.58	-	9±2.00	8±0.00
P.guajava (EO)	11.67±1.5	19±1.7	20±0.00	24±0.58	14.33±1.7
Ampicillin 30 µg	27	25	25	29	24

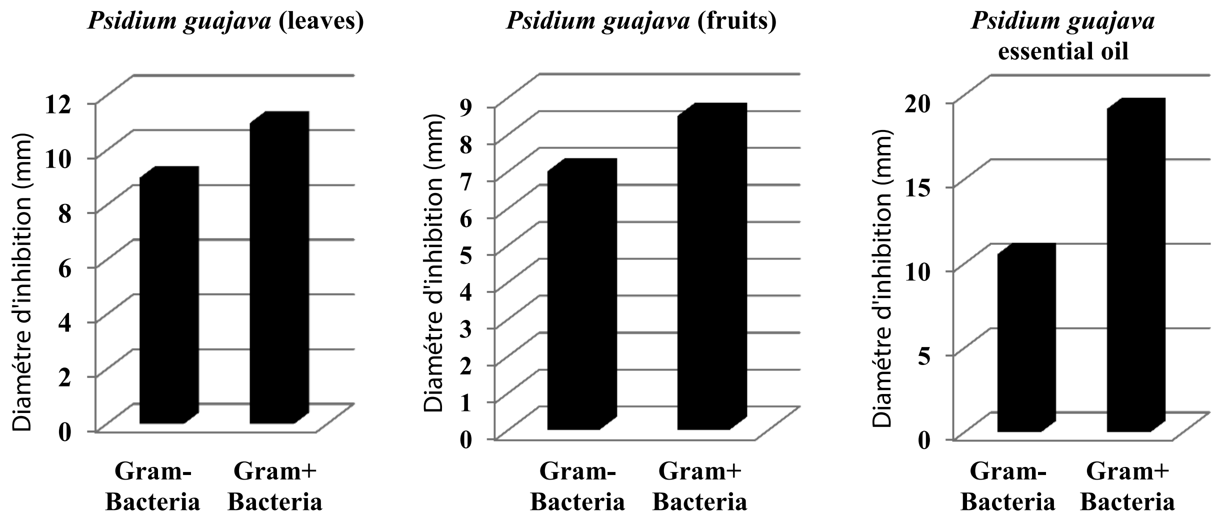

Figure 3. Sensitivity of the bacteria according to their Gram to *P.guajava* extracts

(9.0±0.5mm), and *S. pyrogenes* (11.0±0.6 mm) [37]. *Weli* et al. obtained smaller inhibition diameters, not exceeding 13 mm and concluded that PgEO was characterized by significant antibacterial activity[38].

Concerning to dichloroethanoic extract of *P.guajava* leaves, it should be noted that the results obtained are very similar to those found by *Biswas* et al. [48] when the ethanolic extract showed an inhibition zone of 6.11 mm and 11 mm against *S. aureus* and *B. cereus*, respectively. Indeed, several other studies confirm the antibacterial effect of *P. guajava* extracts, methanolic and ethanolic extracts showed an inhibitory activity against Gram-negative bacteria, known by their resistance as *E. coli* and *Pseudomonas* Sp, and also against Gram positive bacteria like *S. aureus* [39].

Antibacterial activity of *P.guajava* fruits extract was found to be less pronounced than previous extracts (inhibition zone 8-12 mm). Another evaluation of ethanolic extract found very close results, with inhibition zones ranging from 7 to 13 mm [40].

Antifungal activity

The screening of the antifungal activity of *P.guajava* extracts (Table 4) indicates that *P.guajava* fruits extract showed a moderate antifungal activity against all yeasts tested, with an inhibition diameter between 11 mm and 12 mm compared to the standard antifungal used Econazole 30 µg which showed an inhibition diameter between 20 mm and 22 mm. The results obtained for this extract are very similar to those found by *Malaviya* et al., for the alcoholic extract and the aqueous extract of *P. guajava* fruits extract against *Candida albicans* [47] or those obtained by *Panedy* et al., for the methanolic extract, ethanolic and ethyl acetate extract against *Microsporum canis*, *Tripchopythonrubrum*, *Aspergillus niger* and *Candida albicans* [48].

P.guajava essential oil is active against all tested fungi with inhibition diameter between 9 cm and 16 cm. The maximum activity was observed against *Candida albicans* (d=16 mm). Close to that of Econazole, it showed the strongest antifungal activity against *Candida tropicalis* (d=14 cm)known by strong resistance to antifungal drugs.In agreement with numerous studies, which confirmed the antifungal power of *P.guajava* essential oil against *Candida* strains and phytopathogenic fungi (*Curvularia lunata* and *Fusarium chlamydosporum*) [49]. This activity can come to the action of secondary metabolites such as phenolic compounds like ellagic [50]. This antifungal activity obtained in our study remains important given the pathogenicity of the tested yeasts, since they are responsible for several infections and diseases [51,52].

The leaf extract of *P.guajava* is active against *C. albicans* (d=12 mm) and *C. neoformans* (d=10 mm), but inactive against *C. tropicalis*, which is considered a resistant yeast.As shown by several previous researches, which confirmed the antifungal effect of this plant against *C. albicans* and also against *C. krusei*, *C. glabrata* and *M. canis* [53]. In this fact, *P. guajava* essential oil, the only strongly active extract against *Candida tropicalis*, which seems very interesting for the development of anti-*Candida tropicalis* bio-antifungals.

Table 4. Antifungal activity screening of *P.guajava* extracts

Extracts	Inhibition zone diameter (mm)		
	Candida albicans	Candida tropicalis	Cryptococcus neoformans
Psidium guajava (fruits)	12±1.00	11±2,.64	11±0.5
Psidium guajava (leaves)	12±1.5	-	10±1.16
*Psidium guajava (*EO*)*	16±1.73	14±1.04	9±0.76
Econazole 30 µg	20	21	22

On the other hand, the fact that Gram negative bacteria are more resistant than Gram positive bacteria is confirmed by previous results showing greater antibacterial activity of herbal extracts against Grampositive bacteria compared to Gram negative bacteria [41]. This observation can be explained by the difference in bacterial membrane structure between Gram positive bacteria and Gram negative bacteria, the efflux pump system of Gram-negative bacteria that can serve as a mediator for such a difference and also the periplasmatic space of Gram-negative bacteria that can contain enzymes capable of breaking down foreign molecules introduced into the bacterial cell from the outside [42]. This bacterial resistance is caused by the impermeability of the lipopolysaccharide membrane of the bacterium, in the presence of active compounds of *P.guajava*, especially tannins, which have the effect of limiting the multiplication of *S.aureus* by inhibiting the phosphorylation of bacteria to form its cell wall during bacterial multiplication [43]. Also, some bioactive compounds such as: saponins, flavonoids, tannins, alkaloids, phenols and phytosterols, effective against several strains of pathogenic bacteria, may have a protein degradation effect against bacterial proteins [44, 45, 46].

FTIR analysis of *P. guajava* essential oil

The infrared spectrum of *P. guajava* essential oil (Figure 4) shows the following bands: the very broad absorption band observed around 3422 cm^{-1} may be due to the presence of bonded O–H stretching of acids, with another very strong absorption band appearing in the region 1065 cm^{-1} due to C–O stretching vibration. The combination of these two bands indicates the presence of alcohols as linalool, cadinol, santalol, pogostol, muurolol, viridiflorol, spathulenol, cubebool, guaiol, nerolidol [38, 54, 55] and phenols such as durohydroquinone, chavibetol, thymol and 2,5-diethylphenol [55].

Two other bands found at 1454 cm^{-1} and 3076 cm^{-1} associated with C=C and =C-H of aromatic hydrocarbons such as calacorene, calamene, eugenol acetate, phenylethyl butyrate, o-cymene, benzyl benzoate and safrole [55, 56].

In the region between 1705 $cm^{-1}$and 1725 cm^{-1}, A medium intense absorption bandexisted at 1712 cm-1 associated with C=O ketones groupement, which significant the presence of ketones in the essential oil especially the tagetone [57]**.**

The very low absorption band appearing in the region 1634 cm^{-1} is due to C=C stretching vibration of the alkenes group, confirmed by another absorption band of =C-H groupement beyond 3000 cm^{-1}, shows the presence ofterpenes already confirmed in the essential oil of *Psidium guajava*, this includes thuyere, myrcene, limonene, ocimene, copaene, aryphyllene, humuene, amorphene, seychellene, viridiflorene, aromadendene, bisabolene, caryophyllene [38, 54, 55].

The relationship between the chemical composition and biological or chemical activities of *P. guajava* essential oil is confirmed by several researches. Indeed, antimicrobial activities of 1,8-cineole has been demonstrated against *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. faecalis* and *C. albicans* [59].On the other hand, the most abundant esters in the essential oils as bornyl acetate, geranyl acetate, *α*-terpenyl acetate, iso-bornylformateare responsible to the antibacterial effect [60, 61, 62, 63]. However, sabinyl acetate and vinyl crotonate showed a low to moderate antimicrobial activity [64, 65, 66]. Tagetone presented antifungal activities *in vitro* against *Candida lipolityca, Candida parapsilosis, Trichosporon asahii* and *Sphaceloma ampelinum* [67].

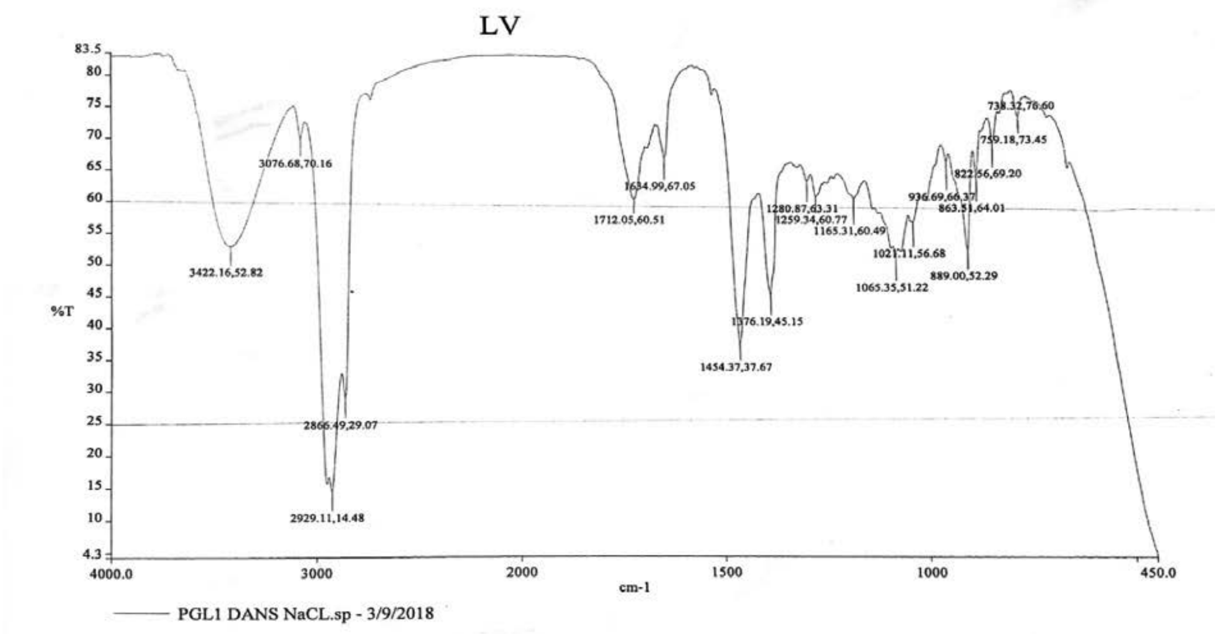

Figure 4. Infrared spectrum of *P.guajava* essential oil

Around the 3000 cm^{-1} region, there are two intense bands of 2929 cm^{-1} and 2866 cm-^{1}, which can be associated with the O=C-H aldehydes grouping, especially the nerol, citronellal, methanal phenylated, benzaldehyde and farnesol [38, 55, 56, 57] and also with the C-H alkanes, such as heptadecane, pristane, octadecane, phytane, phenosane and octacosan[58].

Three bands exist at 1021 cm^{-1}, 1259 cm-1 and 1280 cm^{-1}, possibly related to the C-O ether grouping, such as 1,8-cineol and the C-C esters grouping, including citronellyl acetate, bornyl acetate, geranyl butyrate, terphenyl acetate, dihydrocarveol acetate, isopulyl acetate, isobornylformate, sabinyl acetate and vinyl crotonate [54, 55]**.**

As regarding the terpenes in *.guajava* essential oil, previous studies have shown that β-Myrcene, β-Caryophyllene, α-Humulene, Germacrene, D-Limonene, β-ocimene and viridiflorene had a positive relationship with the antimicrobial activity

[68, 69, 70, 71]. The must alkanes that have shown excellent antimicrobial activity are heneicosane, tetracosane, heptadecane and eicosane [72, 73]. In addition, aldehydes such as cis-citral, farnesol and citronellal, found in essential oil are effective against several bacteria and fungi [63,74,75]. Moreover, others finding confirmed the high antimicrobial activity of aromatic hydrocarbon, especially Eugenol acetate, calamenene, phenylethyl butyrate, o-cymene and safrole showed higher antibacterial and antifungal activities [23, 63, 76, 77, 78, 79], these compounds may act alone or in combination with other compounds as *β*-caryophyllene, thioamide drugs, citral and carvacrol by a synergistic interactions [23, 63, 76, 77]. For alcohols and phenols, they reported the fungicidal and the bacterial effects of linalool, τ-cadinol, *cis*-α-santalol, pogostol muurolol, viridiflorol, spathulenol, trans-nerolidol, cubebol, terpineol against bacteria and fongi such as *E. faecalis, S. aureus*, *E. coli, E. faecalis, C.neoformans* and *candida sp* [80, 81, 82, 83]. Finally, durohydroquinone, chavibetol and thymol are the main phenols in *P. guajava* essential oil that have shown high antibacterial and antifungal properties [84, 85, 86].

CONCLUSIONS

The present study demonstrated that *Psidium guajava* is a valuable source of active compounds with antioxidant and antimicrobial activities. This finding suggests the new use of the fruits and the leaves extracts of this plant in the treatment of bacterial and fungal infections, as well as for the extraction of new antioxidants. Therefore, it is necessary to be carried out in another study to identify the active (s) compound(s) in *P.guajava* essential oil with respect to their mechanisms and synergistic actions.

Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM MOROCCAN FRESH FAVA BEANS PODS (*VICIA FABA L.*)

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ABSTRACT

Background. In Morocco, fava beans are widely used as a main meal or as an ingredient in various traditional recipes, in the form of fresh ripe seeds or dry seeds. In the past, the tender skin of bean pods was also used in certain specific dishes, thus diversifying the diet. However, the peels of the tender bean pods are currently less or not used and considered waste. In Moroccan, fava bean pods peels, traditionally used in food in the past, are today considered as waste. The valorization of fresh fava bean pods could revitalize the use of the specific dishes and diversify the diet. For this reason, the research aimed to assess the nutritional values and biological compounds of the whole fresh fava bean pods (*Vicia faba L.*).

Objective. Evaluate the content of nutrients, total phenolic, flavonoids and tannin contents and antioxidant activity in different extracts of the tender pods of the fava bean (*Vicia faba L.*).

Material and methods. The proximate composition and minerals were determined using AOAC methods. The total phenolic compounds by the *Folin-Ciocalteu* reagent, the total flavonoids were analyzed using aluminum chloride colorimetric method, the tannins by method of vanillin in an acidic medium and the antioxidant activity was evaluated by DPPH method.

Results. The results show that the fresh fava bean pods have a moisture content of 87.31 ± 0.25%, ash 4.67 ± 1.03, and protein 29.11 ± 3.20 g/100 g. The legume samples also contain potassium (1946.8±4.61), phosphorus (483.8 ± 3.14), and calcium (399.6 ±2.25) mg/100 g of dry matter representing at last 40-50% of the RDI. The content of the different extracts of (Vicia faba L) varied from 49.5 to 594.4 mg GAE/ g for the total phenols, from 0.7 mg to 3.4 mg QE/g for flavonoids, and from 4.9 mg to 73.91 mg TAE/g dry weight for tannins. The evaluation of the antioxidant activity in the various extracts revealed a better activity in the methanolic extract (IC50=491.2 µg/mL) compared to others extracts: the MeOH/water extract (IC50=606.61 µg/mL), DCM/ MeOH extract (IC50 = 642.67 µg/mL) and DCM extract below of 50%.

Conclusions. This study shows that fava bean pods, traditionally used in food, are rich in macro and micronutrients and bioactive substances, which demonstrates their potential contribution to human food and nutritional security.

Key words: *Vicia faba, chemical composition, antioxidant activity, total phenolic, flavonoids, mineral analysis*

INTRODUCTION

Legumes are an imperative food supply or bear an important function in traditional diets in many regions of the world [8]. They are served an excellent source of proteins especially lysine and threonine which have high levels, two essential amino acids that are deficient in cereal proteins [1, 31] and they are a good alternative to expensive protein from meat and fish. These nutritional properties make legumes a good supplement that improves the protein and nutritional quality of cereal-based products [10, 15], They are also a source of many nutrients, including starch, dietary fiber, essential fatty acids, vitamins, and trace elements. Moreover, legumes are also considered to be an important source of many secondary metabolites such as phenolic antioxidants [8, 16, 28].

Several studies have reported the content of Fava beans in anti-nutritional components that interfere with

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minerals absorption [10, 14, 32] and whose inhibitory effect is inactivated by cooking or autoclaving[22, 24].

Fava beans are reported with effects on health as they reduce blood sugar and cholesterol and prevent heart disease [21, 25, 33], renal, hepatic dysfunction and eye diseases [17, 19], cancers, and *Parkinson's* diseases [6, 13, 29]. In all these studies, the analyses focused on the fresh or dry seed of the fava bean.

The fava bean (*Vicia faba L.*) is one of the oldest plants in the world which belongs to the Leguminosae family [13, 30]. It is an important winter crop in Mediterranean regions (spring). It has been considered one of the most important plant foods for the people of the Nile [12, 26] and is popular food widely consumed in the Middle East, North Africa, and South America [24, 31]. Indeed, cooked beans are considered one of the most famous dishes over the years in Egypt [14, 18].

In Morocco, fava beans are widely used as a main meal or as an ingredient in various traditional recipes, in the form of fresh ripe seeds or dry seeds. In the past, the tender skin of bean pods was also used in certain specific dishes, thus diversifying the diet. However, the peels of the tender bean pods are currently less or not used and considered waste.

The objective of the present study, therefore, was to evaluate the content of nutrients and bioactive substances in the tender pods of the whole fava bean (*Vicia faba L.*) consumed in Morocco.

MATERIAL AND METHODS

Sampling

The immature fresh pods of fava bean (Vicia faba L) were collected from ten fields of the rural areas (elmechrek and krdid) of the province of Sidi Bennour (Morocco) in spring 2020. Each sample of 1 kg, taken at random from different points of each field. All samples are transported on the same day in airtight bags to the Biotechnology, Biochemistry and Nutrition laboratory of the Faculty of Sciences, at Chouaib Doukkali University in El Jadida city. They are mixed to have a homogeneous sample. A mass of this raw sample was used for moisture measurement and the remaining amount was carefully washed with tap water to remove attached dust particles, then with distilled water and deionized water. They were then sliced and dried in an oven (SM400, MEMMERT, Germany) at 45°C until constant weight. The pool is then ground into a fine powder and stored in airtight containers.

Preparation of crude extract

Maceration for 72 hours, of the powder of the sample, was carried out for the extraction of the phenolic compounds, by different solvents: (MeOH) methanol, (MeOH / H_2O) methanol / water (70% / 30%), (DCM / MeOH) dichloromethane / methanol

a)

b)

Figure 1. The tender pods (a) of (*Vicia faba L*) and in traditional recipes (b)

(50/50) and (DCM) dichloromethane. The four extracts were filtered and the solvent removed by rotary evaporation under pressure and at 45°C and then stored at -4°C until analysis.

Proximate analysis

The moisture and ash content were determined according to AOAC [2]. The total protein content was determined by the Kjeldahl method according to AOAC procedures [4] and calculated by multiplying the total nitrogen content N (%) by the coefficient 6.25.The crude fat content was determined using the Soxhlet extraction technique according to AOAC [5]. The total carbohydrates (including fibre) were calculated by difference [Total carbohydrates (g/100 g) = 100 - (g fat + g protein + g ash)] [5]. Total energy was calculated according to the following equations:

Total energy (kJ) = 17 x (crude protein (g) + total carbohydrate (g)) + 37 x (crude fat (g)).

The mineral content is determined according to AOAC 985.01 method [3], using inductively coupled plasma ICP-AES ULTIMA 2C HORIBA technique. One gram of the plant powder was weighted into a 30-mL glazed porcelain crucible placed into a cool muffle furnace and muffle at 500°C for 2 hours then cooled and added with 3.0 mL of $HN0_3$ (1+1). The sample is heated on a hot plate at 100-120°C until dry. The crucible is placed back into the muffle furnace and muffle at 500°C oven for 1 additional hour and afterward removed from the muffle furnace, cooled, and added with 10 mL HC1 (1+1), and then the sample is transferred to a 50 mL volumetric flask, and diluted to volume with deionized water and well mixed.

The operating parameters were as follows: Radiofrequency power: 1200 W; Detector type: High dynamic range detector (HDD); Nebulizer: Meinhard Type, Plasma gas flow: 14 l/min; Auxiliary gas flow: 3 l/min; sample time delay: 30 sec; Integration time: 5 sec).

Determination of total phenolic content:

The assessment of phenolic compounds is carried out by the spectrophotometric technique using the *Folin-Ciocalteu* reagent [20] (JENWAY 6300 spectrophotometer). The sample concentration in total phenolic compounds is deduced from a standard curve using known gallic acid concentration solutions. The results are expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Determination of total flavonoids content

The determination of the total flavonoids in the extracts was carried out using the spectrophotometer method described by *Dehpour* et al. [11], and the results are expressed in milligrams of quercetin equivalent per gram of dry weight (mg QE/g dw).

