ISSN 0035-7715
eISSN 2451-2311

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE

Quarterly
2025
Volume 76
Number 4 - DECEMBER

PUBLISHER:
NATIONAL INSTITUTE OF PUBLIC HEALTH NIH
– NATIONAL RESEARCH INSTITUTE
Warsaw, Poland

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
(ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE)

Published since 1950

Quarterly, 4 issues in 1 volume per year (No 1 - March, No 2 - June, No 3 - September, No 4 - December)
The journal is devoted to research studies on food and water safety, nutrition, dietetics,environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences

Available at https://roczniki.pzh.gov.pl/

Publisher: National Institute of Public Health NIH - National Research Institute, Warsaw, Poland

Indexed/abstracted in: PubMed/MEDLINE, Scopus, Clarivate Web of Science (all data bases), EMBASE, EBSCO, Agro, DOAJ, Food Science and Technology Abstracts, Global Health, NISC SA Databases, Index Copernicus Int., Polish Medical Bibliography/Central Medical Library, Polish Ministry of Science and Higher Education (MNiSW), CNKI Scholar

Full articles are freely accessible on the journal's website: https://roczniki.pzh.gov.pl/Archive
The printed version of the journal is identical to the online version.

Editorial Office:
National Institute of Public Health NIH - National Research Institute
24 Chocimska Street, 00-791 Warsaw, Poland
e-mail: roczniki.pzh@pzh.gov.pl

ISSN 0035-7715
eISSN 2451-2311

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE

Quarterly
2025
Volume 76
Number 4 - DECEMBER

PUBLISHER:
NATIONAL INSTITUTE OF PUBLIC HEALTH NIH
– NATIONAL RESEARCH INSTITUTE
Warsaw, Poland

Printing house:
Agencja Reklamowa TOP
Chocimska 4, 87-800 Włocławek
tel.: + 48 54 427 09 70
http://www.agencjatop.pl

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
[ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE]

Volume 76 **2025** **Number 4**

OD REDAKTORA NACZELNEGO

Szanowni Państwo,

W bieżącym numerze Roczników Państwowego Zakładu Higieny znajdziecie Państwo trzy artykuły wpisujące się w aktualną tematykę badań nad możliwością wykorzystania naturalnych związków bioaktywnych, w profilaktyce i wspomaganiu leczenia chorób niezakaźnych. N. Nechai i in. w badaniach na zwierzętach potwierdzili ochronną rolę resweratrolu w odniesieniu do stresu oksydacyjnego wywoływanego narażeniem na benzoesan sodu. F. Affane i in. stwierdzili w odniesieniu do gatunków *Olea europaea* L. występujących w Maroku, że stosowanie naparu z liści tej rośliny istotnie obniża ciśnienie krwi w grupie badanej, w porównaniu do grupy kontrolnej przyjmującej wyłącznie leki na nadciśnienie. Z kolei A. Aboukhalaf i in. zbadali wartość odżywczą oraz działanie antyoksydacyjne i przeciwbakteryjne bulw *Arisarum vulgare* O. Targ. Tozz (kleśniec zwyczajny), rośliny od wieków spożywanej w Maroku w okresach głodu. Potwierdzili jednocześnie, że tradycyjna metoda przygotowywania rośliny do spożycia pozwala na usunięcie toksycznych alkaloidów występujących w świeżych bulwach. Trzy kolejne artykuły lokują się w tematyce badań nad sposobem żywienia i stanem odżywienia. Pierwszy z nich, autorstwa I. Haddou i in., opisuje walidację w grupie nastolatków w Maroku, kwestionariusza KIDMED, powszechnie stosowanego do oceny przestrzegania przez dzieci i młodzież diety śródziemnomorskiej. Związek pomiędzy poziomem percepcji i preferencją smaku słodkiego oraz spożyciem żywności i BMI, był oceniony przez R. M. Sankeshwari i in., w grupie dzieci w wieku szkolnym w Indiach. W trzecim artykule z tego zakresu, J. Pastrnáková i in. zbadali związek pomiędzy problemami związanymi ze snem i wybranymi wskaźnikami zdrowia, w tym m.in. BMI i nadciśnieniem tętniczym w grupie osób dorosłych. Ocena wiedzy na temat probiotyków, prebiotyków i mikroflory jelitowej była przedmiotem pilotażowych badań przeprowadzonych przez H. Lerhzouli i in. w grupie pracowników służby zdrowia w Maroku. Wyniki tych badań potwierdzają niedostateczną wiedzę w tym zakresie wykazywaną w innych badaniach, w tym m.in. w Polsce.