Determination of tannins content

The tannins are determined in the extracts by the spectrophotometer method of vanillin in an acidic medium [7]. The results were expressed as the equivalent of tannic acid in milligrams per gram of dry weight (mg TAE/g dw).

UV-Visible spectrophotometer analysis

The UV-visible spectra of the various extracts were obtained after extraction of the sample powders with MeOH / H_2O, MeOH, MeOH / DCM, and DCM (0.1 g/10 ml). The acquisition of spectral data on the UV-visible wavelength (200 - 800 nm) was carried out with solutions of the same dilution using a UV-Visible Shimadzu UV-2450 spectrometer (Kyoto, Japan).

DPPH free radical-scavenging activity

The free radical scavenging potentials of methanolic extracts were evaluated using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method described by *Sánchez-Moreno* et al. [27], using a UV spectrophotometer (Jenway 6300, USA).

The DPPH free radical scavenging activity (AA) in (%) was calculated using the following formula:

$$(AA)\ (\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100.$$

where:

A_{blank} - is the absorbance of the control reaction (containing all reagents except the test compound)

A_{sample} - is the absorbance of the test compound.

The concentration of extract ensuring 50% inhibition (IC50) was calculated from the graph plotted of the inhibition capacity (AA) in (%) as a function of the different concentrations of the extracts with as standards, ascorbic acid and butylated hydroxytoluene (BHT), using Graph Pad Prism 8.0.1 Software.

Statistical analysis was performed by analysis of variance (ANOVA) and differences between means were determined by *Tukey* test using SPSS software version 26. The significant difference threshold is set at $p<0.05$.

RESULTS

Proximate analysis

Table 1 shows the results of the proximate composition. The table shows that the fresh bean pod samples contain 87.31 ± 0.25% of moisture, 4.67 ± 1.03 g /100 g ash, 29.11 ± 3.20 g/100 g of protein, and 1.2 ± 0.05 g/100 g of lipids.

Table 1. Proximate composition of faba bean pods

Proximate	Content
Moisture %	87.31±0.25
Ash (g/100 g)	4.67±1.03
Proteins (g/100 g)	29.11±3.20
Fat (g/100 g)	1.2± 0.05
Total carbohydrate (g/100 g)	65.02±1.42
Energy (kJ)	1644.61

The analysis of the mineral composition (Table 2) shows that the fresh pods of (*Vicia faba L*) have a high content in particular of potassium (1946.8 ± 4.61 mg/100 g), phosphorus (483.8 ± 3.14 mg/100 g), sodium 430.9 ± 8.73, calcium (399.6 ± 2.25 mg/100 g), magnesium (301.0 ± 2.35 mg/100 g) and a considerable content of manganese, iron and copper. These content are considerable as they contribute to the recommended daily intake (RDI) representing 40% for Ca, 45.5% for protein, 48% for phosphorus, 51.2% for potassium, 70.6% for Cu, 71.7% for Mg, 82% for iron, 70% for sodium of RDI [35].

Table 2. Mineral composition of fresh faba bean pods

Element	Content (mg/100 g dry matter)
Ca	399.6±2.25
P	483.8±3.14
K	1946.8±4.61
Mg	301.0±2.35
Na	430.9±8.73
S	212,.±9.75
Fe	6.6±0.09
Mn	13.1±0.21
Cu	1.2±0.04

The results concerning the yield of the various extracts expressed as a percentage relative to 100 g of dry weight are presented in Table 3. The extract with methanol/ water revealed the highest yield (48.10%) followed by the methanol extract (19.55%) then methanol/dichloromethane 16.5% and finally that was obtained with dichloromethane (9.75%) extracts.

Total phenolic content

Table 3 shows the results concerning the total phenolic content in the fresh pods of (Vicia faba L.) extracts. The methanol/water extract reveals the highest levels of total phenolic compounds (594.4 mg GAE/g), followed by methanol extracts, that of methanol/ dichloromethane, and finally dichloromethane extracts (49.5 mg GAE / g).

Total flavonoids content

The total flavonoids contents in the fresh pods of (*Vicia faba L.*) ranged from 0.7 to 7.2 mg QE/g in the methanol/water extract and the methanol extract respectively. Also, the total flavonoids content in the methanol extract was significantly higher than the other extracts.

The tannins content

Table 3 also presents the tannin content in the various extracts of fresh bean pods (*Vicia faba L.*) analyzed. The results show that the tannins contents are ranging from 4.9 mg TAE/g to 73.91 mg TAE /g in the methanol/water and the methanol extracts, respectively.

The highest content in tannins (73.91 mg TAE/g) is found in the methanol extract followed by methanol/ Dichloromethane extract (46.7 mg TAE/g) with no significant difference between the other extracts.

The study carried out by *Baginsky* et al. [8] showed that condensed tannin content in immature seeds of fava bean ranged from 309 to 958 mg catechin equivalent per kilogram.

UV-Visible spectrophotometric analysis

Figure 2 shows the UV-visible spectra of the extracts obtained by MeOH/H_2O, MeOH, MeOH / DCM, and DCM with the characteristics presented in Table 3. The spectra of the four extracts are characterized by the high absorbance intensities in the region 260 - 280 nm, commonly attributed to phenolic and aromatic compounds rich in unsaturated groups of C = C, C = O, and N = N [36]. From this analysis, it appears that the contents of the total phenolic compounds in

Table 3. Content of total phenolic compounds and flavonoids and tannins in different extracts of immature Faba bean pods

Extract	Total phenolic compounds	Total flavonoids	Tannins	Extraction yield (%)
MeOH/water	594.4±18.3	0.7±0.1	4.9±0.2	48.10
MeOH	189.1±5.3[a]	7.2±1.2[a]	73.91±35.1[8]a	19.55
MeOH/DCM	81.4±2.7[a]	3.4±0.1[a]	46.7±0.[9]b	16.5
DCM	49.5±21.5[b]	0.9±0.1[b]	5.9±2.6[b]	9.75

Data presented as means ± standard deviation from the triplicate analysis.

[a] Significant when MeOH/water was compared to the other extracts at $p < 0.05$.

[b] Not Significant when MeOH/water was compared to the other extracts at $p < 0.05$.

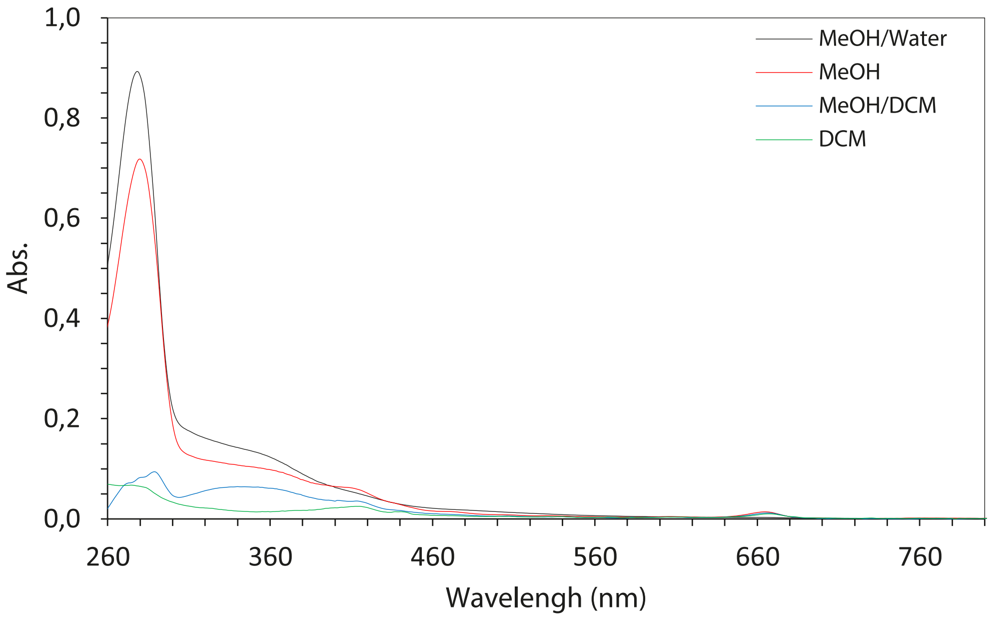

Figure 2. UV-visible spectra of the diluted extracts obtained by MeOH / H_2O, MeOH, MeOH/ DCM, and DCM

the extracts are in agreement with the absorbance intensities observed for all spectra at 285 nm.

DPPH free radical scavenging activity

The scavenging profiles of the extracts and standards ascorbic acid and BHT are presented in Figure 3. The figure shows that all extracts have free radical scavenging potential. The methanol extract was the most active while the dichloromethane extract was the least.

As shown in Table 4, for all the extracts, the IC50 values are significantly different from each other. Also, the IC50 value of the MeOH extract was significantly lower than all the standards. The best activity was recorded for the methanol extract with an IC50 value of 491.2 µg/mL followed by that of the MeOH / water extract and the DCM / MeOH extract with IC50 values of 606.61 µg/mL and 642.67 µg/mL respectively, while the inhibition percentage of the DCM extract of the 2 mg/mL concentration does not exceed 23.53%. Compared to two standards samples, ascorbic acid showed better activity (IC50 value=117.05 µg/mL followed by BHT (IC50 = 235.42 µg/mL).

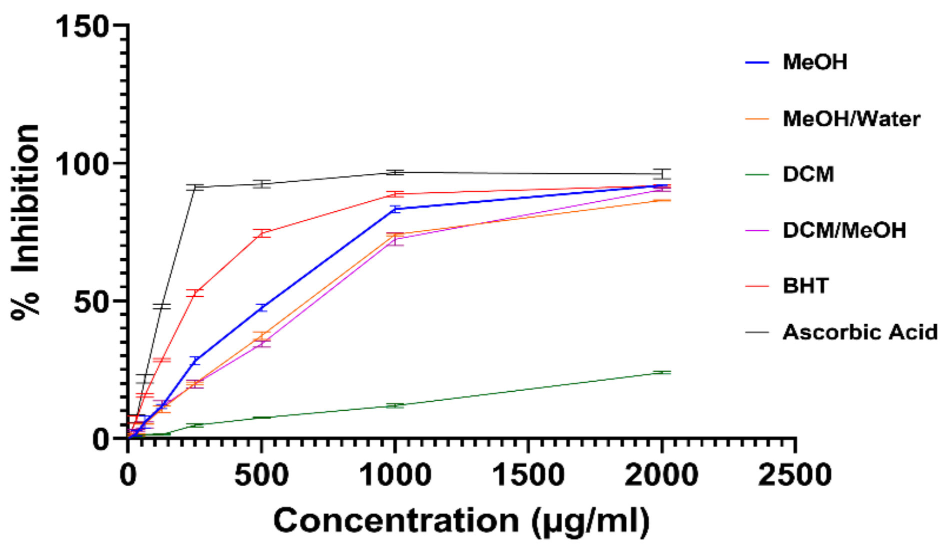

Figure 3. Antiradical activity of extracts of immature pods of (*Vicia faba L*) (each value represents the average of three determinations ± SD).

Table 4. IC50 values for the extracts as well as ascorbic acid and BHT

Extract / standard	IC 50 (µg/ml)
MeOH**	491.2 ± 10.81
MeOH/Water*	606.61 ± 2.36
DCM	ND
DCM/MeOH*	642.67± 26.57
BHT**	235.42± 1.79
Ascorbic acid**	117.05 ±1.51

*Significant level $p < 0.05$, **Significant level $p < 0.01$
ND - not determined

DISCUSSION

This work focused on the evaluation of the nutritional and biological value of fresh pods of (Vicia faba L), including their bark, as traditionally used in recipes consumed by the Moroccan population. The analyzes carried out in this study revealed considerable nutrient contents in a 100 g portion of this legume which can meet a proportion ranging from 40 to 100% of the recommended dietary intake. The study also showed that the nutrient contents found are higher or in line with the values reported for legumes in other studies.

Indeed, the ash, protein and lipid contents obtained in this study are similar to those reported by *Millar* et al. on shelled/split beans (3.40 ± 0.09 g/100 g; 28vg/100 g; 1.57 ± 0.11 g/100 g) dw respectively [23].

The fresh bean pods analyzed contain also a high content of energy (1644.61 kJ) and carbohydrate (65.02 g/100 g). The carbohydrate content found in the present study is similar to that reported by *Berrios* et al. [9] for lentils and chickpeas (62.49–65.7 g/100 g dw).

The minerals values found here were comparable to those previously published by *Millar* et al. [23] for the potassium, iron, calcium, and magnesium contents in the split fava beans. On the other hand, the contents of Ca, K, Mg, Na reported in the present study are higher than those found in the bean seed by *Hacıseferoğulları* et al. [17].

The total phenolic compounds content found in this study samples were higher than those reported previously by Millar *et al.* [23] and *Baginsky* et al. [8] in the split beans as well as in other species of the same family, like common beans (*Phaseolus vulgaris L.*) [34].

Several studies have analyzed flavonoids and reported different contents in legumes. Among these *El-Feky* et al. [13] studies have found 16 flavonoids in the ethyl acetate fraction of the bean peels and in the whole fraction a content of 12.30 mg/g dry weight. Other legumes like common beans (*Phaseolus vulgaris L)* were reported with a content of total flavonoids of 252 mg CE/100 g dw as shown by *Yang* et al. [34].

CONCLUSIONS

The results obtained show that fresh bean pods represent an important nutritional source of protein, energy and trace elements. These bean pods in addition to diversifying diets, their nutritional value testifies to their contribution to meet the RDI and at the same time to fight against nutrient deficiencies and associated malnutrition. They also contain bioactive compounds, in particular polyphenols which confer considerable natural antioxidant power to these legumes.

The nutrients and biological values found in this study are determined in the two parts of the studied legume, namely, the bean kernel and the peels. These parts are used separately or together in traditional Moroccan recipes. Indeed, the peels are used as the main vegetable of traditional recipes which could diversify the diet of the local populations because they are added with other ingredients according to the recipes. By throwing away this part of legumes as is the case today and viewing them as waste, the nutritional and functional values found in this study are also diminished or lost. It is recommended that all these qualities be considered and valued by the revitalization of traditional recipes using them to fight against their abandonment and waste.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

TRENDS IN PREMATURE CEREBROVASCULAR DISEASE MORTALITY IN THE POLISH POPULATION AGED 25-64 YEARS, 2000-2016*

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ABSTRACT

Background. Many scientific reports have shown a decrease in total cerebrovascular disease (CeVD) mortality over the past few decades, but too little attention has been paid to premature mortality. CeVD accounted for 22.5% and 17.8% of premature cardiovascular disease deaths in Poland, in 2000 and 2016, respectively.

Objective. The aim of the study was to analyse premature CeVD mortality in the Polish population in the recent years, the dynamics of its changes and the potential factors that may have contributed to the decline in mortality. The main goal of the study was to overview the levels and trends in premature CeVD mortality with an emphasis on haemorrhagic, ischaemic and unspecified (not specified as haemorrhagic or ischaemic) stroke.

Material and methods. The analysis was based on a database of the Central Statistical Office of Poland and included data from 2000-2016 on premature cerebrovascular deaths occurring between 25 and 64 years of age (N=104,786). CeVD and haemorrhagic, ischaemic or unspecified stroke were coded with ICD-10 codes I60-I69, I61-I62, I63 and I64, respectively. The analysis included assessment of CeVD deaths distribution and evaluation of age-specific mortality rates in 10-year age groups and age-standardised mortality rates (SMR) in the age group 25-64 years, separately for men and women. Trends in SMRs have been studied in the period 2000-2016.

Results. The number of CeVD deaths decreased by 32.8% in men and 48.8% in women. There was a two-fold decline in CeVD mortality: from 59 to 29 male and from 30 to 12 female per 100,000. In addition, a 2-year increase in the median age of CeVD death was observed (Men: 56.4 to 58.4 years, Women: 56.4 to 58.7 years, $p<0.001$). A statistically significant decline in mortality (per 100,000) was also noticed for haemorrhagic stroke (Men: 18.7 to 10.4; Women: 9.6 to 3.8), ischaemic stroke (Men: 11.8 to 8.4; Women: 4.7 to 3.0) and unspecified stroke (Men: 19.7 to 3.5; Women: 9.1 to 1.3).

Conclusions. A substantial decline in premature CeVD mortality was observed in the period 2000-2016. Additionally, the number of deaths that could not be classified as haemorrhagic or ischaemic stroke death decreased significantly. The increasingly widespread use of new post-stroke therapies and their availability make it possible to expect a further decrease in CeVD mortality. However, the necessary actions should be taken to compensate for the disparities in CeVD mortality between men and women.

Key words: *cerebrovascular disease, stroke, premature mortality, Poland, mortality trends*

STRESZCZENIE

Wprowadzenie. W wielu doniesieniach naukowych pokazano spadek umieralności ogólnej z powodu chorób naczyń mózgowych (ChNM) w ciągu ostatnich kilku dekad, ale zbyt mało uwagi poświęcono przedwczesnej umieralności. ChNM odpowiadały za 22.5% i 17.8% przedwczesnych zgonów z powodu chorób układu krążenia w Polsce, odpowiednio w 2000 i 2016 roku.

Cel pracy. Niniejsza praca miała na celu analizę przedwczesnej umieralności z powodu ChNM w populacji polskiej w ostatnich latach, dynamikę jej zmian oraz potencjalne czynniki, które mogły przyczynić się do spadku umieralności. Głównym celem pracy był przegląd poziomów i trendów zmian przedwczesnej umieralności z powodu ChNM

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ze szczególnym uwzględnieniem udarów krwotocznego, niedokrwiennego i nieokreślonego jako krwotoczny lub niedokrwienny.
Materiał i metody. Analiza obejmowała dane oficjalnej statystyki państwowej z lat 2000-2016 dotyczące przedwczesnych zgonów z powodu ChNM pojawiających się pomiędzy 25 a 64 r.ż. (N=104,786). Choroby naczyń mózgowych oraz udary krwotoczny, niedokrwienny i nieokreślony jako krwotoczny lub niedokrwienny były kodowane zgodnie z ICD-10 odpowiednio jako I60-I69, I61-I62, I63 i I64. Analiza obejmowała ocenę rozkładu liczby zgonów spowodowanych ChNM, obliczenia współczynników umieralności w 10-letnich grupach wieku i standaryzowanych współczynników umieralności (SMR) w grupie wieku 25-64 lata, osobno dla mężczyzn i kobiet. Badano też trendy zmian standaryzowanych współczynników umieralności w okresie 2000-2016.
Wyniki. Liczba zgonów z powodu ChNM obniżyła się o 32.8% u mężczyzn i o 48.8% u kobiet. Zaobserwowano 2-krotne zmniejszenie się umieralności z powodu ChNM: z 59 do 29 mężczyzn i z 30 do 12 kobiet na 100,000. Dodatkowo zaobserwowano wzrost mediany wieku zgonu z powodu ChNM o 2 lata (mężczyźni: z 56.4 do 58.4 lat, kobiety: z 56.4 do 58.7 lat, p<0.001). Statystycznie istotny spadek umieralności (na 100,000) został również odnotowany dla udaru krwotocznego (mężczyźni: 18.7 do 10.4; kobiety: 9.6 do 3.8), udaru niedokrwiennego (mężczyźni: 11.8 do 8.4; kobiety: 4.7 do 3.0) oraz udaru o nieokreślonej jako krwotoczna lub niedokrwienna etiologii (mężczyźni: 19.7 do 3.5; kobiety: 9.1 do 1.3).
Wnioski. Znaczący spadek przedwczesnej umieralności z powodu ChNM został zaobserwowany w okresie 2000-2016. Ponadto istotnie zmniejszyła się liczba zgonów, których nie można sklasyfikować jako spowodowane udarem krwotocznym lub niedokrwiennym. Coraz bardziej rozpowszechnione użycie nowych poudarowych terapii i ich dostępność pozwalają się spodziewać dalszego spadku umieralności z powodu ChNM. Jakkolwiek niezbędne działania powinny zostać podjęte w celu wyrównania różnic pomiędzy przedwczesną umieralnością mężczyzn w stosunku do kobiet.

Słowa kluczowe: *choroby naczyń mózgowych, udar, przedwczesna umieralność, Polska, trendy umieralności*

INTRODUCTION

Cardiovascular disease (CVD) has been the main cause of death in the Polish population for many years, and although CVD mortality has been declining since the 1990s [15, 38], it still accounts for more than 40% of total deaths annually [2, 37]. A particularly disturbing phenomenon in Poland is premature CVD mortality. According to our calculations, CVD accounted for 31.0% and 25.5% of total deaths in people aged 25-64 years, in 2000 and 2016, respectively. Globally, ischaemic heart disease (IHD) and cerebrovascular disease (CeVD) were the two leading causes of CVD death and years of life lost (YLLs) in 2016 [8]. We calculated that IHD accounted for 30.2% and CeVD for 17.8% of CVD premature deaths in 2016 in Poland.

Many scientific reports have shown a decrease in total cerebrovascular disease mortality over the past few decades [8, 9], but too little attention has been paid to premature mortality. However, in Poland, premature mortality (e.g. from CVD, malignant neoplasm, disease of the respiratory system) is reported annually by the National Institute of Public Health NIH - National Research Institute (formerly Institute of Public Health - National Institute of Hygiene) [37].

The aim of the study was to analyse premature CeVD mortality in the Polish population in the recent years, the dynamics of its changes and the potential factors that may have contributed to the decline in mortality. The main goal of the study was to overview the levels and trends in premature CeVD mortality with an emphasis on haemorrhagic, ischaemic and unspecified (not specified as haemorrhagic or ischaemic) stroke.

MATERIAL AND METHODS

The analysis covered the official state statistics on cerebrovascular deaths in Poland from 2000 to 2016 obtained from Statistics Poland (formerly Central Statistical Office). In this report, to cover the majority of the professionally active population, premature mortality occurring between 25 and 64 years of age was considered (N=104,786).

For the purpose of the study cerebrovascular disease was coded in accordance with the International Statistical Classification of Diseases and Related Health Problems, 10th Revision [ICD-10] by I60-I69 codes. Cerebral haemorrhage (haemorrhagic stroke) included intracerebral haemorrhage (ICD-10 code I61) and other nontraumatic intracranial haemorrhage (ICD-10 code I62), while cerebral infarction (ischaemic stroke) and unspecified stroke (not specified as haemorrhage or infarction) were identified by ICD-10 codes I63 and I64, respectively.

The analysis included assessment of CeVD deaths distribution and evaluation of age-specific mortality rates (MR) and age-standardised mortality rates (SMR). SMRs were calculated using the direct method according to the European Standard Population ESP2013 [7, 23]. Mortality rates were calculated in 10-year age groups (25-34, 35-44, 45-54 and 55-64 years), while SMRs were calculated in the age group 25-64 years, separately for men and women.

Trends were determined by fitting annual SMRs data in the unobserved components model (UCM) and the piecewise polynomial spline model (PPSM). An UCM is widely used in econometrics to analyse time series as it allows for decomposition the time series into components such as trend, seasonal, cycle, and random, as well as for improving forecast accuracy, especially when there are outliers in the data. Unlike UCM, the spline model can provide an equally good fit to the data, but if there is seasonality and cyclicality, the forecast results are worse. The *Cochran-Armitage* trend test and the *Jonckheere-Terpstra* test were used to determine a statistically significant trend between the number of deaths in subsequent years, while fit statistics based on residuals were applied to test trend significance for SMRs and median age at death.

Additionally, the male to female ratio (MFR) and percentage change (PC) in all mortality indices (number of deaths, MR, SMR) were calculated. The significance level of 0.05 was assumed to verify the statistical hypotheses. The statistical analysis was performed in SAS9.4 (SAS Institute, Cary, North Carolina, USA). The study was in accordance with the ethical standards of the Bioethics Committee at the Institute of Cardiology (approval no.1537/2016).

RESULTS

A substantial decline in premature CeVD mortality has been noticed during the years observed. The number of CeVD deaths decreased by 32.8% in men and 48.8% in women between 2000 and 2016 (Table 1).

The share of CeVD deaths in CVD deaths changed from 19.4% to 16.2% in males and from 31.3% to 22.8% in females in the same period, and although this trend seems to be optimistic, the increase in male towards female deaths is worrying (Men:63.3% MFR=1.7 in 2000, Men:69.4% MFR=2.3 in 2016, $p<0.001$ for trend). An increasing percentages of male deaths or MFR were also observed for cerebral haemorrhage (Men:63.8% MFR=1.8 in 2000, Men:71.5% MFR=2.5 in 2016, $p<0.001$ for trend) and for unspecified stroke (Men:65.8% MFR=1.9 in 2000, Men:71.1% MFR=2.5 in 2016, $p<0.001$ for trend). The same tendency was noticed for ischaemic stroke, but it was not statistically significant (Men:68.5% MFR=2.2 in 2000, Men:72.1% MFR=2.6 in 2016, $p=0.09$ for trend).

The highest decline in the number of stroke deaths was observed for an unspecified stroke (PC=74.8% for men, PC=80.3% for women) that was associated with a more precise diagnosis of the cause of death and resulted in changes of CeVD deaths distribution.

Thus, the greatest changes in the distribution of CeVD deaths took place within unspecified and ischaemic stroke deaths, while the share of haemorrhagic stroke death in CeVD death was almost constant in 2000-2016 (Table 1). Generally, stroke (combined haemorrhagic, ischaemic and unspecified) contributed to 84.5% and 76.4% of male CeVD deaths, as well as 76.5% and 68.3% of female CeVD deaths, in 2000 and 2016.

Trends in CeVD deaths and in haemorrhagic, ischaemic or unspecified stroke deaths in 10-year age groups are shown in Figure 1. The lowest number of deaths was recorded in the age group 25-34 years. The average number of CeVD deaths was less than 90 and 50 per annum and the average number of haemorrhagic stroke deaths was less than 40 and 20 per annum, respectively for men and women. For ischaemic and unspecified stroke the average number of deaths was less than 10 for both genders. A small number of ischaemic and unspecified stroke deaths was also reported in the age group 35-44 years. For all cerebrovascular diseases the most numerous were deaths in the age group 55-64 years.

These findings were confirmed by observation of MR levels – the lowest mortality rates were in the age group 25-34 years, while the highest in the age group 55-64 years (Table 1). For all 10-year age groups CeVD mortality rates decreased in the period 2000-2016. The detailed information on all MR levels is presented in Table 1, while trends in age-specific mortality rates are shown in Figure 2.

The results of our study revealed a two-fold decrease in age-standardised mortality rate of CeVD in the Polish population aged 25-64 years in the period 2000-2016. SMR of CeVD decreased from 59 to 29 and from 30 to 12 per 100,000 in men and women, respectively. A considerable decrease in mortality was also obtained for cerebral haemorrhage (men: 1.8 times decrease, women: 2.5 times decrease), cerebral infarction (men: 1.4 times decrease, women: 1.6 times decrease) and unspecified stroke (men: 5.7 times decrease, women: 7.0 times decrease).

Trends in CeVD mortality in the years 2000-2016 are shown graphically in Figure 3. There were fitted in two models: unobserved components model and piecewise polynomial spline model, which was defined as a combination of two first degree polynomials (linear polynomials) with joinpoint detected by the model in 2008 (Figure 4). The observed decline in mortality was statistically significant ($p<0.001$). A better fit to the SMR data was found in the piecewise polynomial spline model (higher values of R^2 for PPSM than for UCM indicate a better fit of the model to the sample). However, the use of both models shows that the statistical methods used in econometrics can be applied in the analysis of medical data.

The expected SMR of CeVD in 2020, estimated in the unobserved components model, was 21.0 (95% CI: 16.2-25.8) per 100,000 in men and 7.8 (95% CI: 6.2-9.4) per 100,000 in women, assuming the same dynamics of change.

For indicative purposes, the percentage change of SMRs in two time periods, 2000-2008 and 2008-2016,

Table 1. Cerebrovascular disease (CeVD) mortality in Polish population aged 25-64 years by sex, 2000-2016

Year	Men			Women		
	2000	2016	PC	2000	2016	PC
Deaths						
CeVD	4582	3077	-32.8	2652	1359	-48.8
haemorrhagic stroke	1530	1083	-29.2	867	431	-50.3
ischaemic stroke	864	896	3.7	397	347	-12.6
unspecified stroke	1474	371	-74.8	765	151	-80.3
Share of CeVD [%]						
haemorrhagic stroke	33.4	35.2	1.8	32.7	31.7	-1.0
ischaemic stroke	18.9	29.1	10.2	15.0	25.5	10.5
unspecified stroke	32.2	12.1	-20.1	28.8	11.1	-17.7
other	15.5	23.6	8.1	23.5	31.7	8.2
Mortality Rate [1/100,000]						
CeVD						
25-34 yrs	3.85	2.32	-39.7	2.18	1.35	-38.1
35-44 yrs	15.79	8.03	-49.1	9.67	2.99	-69.1
45-54 yrs	54.77	29.10	-46.9	29.93	12.13	-59.5
55-64 yrs	167.93	79.36	-52.7	81.91	32.52	-60.3
haemorrhagic stroke						
25-34 yrs	1.85	1.05	- *	1.03	0.67	- *
35-44 yrs	7.98	4.13	-48.2	3.59	1.20	-66.6
45-54 yrs	21.38	12.94	-39.5	11.56	4.17	-63.9
55-64 yrs	44.49	23.76	-46.6	22.85	9.54	-58.2
ischaemic stroke						
25-34 yrs	0.15	0.29	- *	0.08	0.07	- *
35-44 yrs	1.40	0.97	- *	0.91	0.24	- *
45-54 yrs	8.20	6.24	-23.9	3.41	2.65	-22.3
55-64 yrs	39.41	27.11	-31.2	15.16	9.47	-37.5
unspecified stroke						
25-34 yrs	0.54	0.16	- *	0.12	0.00	- *
35-44 yrs	2.66	0.40	- *	1.13	0.24	- *
45-54 yrs	16.10	2.46	-84.7	6.71	1.01	-84.9
55-64 yrs	62.48	11.29	-81.9	30.04	4.13	-86.3
Age-standardised Mortality Rate [1/100,000]						
CeVD	58.98	29.04	-50.8	30.05	11.89	-60.4
haemorrhagic stroke	18.72	10.37	-44.6	9.59	3.80	-60.4
ischaemic stroke	11.77	8.35	-29.1	4.66	2.98	-36.1
unspecified stroke	19.74	3.45	-82.5	9.06	1.29	-85.8
Subperiod I (2000-2008)	2000	2008	PC	2000	2008	PC
CeVD	58.98	47.59	-19.3	30.05	20.42	-32.0
haemorrhagic stroke	18.72	16.78	-10.4	9.59	6.87	-28.4
ischaemic stroke	11.77	11.89	1.0	4.66	4.29	-7.9
unspecified stroke	19.74	10.11	-48.8	9.06	3.65	-59.7
Subperiod II (2008-2016)	2008	2016	PC	2008	2016	PC
CeVD	47.59	29.04	-39.0	20.42	11.89	-41.8
haemorrhagic stroke	16.78	10.37	-38.2	6.87	3.80	-44.7
ischaemic stroke	11.89	8.35	-29.8	4.29	2.98	-30.5
unspecified stroke	10.11	3.45	-65.9	3.65	1.29	-64.7

PC – percentage change; for deaths, mortality rates and age-standardised mortality rates percentage change means relative percentage change

* – not reported due to possible errors resulting from too low mortality rates level and too low dynamics of their changes

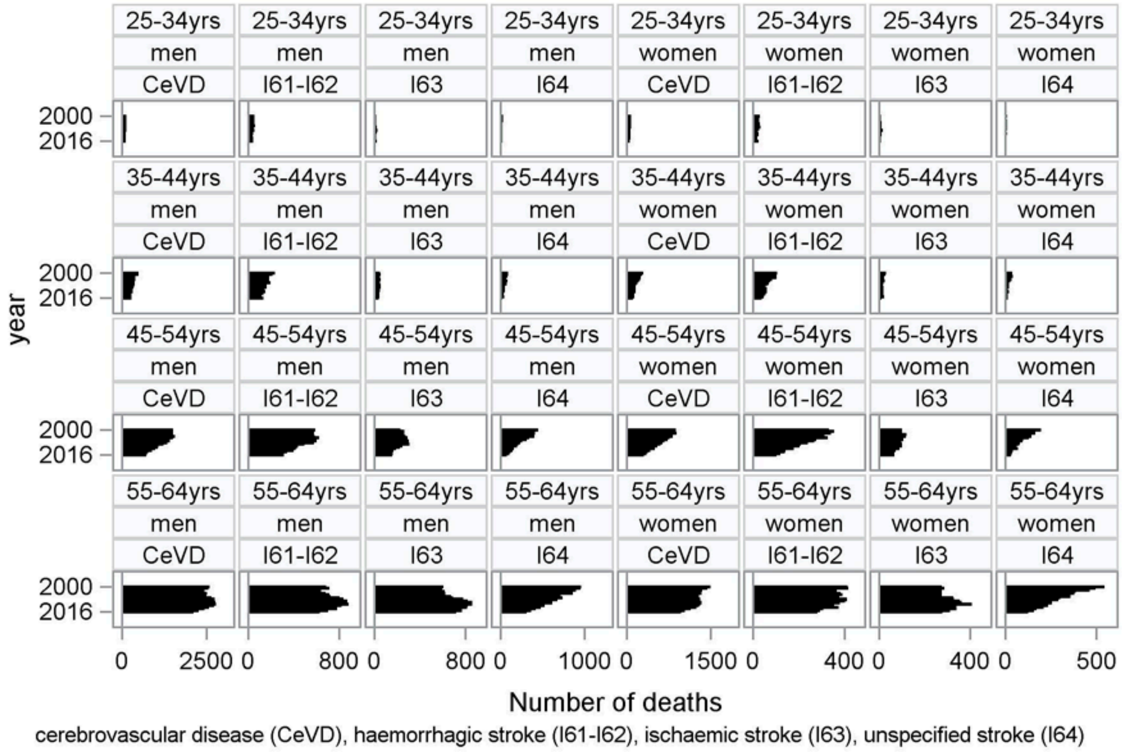

Figure 1. Number of cerebrovascular disease (CeVD), cerebral haemorrhage (I61-I62), cerebral infarction (I63) and unspecified stroke (I64) deaths in men and women aged 25-64 years by 10-year age groups, 2000-2016

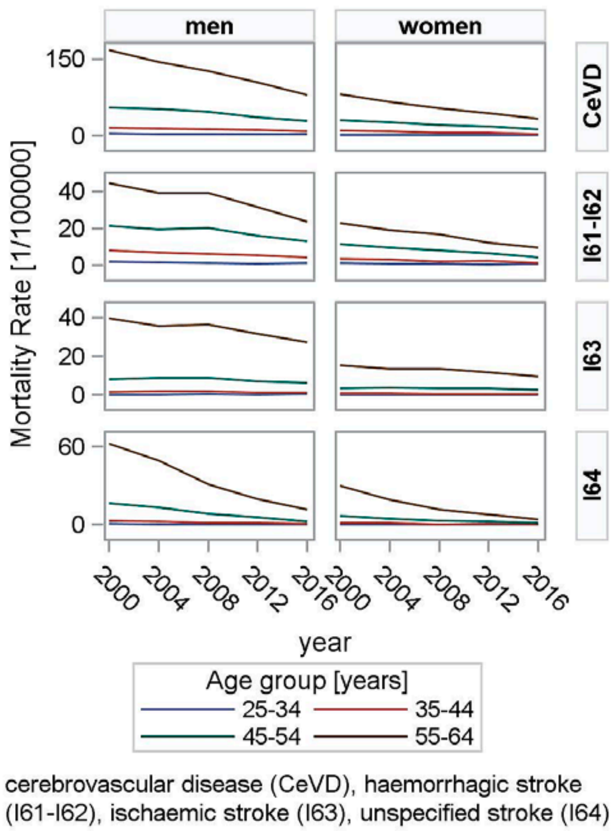

Figure 2. Age-specific mortality rates of cerebrovascular disease (CeVD), cerebral haemorrhage (I61-I62), cerebral infarction (I63) and unspecified stroke (I64) in men and women aged 25-64 years, 2000-2016

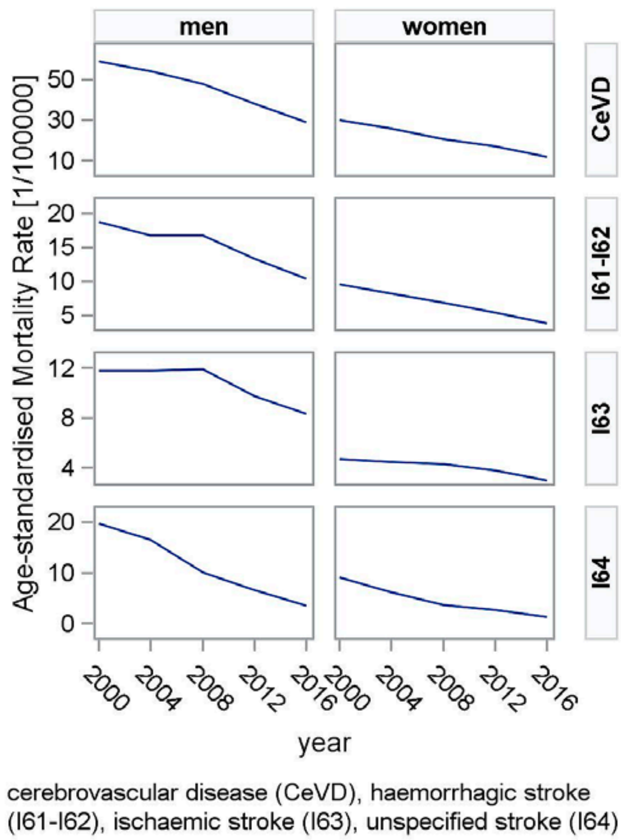

Figure 3. Age-standardised mortality rates of cerebrovascular disease (CeVD), cerebral haemorrhage (I61-I62), cerebral infarction (I63) and unspecified stroke (I64) in men and women aged 25-64 years, 2000-2016

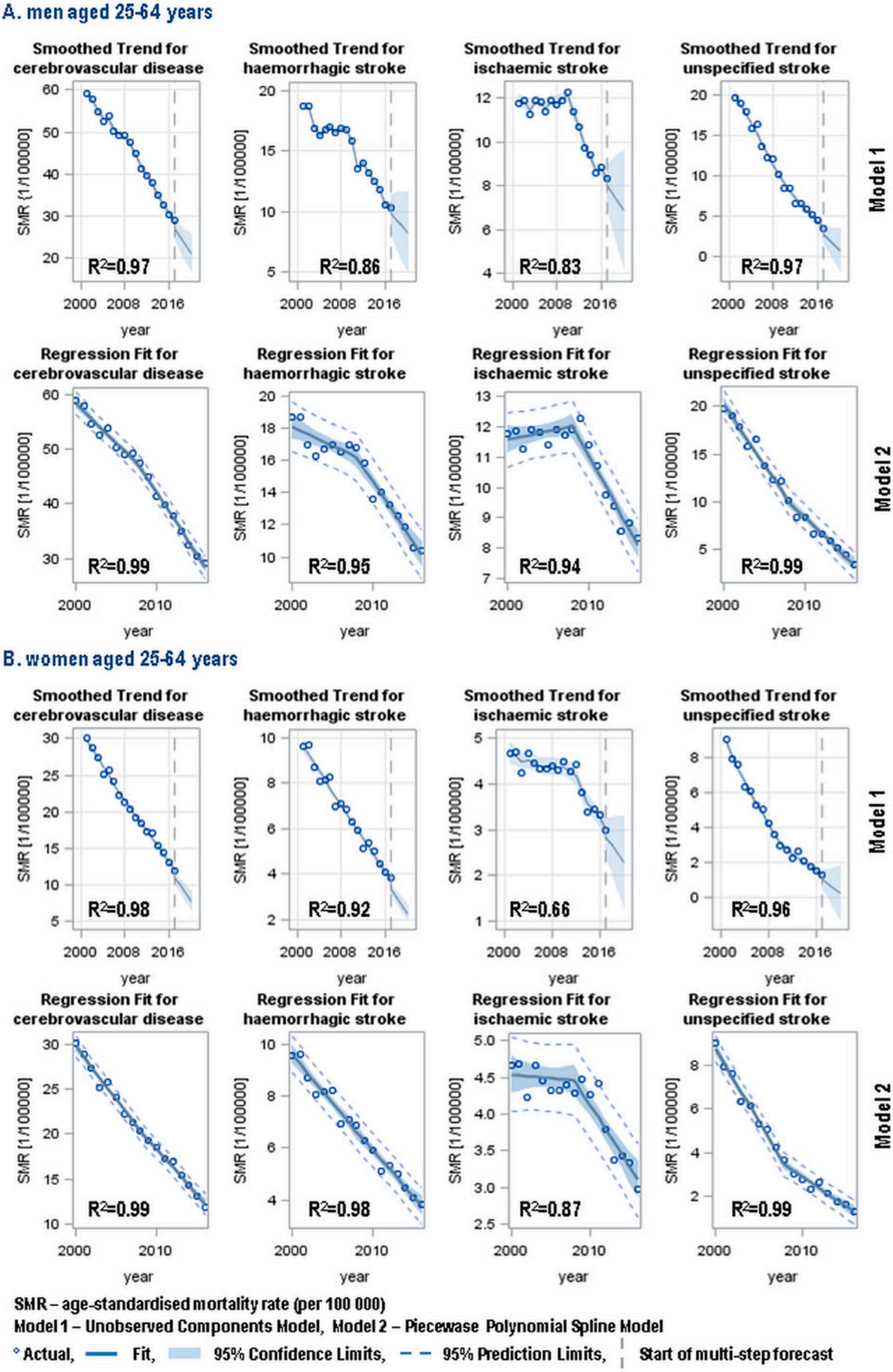

Figure 4. Trends of age-standardised mortality rates of cerebrovascular disease (CeVD), haemorrhagic stroke (I61-I62), ischaemic stroke (I63) and unspecified stroke (I64) in men and women aged 25-64 years, 2000-2016

was also estimated. In general, the lower decrease in CeVD mortality was observed in the period 2000-2008 (PC_{Men}=19.3%, PC_{Women}=32.0%), while the higher in 2008-2016 (PC_{Men}=39.0%, PC_{Women}=41.8%). The detailed information referred to all types of stroke is summarized in Table 1.

An additional finding of our study was a clear shift toward an older median age at CeVD death (Men: 56.4 to 58.4 years, $p<0.001$; Women: 56.4 to 58.7 years, $p<0.001$). The same tendency was observed for cerebral haemorrhage (Men: 53.6 to 56.6 years, $p=0.007$; Women: 54.4 to 57.8 years, $p<0.001$), ischaemic stroke (Men: 59.5 to 60.2 years, $p=0.013$; Women: 58.9 to 60.4 years, $p=0.001$) and unspecified stroke (Men: 58.2 to 60.2 years, $p=0.001$; Women: 59.8 to 60.1 years, $p=0.046$).

A 2-year increase in the median age of any CeVD death was considered clinically relevant. This result was achieved for haemorrhagic stroke and total CeVD.

DISCUSSION

The overall trend of decreasing the number of premature CeVD deaths was in accordance with the trend of premature CVD deaths decline, although according to our calculations the reduction in premature CVD deaths was smaller (men: 19.7%, women: 29.7%) in 2000-2016.

Declining premature CeVD mortality trend is consistent with the general trend in decreasing CeVD mortality in the total Polish population by 21% in the period 1990-2015 [22], despite the high number of total CeVD deaths (above 29 thousands in 2016). These results are also in line with our earlier reports [5, 13].