Zapraszam do czytania i publikowania w Rocznikach PZH. Jednocześnie uprzejmie informuję, że od 2026 roku Roczniki PZH nie będą się już ukazywały w formie papierowej.

Z poważaniem,

Prof. dr hab. Hanna Mojska
Redaktor naczelna
Roczników Państwowego Zakładu Higieny

EDITORIAL INTRODUCTION

Ladies and Gentlemen,

In the current issue of the journal Roczniki Państwowego Zakładu Higieny (Annals of the National Institute of Hygiene) you will find three articles that fit into the current research topic on the possibility of using natural bioactive compounds in the prevention and support of the treatment of non-communicable diseases. N. Nechai et al. in animal studies confirmed the protective role of resveratrol in relation to oxidative stress induced by exposure to sodium benzoate. F. Affane et al. found that, with respect to the *Olea europaea* L. species found in Morocco, the use of an infusion of the leaves of this plant significantly reduced blood pressure in the study group, compared to the control group taking only antihypertensive drugs. In turn, A. Aboukhalaf e al. examined the nutritional value, antioxidant and antibacterial activity of the tubers of *Arisarum vulgare* O. Targ. Tozz (friar's cowl), a plant that has been consumed in Morocco for centuries during periods of famine. They also confirmed that the traditional method of preparing the plant for consumption allows for the removal of toxic alkaloids present in fresh tubes. The next three articles focus on research on dietary habits and nutritional status. The first, by I. Haddou et al., describes the validation of the KIDMED questionnaire, commonly used to assess adherence to the Mediterranean diet among children and adolescents, in a Moroccan adolescent cohort. The relationship between the level of perception and preference for sweet taste and, among others, food consumption and BMI was assessed by R. M. Sankeshwari et al., in a group of school-age children in India. In the third article in this field, J. Pastrnáková et al. examined the relationship between sleep problems and selected health indicators, including BMI and hypertension in a group of adults. A pilot study by H. Lerhzouli et al. assessed knowledge about probiotics, prebiotics, and intestinal microbiota among healthcare workers in Morocco. The results confirm the insufficient knowledge in this area demonstrated in other studies, including those from Poland.

I invite you to read and publish in the journal Roczniki Państwowego Zakładu Higieny. Additionally, I would like to inform you that, from 2026, this journal will no longer be published in paper form.

Kind regards,

Prof. Hanna Mojska, PhD
Editor-in-Chief
Roczniki Państwowego Zakładu Higieny

Rocz Panstw Zakl Hig 2025;76(4):285-291
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/218624

ORIGINAL ARTICLE

PROTECTIVE EFFECT OF RESVERATROL ON OXIDATIVE STRESS IN RATS EXPOSED TO ELEVATED DOSES OF SODIUM BENZOATE

Nataliia Nechai[1], *Vladislav Fira*[1], *Oksana Kachur*[2], *Lyudmila Fira*[1]

[1]Department of Pharmacy, I. Horbachevsky Ternopil National Medical University, Ukraine
[2]Department of General Chemistry, I. Horbachevsky Ternopil National Medical University, Ukraine

ABSTRACT

Background. Sodium benzoate is a widely used food preservative permitted in Ukraine with an acceptable daily intake of 5 mg/kg body weight. However, exposure to elevated doses may induce oxidative imbalance and metabolic disturbances. Therefore, the search for effective protective agents against sodium benzoate–induced oxidative stress remains relevant. Resveratrol is a natural polyphenolic compound known for its antioxidant, anti-inflammatory, and cytoprotective properties.

Objective. To evaluate the effect of resveratrol on free radical processes and antioxidant defense parameters in rats exposed to elevated doses of sodium benzoate.

Material and Methods. The study was conducted on 42 adult male rats. Sodium benzoate was administered intragastrically at a dose of 30 mg/kg body weight daily for 28 days. Resveratrol was administered intragastrically at a dose of 20 mg/kg body weight. Animals were euthanized on days 14, 21, and 28. Oxidative stress and antioxidant status were assessed by measuring thiobarbituric acid reactive substances (TBARS), products of oxidative modification of proteins, ceruloplasmin content, reduced glutathione levels, and catalase activity in blood serum and liver tissue.