As mentioned previously, the decline in the number of premature CeVD deaths between 2000 and 2016 was over 32% in men and over 48% in women. A similar decrease was observed in the United Kingdom (31.3%) and Switzerland (34.3%) in men and in Bulgaria (43.8%) and Portugal (54.1%) in women (Figure 5) [6, 12]. In turn, the highest percentage change in CeVD deaths was observed in Estonia (men: -72.4%, women: -82.0%), the lowest for Lithuania in men (-3.8%) and for France in women (-19.9%), while for Slovakia, after an initial period of declines until 2006, an increase in cerebrovascular deaths was revealed (men: 25.1%, women 31.8%). Among the countries featured in the paper, Switzerland had the lowest percentage of premature CeVD deaths in all premature deaths and they remained almost unchanged in the observed period (men: 2.4% to 2.1%, women: 2.9% to 2.5%). In turn, the percentage of premature CeVD deaths in all premature deaths in Poland changed from 5.9% to 4.3% and from 8.1% to 4.6%, for men and women, respectively. The highest reported shares of CeVD deaths were in Bulgaria and Romania in 2000, as well as in 2016.

Noteworthy is a better diagnosis of stroke type as a cause of CeVD death, in the observed 17 years unspecified stroke deaths reduced from about 30% to slightly above 10%. Developing earlier and more precise CeVD diagnostics also resulted in better choice and early application of treatment, that could reduce mortality.

The last two decades brought a new approach to stroke treatment options. In 1997 in Poland the network of stroke specialised units started to be built, and in 2000 there were 23 such units [3]. Furthermore, the principle that "time is brain" was adopted. The most effective method of ischaemic stroke acute phase treatment is a thrombolytic intravenous therapy with recombinant tissue plasminogen activator (rtPA), which was started in Poland in 2003, initially in selected centres within the framework of the National Programme of Prevention and Treatment of Stroke (Polish abbreviation POLKARD), and from 2009 in all stroke treatment units. There were 686 (1.8%) and 7568 (15.7%) hospitalizations with thrombolytic treatment, in 2009 and 2016, respectively, of which 228 (2.8%) and 1538 (17.9%) in the age group 18-60 years [19]. Currently about 20% of patients with ischaemic stroke are treated with rtPA [10, 14].

In addition, a substantial decrease in ischaemic stroke mortality after 2008 could be a consequence of better medical management following the introduction of new recommendations of the European Stroke Organization for treating ischaemic stroke and transient ischaemic attack in 2008 [32].

Based on current knowledge driven by a series of randomised controlled trials, an opinion exists that mechanical thrombectomy (MT) is the most effective method of treatment in selected patients with acute ischaemic stroke [4, 31, 35]. However, it seems not plausible that MT influenced the results of our analysis significantly because this new invasive procedure was not reimbursed by public resources and the number of MT procedures performed up to 2016 in Poland was very minimal, 585 in the period 2012-2016 [21, 25, 36].

There are still disparities in CeVD mortality trends between men and women. These results are consistent with previous studies and surveys [16, 26, 30]. A higher CeVD mortality rate for men than for women is observed in many countries, although not all differences are as large as in Poland [1, 11, 17, 33, 39].

The average life expectancy in Poland in 2016 was estimated to be 45.0 and 52.5 years, respectively, in 30 year old men and women [27]. In this context, it seems important to lead a healthy life without the burden of a past stroke. The previous studies in the Polish population revealed that CeVD was one of the leading single disease entities resulting in life lost years, for

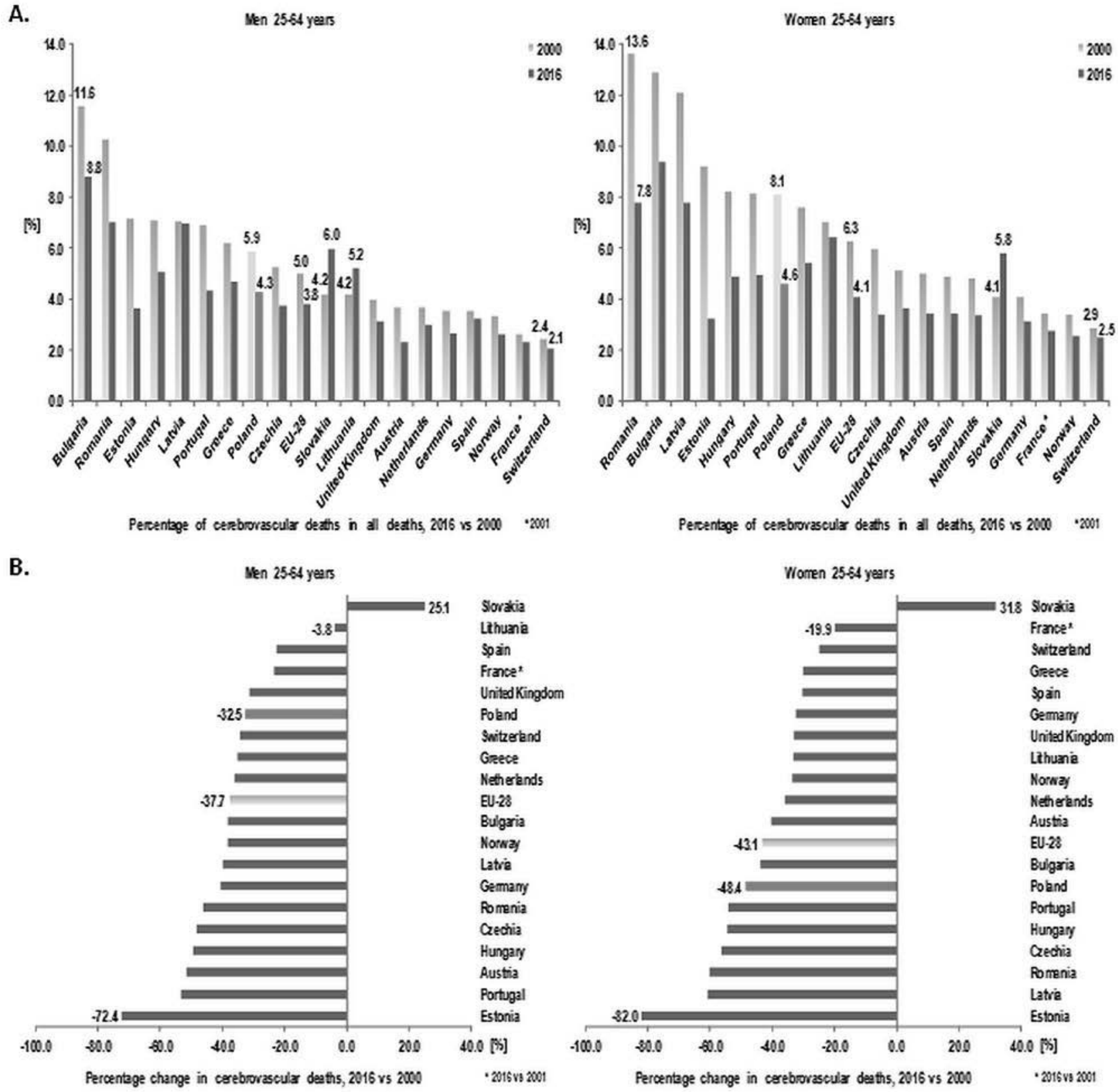

Figure 5. Comparison of cerebrovascular deaths in men and women aged 25-64 years in selected European countries, 2000 versus 2016. (Source: own calculation on Eurostat data)

instance in 2011, CeVD accounted for 4.6 YLLs per 1000 men (fourth position, after IHD, lung cancer and suicide) and 3.8 YLLs per 1000 women (first position, before IHD and heart failure) [18].

Our finding related to the significant shift towards older median ages of CeVD deaths is consistent with the decreasing trend for life years lost due to CeVD for both genders in 2000-2014 [24].

A survey conducted by the World Stroke Organization revealed that the burden of stroke should be reduced simultaneously in several domains: stroke diagnostics, stroke management, stroke care services, stroke rehabilitation, and stroke prevention [28]. The latter one gives the widest scope for further action.

In conclusion, although our study showed a progressive reduction in premature CeVD mortality in both sexes, every effort should be made to increase awareness of stroke prevention, especially among men, because in 2017 stroke was still the second cause of mortality and the fifth cause of disability in the European Union countries, and in Poland mortality and disability due to stroke were definitely higher than the corresponding average indices for the European Union [29]. In fact, primary prevention of stroke ought to be related to modifiable lifestyle risk factors and aimed at tobacco smoking cessation, healthy nutrition, increase in physical activity, avoiding obesity, and chronic stress.

Of course, population-wide detection and effective treatment of hypertension is of particular importance for reducing CeVD morbidity and mortality [20].

In conclusion, a stroke prevention framework (e.g. awareness-raising programs) should be continued, developed and improved as soon as possible because despite the general declining trend in stroke mortality, the mean global lifetime risk of stroke increased from 22.8% in 1990 to 24.9% in 2016 due to accumulation of risk factors and the aging of the population, and further growth is expected in the coming years [34].

CONCLUSIONS

The results of our study revealed an expected decrease in premature CeVD mortality in the period 2000-2016. They have also shown an acceleration in the decline in premature ischaemic stroke mortality after 2008 possibly related to the introduction of thrombolytic intravenous therapy as an effective and widely available therapy in all stroke treatment units all over the country. A further decrease in CeVD mortality is expected after the implementation of mechanical thrombectomy as a standard procedure in specialised stroke units. Additionally, this report indicated that the number of cerebrovascular deaths that could not be classified as haemorrhagic or ischaemic stroke deaths decreased significantly.

Although a substantial decline in premature CeVD mortality is a positive phenomenon, the necessary actions should be taken to compensate for the disparities in CeVD mortality between men and women.

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Conflicts of interest

None.

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ORIGINAL ARTICLE

ANALYSIS OF DIABETIC PATIENTS HOSPITALIZATIONS IN POLAND BY GENDER, AGE AND PLACE OF RESIDENCE

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ABSTRACT

Background. There are few updated data on rates of hospital mortality of diabetic patients and length of their hospital stay on a country level. To determine such rates we provided analysis using claims data from a Nationwide General Hospital Morbidity Study carried out by the National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) in Warsaw from 2010 to 2018.

Objective. The aim of the study was to analyze the nine years changes of in-hospital morbidity and mortality in diabetic patients and length of hospital stay using a comparative approach by gender, age and place of residence.

Material and methods. The data on all patients from general hospitals in Poland treated because of diabetes were taken from a nationwide database, kept since 1979 by the Department for Monitoring and Analyses of Population Health of NIPH-NIH. This database contains information gathered under the Statistical Research Program of Public Statistics. Hospitalization rates were used to evaluate the 'hospitalized' incidence of diabetes (number of hospitalization cases due to diabetes per year by the analyzed unit of population). In-hospital mortality was calculated as the percentage of deceased patients out of all patients hospitalized due to diabetes.

Results. The number of cases and hospitalization rates of diabetic patients was rapidly declining by 18.8% for type 2 (E11) and 23.7% for type 1 (E10) diabetes. The downward tendency in the scope of hospitalization affected mainly older women and rural residents. Hospital mortality due to diabetes rose up dangerously to 3.77% exceeding the rates recorded eight years earlier.

Conclusions. The recent reduction in hospitalization rates of people with diabetes in Poland may be associated with an unexpected increase in hospital mortality.

Key words: *epidemiology, inpatient diabetes mellitus, mortality, length of hospital stay*

STRESZCZENIE

Wprowadzenie. Niewiele jest aktualnych danych na temat śmiertelności szpitalnej pacjentów z cukrzycą i długości ich pobytu w szpitalu w skali kraju. W celu określenia takich wskaźników przeprowadzono analizę z wykorzystaniem danych z Ogólnopolskiego Badania Chorobowości Szpitalnej Ogólnej prowadzonego przez Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny PIB w ramach Programu Badań Statystycznych Statystyki Publicznej w Polsce w latach 2010-2018.

Cel. Celem pracy była analiza dziewięcioletnich zmian chorobowości i śmiertelności szpitalnej pacjentów z cukrzycą i długości pobytu w szpitalu, stosując podejście porównawcze z uwzględnieniem płci, wieku i miejsca zamieszkania.

Materiał i metody. Dane o wszystkich pacjentach leczonych z powodu cukrzycy ze szpitali ogólnych w Polsce zaczerpnięto z ogólnopolskiej bazy danych, prowadzonej od 1979 r. przez Zakład Monitorowania i Analizy Stanu Zdrowia Ludności NIZP-PZH PIB. Baza ta zawiera informacje zebrane w ramach Programu Badań Statystycznych Statystyki Publicznej. Do oceny zachorowalności na cukrzycę „w szpitalu" (liczba hospitalizacji z powodu cukrzycy w ciągu roku w analizowanej jednostce populacji) wykorzystano wskaźniki hospitalizacji, śmiertelność szpitalną wyliczono jako odsetek zmarłych spośród wszystkich pacjentów hospitalizowanych z powodu cukrzycy.

Wyniki. Liczba zachorowań i hospitalizacji pacjentów z cukrzycą gwałtownie spadała: E11, typ 2 o 18,8% i E10, typ 1 cukrzycy o 23,7% w okresie objętym analizą. Tendencja spadkowa w zakresie hospitalizacji dotyczyła głównie starszych kobiet i mieszkańców wsi. Śmiertelność szpitalna z powodu cukrzycy wzrosła niebezpiecznie do 3,77% przekraczając wskaźniki notowane osiem lat wcześniej.

Wnioski. Obserwowane ostatnio zmniejszenie liczby hospitalizacji osób chorych na cukrzycę w Polsce przypuszczalnie wiąże się z nieoczekiwanym wzrostem śmiertelności szpitalnej.

Słowa kluczowe: *epidemiologia, cukrzyca, leczenie cukrzycy w szpitalu, śmiertelność szpitalna, długość pobytu w szpitalu*

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, resulting from a defect in insulin secretion or action, and can damage many organs. Diabetes is recognized by the World Health Organization as a social illness, due to its spread and long-term consequences for affected people [10, 13, 20] .

According to the estimates of the National Health Fund, from 1,800 up to 2,400 k people suffer from diabetes in Poland [18]. The analysis of the frequency of hospitalization of people with diabetes in Poland is based on data from a Nationwide General Hospital Morbidity Study carried out by the National Institute of Public Health National Institute of Hygiene (NIPH - NIH) from 2010 to 2018. The analysis takes into account the E10-E14 diagnoses, particularly E10 (type 1 diabetes) and E 11 (type 2 diabetes), according to revision 10 of the International Statistical Classification of Diseases and Health Problems (ICD-10). This work is a continuation of the analysis of hospitalization of diabetic patients conducted by NIPH – NIH in the years 1980-1999 [14] and 2005-2009 [7]

AIMS

1. The assessment of the dynamics of changes in the frequency of hospitalization and the length of hospital stay of patients with diabetes in Poland in 2010-2018.
2. The analysis of hospitalization by sex and age.
3. The assessment of the dynamics of changes in hospital mortality of people hospitalized in 2010-2018 due to diabetes.
4. The assessment of territorial differentiation of hospital morbidity

MATERIAL AND METHODS

The data on patients hospitalized in Poland because of diabetes were taken from a nationwide database, kept since 1979 by the Department for Monitoring and Analyses of Population Health of the National Institute of Public Health – National Institute of Hygiene in Warsaw. This database contains information gathered under the Statistical Research Program of Public Statistics. The data come from all general hospitals in Poland. The analyzed data relate to patients for whom the main cause of hospitalization was diabetes, as classified in the International Classification of Diseases and Health Problems ICD-10: E10 (insulin-dependent diabetes mellitus), E11 (non-insulin-dependent diabetes mellitus), E12 (malnutrition-related diabetes mellitus), E13 (other specified diabetes mellitus) and E14 (unspecified diabetes mellitus). Hospitalization rates were used to evaluate the 'hospitalized' incidence of diabetes (number of hospitalization cases due to diabetes per year by the analyzed unit of population). In-hospital mortality was calculated as the percentage of deceased patients out of all patients hospitalized due to diabetes.

RESULTS

Diabetes Mellitus: hospitalization rates in Poland

Hospitalization frequency due to diabetes mellitus (E10-E14) in 2018 is much lower than in 2010 and 2014. Hospitalization rates per 100 k population were 231.5 in 2010, then 196.3 in 2014 and 188.1 in 2018. During the 9 analyzed years, these rates decreased by 18.8%.

Adult patients diagnosed with type 2 diabetes (E11) constitute the largest group of patients treated in hospital among diabetics, with 48,866 cases in 2010. Among these patients, the hospitalization rate decreased by 18.8%.

Another large group of hospitalized patients (37,215 people in 2010) is patients with type 1 (E10) diabetes (Table 1, Figure 1). Within 9 years, the frequency of hospitalization of these patients decreased by as much as 23.7%.

The remaining types of diabetes, E12-E14, concern smaller groups of patients (a total of 2323 cases in 2010) and, except for diabetes associated with malnutrition (E12), there was no downward trend in the frequency of their hospitalizations.

Diabetes-associated hospitalizations by age and gender groups

During the 9 years analyzed, serious discrepancies in hospital treatment between women and men were observed. The negative percentage difference in the frequency of hospitalization of women was 25.5%. At that time, the frequency of male hospitalizations decreased by only 11.6%. The change in the hospital admission management of people with diabetes is therefore most visible for women. This trend is especially observed in the most numerous group of patients with type 2 diabetes (E11). Over the nine-year period, the difference in admission rates was 18.8%; at that time, the hospital admission of women decreased significantly by 24.9%, while that of men only by 11.7%.

The frequency of hospitalizations for type 2 diabetes increases very slowly with age, and rises after the age of 40 (Table 2, Figure 2). For example – the rate for women before the age of 40 was 39.1 per 100 k, while after the age of 80 – as many as 659.7.

In 2010-2018, there was a strong downward tendency in the frequency of hospitalization of patients with type 2 diabetes, especially in people over the age of 30, as younger patients were hospitalized more often

Table 1. Hospitalization trends in different types of diabetes mellitus in 2010, 2014 and 2018. Hospitalization rates per 100 k inhabitants

Type of diabetes	2010 N = 88409			2014 N = 75560.0			2018 N = 72240			Percentage difference 2010-2018		
	Overall	M	F	Overall	M	F	Overall	M	F	Overall	M	F
Overall	231.54	232.49	230.66	196.3	205.8	187.5	188.1	205.4	171.9	**-18.8**	**-11.7**	**-25.5**
E10	97.5	103.5	91.8	80.4	89.2	72.1	74.4	85.9	63.6	**-23.7**	**-17.0**	**-30.7**
E11	128.0	121.6	134	107.3	106	108.4	103.9	107.4	100.6	**-18.8**	**-11.7**	**-24.9**
E12	0.19	0.23	0.15	0.2	0.3	0.2	0.1	0.2	0.1	**-47.4**	**-13.0**	**-33.3**
E13	3.09	4.13	2.11	4.5	5.5	3.5	4.3	5.6	3.1	39.2	35.6	46.9
E14	2.83	3.07	2.59	4	4.8	3.3	5.3	6.2	4.4	87.3	102.0	69.9

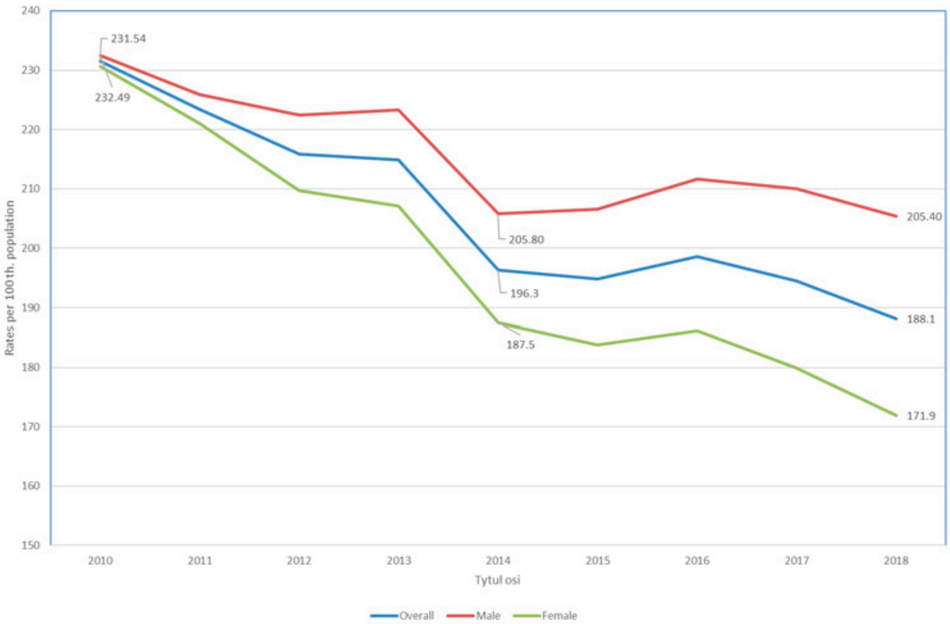

Figure 1. Hospitalization trends in different types of diabetes (E10-E14 overall) in 2010-2018. Hospitalization rates per 100 k inhabitant

Table 2. Hospitalization trends in type 2 diabetes mellitus (E11) by age and gender (2010-2018). Hospitalization rate per 100 k inhabitants

Age groups (in years)	2010			2014			2018			Percentage difference 2010-2018		
	Overall	M	F	Overall	M	F	Overall	M	F	Overall	M	F
Overall	128.0	121.6	134.0	108.4	107.3	106	100.6	103.9	107.4	33.2	23.2	13.2
0-9	0.9	0.8	0.9	0.4	0.4	0.3	1.2	0.9	0.7	**-25.0**	**0.0**	**14.3**
10-19	2.9	2.3	3.5	3.1	3.1	3.1	2.8	3.3	3.8	**25.0**	**-12.1**	**-39.5**
20-29	4.4	5.1	3.7	3.6	4.8	5.9	5.7	6.3	6.9	**-35.1**	**-30.2**	**-26.1**
30-39	15.4	20.8	9.9	8.0	12.8	17.6	8.6	15.2	21.5	15.1	1.3	-3.3
40-49	62.2	85.3	39.1	27.9	49.4	70.8	24.4	44.1	63.6	60.2	41.0	34.1
50-59	176.0	226.2	129.7	98.1	134.6	173.1	80.0	115.6	152.6	62.1	52.2	48.2
60-69	353.3	388.2	325.0	227.1	264.5	309.2	180.2	228.9	286.6	80.4	54.3	35.5
70-79	533.6	484.8	564.3	435.0	423.4	405.4	389.2	402.0	420.8	45.0	32.7	15.2
80+	639.0	590.7	659.7	551.7	544.3	527.6	508.6	502.6	489.2	29.7	27.1	20.7

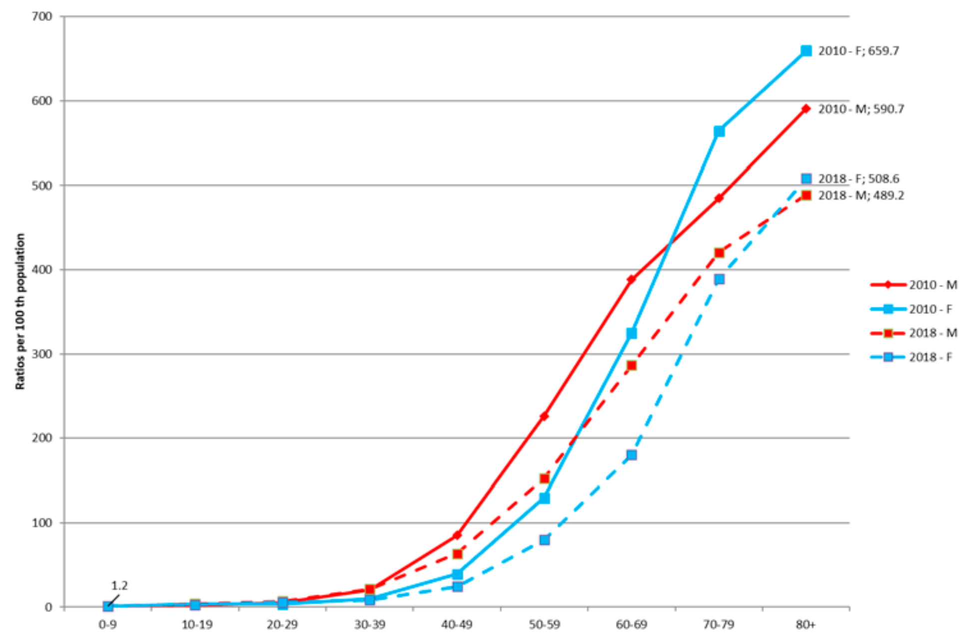

Figure 2. Hospitalization trends in type 2 diabetes mellitus (E11) by age and gender (2010-2018). Hospitalization rate per 100 k inhabitants

in 2010 compared to 2018. Both in 2010 and 2018, men were hospitalized more often than women, except for the oldest age groups (Table 3).