Results. Sodium benzoate exposure resulted in a significant increase in lipid peroxidation and protein oxidative modification, accompanied by a decrease in reduced glutathione levels and catalase activity in both blood serum and liver tissue. By the end of the experiment, TBARS levels increased 5.1-fold in serum and 3.7-fold in liver tissue compared to controls. Resveratrol administration significantly attenuated oxidative damage, restored reduced glutathione levels to near-control values, and increased catalase activity throughout the experimental period ($p \leq 0.05$).

Conclusions. Resveratrol effectively reduces sodium benzoate–induced oxidative stress and restores antioxidant defense mechanisms in rats, indicating its potential protective role against preservative-associated oxidative damage.

Keywords: *lipid peroxidation, resveratrol, sodium benzoate, antioxidant defense system, oxidative stress*

INTRODUCTION

Sodium benzoate (E211), the sodium salt of benzoic acid, is one of the most commonly used preservatives in the food industry. It is widely applied to inhibit microbial growth and prolong the shelf life of beverages, meat and fish products, confectionery, sauces, and fruit-based foods. According to international and national regulations, sodium benzoate is permitted for use only within strictly defined concentrations, and its acceptable daily intake is set at 5 mg/kg body weight. Nevertheless, concerns remain regarding the potential biological effects associated with long-term or excessive intake of this preservative [1, 2].

Experimental and clinical studies indicate that sodium benzoate may exhibit prooxidant activity, leading to the activation of free radical processes and the development of oxidative stress. Excessive generation of reactive oxygen species has been shown to impair mitochondrial function, disrupt cellular metabolism, and induce oxidative damage to lipids and proteins. In addition, sodium benzoate has been reported to interfere with amino acid metabolism, particularly glycine availability, and to exert neuromodulatory effects under certain conditions [3-5].

Of particular concern is the ability of sodium benzoate to participate in benzene formation when combined with ascorbic acid (E300). This reaction may occur during storage and is promoted by exposure to light and elevated temperatures [6]. Benzene is a well-known carcinogen, and chronic exposure has been associated with hematological disorders and an increased risk of leukemia. Although sodium benzoate is generally considered safe within recommended intake limits, combined exposure from

Corresponding author: Oksana Kachur, Department of General Chemistry, I. Horbachevsky Ternopil National Medical University, Ukraine; email: oksana.kachur2012@gmail.com

Publisher: National Institute of Public Health NIH - National Research Institute

multiple sources or prolonged intake of elevated doses may pose potential health risks [6, 7].

Sodium benzoate is used not only in food products but also in pharmaceuticals, cosmetics, personal care products, and animal feed, increasing the likelihood of cumulative exposure. Long-term intake may promote its accumulation in organs such as the liver and kidneys, contributing to metabolic disturbances and oxidative imbalance [8, 9]. Therefore, investigating the mechanisms of sodium benzoate–induced toxicity and identifying effective protective strategies remains an important task in food hygiene and public health research [10-12].

Resveratrol is a natural polyphenolic compound widely distributed in grapes, berries, and red wine. It has attracted considerable scientific interest due to its antioxidant, anti-inflammatory, anti-aggregant, and cytoprotective properties [13-15]. Resveratrol is capable of scavenging reactive oxygen species, modulating antioxidant enzyme activity, and supporting endogenous antioxidant defense systems. Previous studies have demonstrated its protective effects against oxidative damage induced by various xenobiotics and environmental stressors [16-18].

Our previous investigations showed that subchronic administration of sodium benzoate at a dose of 30 mg/kg body weight induces pronounced oxidative stress in experimental animals. Based on these findings, the present study aimed to evaluate the ability of resveratrol to attenuate oxidative stress and restore antioxidant defense parameters in rats exposed to elevated doses of sodium benzoate.

MATERIALS AND METHODS

Animals and experimental design

The study was conducted on 42 adult male outbred white rats weighing 170-180 g. Animals were housed under standard vivarium conditions with free access to food and water and maintained on a standard laboratory diet throughout the experiment.

Rats were randomly divided into three experimental groups:

1. intact control group (n = 6);
2. rats receiving sodium benzoate at a dose of 30 mg/kg body weight (n = 18);
3. rats receiving sodium benzoate (30 mg/kg body weight) in combination with resveratrol (20 mg/kg body weight) (n = 18).

All substances were administered intragastrically once daily. The duration of the experiment was 28 days. Animals from the study groups were withdrawn from the experiment in equal numbers on days 14, 21 and 28 after the start of substance administration.