In the less common type 1 diabetes (E10), the incidence of hospitalization increases with age in both men and women, except for a decrease in the 20-29 age group. A significant increase in the number of hospitalizations occurs among men over 40 and among women over 50. There was a strong downward trend in the frequency of hospitalizations of patients with type 1 diabetes in 2010-2018, when the rate decreased by 33%: from 98 per 100 k down to 64 per 100 k population. However, this reduction only applies to people over the age of 30. In younger patients treated for type 1 diabetes, an increase in the frequency of hospitalizations in those years was observed (Figure 3).

Comorbidities

People with diabetes are known to suffer from other concomitant chronic diseases, which is confirmed in our material on hospitalized patients: every second person with type 2 diabetes and every fourth person with type 1 diabetes is also treated for cardiovascular disease. Gender does not differentiate these frequencies (Table 4).

Table 3. Hospitalization trends in type 1 diabetes mellitus (E10) by age and gender (2010-2018). Hospitalization rate per 100 k inhabitants

Age groups (in years)	2010 N = 37215			2014			2018			Percentage difference 2010-2018		
	Overall	M	F	Overall	M	F	Overall	M	F	Overall	M	F
Overall	97.5	103.5	91.8	80.4	89.2	72.1	74.4	85.9	63.6	31.0	20.5	44.3
0-9	54.4	51.6	57.4	54.4	53.8	55.1	58.4	58.8	58.0	-6.8	-12.2	-1.0
10-19	123.9	119.4	128.6	131.7	128.5	135.0	159.4	159.1	159.8	-22.3	-25.0	-19.5
20-29	40.7	40.4	41.1	38.5	38.2	38.7	41.7	46.8	36.3	-2.4	-13.7	13.2
30-39	46.9	57.0	36.5	39.3	47.8	30.5	40.8	49.5	32.0	15.0	15.2	14.1
40-49	62.1	83.9	40.3	52.6	69.0	36.0	43.1	59.0	27.0	44.1	42.2	49.3
50-59	104.7	137.8	74.1	78.8	106.6	52.4	60.6	83.6	38.5	72.8	64.8	92.5
60-69	179.8	211.6	154.0	121.5	158.1	90.9	91.4	125.7	62.4	96.7	68.3	146.8
70-79	229.0	231.6	227.4	164.2	178.4	155.0	132.8	155.0	117.8	72.4	49.4	93.0
80+	242.8	235.6	245.9	181.4	191.3	177.0	140.8	160.3	132.1	72.4	47.0	86.1

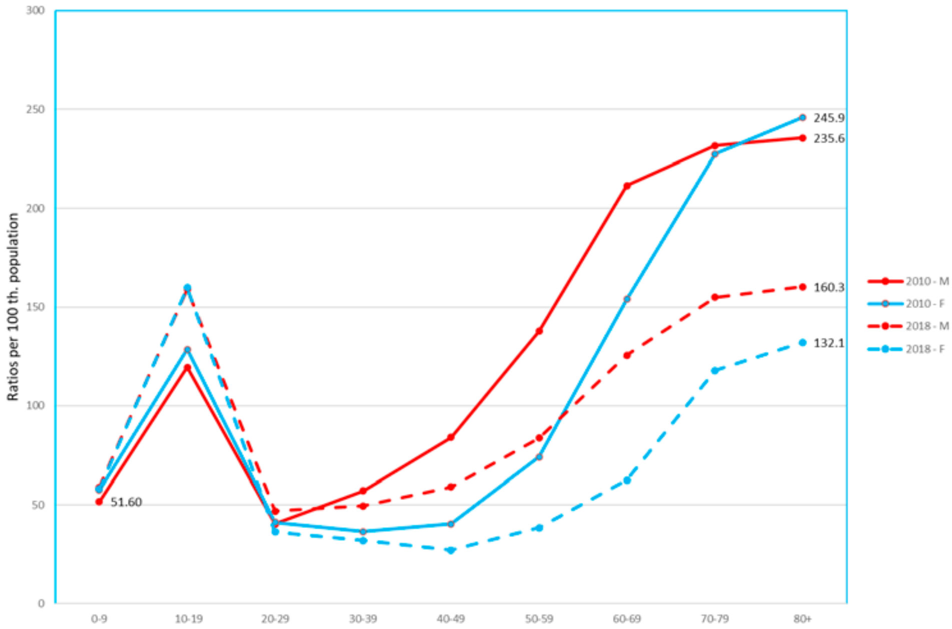

Figure 3. Hospitalization trends in type 1 diabetes mellitus (E10) by age and gender (2010-2018). Hospitalization rate per 100 k inhabitants

Table 4. The frequency of predominant concomitant diseases among hospitalized patients with diabetes mellitus in 2009 by gender and type of diabetes

	Type 1 diabetes (E10)			Type 2 diabetes (E11)		
Concomitant diseases	Overall	M	F	Overall	M	F
E00-E90	27.3	23.7	31.4	21.4	21.7	21.4
I00-I99	26.9	27.7	26.0	39.4	40.5	39.4
L00-L99	9.0	11.4	6.2	4.6	3.3	4.6
N00-N99	8.9	8.2	9.7	10.8	11.1	10.8
K00-K93	6.8	7.8	5.7	5.2	4.6	5.2
J00-J99	3.3	3.4	3.1	3.5	3.4	3.5
D50-D89	2.8	2.7	3.0	2.8	2.7	2.8
Other diseases	15.0	15.0	15.0	12.4	12.6	12.4

Average length of hospital stay

The length of hospital stay in days is presented in Table 5 and Figure 4.

A very young patient usually stays less than a week. People aged 10-19 years stay in hospital for the shortest time: 4.5 days. The observation period does not change this trend. Each subsequent age group is hospitalized longer than the younger one. During the analyzed 9 years, the hospitalization of patients treated for the most common type 2 diabetes (E11) became significantly shorter, starting from the 20-29 age group. Nevertheless, most significant reductions in terms of hospital stay in the period 2010-2018 mainly affected women over the age of 70.

Hospitalization of city and country dwellers

Data from Polish hospitals reveal that the place of residence is clearly related to the chance of inpatient treatment of the most common type 2 diabetes. In 2010-2018, the hospitalization rate decreased among urban patients by 17.5%, while among patients living in the countryside by as much as 20.8 % (Table 6, Figure 5).

Territorial differences of diabetic patient hospitalization

In order to analyze the territorial differentiation of diabetic patient hospitalizations, the crude and standardized rates of hospitalization by voivodship and sex of patients treated in hospitals in 2010 and 2018 were calculated. It was found that men were hospitalized more often than women in all voivodships.

Table 5. The average length of hospital stay (in days) by age groups among diabetic patients (2010, 2014, 2018)

Age	2010						2014						2018					
	Overall		M		F		Overall		M		F		Overall		M		F	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Total	8.0	7.7	8.1	8.2	8.0	8.3	7.6	8.7	7.8	8.9	7.4	8.5	7.7	7.9	7.7	8.1	7.6	7.7
0-9	5.3	5.5	5.4	5.7	5.3	5.3	5.0	5.1	5.2	5.1	4.9	5.1	5.3	5.3	5.1	5.4	5.4	5.3
10-19	4.6	4.4	4.6	4.4	4.7	4.4	4.1	3.7	4.0	3.7	4.1	3.7	4.5	3.8	4.5	3.9	4.5	3.7
20-29	6.0	4.0	6.1	3.9	6.0	4.2	5.3	4.0	5.4	3.8	5.2	4.2	5.5	3.9	5.8	4.2	5.3	3.4
30-39	6.9	6.1	6.9	5.8	6.8	6.6	6.3	6.3	6.5	6.7	5.9	5.3	6.3	6.3	6.4	5.7	6.1	7.3
40-49	7.7	7.3	7.8	7.4	7.5	7.1	7.0	6.8	7.2	7.0	6.5	6.0	7.1	6.9	7.2	7.2	6.9	6.1
50-59	8.2	7.9	8.3	8.5	8.0	7.0	7.9	8.1	8.3	8.8	7.1	6.3	7.8	7.7	7.9	8.1	7.5	7.0
60-69	8.7	8.4	9.1	9.5	8.4	7.1	8.4	9.4	8.9	10.2	7.7	8.0	8.3	8.5	8.5	9.3	8.1	7.4
70-79	8.8	10.4	9.1	8.6	8.7	11.3	8.6	9.4	9.1	9.8	8.2	9.1	8.4	7.6	8.7	8.4	8.2	7.0
80+	8.6	7.5	8.7	8.3	8.6	7.2	8.8	11.0	9.0	10.6	8.7	11.1	8.5	9.8	8.8	9.0	8.5	10.2

M – Male; F – Female

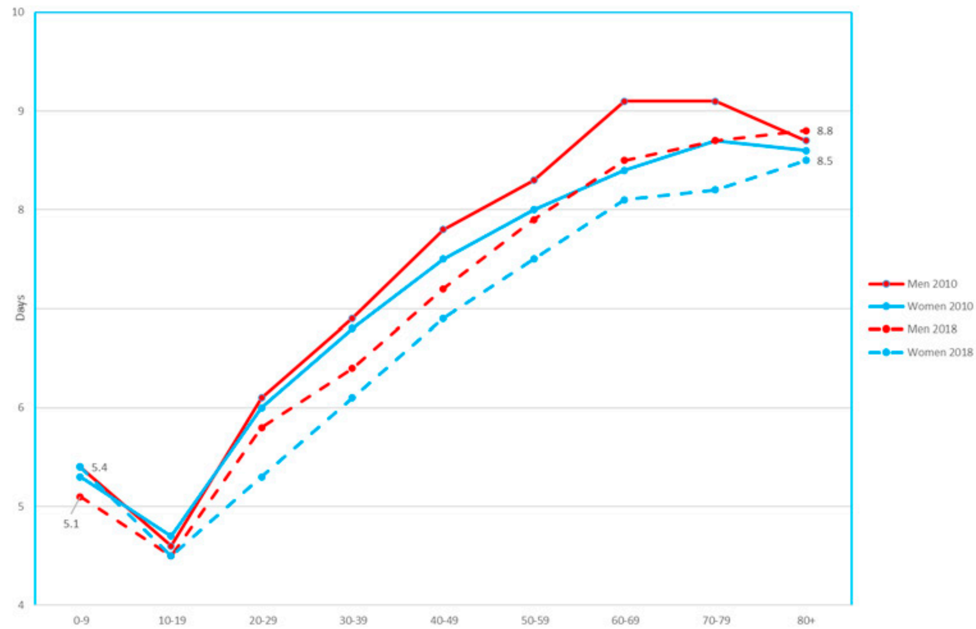

Figure 4. The average length of hospital stay (in days) by age groups among diabetic patients (2010 and 2018)

Table 6. Hospitalization trends in type 1 (E10) and type 2 (E11) diabetes mellitus by place of residence (2010-2018). Hospitalization rates per 100 k inhabitants

Item	2010			2014			2018			Percentage difference		
	M	F	Overall	M	F	Overall	M	F	Overall	M	F	Overall
Urban												
E10	108.7	92	99.9	84.7	72.2	82.5	89.8	63.9	76.2	18.9	28.1	23.7
E11	128.4	130.1	129.3	112.3	107.4	109.7	114.5	99.7	106.7	13.9	30.4	22.6
Rural												
E10	95.8	91.6	93.7	81.9	72	77.2	80	63.2	71.8	15.8	28.4	21.9
E11	111.5	140.3	126	96.4	110.2	103.6	96.8	102.1	99.8	14.7	38.2	26.2

M – Male; F – Female

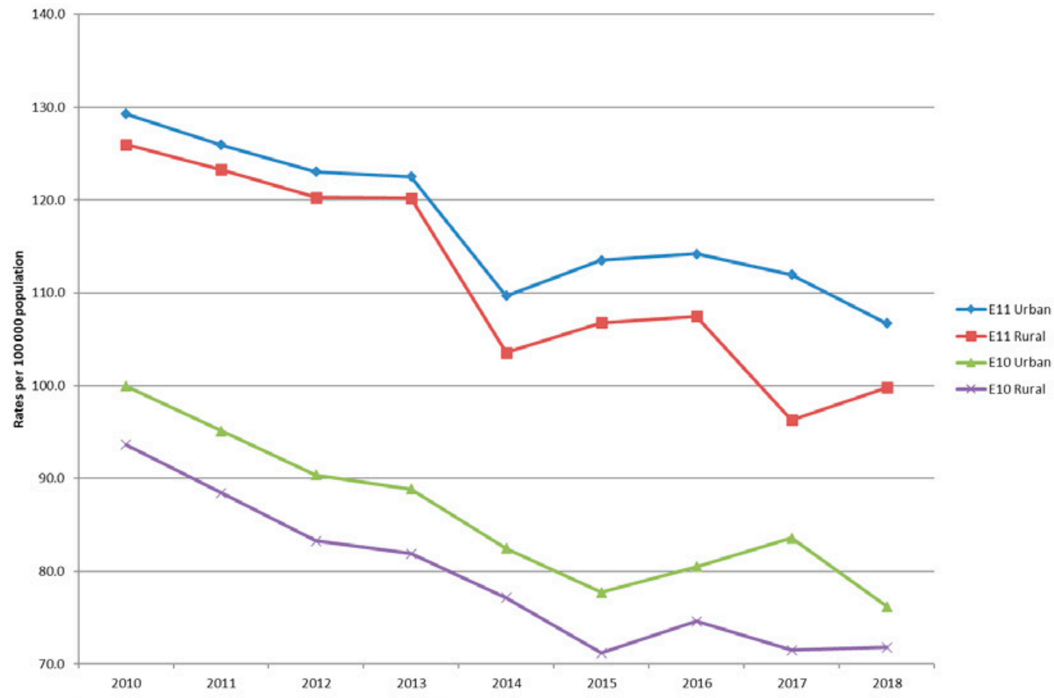

Figure 5. Hospitalization trends in type 1 (E10) and type 2 (E11) diabetes mellitus by place of residence (2010-2018). Hospitalization rates per 100 k inhabitants

For the standardized coefficients, this difference was on average 23% for type 1 diabetes and 22% for type 2 diabetes.

In 2018, this difference increased and for all voivodships it averaged 36% and 34% respectively for type 1 and type 2 diabetes. There were significant differences in the frequency of hospitalizations due to diabetes between voivodships both in 2010 and 2018

In 2010, patients with type 1 diabetes were hospitalized most often in the Lubuskie voivodship (132 per 100,000) and least often in the Śląskie voivodship (61 per 100,000), so the difference was more than twofold.

In 2018, patients with type 1 diabetes were most often hospitalized in the Lubuskie voivodship, but the rate of hospitalization was already lower (108.2 per 100,000). The least frequent hospitalizations were in the Małopolskie voivodship (45 per 100,000).

Among patients with type 2 diabetes, an equally high variability range of hospitalization rates is observed – in 2010, hospitalization rates were the highest in the Łódzkie voivodship (196 per 100,000), and the lowest in the Pomeranian voivodship (56 per 100,000). In 2018, the hospitalization variability for type 2 diabetes remained also very high. The most frequent hospitalizations were still in the Łódzkie

Table 7. Hospital mortality rates (%) due to type 1 and type 2 diabetes mellitus by age and gender (2005-2009)

Item	Type 1 diabetes mellitus				Type 2 diabetes mellitus			
	2010		2018		2010		2018	
	M	F	M	F	M	F	M	F
Overall	1.49	1.86	2.20	2.58	1.90	2.37	2.76	3.77
0-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10-19	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
20-29	0.08	0.00	0.09	0.23	0.00	1.06	0.00	0.00
30-39	0.60	0.68	1.02	0.40	0.00	0.11	1.06	0.75
40-49	0.99	0.72	1.06	0.55	0.53	0.51	0.11	1.22
50-59	1.07	0.76	1.66	1.68	0.68	1.26	0.51	1.16
60-69	1.79	1.83	3.03	3.56	1.38	2.18	1.26	2.05
70-79	3.56	2.91	5.75	4.29	3.21	5.58	2.18	2.78
80+	7.89	6.52	12.47	10.64	5.94	2.37	5.58	7.75

M – Male; F – Female

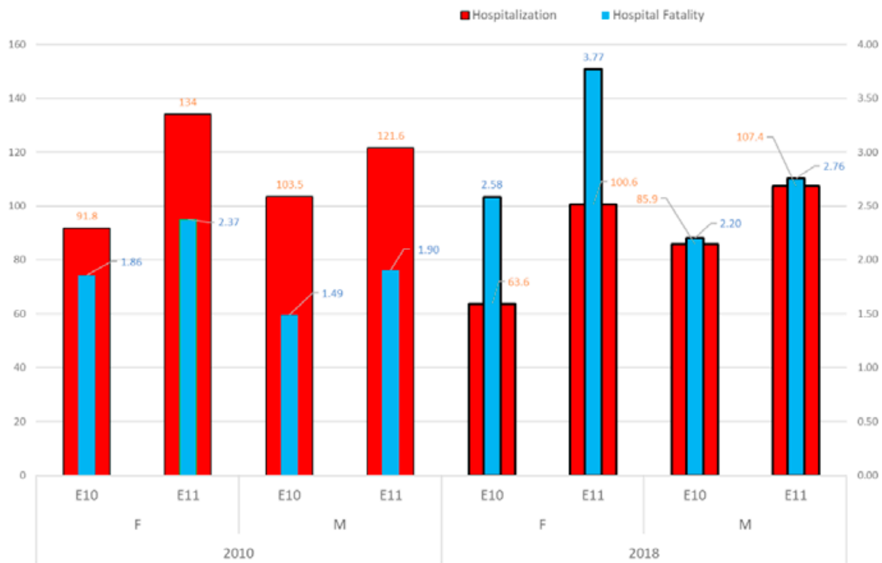

Figure 6. Hospitalization and hospital mortality due to diabetes in Poland in 2010 and 2018

voivodship (111 per 100,000), while the least frequent in the Zachodniopomorskie voivodship (30 per 100,000).

Hospital mortality rate

During the analyzed nine years, there was a dangerous significant change in terms of total hospital mortality, both among men and women. Particular attention should be paid to the significant increase in hospital mortality in these patients with age, especially after the age of 60, both for men and women. Among people aged 80 and over, the mortality rate even exceeds 10%. The mortality percentage of women admitted to hospital for type 2 diabetes (E11) increased sharply from 2.37 to 3.77 over the 9 years analyzed (Table 7, Figure 6).

DISCUSSION

The prevalence of diabetes in Poland. Diabetic patient hospitalization rates. Comparison between 1980 and 2018

According to the results of the presented analysis, over the nine-year period, the number of cases and hospitalization rates of diabetes per 100,000 population was rapidly declining. A comparison with the years 2005-2009 reveals a completely different picture of the situation – then, the number of hospitalized patients was increasing and the rates per 100,000 rose every year [7]. An even earlier study covering the years 1980-1999, due to the longest follow-up period, allowed for the conclusion that “after a decrease in the frequency of hospitalizations at the beginning of the 1980s, for the next 10 years, there was a constant increase” [14].

In the general population, type 2 diabetics constitute 85-90% of all diabetic cases [1, 2, 4, 17]. Among hospitalized cases, these are also the most numerous group of diabetic patients, but their percentage is smaller: 55.2. Other patients with type 1 diabetes and other types are represented more often than outside hospital. Therefore, hospitals are struggling with very risky situations: people who are undiagnosed before their hospital admission, patients with complications such as cardiovascular complications, retinopathy, diabetic foot syndrome, and patients who have to stay in the Intensive Care Unit [3, 9, 11, 12].

Territorial differences

The downward tendency in the scope of hospitalization affected rural residents more strongly than urban residents. This inequality persists despite the passage of time, which is not justified in the epidemiological situation and indicates a lack of control over the hospital treatment availability.

The picture of voivodship variability in the analyzed years is “mosaic” in nature and is very difficult to interpret. An objective assessment of the situation of the selected area requires taking into account regional diabetes prevention and treatment programs, local material and human resources, local government activity, the existence of patient associations, and other variables, which does not constitute the subject matter of our work.

When assessing the inter-voivodship diversification of diabetic patient hospitalizations, it can be concluded that the reduction in the frequency of diabetic patient hospitalizations recommended by diabetes specialists and health care organizers [15, 16, 17, 22] is implemented in Polish hospitals in all voivodships, which can be seen from the comparison of standardized hospitalization rates in 2010 and 2018. More than twice as serious a concern is caused by epidemiologically unjustified. differences between extreme voivodships in terms of the frequency of hospitalization of patients with type 1 diabetes and type 2 diabetes, which applies to both 2010 and 2018.

Comorbidities, coronary heart disease

Diabetic patients are also treated in hospital for comorbidities, mainly ischemic heart disease. This most common health burden can lead to a diabetic patient dying prematurely. According to diabetologists, "the overriding goal of treating diabetes is to reduce the risk of cardiovascular complications and extend the life of patients" [5].

Shortening the period of inpatient treatment as a case of the general trend of financing in hospitals [2, 6]. The total length of hospital stay in Poland for all reasons is one of the shortest in the European Union countries [21]. According to *Gajewska's* analysis [7], the length of hospital stay decreased from 9.1 days to 8.1 days. In the following years, we observed a reduction in the hospital treatment of diabetic patients to 7.7 days, which has remained essentially unchanged

High mortality and decreasing hospitalization rates over a 9-year follow-up

In 2010-2018, a shocking change took place, as shown in Figure 6. Hospital mortality due to diabetes rose dangerously in 2018, exceeding the rates recorded ten years earlier. The most common reason for hospitalization in the group of diabetic patients, type 2 diabetes (E11), is an area where there is a record increase in hospital mortality rate among women from 2.37 to 3.77% [1]. A similar process accelerated in a group of patients hospitalized for type 1 diabetes (E10). Mortality among people treated with type 1 diabetes increased the severity among women from 1.86 to 2.58% and among men from 1.49 to 2.20%.

The background of these unexpected, negative effects of diabetes hospitalization in 2010-2020 is the inexorable reduction in hospital admissions, shown in the same Figure 6. The hospitalization mortality rates decreased year by year, among women more dynamically than among men.

CONCLUSIONS

The recent drastic reduction in hospitalization rates of people with diabetes in Poland is associated with an unexpected increase in hospital mortality among these patients and the disclosure of evident inequalities in access to hospital treatment among women, inhabitants of rural areas and some voivodships.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

COMPARISON OF URINARY BIOMARKERS CONCENTRATIONS IN EXPOSED AND NON-EXPOSED PETROL STATION WORKERS IN THE EASTERN ECONOMIC CORRIDOR (EEC), THAILAND

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ABSTRACT

Background. The Thai government has been developing its Eastern Economic Corridor (EEC), which spans three provinces, with the aim of improving connections with other Asian nations. Since this strategic development, the number of trucks, private car, and passenger car registrations have continued to grow, with a corresponding increase in related to benzene, toluene, ethylbenzene, and xylene (BTEX).

Objectives. This study aims to compare the levels of trans, trans-muconic acid (t, t MA); toluene (TU); mandelic acid (MA); and methyl hippuric acid (MHA) in the urine of gas station employees, considering demographic and occupational factors.

Material and methods. These employees worked either near or away from the fuel dispenser, and there 100 people in each group. Data were collected using interviews and testing environmental air and urine samples for benzene, toluene, ethyl benzene, and xylene (BTEX).

Results. The results showed that BTEX concentrations were just detectable in all 200 cases (100%). The mean (±SD) urine level of t, t MA was 449.28 (±213.32) µg/g creatinine, while the median (min-max) was 428.23 (95.58-1202.56) µg/g creatinine. The mean TU was 0.011 (0.001) mg/L, while the median (min-max) was 0.011 (0.010-0.013) mg/L. MA levels were higher inside the pollution control zone than they were outside the zone (p=.009). Employees who practiced poor personal hygiene had relatively high urinary toluene and MHA levels (p=.009) and those who did not wear personal protective equipment (PPE) had relatively high MA levels (p=.040).

Conclusion. The results of this study revealed statistically significant biomarkers influencing the levels of t, t MA; TU; MA; and MHA in urine. The recommendations of this study are that employers should provide their employees with suitable PPE, check regularly to ensure that it is worn, and strongly encourage employees to take care of their sanitation. Employees should take appropriate breaks and days off to minimize their exposure to BTEX.

Key words: *exposure to gasoline, trans trans-muconic acid, toluene, mandelic methyl hippuric acid, urinary biomarkers*

INTRODUCTION

Since 2018, the Thai government has been developing its Eastern Economic Corridor (EEC), which spans three provinces (Chachoengsao, Chonburi, and Rayong) [1]. As a result, the EEC region has experienced high population growth. According to the EEC office, the number of residents is projected to grow from 4 million to more than 6 million by 2037 [2]. This national policy has led to a greater demand for energy, including demand for transportation fuels, in this region than in others [3, 4, 5].

Petrol stations exist in many settings, including in car wash facilities and adjacent to convenience stores, and are manned by attendants, cashiers, and fuel loading personnel [6, 7]. Pump attendants providing fuels are at risk of benzene, toluene, ethylbenzene, and xylene (BTEX) exposure, with benzene being the most toxic to humans [8]. Volatile organic compounds (VOC) can enter the human body *via* inhalation [9, 10],

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the skin, and ingestion [11]. The most common form of occupational exposure to chemical vapors is inhalation, with workers at gas stations across the world all being at risk [12].

BTEX exposure affects multiple body systems [13, 14, 15], leading to both serious health and cognitive and behavioral problems in occupationally exposed groups [8]. Benzene is the most dangerous substance. Even at low concentrations, long-term exposure to benzene increases the risk of leukemia and aplastic anemia in humans. Benzene has been classified as carcinogenic to humans (IARC group 1) [16]. The assessment of biomarkers of exposure, such as urinary levels of benzene and toluene [17, 18], would facilitate occupational health surveillance. For example, trans, trans-muconic acid (t, t MA) and s-phenyl mercapturic acid (SPMA) [18] can be used as markers for benzene, mandelic acid (MA) can be used as a marker for ethylbenzene, and methyl hippuric acid (MHA) can be used as a marker for xylene [17]. The biological monitoring of urinary excretion levels of such solvents or products is of fundamental importance in terms of the health surveillance of fuel distributors.

Employees working at fuel service stations may be exposed to BTEX, with their risk level dependent on factors such as the working environment (e.g., whether they work inside or outside a building), the nature of their work, and their work duration [19]. Additionally, personal behaviors, such as the consumption of alcohol and smoking, can aggravate health problems [20], while the use of personal protective equipment (PPE) and wearing a facemask can reduce substance exposure [21]. Demographic characteristics such as sex, age [22], and educational level [23] may also be related to the level of BTEX in the body.

Previous studies in Thailand have examined factors affecting urinary t, t MA levels in gas station laborers [6] assessed health risks related to benzene; assessed [24] associations between BTEX and biological parameters in petrol attendants; and conducted urinary t, t MA assessments in gas station attendants [25]. Previous studies from other countries include Egyptian research on urinary t, t MA levels in gas station workers [26] and BTEX exposure assessments before and after work in gas station attendants [18, 26]. BTEX levels in ambient air in Sri Lanka have also been studied and related research has been conducted in Iran in petrol pumps station workers [27] and Brazil in gas station attendants [26]. In addition, research based in Portugal found high BTEX exposures in residents near fuel service stations [28], and in Malaysia BTEX exposure was linked to adverse health effects in petrol attendants [29].

There remains a lack of comparative studies in this field that have measured the levels of t, t MA; TU; MA; and MHA in fuel service workers based both inside and outside dispenser areas. The aim of this study is to look for comparisons between the levels of urine t, t MA; TU; MA; and MHA and the personal and occupational factors of fuel service employees in EEC gas stations. This fundamental knowledge would help us to create healthcare policies for employees in high-risk groups.

MATERIAL AND METHODS

Study site and population

This analytical study included an exposure group of 100 Rayong Province fuel service attendants who were exposed to BTEX for at least three months and a non-exposure group of 100 employees who worked in fuel stations but were not involved directly in fuel service duties.

Each of the participants was recruited from one of three provinces - Chonburi, Rayong, and Chachoengsao - with 100 locations in total. A multistage sampling method was used. Initially, data from the Thai Department of Energy Business were used to select the top three large fuel service companies and several of their stations [5]. Following this, a survey revealed that company employees' performance characteristics were similar in all three EEC provinces, so the analysts chose to use the sampling group in Rayong. The researchers then communicated with the municipal authorities for public relations and randomly selected fuel service stations near main roads in Rayong province using simple random sampling. The stations were located on main roads (n=2), on by-pass roads (n=3), and on interdistrict roads (n=3). The owners of each of the eight stations were contacted to obtain permission to collect data in the operation area and to recruit filling attendant employees using cluster random sampling. All employees who agreed to participate and met the inclusion criteria were included in the study. Rayong city is currently divided into two sections: a pollution control zone and the surrounding area [30].

Sample size determination

The researchers used the G*Power software to calculate the sample size using an effect size of 0.20 in Cohen's formula and a statistical power of 0.84. G*Power calculation [31] indicated a sample size of 176 people. Twenty-four (15%) extra people were added to this sample to allow for attrition, thus making the total sample size 200. Gas station employees aged between 18 and 60 years old who were able to read, listen, write Thai, and work on the day of data collection for 8 hours were eligible for inclusion in the study.

Research ethics

This study was conducted in accordance with the Declaration of Helsinki. Burapha University Institutional Review Board for Protection of Human Subjects in Research (BUU-IRB) approved this research under certificate no. 019/2020.

Data collection tools

The research tools and equipment used in this study included interview forms, and urine specimen collection equipment. These are described as follows:

The interview form

The interview form was divided into four parts, with 30 questions in total, and was completed by selecting responses and adding text. In part 1, ten demographic questions concerning gender, age, weight, height, marital status, smoking history, and alcohol use were included. A total of nine questions in part 2 were on personal hygiene care, five of which were of a positive nature - such as hand washing before eating. Four questions of a negative nature such as drinking water in the workplace, wearing un-washed work clothes, and others. Part 3 (six items) focused on whether the respondent practiced personal protective behaviors such as wearing a nose mask, gloves, boots or sneakers, pants, or long-sleeved shirts. Part 4 asked about work history, with five items on the number of years of work experience the employee had, the hours of work they completed per day, their days of work per week, their hours of overtime per week, and their hours sleep each night. The quality of the interview questions was assessed based on the content validity, structure, alignment with the objectives of the research, and suitability of the language using the tools created for this purpose by three experts. The item–objective congruence index (IOC) showed that each item had an IOC greater than 0.5 and a *Cronbach's* alpha coefficient of 0.88, indicating their reliability.

Biological collection

Biological collection cards with 50 mL polyethylene tubes were used for urine specimen collection. The purpose of urine collection was to evaluate the levels of t, t MA; TU; MA; and MHA using the data interpretation criteria recommended by the American Conference of Governmental Industrial Hygienists [32].

Data collection

Following ethical approval (see below) and prior to data collection, the researchers communicated with managers at each site to request cooperation with the study and to clarify the study objectives and data collection details and gain permission to collect the data. The study analysts then met with the managers to confirm their permission to collect data and to schedule the data collection. The research team then conducted data collection, which included interviews, and urine specimen collection, as described below.

Interview

The researchers explained the questions carefully to the research assistants to ensure that they understood. Arrangements were made with the managers at each gas station to interview each participating individual in the private office area of each gas station. The interview form was collected by the researcher upon the completion of the interview, which lasted approximately 15 minutes.

Urine sample collection

The researchers provided urine sample containers to employees to carry during their working day and explained that they should be used to collect urine after work. The employees were instructed 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; TU concentration for toluene; MA concentration for ethylbenzene; and MHA concentration for xylene [33, 34]. The urine samples were analyzed using HPLC following the method described as these studies [33, 34].

Statistical analysis

The researchers checked the accuracy of the data and coded for them analysis using the Statistical Package for The Social Sciences/Personal Computer (SPSS/PC) software version 20.

The statistical data were divided into two sections: 1) descriptive statistics in tabular form, including frequency, percentage, mean, standard deviation, geometric mean and standard deviation GM (GSD), median, minimum, and maximum values. 2) Inferential statistics including comparative analyses of urine results based on 17 independent variables were collected from interview data, while dependent variables were the urinary t, t MA; TU; MA; and MHA volumes. Comparisons between the two groups were made using an independent samples t-test, while comparisons between more than two groups were made using a one-way analysis of variance (ANOVA). P values less than 0.05 were considered to be statistically significant.

RESULTS

Demographic data

Of the 200 subjects, most (68.5%) were female. The mean age (SD) of the sample was 30.25 (11.02) years, with the majority of the employees, 73%, being ≤35

Table 1. Demographic information

Factors	N (200)	%
Gender		
Male	63	31.5
Female	137	68.5
Age (years)		
≤ 35	146	73.0
> 35	54	27.0
Mean ± standard deviation	30.25±11.015	100
Body Mass Index (kg/m³)		
≤22.9	112	56.0
>22.9	88	46.0
Mean ± standard deviation	23.63±2.259	100
Marital status		
Single	105	52.5
Partner/Married	86	43.0
Widowed / Divorced / Separated	9	4.5
Education level		
Illiterate	6	3.0
Primary	58	29.0
Secondary	105	52.5
Higher than secondary	31	15.5
Income (baht) (n = 169)		
Less or equal to 10,000	56	33.1
10,001 – 20,000	98	58.0
More than 20,000	15	8.8
Mean ± standard deviation	12053.25±4406.189	
Smoking		
Non-smoking	68	34.0
Currently smoke	132	66.0
Alcohol consumption		
Do not drink alcoholic beverages	91	45.5
Currently drink	109	54.5

years old. In terms of education, the majority (52%) had completed secondary school. Of the 200 subjects, 34% were non-smokers and 66% were smokers, as detailed in Table 1.

Personal hygiene and work history

Interview data showed the frequency of personal hygiene practices in workers stationed at and away from fuel dispensers, with 84% practicing personal hygiene in the workplace and 30.5% wearing personal protective equipment (PPE) for 3 hours or more. Most subjects (73.5%) had work experience of 1 year or more, 56% worked >8 hours per day, and 13.5% worked ≥six days per week. These data are shown in Table 2.

Table 2. Employment history and occupational exposure of gasoline station workers to BTEX compounds at fuel service stations

Work history and exposure to BTEX compounds	N	%
Working area		
At fuel dispenser	100	50
Outside the fuel dispenser	100	50
Personal hygiene		
Do not practice	32	16.0
Practice	168	84.0
Wearing personal protective equipment (PPE)		
Do not use / use for 1-3 hours	139	69.5
Use for >3 hours or more	61	30.5
Wearing safety glasses		
Do not use	190	95.0
Use	10	5.0
Wearing a mask		
Do not use	32	16.0
Use	168	84.0
Wearing gloves		
Do not use	158	79.0
Use	42	21.0
Using boots / shoes		
Do not use	102	51.0
Use	98	49.0
Wearing a long-sleeved shirt		
Do not use	142	71.0
Use	58	29.0
Wearing long pants		
Do not use	39	19.5
Use	161	80.5
Work experience (years)		
Less than 1 year	53	26.5
1 year or more	147	73.5
Mean ± standard deviation	2.44±4.063	
Hours of work per day		
8 hours	88	44.0
> 8 hours	122	56.0
Mean ± standard deviation	9.05±1.568	
Number of workdays per week		
6 days	111	55.5
7 days	89	44.5
Mean ± standard deviation	6.31±0.477	
Overtime (Hour / week)		
<6 hours	173	86.5
≥6 hours	27	13.5

Metabolite concentration

The results of the analysis of the t, t MA concentration showed that the mean (SD) was 449.28 (213.32) µg/g creatinine, with a median (min-max) value of 428.23 (95.58-1202.56) µg/g. The TU concentration showed a mean (SD) of 0.011 (0.001) mg/L, with a median (min-max) of 0.011 (0.010-0.013) mg/L. The mean (SD) MA concentration was 0.061 (0.012) g/g creatinine and the median (min-max) was 0.060 (0.04-0.09) g/g creatinine, both calculated from all 200 samples (100%).

These levels are below the standard (<0.15 g/g creatinine). The results of the analysis of the concentration of MHA levels of workers at and away from fuel dispensers were below the standard of 1.5 g/g creatinine, with a mean (SD) value of 0.43 (0.112) g/g creatinine and a median (min-max) value of 0.410 (0.25-0.88) g/g creatinine, as detailed in Table 3.

Comparison of various factors with urinary BTEX levels

The results of a comparative analysis focusing on a range of factors and the urinary t, t MA; TU; MA; and MHA values of gas station workers showed statistically significant ($p<0.05$) associations in urinary t,t MA; TU; MA; and MHA levels. Being male, having a low body mass index, having a working duration of more than 8 hours per day, and having a level of overtime of 6 hours per week or more were the factors that raised the t, t MA levels. Ineffective hygiene practices were linked to a higher TU level. The absence of PPE was related to higher MA, while male gender and body

Table 3. Occupational data and level of exposure to t, t muconic acid; toluene; mandelic acid; and methyl hippuric acid. SD=standard deviation; IQR=inter-quartile range; GM=geographic mean; GSD=geographic standard deviation

Factor	t,t- muconic acid (µg/g creatinine)	Toluene in urine (mg/L)	Mandelic acid (g/g creatinine)	Methyl hippuric acid (g/g creatinine)
Work in the area of the fuel dispenser (n=100)				
- Mean (SD)	449.28 (213.323)	0.0016 (0.002)	0.061 (0.012)	0.43 (0.112)
- GM (GSD)	407.38 (1.595)	0.0011 (1.798)	0.060 (1.233)	0.41 (1.279)
- Median (IQR)	428.23 (256.973)	0.0010 (0.0000)	0.060 (0.167)	0.41 (0.150)
- Max-Min	1202.56 -95.58	0.0138 - 0.0010	0.091- 0.035	0.88 - 0.25
Work outside area of fuel dispenser (n=100)				
- Mean (SD)	413.17 (252.200)	0.0020 (0.003)	0.063 (0.017)	0.40 (0.094)
- GM (GSD)	346.73 (1.803)	0.0013 (2.094)	0.060 (1.333)	0.38 (1.267)
- Median (IQR)	375.57 (256.983)	0.0010 (0.0000)	0.062 (0.260)	0.39 (0.110)
- Max-Min	1482.46 -59.71	0.0133 - 0.0010	0.108 - 0.030	0.72 - 0.20
Total (n = 200)				
- Mean (SD)	431.23 (233.68)	0.0018 (0.002)	0.062 (0.015)	0.41 (0.015)
- GM (GSD)	380.18 (1.706)	0.0012 (1.949)	0.060 (1.285)	0.398 (1.276)
- Median (IQR)	393.40 (244.59)	0.0010 (0.0000)	0.061 (0.228)	0.40 (0.130)
- Max-Min	779.98 - 95.58	0.0138 - 0.0010	0.108 -0.030	0.88 - 0.20
Gas station locations in the pollution control zone (n = 137)				
- Mean (SD)	450.87 (260.571)	0.0015 (0.002)	0.064 (0.015)	0.41 (0.095)
- GM (GSD)	389.04 (1.778)	0.001 (1.733)	0.061 (1.276)	0.398 (1.256)
- Median (IQR)	406.93 (253.905)	0.0010 (0.0000)	0.063 (0.210)	0.40 (0.125)
- Max-Min	1482.46-59.71	0.0133 - 0.0010	0.108 - 0.030	0.72 - 0.22
Gas station locations outside the pollution control zone (n = 63)				
- Mean (SD)	388.51 (153.734)	0.0024 (0.003)	0.058 (0.014)	0.42 (0.120)
- GM (GSD)	354.81 (1.545)	0.0014 (2.3550)	0.056 (1.288)	0.41 (1.315)
- Median (IQR)	378.05 (219.660)	0.0010 (0.0000)	0.056 (0.230)	0.41 (0.150)
- Max-Min	779.98 - 95.58	0.0138 - 0.0010	0.090 - 0.035	0.88 - 0.20
Total (n = 200)				
- Mean (SD)	431.23 (233.686)	0.0018 (0.002)	0.062 (0.015)	0.41 (0.015)
- GM (GSD)	380.18 (1.706)	0.0012 (1.949)	0.060 (1.285)	0.398 (1.276)
- Median (IQR)	393.40 (244.59)	0.0010 (0.0000)	0.061 (0.228)	0.40 (0.130)
- Max-Min	1482.46 - 59.71	0.0138 - 0.0010	0.108 - 0.030	0.88 - 0.20

Table 4. Comparison of various factors and BTEX exposure in urine (benzene, toluene, ethylbenzene, xylene, BTEX). GM=geographic mean; GSD=geographic standard deviation