Chemicals and reagents

Sodium benzoate (E211) was used as the toxic agent and administered at a dose of 30 mg/kg body weight. Resveratrol was administered at a dose of 20 mg/kg body weight in the form of the phytocomplex Resverazin, containing resveratrol, red wine extract, and grape seed extract. Thiopental sodium was used for anesthesia during euthanasia procedures.

Sample collection

Animals were euthanized under thiopental sodium anesthesia (60 mg/kg body weight). Blood samples were collected by cardiac puncture and centrifuged at 3000 rpm for 30 min at room temperature (22-24°C) using a CM-6M centrifuge (Elmi Ltd., Riga, Latvia) to obtain serum. Liver tissue samples were excised, perfused with physiological saline, and homogenized (250 mg tissue) using a Silent Crusher S magnetic homogenizer.

Biochemical analysis

Thiobarbituric acid reactive substances (TBARS)

The level of thiobarbituric acid reactive substances (TBARS) was determined according to the method of Ohkawa et al. (1979), based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) under acidic conditions and high temperature, forming a colored trimethine complex. Briefly, samples were mixed with 0.8% TBA in 1.5% trichloroacetic acid and heated in a boiling water bath (95-100°C) for 60 min. After cooling, the mixture was centrifuged to remove precipitated proteins. The absorbance of the supernatant was measured at 532 nm. A calibration curve was constructed using 1,1,3,3-tetramethoxypropane in the concentration range of 0-10 μmol/L [19].

Oxidative modification of proteins (OMP)

Protein carbonyl content was determined according to Levine et al. (1990), based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) to form stable hydrazone derivatives. Samples were incubated with 0.1% DNPH in 2 M HCl for 60 min at room temperature in the dark. Proteins were precipitated with trichloroacetic acid and washed with an ethanol-ethyl acetate mixture. The final precipitate was dissolved in 8 M urea. Optical density was measured at 370 nm (ketone derivatives) and 430 nm (aldehyde derivatives). The calibration range corresponded to 0-5 nmol carbonyl μmol/g protein [20].

Reduced glutathione (GSH)

Reduced glutathione content was determined by the Ellman method (Ellman, 1959), based on the reaction of sulfhydryl groups with 5,5'-dithiobis(2-

nitrobenzoic acid) (DTNB), forming a yellow-colored 5-thio-2-nitrobenzoic acid anion. Samples were mixed with phosphate buffer (pH 7.4) and DTNB reagent and incubated for 10 min at room temperature. Absorbance was measured at 412 nm. A calibration curve was prepared using standard glutathione solutions in the range of 0-100 μmol/L [21].

Catalase activity

Catalase activity was determined spectrophotometrically according to Korolyuk et al. (1988), based on the decomposition of hydrogen peroxide (H_2O_2). The residual hydrogen peroxide forms a stable yellow complex with ammonium molybdate. After incubation of samples with H_2O_2 at 37°C for 10 min, the reaction was stopped by adding 4% ammonium molybdate. Absorbance was measured at 410 nm against a control sample [22].

Ceruloplasmin

Ceruloplasmin concentration was determined by the p-phenylenediamine oxidation method according to Ravin (1961). The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped with sodium azide. Absorbance was measured at 540 nm [23].

Instrumentation

All spectrophotometric measurements were performed using a ULAB-108UA spectrophotometer (ULAB, Ukraine).

Statistical analysis

Statistical analysis was performed using STATISTICA 13 software (TIBCO Software Inc., Palo Alto, CA, USA). Data are presented as mean ± standard deviation (M ± SD). Differences between independent groups were evaluated using the Mann-Whitney U test. Results were considered statistically significant at $p < 0.05$.

Ethics approval

All experimental procedures involving animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123) and were approved by the Bioethics Committee of Horbachevsky Ternopil National Medical University (No. 4 25.10.2024). All efforts were made to minimize animal suffering and to reduce the number of animals used.

RESULTS

Exposure of rats to sodium benzoate at a dose of 30 mg/kg body weight resulted in a pronounced activation of lipid peroxidation processes in both blood serum and liver tissue. As shown in Table 1, TBARS levels in blood serum increased significantly throughout the experimental period. On day 14, TBARS concentration was elevated compared to the control group, and a further progressive increase was observed on days 21 and 28. By the end of the experiment, TBARS levels in blood serum exceeded control values by 5.1-fold ($p \leq 0.05$).

In rats exposed with sodium benzoate, a progressive and significant ($p \leq 0.05$) increase in the content of TBARS in the blood serum was observed throughout the study period. On the 28th day of the experiment, this indicator exceeded the level of intact animals by 5.1 times. A similar trend was observed in the liver:

Table 1. TBARS levels in blood serum (μmol/L) and liver tissue (μmol/kg) of rats exposed to sodium benzoate and treated with resveratrol (M ± SD, n = 42)

Animal groups	Research time, days		
	14	21	28
Blood serum			
Control group	2.74 ± 0.14		
Sodium benzoate (30 mg/kg)	11.81 ± 0.42*	13.46 ± 0.36*	14.06 ± 0.31*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	11.35 ± 0.21	10.02 ± 0.24**	7.91 ± 0.26**
Liver homogenates			
Control group	1.43 ± 0.09		
Sodium benzoate (30 mg/kg)	3.49 ± 0.19*	4.4 6 ± 0.14*	5.27 ± 0.11*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	3.30 ± 0.24	1.97 ± 0.09**	1.57 ± 0.06**

*$p \leq 0.05$ compared to the control group; **$p \leq 0.05$ compared to the sodium benzoate-exposed group

on the 28th day, the content of TBARS was 3.7 times higher compared to the control.

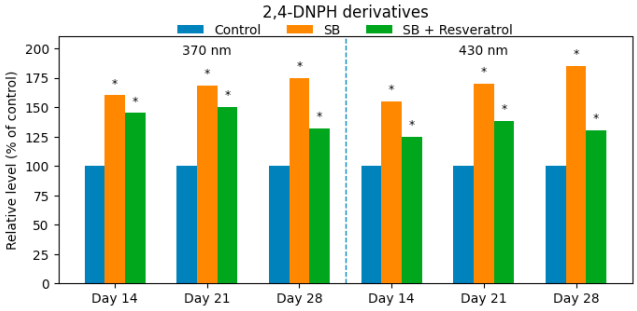
The use of resveratrol was accompanied by a decrease in the level of lipoperoxidation products. On the 14th day, only a tendency to decrease the indicator without statistical significance was noted. A significant decrease ($p \leq 0.05$) in the content of TBARS in the blood serum and liver was observed on the 21st and 28th days of the experiment. At the end of the study, the content of TBARS in the blood serum decreased by 1.8 times, and in the liver - by 3.4 times compared to the group of rats exposed to sodium benzoate.

Activation of free radical oxidation processes leads to the action of reactive oxygen species (ROS) and toxic metabolic products on the protein components of membranes and other proteins of the body, which causes their degradation and changes in structure. Along with the activation of lipoperoxidation processes, processes of oxidative modification of proteins occur.

The results of determining the content of 2,4-dinitrophenylhydrazones (2,4-DNPH) of neutral (370 nm) and basic (430 nm) nature in blood serum are given in Table 2.

In rats after administration of sodium benzoate at a dose of 30 mg/kg body weight, a significant ($p \leq 0.05$) increase in the content of both keto- and aldehyde-derived proteins in blood serum was noted throughout the experiment. The highest values of both fractions of 2,4-DNPH were recorded on the 28th day of the study.

The use of resveratrol led to a decrease in the levels of oxidative modification of proteins. A significant decrease in the content of 2,4-DNPH of a neutral nature was observed only on the 28th day of the experiment,



*$p \leq 0.05$ compared to the control group

Figure 1. Content of oxidative modification products of proteins (2,4-dinitrophenylhydrazones of neutral and basic nature) in liver tissue of rats exposed to sodium benzoate and treated with resveratrol

when this indicator was 1.3 times lower compared to the group of affected animals. The content of 2,4-DNPH of a basic nature was significantly reduced throughout the entire study period.

In the liver of rats after intoxication with sodium benzoate, a significant increase in the content of both fractions of modified proteins was also noted (Figure 1). After the use of resveratrol, their content decreased: on the 28th day, the level of 2,4-DNPH(370) was 49%, and 2,4-DNPH(430) was 43% lower than in the group of affected animals.