Factor	t,t-muconic acid (t,tMA) (μg/g creatinine)		Toluene in urine(TU) (mg/L)		Mandelic acid (MA) (g/g creatinine)		Methyl hippuric acid (MHA) (g/g creatinine)	
	GM(GSD)	*p*-value	GM(GSD)	*p*-value	data	GM(GSD)	*p*-value	GM(GSD)
Gender								
Male	416.87 (1.714)	0.048	0.0012 (1.854)	0.598	0.062 (1.271)	0.407	0.43 (1.297)	0.031
Female	354.81 (1.694)		0.0012 (1.995)		0.060 (1.294)		0.39 (1.259)	
Age (years)								
≤35	380.19 (1.694)	0.624	0.0012 (1.862)	0.322	0.060 (1.291)	0.533	0.40 (1.276)	0.166
> 35	363.08 (1.746)		0.0013 (2.183)		0.062 (1.271)		0.42 (1.274)	
BMI (kg/m^3)								
≤22.9	407.38 (1.663)	0.009	0.0012 (1.950)	0.995	0.060 (1.291)	0.986	0.42 (1.265)	0.005
>22.9	338.84 (1.734)		0.0012 (1.959)		0.060 (1.279)		0.38 (1.276)	
Marital status								
Single	398.11 (1.652)	0.049	0.0011 (1.782)	0.213	0.060 (1.276)	0.689	0.40 (1.265)	0.106
Partner/Married	363.08 (1.766)		0.0013 (2.051)		0.062 (1.291)		0.40 (1.288)	
Widowed/divorced/Separ.	257.04 (1.563)		0.0017 (2.838)		0.058 (1.358)		0.48 (1.208)	
Income (baht)								
≤10,000	331.13 (1.644)	0.051	0.0012 (1.901)	0.740	0.060 (1.274)	0.918	0.40 (1.274)	0.803
>10,001 – 20,000	407.38 (1.702)		0.0011 (1.770)		0.060 (1.279)		0.41 (1.276)	
>20,000	371.54 (1.462)		0.0010 (1.000)		0.062 (1.358)		0.41 (1.180)	
Smoking								
Do not smoke	380.19 (1.690)	0.696	0.0013 (2.109)	0.411	0.062 (1.256)	0.318	0.42 (1.276)	0.254
Currently smoke	371.54 (1.718)		0.0012 (1.871)		0.059 (1.300)		0.40 (1.274)	
Alcohol drinking								
Do not drink	398.11 (1.675)	0.245	0.0011 (1.750)	0.229	0.060 (1.276)	0.625	0.41 (1.291)	0.256
Currently drink	363.08 (1.734)		0.0013 (2.109)		0.060 (1.294)		0.40 (1.259)	
Personal sanitation								
Do not practice	371.54 (1.637)	0.756	0.0010 (1.000)	0.002	0.049 (1.318)	0.060	0.47 (1.169)	0.009
Practice	354.81 (1.531)		0.0015 (2.506)		0.057 (1.276)		0.39 (1.327)	
Wearing personal protective equipment								
Do not use / use 1-3 hours	380.19 (1.706)	0.581	0.0012 (1.884)	0.511	0.062 (1.282)	0.040	0.41 (1.274)	0.786
Use 4 hours or more	363.08 (1.718)		0.0013 (2.104)		0.058 (1.288)		0.40 (1.279)	
Work experience								
Less than 1 year	389.05 (1.626)	0.570	0.0011 (1.786)	0.481	0.060 (1.239)	0.962	0.39 (1.285)	0.237
Over 1 year	371.54 (1.738)		0.0012 (2.009)		0.060 (1.303)		0.41 (1.271)	

Work per day								
8 hours	380.19 (1.722)	0.690	0.0011 (1.578)	0.026	0.059 (1.279)	0.401	0.41 (1.262)	0.475
More than 8 hours	371.54 (1.702)		0.0013 (2.198)		0.062 (1.291)		0.40 (1.285)	
Overtime								
Less than 6 hs	363.08 (1.702)	0.007	0.0012 (2.004)	0.364	0.060 (1.291)	0.686	0.40 (1.285)	0.466
6 hs or more	489.78 (1.626)		0.0011 (1.574)		0.059 (1.259)		0.42 (1.211)	
Sleep duration								
Less than 8 hs	371.54 (1.698)	0.762	0.0012 (1.897)	0.709	0.062 (1.300)	0.162	0.41 (1.262)	0.844
Over 8 hours	380.19 (1.722)		0.0012 (2.018)		0.059 (1.268)		0.40 (1.294)	
Work Area								
At fuel dispenser	407.38 (1.596)	0.054	0.0011 (1.799)	0.301	0.060 (1.233)	0.625	0.42 (1.279)	0.110
Away from fuel dispenser	349.74 (1.803)		0.0013 (2.094)		0.060 (1.334)		0.39 (1.268)	
Gasoline station location								
Inside control zone (in city)	389.05 (1.778)	0.343	0.0011 (1.734)	0.031	0.062 (1.276)	0.009	0.40 (1.256)	0.872
Outside control zone	354.81 (1.545)		0.0014 (2.355)		0.056 (1.288)		0.41 (1.315)	
Number of trucks refueled								
≤ 10 cars	371.54 (1.718)	0.239	0.0013 (2.042)	0.058	0.060 (1.288)	0.513	0.40 (1.268)	0.146
> 10 cars	407.38 (1.656)		0.0011 (1.496)		0.059 (1.274)		0.43 (1.306)	

mass index were related to MHA. Details are shown in Table 4.

DISCUSSION

Our finding that the majority of fuel station employees (68.5%) were female contradicts the findings of Tunsaringkasm et al [35], who studied the characteristics of gas station attendants in Bangkok and found most to be male workers, as well as the findings of a study conducted in Brazil, which found that the vast majority of employees were male (90.5%) [19].

The average age of participants in the present study, 30.25 (± 11.015) years, is in better agreement with the results of a previous study [35], which investigated the characteristics of gas station attendants in Bangkok and discovered that the average age was 29.8 years, as well as the results of similar research conducted in Indonesia [36] and Brazil [19]. In the present study, 52.5% of workers had completed secondary school, which is consistent with the results of a study conducted in Brazil, which found that 50.2% had graduated with secondary education [19].

Our finding that around half of the gas station employees in this sample were smokers or alcohol drinkers is similar to previous findings in Thailand [35] and highlights potential factors that increase body exposure to BTEX. Similarly, *Chambers* et al [37] stated that smoking was a crucial source of benzene, styrene, toluene, and xylene exposure in US citizens. Our results could not identify a link between smoking and BTEX compound levels, contradicting a study by *Lovrglio* et al [38] which evaluated benzene exposure in 137 people. The estimated benzene exposure in smokers was 10% higher than that in non-smokers [39]. The blood levels of BTEX in 151 fuel pump attendants exposed to BTEX were statistically significantly higher after an average of five cigarettes smoked within five hours than those in non-smokers [40].

Biological monitoring and assessment

We assessed exposure to urinary t, t MA; TU; MA; and MHA in an exposed group and a non-exposed group after they finished their shifts, according to the recommendations of the ACGIH [32]. The average concentration of t, t MA was higher in employees working at the fuel dispenser than in those working away from it. These levels were lower than those of the *Chaiklieng* et al. [6] study, with 25% of t, t MA exceeding the standard µg/g creatinine. The *Geraldino* et al. [26] study conducted in Brazil found t, t MA to be higher in fuel station workers than in office workers, with lower levels still found in convenience store workers. The results of this study were consistent with research on workers at gas stations in Iran with a low risk of TU exposure [8, 32]. Therefore, the regular health surveillance of fuel service attendants should include analyses of BTEX exposure.

The comparison of various factors with levels of urinary t, t muconic acid; toluene; mandelic acid; and methyl hippuric acid. A range of factors were found to be significantly linked to higher t, t MA; TU; MA; an MHA levels, as discussed below. Near or away from the fuel dispenser. The concentrations of t, t MA; TU; MA; and MHA were statistically similar in employees working at or away from the fuel dispenser, although the comparison was close to significance (p=0.054). This finding is inconsistent with that of *Chaiklieng* et al. [6], who found that the risk was 93.7 times higher in the exposed group than in a control group. Differences in the study settings and designs may account for this lack of agreement.

Gas station workers working near to or away from the fuel dispenser are exposed to a variety of solvents at different levels [10]. Employees working outside the dispenser area, such as in office employees, coffee shop staff, and convenience store workers, are often in a closed building environment, but the high frequency of door opening and closure in these environments allows BTEX compounds to enter. Employees in other areas, such as car wash facilities, were further away from the source of the emissions but were still at risk of BTEX due to the spread of vapor from the emissions [26].

Employees engaged in fueling service activities were unavoidably exposed to fuel sources, although operators outside the dispenser were more likely to be exposed to BTEX. *Fakhrinnur* et al. [36] found that exposed employees' urine t, t MA levels were significantly higher than those of office workers. This result was similar to the findings of *the Geraldino* et al. [26] study, which discovered that the exposed group had statistically significantly higher t, t MA levels than the control group.

Within and outside of the pollution control zone, Rayong province districts are currently divided into two groups: those within and those outside of the pollution control zone [30]. While the concentrations of t, t MA were higher within than outside of the pollution control zone, the difference was not quite statistically significant (p-value = 0.054). The concentrations of urine MA and TU in individuals within the pollution control zone, however, were significantly higher than in those outside the zone. This finding is inconsistent with the study of *Tunsaringkarn* et al. [14].

Personal factors

Gender. In this study, males had higher t, t MA levels than females, which is inconsistent with the results *Fakhrinnur* et al. [36], who found that sex was not associated with urinary t, t MA levels. In addition, the present study found higher MHA in males than in females, consistent with the findings of *Ernstgrd* et al. [41].

Marital Status. The relationship between marital status and work has attracted interest from researchers and national policy leaders [42]. Research has reported that single parents tend to work more than married couples to increase their income and due to family responsibilities [41]. Thus, it is important to include single status as well. In addition, *McManus* et al. [43] found that family income might vary depending on marital status. This study found that a single status factor affecting t, t MA arises because single people had more free time and needed income to take care of family members. These factors encouraged them to complete more work over long working hours, putting them at greater risk of exposure to benzene in the body. However, this study not only examined the metabolites of benzene as t, t MA but also looked at the levels of toluene in urine; the metabolites of ethyl benzene and xylene were found to be mandelic acid and methyl hippuric acid in urine.

Body mass index (BMI). The results showed that low body mass index (BMI) was a factor affecting t, t MA and MHA in urine. The case study group had an average BMI of just over 23 kg/m^3, with 44% considered overweight/obese [44, 45, 46]. This finding is in agreement with previous research [44] carried out in gas stations in Bangkok and suggests that employees should receive healthcare promotions encouraging weight control and the prevention of non-communicable diseases [45, 47]. The present findings show that a BMI within the standard range was a factor in the urinary elevation of t, t MA and MHA. It is possible that participants with a standard body mass index and without obesity were able to move their body and perform more activities in fuel services than those with a high BMI, therefore increasing their chance of accumulating benzene and xylene in their bodies. The results of this study are consistent with those of *Fakhrinnur* et al. [36], indicating that individuals within the standard BMI range were more likely to have increased urinary t, t MA levels, although the results were not statistically significant.

Work history factors

Working hours per day and overtime. Our results show that most employees in our sample worked more than 40 hours per week and over 6 days per week, exceeding the limits set by the Labor Protection Act, 1998, section [28]. Employees should prioritize adequate sleep and rest to reduce their risk of toluene exposure. The participants worked more hours per day than those assessed in a Brazilian study, which found that most fuel service employees worked 6 hours per day, or 8 hours per day for those working overtime [19].

In this study, a higher number of working hours per day contributed to higher urinary toluene exposure, while working six or more hours of overtime per week contributed to higher t, t MA levels. A previous report found that longer working hours are associated with higher BTEX exposure and an increased risk of benzene in the body [49], among other findings. *Fakhrinnur* et al. [36] also found that the duration of the fueling service affected the urinary t, t MA levels (p=0.000), suggesting that workers should have reasonable break periods to limit their BTEX absorption [12].

In this study, the time taken to refuel each vehicle was not evaluated, but the number of cars refueled per day was recorded. The latter was not a significant factor in the urinary levels of t, t MA, TU, MA, and MHA. As benzene is known to be a carcinogen [16], developing strategies to assist employees in reducing benzene exposure in fuel is critical, since this study found that the duration of exposure to chemicals may be a factor associated with chemical accumulation in employees. The duration of chemical exposure may also be a factor in chemical accumulation in living organisms.

Personal hygiene. We found that inappropriate personal hygiene practices influenced urinary TU and MHA levels. These habits may increase BTEX exposure due to the unintentional contamination of water and food, as well as through dermal benzene exposure, which can occur if fuel spills on the employee's skin [50]. Therefore, fuel service employees working both within and away from the dispenser area should be advised about methods to maintain personal cleanliness, such as washing hands before eating or drinking water, avoiding drinking water or eating in the work area, and not repeatedly wearing used clothes. The findings of this study emphasize the importance of PPE and establish a standard for personal hygiene guidance based on the research of *Wiwanitkit* et al. [25]. Additionally, the BTEX exposure level may be reduced by providing sinks near the fueling area where employees can wash their hands.

Wearing personal protective equipment

The results revealed that wearing PPE caused urine MA levels to rise more than they rose when not wearing PPE (p=0.040). As a result, employers should advise their staff to wear a mask at all times while working to avoid BTEX exposure, including exposure to ethylbenzene, consistent with the results of *Chang* et al. [21], who found that the concentration of solvent was different in air outside compared to inside the mask.

The results revealed that many employees performed multitasking operations while wearing PPE. Most (84%) employees wore masks, while 49% wore sneakers rather than protective boots. This study collected data during the coronavirus 19 pandemic, so the study group wore fabric masks more than usual, and these may have been ineffective against BTEX exposure. The study collected samples between October and December 2020, during which Thailand launched a campaign for the universal wearing of PPE. This is consistent with research carried out in other areas of Thailand [6] that studied the relationship between the use of personal protective equipment and t, t MA in gas station employees in Khonhaen province before the coronavirus pandemic.

The findings of this study are inconsistent with those of a study conducted in Brazil, which found that almost all employees wore boots, while only 6.3% wore short-sleeved t-shirts and trousers [19]. According to one study, using all forms of PPE during all work shifts reduced BTEX contamination [46]. Since BTEX, particularly benzene, is a highly hazardous chemical, employees should take precautions while performing work-related tasks. In addition to the control of BTEX using various technologies, exposure reduction could be accomplished by ensuring that PPE is worn to reduce BTEX exposure and transmission into the body [51].

CONCLUSION AND RECOMMENDATIONS

The results of this study revealed statistically significant biomarkers influencing the levels of t, t MA; TU; MA; and MHA in urine. The recommendations of this study are that employers should provide their employees with suitable PPE, check regularly to ensure that it is worn, and strongly encourage employees to take care of their sanitation. Employees should take appropriate breaks and days off to minimize their exposure to BTEX. Future research should investigate strategies to prevent BTEX exposure among employees working as fuel service attendants in EEC areas.

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Authors' contributions

Anamai Thetkathuek, Chan Pattama Polyong, and Jintana Sirivarasai decided to conduct this study and *collected data. Anamai Thetkathuek wrote the first draft of the manuscript. Anamai Thetkathuek and Wanlop Jaidee planned the design of the study. Wanlop Jaidee also helped with the research methodology. All authors read and approved the final manuscript.*

Disclosure statement

All authors declare that they have no competing interests in this work.

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ORIGINAL ARTICLE

INVESTIGATION OF KIDNEY FUNCTION CHANGES IN SEA SALT WORKERS DURING HARVEST SEASON IN THAILAND

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ABSTRACT

Background. Occupational factors have previously been mentioned as contributing to decreased kidney function and the development of chronic kidney disease of unknown cause. Sea salt workers are one of the occupations facing high outdoor temperatures and a highly, intensive workload.

Objective. The purpose of the study was to examine whether the kidney function of sea salt workers at the beginning of the harvest season differs from kidney function at the end of the harvest season and to identify factors that can predict the change of kidney function.

Material and methods. Data were collected from salt workers (n=50) who were between 18–60 years of age without hypertension, diabetes, and kidney disease in Samut Sakhon province, Thailand. Urine specific gravity (USG) was used for hydration status and the estimated glomerular filter rate (eGFR) was used to measure kidney function. The mixed model was used to find differences over the harvest season and prediction of factors.

Results. On average, the eGFR was estimated to decrease by 15.2 ml/min/1.73 m2 over the harvest season. The decline in eGFR of sea salt workers with moderate and heavy workloads were significantly faster than their light workload counterparts after controlling for other covariates. Similarly, dehydration (USG $\geq$ 1.030) significantly accelerated the rate of kidney function loss.

Conclusions. Our study confirmed exposure to heat over the harvest season leads to decreased eGFR in sea salt workers. The rate of change of eGFR could be predicted by workload and hydration status. Workers with dehydration who performed medium to heavy workloads in farms showed faster kidney function decline than those who performed light workload.

Key words: *heat exposure, agricultural work, kidney function change*

INTRODUCTION

Chronic kidney disease (CKD) is a global disease and one of the three causes of death that has increased in the last 20 years [1, 2]. Common causes of CKD are hypertension, diabetes, and obesity, among others [3]. However, CKD of unknown etiology or CKDu has emerged and received considerable attention in many countries particularly in coastal areas [4]. Kidney biopsies in male patients with CKDu were studied, and it was noted that the specimens were not similar to other normal kidney diseases [5]. The causes of CKDu are not yet established, but research has suggested it may relate to occupational factors, such as prolonged heat exposure, heavy workload, and dehydration [6]. Prolonged heat exposure can cause insensible loss of water and electrolytes in the body, which can lead to substantial fluid deficits and vasoconstriction, which are associated with kidney injury. High physical workload with repeated dehydration can contribute to muscle breakdown and rhabdomyolysis, causing hyperuricemia, which may lead to glomerular hypertension and renal tubular injury [7]. Dehydration is related to kidney function, with an increase in fluid intake associates with a lower risk of CKD and slower kidney function decline [8].

Reports of CKDu among outdoor workers, farmers, and agricultural workers have increased in Thailand and other countries (e.g., Egypt, Sri Lanka, Bangladesh, India, Mexico, Guatemala, El Salvador, Nicaragua, and Costa Rica [9, 10, 11]. CKDu is commonly found among those working at sea level, such as sugarcane workers, whereas the rate of CKDu is lower among those working at high altitudes, such as coffee plantations [10, 11]. The highest prevalence rate of CKDu occurred in sugarcane workers [11], while the

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rate is growing for other agricultural workers, such as corn and cotton workers, as well as in industrial settings, such as construction sites and mines [11]. Agricultural workers face high ambient temperatures in the working environment as their tasks are mostly outdoors in direct sunlight. They are more vulnerable to heat stress, often combined with dehydration. The heat load in the human body is influenced by direct exposure to the sun and high physical exertion. To maintain normal body temperature, sweat is released. This can lead to loss of water and electrolytes, such as sodium and chloride, which may cause acute kidney injury or chronic decreased kidney function. Several studies in the sugarcane farming field found relationships between heat exposure, dehydration, and kidney health. A study from El Salvador reported a change in the estimated glomerular filtration rate (eGFR) over the shift day consistent with repeated dehydration from a high workload in a hot and humid environment [12]. A study from Nicaragua found the eGFR decreased by 10 ml/min/1.73 m^2 in nine weeks during harvest season and decreased in eGFR associated with increase in serum creatinine which may be caused by dehydration [13]. Another study in Nicaragua showed the eGFR decreased during the harvest season in various job tasks among sugarcane workers [14].

Thailand is a country located in the tropical zone and numerous people in Thailand are engaged in agriculture. A study of health impacts of climate change on occupational health and productivity found a temperature of 34.6°C in a vegetable field, which is classified as an extreme cause for caution or as a danger for heat-related illness [15]. A temperatures of 30.6 °C was measured in a sugarcane field [16], and the average temperature of a sea salt working area was found to be 33.83 ± 0.95°C with a high of 35.6°C [17]. A study about occupational heat stress and kidney disease in Thailand showed a high prevalence of heat stress in those with lower income and education levels, and heat stress was more common in men [18]. The harvest season in Thailand starts from January to May when the weather is hot and dry. Sea salt workers cannot harvest in other seasons because sea salt production needs sunlight to evaporate the seawater, which is then left to dry naturally. Sea salt workers work outdoors with intense workloads, consecutively putting in 2–10 hours a day for consecutive days. No shade is provided, and their payment depends on how much they work.

To date, only a few studies have investigated heat exposure, dehydration, and kidney health among agricultural workers in Thailand. One study reported that sugarcane workers were at high risk for heat stress and heat-related symptoms and concluded that an extremely hot environment with a high workload was associated with acute health effects [19]. Another study showed that sugarcane workers who did not drink enough and faced heat stress were more likely to have acute kidney injury [20]. A study on sea salt workers found a strong positive correlation between temperature and a hydration biomarker (i.e., urine specific gravity) [17]. To our knowledge, previous studies have not investigated the change of kidney function among sea salt workers in Thailand. Given that sea salt workers are vulnerable to extreme heat exposure that may lead to kidney disease, it is important to investigate whether Thai sea salt workers are facing risks from their work.

The present study aimed to examine the change in kidney function measured by the eGFR among sea salt workers in Thailand from the beginning of the harvest season to the late season. We hypothesized that the eGFR significantly worsened over the period of approximately three months. Additionally, we explored whether demographic characteristics (i.e., age, gender, and BMI), hydration status (measured by the urine specific gravity), and work-related factors (i.e., the amount of workload, working experience, and hours spent working) could explain the individual changes in kidney function.

MATERIAL AND METHODS

Study design, population and heat exposure

This longitudinal study was conducted during the harvest season of a sea salt farm February–May 2020. A two-time measurement was performed after work. The first examination was held at the beginning of the harvest season in February 2020 (Time 1) and then repeated three months later, near the end of the harvest season in May 2020 (Time 2). More information regarding Time 1 can be found in paper *Luangwilai* et al [21]. Data were collected at Samut Sakhon, Thailand where a sea salt learning center has been established. The chairman of the center provided a list of sea salt workers and the protocol of this study was described to the sea salt workers by our research team. Eighty sea salt workers in this area were screened. Men and women aged between 18–60 years who had worked on sea salt farms for at least one year were randomly selected. Thirty participants who reported having hypertension, diabetes, or kidney disease and not willing to participated were excluded, such that there were 50 sea salt workers in Time 1. Seven participants were lost to follow-up, resulting in 43 sea salt workers in Time 2. The workers in this study area were separated into five tasks including farm owners, deliverers, extractors, pilers, and scoopers (Figure 1). Farm owners usually drive a compactor, check the sea level in each pond, and verify the salinity of salt. Extractors are generally both male and female workers who break the salt out of the ground using a wooden

Farm owners

Extractors

Pilers

Scoopers

Deliverers

Figure 1**.** Working tasks of sea salt workers

tool. Pilers drag the salt into a triangular pile. Scoopers help the delivery persons to scoop the salt into the carts. Lastly, workers who serve as deliverers are men who scoop the salt from the pile into the cart and shove the cart to the storeroom. They work as a team and do not switch roles. Concerning the workload [22], deliverers and extractors perform "heavy work" (> 350 kcal/hr.), pilers and scoopers perform "moderate work" (200–350 kcal/hr.), and farm owners perform "light work" (<200 kcal/hr.).

Heat exposure was measured by the researchers using a standardized wet-bulb globe temperature (WBGT) monitor, model ISO 7243 (3M QUEST temp 32^{o}C), following the guidelines. WBGT data collection on two observation days at each time point, that is, Time 1 and Time 2, started when the workers began to work outdoors on the sea salt farms. The monitor was set up as close to the workers as possible and calibrated before use. Digital data were recorded every hour, and five parameters were measured, namely, dry-bulb temperature, wet-bulb temperature, globe-bulb temperature, WBGT outdoor, and relative humidity.

This study was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Thailand (COA No. 035/2020). All participants provided their informed consent before participation.

Questionnaire and urine collection after work shift

Face-to-face interviews by the researcher team using validated questionnaires were performed after work at the sea salt learning center. At Time1, the questionnaire included questions on personal characteristics, including age, alcohol consumption, and smoking. Exposure factors and work-related factors, including water and other beverage intakes, frequency of urination, work experience on sea salt farms, and hours spent working were asked at Time 1 and Time 2.

After the work shift, each participant provided 20–50 ml. of mid-steam urine in standard polypropylene specimen containers. For the urinalysis, the samples were sent immediately to a certified laboratory and the urine specific gravity (USG) was recorded, which indicates the hydration status. USG $\geq$ 1.030 was marked as dehydration [23].

Kidney function

Blood pressure, pulse rate, and body weight were measured before blood collection at each time point after the sea salt workers finished working. Two, three ml samples of blood was drawn by a medical technician from a Tambon health promoting center to test the levels of serum creatinine (Scr). Blood samples were packed on ice and immediately sent to a certified laboratory. *Jaffe's* kinetic method was used for Scr kidney biomarkers and the eGFR in this study was calculated using the CKD-EPI equation [24].

Data analysis

We used descriptive statistics including frequencies, percentages, averages, and standard deviation. Moreover, we employed a series of *Wilcoxon* signed-rank tests to compare the biomarkers of sea salt workers between the two time points. To test whether the eGFR changed over time, we implemented a random intercept mixed model with the restricted maximum likelihood (REML) and the *Kenward–Roger* correction for a small sample size. The *Time* variable, indicating whether the outcome was collected near the beginning of the harvest season (Time 1, coded as 0) or the end of the harvest season (Time 2, coded as 1), was included as a sole fixed effect predictor. The mixed model was chosen because it allowed individuals to have different levels of kidney function values (beyond the random error). The mixed model also properly handled missing data (assuming the missing data mechanism was *missing at random*). That is, the mixed model could incorporate available information from a case with missing data on Time 2 without removing the whole case (i.e., removing both Time 1 and 2 observations) from the model. Finally, there was an assessment of what predicted patterns of change.

To investigate whether the demographic characteristics, hydration status, and work-related factors could explain interindividual differences in changes in the eGFR, we fitted a random intercept mixed model by regressing the eGFR on time and other fixed-effect predictors (i.e., age, gender, BMI, hydration status, workload, work experience, and hours spent working). The two-way interactions between the

Table 1. Characteristics of sea salt workers at two study time points in a sea salt farm in Thailand

Factors	Study Time Points	
	Beginning of the Harvest Season Mean ± SD, Median, n (%)	End of Harvest Season Mean ± SD, Median, n (%)
Participants (n)	50	43
Gender		
Male	29 (58.0)	24 (55.8)
Female	21 (42.0)	19 (44.2)
Age	47.16 ± 11.28, 51.00	49.51 ± 10.05, 53.00
< 29	6 (12.0)	3 (6.0)
30–39	5 (10.0)	3 (6.0)
40–49	12 (24.0)	(20.0)
≥ 50	27 (54.0)	27 (54.0)
Weight (kg.)	68.64 ± 14.96, 65.50	65.88 ± 13.13, 65.00
Height (cm.)	164.30 ± 9.47, 163.50	162.72 ± 8.19, 163.0
BMI	25.57 ± 5.76, 25.54	25.53 ± 5.70, 24.84
Underweight (< 18.50)	3 (6.0)	3 (7.0)
Normal range (18.50–24.99)	22 (44.0)	20 (46.5)
Overweight (25–29.99)	15 (30.0)	11 (25.6)
Obesity (≥ 30)	10 (20.0)	9 (20.9)
Systolic BP (mm Hg.)	138.54 ± 21.61, 134.0	135.40 ± 15.80133.0
Diastolic BP (mm Hg.)	87.98 ± 15.01, 87.0	83.0 ± 9.16, 82.0
Pulse rate (per minute)	86.92 ± 13.79, 83.50	86.42 ± 13.76, 83.0
Herbal supplement		
Did Not Use	47 (94.0)	40 (93.0)
Uses	3 (6.0)	3 (7.0)
Alcohol drinking status		
Did Not Drink	30 (60.0)	27 (62.8)
Drank	20 (40.0)	16 (37.2)
Smoking status		
Did Not Smoke	34 (68.0)	28 (65.1)
Smoke	16 (32.0)	15 (34.9)
Water intake, ml.	1530 ± 665.55, 1500	1883.72 ± 705.73, 2000
Beverage intake		
Did not drink	15 (30.0)	10 (23.3)
Did drink	35 (70.0)	33 (76.7)
Energy drink	15 (30.0)	10 (23.3)
Electrolyte drink	5 (10.0)	1 (2.3)
Sugary drink	9 (18.0)	7 (16.3)
Coffee	6 (12.0)	5 (11.6)
Combined	-	10 (23.2)
Hours spent working	5.16 ± 2.27, 5.0	5.07 ± 2.32, 4.00

time and each predictor (e.g., *Time x Workload*) were separately added to the mixed model one by one, to see whether the predictor could predict the rate of change in the eGFR. The final model contained statistically significant predictors and lower terms of significant interaction(s). To gauge the robustness of our findings, we also examined several other configurations. All data analyses were conducted using SPSS version 25 for Windows.

RESULTS

Characteristics and work-related factors

Characteristics of sea salt workers at the two study time points are shown in Table 1. There were more male participants than female in the study. The mean age of sea salt workers in Time 1 and Time 2 was 47.16 and 49.51 years, respectively. The participants BMI was in the normal range. Most participants reported they did not use herbal supplements, around half smoked and drank alcohol. The average water intake reported at the beginning of the harvest season was about 1.5 L and increased slightly to 1.8 L in the late harvest season. Besides water, more than 70% of participants drank other beverages (e.g., energy drink). Participants spent about five hours working, on average.

Heat exposure, dehydration and kidney function

The heat exposure measurements using WBGT at two time points are presented in Figure 2. All parameters increased during the late harvest season. On average, the outdoor WBGT was estimated to increase by 4.62°C, from 28.84°C at the beginning of the harvest season to 33.46°C during the late harvest season.

The biomarkers of sea salt workers are presented in Table 2. The mean of the USG was 1.024 at Time 1 and 1.027 at Time 2, with a significantly difference over the season (p-value = 0.015). In this study, the cut point for dehydration was USG ≥ 1.030; 58% of the participants were dehydrated after work at Time1 and 60% were dehydrated at Time 2. Serum creatinine increased substantially from 0.95 mg/dL to 1.098 mg/dL over the season (p-value <0.001). The eGFR decreased significantly from 86.56 ml/min/1.73 m^2 at Time 1 to 71.91 ml/min/1.73 m^2 at Time 2 (p-value < 0.001).

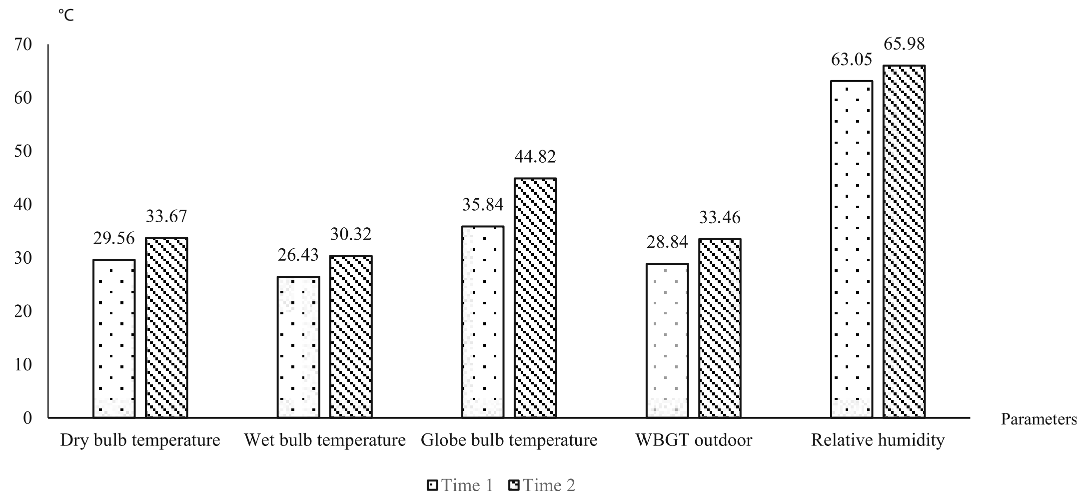

Figure 2. Wet bulb globe measurement between two time measurements

Table 2. Biomarkers in sea salt workers at two study time points in thailand

Study time points	Beginning of the harvest season Mean ± SD, Median n (%)	End of harvest season Mean ± SD, Median n (%)	Comparison of two seasons[a] (*P*-value)
Participants (n)	50	43	
Urine biomarker			
pH	5.26 ± 0.52, 5.00	5.14±0.35, 5.00	0.153
Urine specific gravity	1.024 ± 0.007, 1.025	1.027 ± 0.006,1.03	0.015*
Serum biomarker			
Serum creatinine (mg/dL)	0.95 ± 0.20, 0.9	1.098 ± 0.22, 1.10	<0.001*
Kidney Function			
eGFR-EPI, ml/min/1.73 m^2	86.56 ± 16.47, 90.60	71.91 ± 16.92, 69.60	<0.001*

Notes. [a] Wilcoxon Signed Rank Test, *Association significant at p-value < 0.05

Kidney function

Change Over Time. The result from the random intercept mixed model with time as a single predictor showed a significant decline in the eGFR from Time 1 to Time 2, b = -15.2, $t(44.135) = -5.687$ $p < .001$, 95% CIs [-20.547, -9.796], supporting our hypothesis. Specifically, on average, the eGFR was estimated to decrease by 15.2 ml/min/1.73 m^2, that is, from 86.6 ml/min/1.73 m^2 at the beginning of the harvest season (SD=11 ml/min/1.73 m^2) to 71.4 ml/min/1.73 m^2 at the end of the harvest season.

Predictors of differences in change in the kidney function.

As shown in Table 3, all predictors were included (without interaction effects) to predict the eGFR (Model 1). This model showed that time was strongly predictive of the eGFR after adjusting for other predictors. One interaction at a time was then introduced to the model. Among the two-way interactions, it was found that only *Time x BMI*, *Time x USG*, and *Time x Workload* were significant (Models 2–4). However, only *Time x USG* was significant when including all three interactions in the same model (Model 5). After excluding *Time x BMI*, both *Time x USG* and *Time x Workload* were significant (Model 6). The nonsignificant predictors were further excluded. The final random intercept mixed model (Model 7) included time, age, workload, hydration status, and two interactions (i.e., *Time x USG* and *Time x Workload*). This model suggested that the rate of change in the eGFR could be predicted by workload and hydration status after controlling for other predictors, meaning that the kidney function of sea salt workers with moderate and heavy workloads declined significantly faster than their light workload counterparts with similar characteristics. Similarly, dehydration (USG ≥ 1.030) significantly accelerated the rate of kidney function loss. Additionally, the result showed that older people had significantly lower eGFR compared to their younger peers with similar characteristics.

Additional analyses

It was found that one sea salt worker who was a farm owner had a noticeable increase in eGFR from 75.7 at Time 1 to 136.5 at Time 2 (Figure 3), meaning that the kidney function of the worker greatly improved. Although there was no evidence of any error in the laboratory result or the coding procedure, we examined the robustness of the findings by removing the eGFR value (i.e., 136.5) and the corresponding value of serum creatinine (i.e., 0.5) that was used in the calculation of the eGFR, from the data. The mixed model (using the same predictors as Model 7) indicated that the interaction between time and workload was marginally significant (p=0.098) and the interaction between time and hydration status was significant (p=0.039) after controlling for age. Moreover, when serum creatinine was included as the dependent variable instead of the eGFR, the

Table 3. Results of random intercept mixed models predicting the eGFR

Variable	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
	b	*b*	*b*	*b*	*b*	*b*	*b*
Intercept	94.05***	95.98**	89.94**	86.96***	85.73***	82.19***	77.84***
Time (baseline)	-13.46***	-20.95	-4.54	-0.68	0.31	9.39	8.54
Gender (male)	-0.59	1.59	1.24	2.11	1.22	1.42	
Age	1.92**	-0.57*	-0.54*	-0.57**	-0.51*	-0.52*	-0.49**
BMI (< 25)	-5.19	-11.14	-5.06	-5.12	-8.77	-5.00	
USG (< 1.030)	-2.71	-1.08	4.92	-1.91	6.41	6.29	7.59
Workload (light)	-5.43	-4.75	-4.03	2.44	2.47	4.34	6.68
Working experience	-0.01	-0.01	-0.05	-0.02	-0.06	-0.06	
Hours spent working	0.59	0.14	0.34	0.79	0.21	0.53	
Time x BMI		13.75*			8.68		
Time x USG			-16.00*		-15.41*	-16.87**	-17.05**
Time x Workload				-16.00*	-12.48	-16.94*	-16.28*
Residual Variance	171.56***	145.62***	155.23***	151.34***	125.09***	131.21**	128.03***
Intercept Variance	87.82	107.27*	93.66*	103.84*	117.55*	113.22*	109.64*

Notes. Total N = 93 (N = 50 from Time 1 and N = 43 from Time 2). Age, working experience, and hours spent working were centered at their means (i.e., 47 years old, 16 years, and 5 hours, respectively). A group in brackets refer to the reference group (coded as 0). *p < .05, **p < .01, ***p < .001.

interactions between time and workload (p=0.049) and time and hydration status (p =0.039) remained statistically significant after controlling for age. We concluded that the findings from additional analyses were consistent with the original results, showing that the kidney function of sea salt workers with a light workload or without a sign of dehydration tended to decline at a slower rate.

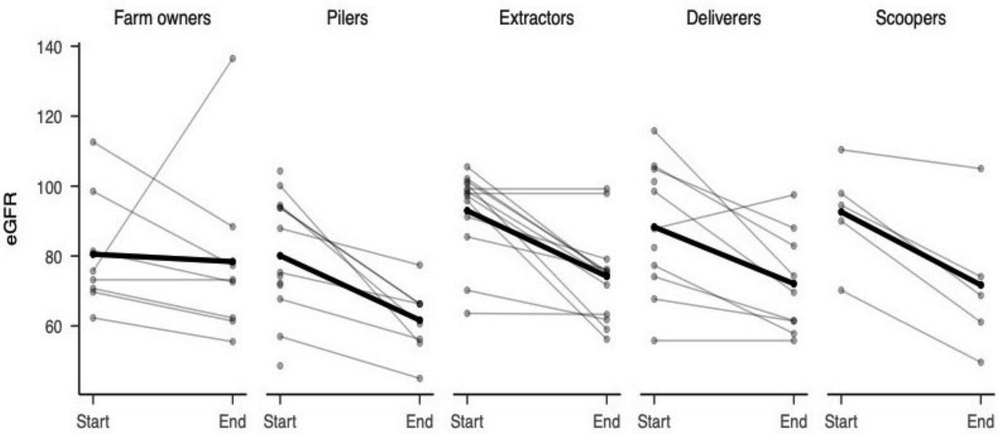

Figure 3. Change of eGFR for all 50 participants grouped by position. Black lines represent the averages of the eGFR across participants

DISCUSSION

Sea salt workers who faced heat exposure over the harvest season can have changes in kidney function. This study aimed to examine the changes in kidney function and investigate factors that explained these individual changes in kidney function from the beginning of the harvest season to close to the end of the harvest season. We found that sea salt workers in Thailand were at the risk of rapid kidney function decline. On average, the eGFR decreased by 15.2 ml/min/1.73 m^2 in three months, which was higher than our expected outcome and higher than found in previous studies. For example, a study from Nicaragua on 51 cane cutters found the average eGFR decreased by 3 ml/min/1.73m^2 over five months [25] and another study in the same country found that the mean eGFR of 29 sugarcane workers decreased by 10 ml/min/1.73m^2 in nine weeks [13]. We were not aware of any relevant studies that found the reduction in eGFR over 10 ml/min/1.73m^2 in three months. This may be because of the characteristics of job tasking of sea salt workers, which include human labor and high-intensity work in combination with the working environment factors such as heat exposure, open land fields, lack of shade, and the sunlight needed for sea salt production which contrasts with heat prevention guidelines [26]. Another reason maybe because age is associated with kidney function. More than half of our participants were older than 50 years. Yet, our participants still had lower average eGFR compared to sugarcane cutters in Thailand with similar age range [20]. Some studies revealed that NSAIDs and herbal supplement were associated with a decrease in eGFR [6], but this study showed only approximately 6% of sea salt workers took paracetamol for pain release and 7% took herbal supplement. Future investigations through follow-up or cohort studies will provide clearer information as this study was conducted only over a harvest season in a salt farm.

The rate of change in the eGFR could be predicted by workload and hydration status. Workers with dehydration who performed moderated and heavy workload showed a faster kidney decline. Working as a sea salt worker required strenuous labor in a hot and humid environment. Sea salt workers perform outdoor work for about two to 10 hours a day, depending on how many salt ponds are ready to be harvested, with an average of about five hours spent working. The sea salt harvest season period is between January to May every year. Our assessment of heat exposure showed that the average WBGT was estimated to increase by 4.62°C, from 28.84°C at the beginning of the harvest season to 33.46°C at the end of the harvest season. At the beginning of the harvest season, all sea salt workers did not work in extreme conditions according to the Thailand regulations in place for working in hot conditions. However, workers with moderate and heavy workloads worked in conditions that were too hot in the late harvest season (workers with light, moderate, and heavy workloads should not work in temperatures over 34°C, 32°C, and 30°C, respectively) [27]. Recent literature among 25 male paddy farmers stated that when engaging in physical work or high-intensity manual work, uric acid increased after muscle breakdown, following which kidney injury could happen through hyperuricemia [28].

It was hypothesized that working in hot and humid conditions could lead to dehydration. A study conducted in the summer in Japan reported 42% of construction workers were dehydrated after work [29]. In our study, we found that dehydration could predict kidney decline. We examined the USG for hydration status. USG significantly increased over the season (Table 2) and 58% of participants were dehydrated after work at the beginning of the harvest season while up to 60% were dehydrated at the end of the harvest season. This is about 10% higher than what was found in a recent study among sugarcane workers in Thailand [16]. Sea salt workers engage in intense manual labor in direct sunlight. Workers in high heat can increase their core temperature, then observe an increase in evaporation through the skin to cool down their body. In that state, water loss from evaporation and inadequate water intake can cause dehydration. American conference of government industrial hygienist (ACGIH) recommended consuming about 235 ml. of water every 20 min during work in hot conditions [29, 30]. Sea salt workers reported a mean water intake of about 1.5 L at the beginning of the harvest season and 1.8 L during late harvest season, which was lower than the recommendation. This may be because of longer distances to toilets and payments which affect the entire team. This encourages them to drink less water and take less rest periods similar to a previous study among 97 crop workers [31]. Studied in sugarcane workers in hot environments with low water intake had inferior kidney biomarkers compared to those who drank enough water [20]. Sugarcane cutters from El Salvador reported temperatures of 34–36°C in the workday, whereas their water intake was 0.8 L per hour. The mean USG increased after work, and eGFR was reduced in 14% of workers. This study concluded that dehydration from high workloads in hot environments is an important factor for kidney function decline [32]. Field workers were at greater risk of kidney function decline over the harvest season compared to non-field workers, which is associated with reduced kidney function [33]. Physical work with heat exposure and dehydration is a common predictor for kidney function decline [34]. Previous studies among paddy farm workers also mentioned that the pathophysiological pathway of dehydration could lead to kidney function decline. Prolonged heat stress exposure in physical jobs with repeated dehydration can cause acute kidney injury, developing into CKDu [28].

This study has several limitations. One major limitation is that only one research area was selected, so that the sample size was small. Second, heat measurement was assessed by WBGT for only two days at each time point. Furthermore, heat exposure can be assessed by standard tools, but it would be more beneficial to measure individual body core temperatures in future studies. Third, data was collected from only two time points in this study, which is a limited statistical technique. Lastly, most of participants in this study were over 50 years. Our results cannot be generalized to younger populations. Prospective studies should collect data for more than two times points with continuous monitoring environment exposure and biomarkers. Investigation about causal factors which could be associated with kidney function declined among high heat exposure workers in Thailand are the need for more research.

CONCLUSIONS

This study found that exposure to heat over the harvest season in sea salt workers leads to decreased eGFR. The rate of change of eGFR could be predicted by the workload and hydration status. Workers with dehydration who performed as deliverers, extractors, pilers, and scoopers at the sea salt farms showed a faster kidney function decline than those who had a light workload. Training or education programs about preventing heat illness at the individual or community levels may be beneficial for sea salt workers in Thailand.

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Conflict of interest

The authors declare no conflict of interest.

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

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