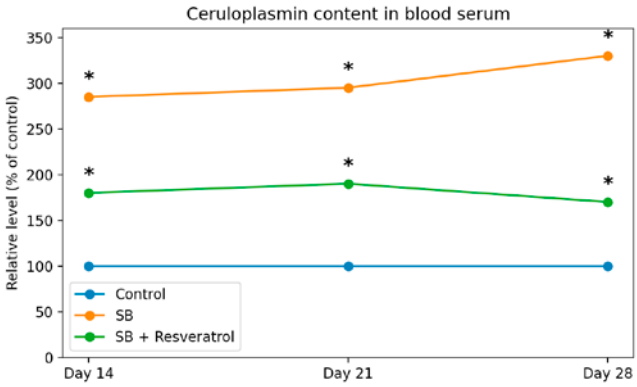
In our experiments, we investigated the content of ceruloplasmin – a protein with enzymatic activity, which is one of the first to enter the body's defense against free radicals (Figure 2).

The content of ceruloplasmin in the blood serum of rats exposed with sodium benzoate significantly ($p \leq 0.05$) increased throughout the experiment (Figure 2). On the 28th day of the study, this indicator

Table 2. Content of oxidative modification products of proteins in blood serum (μmol/g protein) of rats exposed to sodium benzoate and treated with resveratrol (M ± SD, n = 42)

Animal groups	Research time, days		
	14	21	28
2,4-DNPH(370) of neutral nature (keto derivatives)			
Control group	0.118 ± 0.005		
Sodium benzoate (30 mg/kg)	0.217 ± 0.007*	0.235 ± 0.006*	0.275 ± 0.008*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	0.211 ± 0.014	0.219 ± 0.005	0.204 ± 0.006**
2,4-DNPH(430) of basic nature (aldehyde derivatives)			
Control group	0.198 ± 0.010		
Sodium benzoate (30 mg/kg)	0.308 ± 0.010	0.333 ± 0.007*	0.380 ± 0.009*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	0.243 ± 0.012**	0.277 ± 0.005**	0.247 ± 0.006**

*$p \leq 0.05$ compared to the control group; **$p \leq 0.05$ compared to the sodium benzoate-exposed group



*p ≤ 0.05 compared to the control group

Figure 2. Ceruloplasmin content in blood serum of rats exposed to sodium benzoate and treated with resveratrol

exceeded the control level by 233%. The use of resveratrol contributed to a decrease in the content of ceruloplasmin during all periods of observation, but its values remained higher than the level of intact animals (by 65% on the 28th day).

The content of reduced glutathione in the blood serum and liver of rats exposed with sodium benzoate significantly ($p \leq 0.05$) decreased throughout the experiment (Table 3). By day 28, the content of reduced glutathione decreased by 4.2 times in serum and 1.7 times in liver. The use of resveratrol contributed to the restoration of the level of this indicator, which practically reached the control values.

Catalase activity in blood serum and liver tissue of rats exposed to sodium benzoate was significantly reduced (Table 4). The most pronounced decrease was

Table 3. Reduced glutathione levels in blood serum (μmol/L) and liver tissue (μmol/kg) of rats exposed to sodium benzoate and treated with resveratrol (M ± SD, n = 42)

Animal groups	Research time, days		
	14	21	28
Blood serum			
Control group	1.48 ± 0.06		
Sodium benzoate (30 mg/kg)	0.82 ± 0.03*	0.54 ± 0.03*	0.35 ± 0.03*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	1.32 ± 0.09**	1.42 ± 0.09**	1.50 ± 0.08**
Liver homogenates			
Control group	1.77 ± 0.06		
Sodium benzoate (30 mg/kg)	1.52 ± 0.03*	1.29 ± 0.06*	1.03 ± 0.06*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	1.60 ± 0.07	1.65 ± 0.09**	1.69 ± 0.10**

*p ≤ 0.05 compared to the control group; **p ≤ 0.05 compared to the sodium benzoate-exposed group

Table 4. Catalase activity in blood serum (μkat/L) and liver tissue (μkat/kg) of rats exposed to sodium benzoate and treated with resveratrol (M ± SD, n = 42)

Animal groups	Research time, days		
	14	21	28
Blood serum			
Control group	1.59 ± 0.16		
Sodium benzoate (30 mg/kg)	1.00 ± 0.06*	0.76 ± 0.06*	0.78 ± 0.07*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	1.20 ± 0.07	1.36 ± 0.09**	1.47 ± 0.08**
Liver homogenates			
Control group	2.40 ± 0.14		
Sodium benzoate (30 mg/kg)	1.62 ± 0.07*	0.90 ± 0.09*	0.58 ± 0.05*
Sodium benzoate (30 mg/kg) + resveratrol (20 mg/kg)	2.29 ± 0.17**	2.14 ± 0.20**	2.45 ± 0.17**

*p ≤ 0.05 compared to the control group; **p ≤ 0.05 compared to the sodium benzoate-exposed group

observed on day 28, when catalase activity in blood serum was approximately threefold lower than control values, while in liver tissue it was reduced by 4.1-fold compared to intact animals. Resveratrol administration resulted in a significant increase in catalase activity; in liver tissue, normalization of this parameter was observed as early as day 14 of the experiment.

DISCUSSION

Our results confirm that prolonged administration of sodium benzoate at a dose of 30 mg/kg body weight leads to the development of oxidative stress, which is manifested by the activation of lipoperoxidation processes, increased oxidative modification of proteins and inhibition of enzymatic and non-enzymatic links of the antioxidant system. The established changes are progressive in nature and increase with increasing duration of exposure, which indicates a cumulative effect of the toxicant [3, 4, 8, 9].

Literature data indicate that the effect of sodium benzoate on the prooxidant-antioxidant balance is dose-dependent. Under the conditions of administration of doses close to the permissible daily intake, changes in oxidative stress indicators are moderate or compensatory. At the same time, the use of increased doses (25-50 mg/kg and more) is accompanied by a significant increase in the level of TBARS the accumulation of oxidatively modified proteins and a decrease in catalase activity and glutathione content. Our results are consistent with these observations, since activation of lipoperoxidation was recorded already on the 14th day of the experiment, and maximum depletion of the antioxidant system on the 28th day [17, 18, 24, 25].

The results obtained may be associated with increased generation of reactive oxygen species, impaired mitochondrial respiration, and depletion of the glutathione detoxification pathway. A decrease in catalase activity and reduced glutathione levels indicates insufficient enzymatic neutralization of hydrogen peroxide and other reactive metabolites. An increase in ceruloplasmin levels is likely to be compensatory and reflects activation of the antioxidant defense system in response to excessive production of free radicals [13, 15, 17].

The use of a resveratrol-containing agent led to a significant decrease in the intensity of lipoperoxidation and oxidative modification of proteins, as well as to the restoration of antioxidant system parameters. A significant increase in catalase activity and a decrease in ceruloplasmin content in the studied tissues were observed. The use of resveratrol also contributed to the restoration of the functional activity of the glutathione system by increasing the content of reduced glutathione in the blood serum of rats affected by sodium benzoate [26-28].

Our results are consistent with the data of other researchers who have demonstrated that resveratrol-containing drugs have the ability to repair damage caused by active forms of reactive oxygen species. The regenerative effect of resveratrol is probably due to the combination of its direct antiradical activity and the ability to modulate the endogenous antioxidant system. As a polyphenolic compound, resveratrol is able to neutralize reactive oxygen species and interrupt the chain reactions of lipoperoxidation. This is consistent with our data on the normalization of the glutathione pool and catalase activity under the toxic effects of sodium benzoate.

CONCLUSIONS

Subchronic administration of sodium benzoate at a dose of 30 mg/kg body weight induces significant oxidative stress in rats, as evidenced by enhanced lipid peroxidation, increased oxidative modification of proteins, and disruption of antioxidant defense mechanisms in blood serum and liver tissue.

Resveratrol administration significantly attenuates sodium benzoate–induced oxidative damage by reducing lipid peroxidation and protein oxidation and by restoring reduced glutathione levels and catalase activity. These findings indicate the protective potential of resveratrol-containing formulations against preservative-induced oxidative stress.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Mohammed D, Issa H. Mini-review on sodium benzoate: its uses, adverse effects, and environmental impact as a pharmaceutical product. ChemRxiv [Preprint]. 2024. doi: 10.26434/chemrxiv-2024-zvn5k.
2. EFSA Panel on Food Additives and Flavourings (FAF). Re-evaluation of sodium benzoate (E211) as a food additive. EFSA J. 2020;18(3):6051. doi: 10.2903/j.efsa.2020.6051.
3. Khodaei F, Kholghipour H, Hosseinzadeh M, Rashedinia M. Effect of sodium benzoate on liver and kidney lipid peroxidation and antioxidant enzymes in mice. J Rep Pharm Sci. 2019;8(2):e147382. doi: 10.4103/jrptps.JRPTPS_68_18.
4. Asejeje FO, Ajayi BO, Abiola MA, Samuel O, Asejeje GI, Ajiboye EO, Ajayi AM. Sodium benzoate induces neurobehavioral deficits and brain oxido-inflammatory stress in male Wistar rats: ameliorative role of ascorbic acid. J Biochem Mol Toxicol. 2022;36(5):e23010. doi: 10.1002/jbt.23010.

5. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21(7):363-83. doi: 10.1038/s41580-020-0230-3.
6. Seetharam JC, Maiti R, Mishra A, Mishra BR. Efficacy and safety of add-on sodium benzoate, a D-amino acid oxidase inhibitor, in treatment of schizophrenia: a systematic review and meta-analysis. Asian J Psychiatr. 2022;68:102947. doi: 10.1016/j.ajp.2021.102947.
7. McHale CM, Zhang L, Smith MT. Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis. 2012;33(2):240-252. doi: 10.1093/carcin/bgr297.
8. Kohli SK, Khanna K, Bhardwaj R, Corpas FJ, Ahmad P. Nitric oxide, salicylic acid and oxidative stress: is it a perfect equilateral triangle? Plant Physiol Biochem. 2022;184:56-64. doi: 10.1016/j.plaphy.2022.05.017.
9. Khan IS, Dar KB, Ganie SA, Ali MN. Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. Drug Chem Toxicol. 2022;45(3):1345-1354. doi: 10.1080/01480545.2020.1825472.
10. Sambu S, Hemaram U, Murugan R, Alsofi AA. Toxicological and teratogenic effect of various food additives: an updated review. Biomed Res Int. 2022;2022:6829409. doi: 10.1155/2022/6829409.
11. Mohammadzadeh-Aghdash H, Akbari N, Esazadeh K, Ezzati Nazhad Dolatabadi J. Molecular and technical aspects on the interaction of serum albumin with multifunctional food preservatives. Food Chem. 2019;293:491-498. doi: 10.1016/j.foodchem.2019.04.119.
12. Shahmohammadi M, Javadi M, Nassiri-Asl M. An overview on the effects of sodium benzoate as a preservative in food products. Biotechnol Health Sci. 2016;3:7-11. doi: 10.17795/bhs-35084.
13. Ren B, Kwah MX, Liu C, Ma Z, Shanmugam MK, Ding L, et al. Resveratrol for cancer therapy: challenges and future perspectives. Cancer Lett. 2021;515:63-72. doi: 10.1016/j.canlet.2021.05.001.
14. Zhou DD, Luo M, Huang SY, Saimaiti A, Shang A, Gan RY, Li HB. Effects and mechanisms of resveratrol on aging and age-related diseases. Oxid Med Cell Longev. 2021;2021:9932218. doi: 10.1155/2021/9932218.
15. Zhang LX, Li CX, Kakar MU, Khan MS, Wu PF, Amir RM, et al. Resveratrol (RV): a pharmacological review and call for further research. Biomed Pharmacother. 2021;143:112164. doi: 10.1016/j.biopha.2021.112164.
16. Zhang J, Zhang Y, Wang J, Xia Y, Zhang J, Chen L. Recent advances in Alzheimer's disease: mechanisms, clinical trials and new drug development strategies. Signal Transduct Target Ther. 2024;9(1):211. doi: 10.1038/s41392-024-01911-3.
17. Kachur O, Fira L, Lykhatskyi P, Fira D, Kramar S. State of humoral immunity and cytokine profile in rats under experimental carcinogenesis with applying enterosorporation chemotherapeutic factors. Pol Merkur Lekarski. 2020;48(288):431-436.
18. Jin Q, Liu T, Qiao Y, Liu D, Yang L, Mao H, et al. Oxidative stress and inflammation in diabetic nephropathy: role of polyphenols. Front Immunol. 2023;14:1185317. doi: 10.3389/fimmu.2023.1185317.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358. doi: 10.1016/0003-2697(79)90738-3.
20. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990;186:464-478. doi: 10.1016/0076-6879(90)86141-H.
21. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-77. doi: 10.1016/0003-9861(59)90090-6.
22. Hadwan MH. Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochem. 2018;19:7. doi: 10.1186/s12858-018-0097-5.
23. Ravin HA. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med. 1961;58:161-168.
24. Sahoo DK, Heilmann RM, Paital B, Patel A, Yadav VK, Wong D, Jergens AE. Oxidative stress, hormones, and effects of natural antioxidants on intestinal inflammation in inflammatory bowel disease. Front Endocrinol (Lausanne). 2023;14:1217165. doi: 10.3389/fendo.2023.1217165.
25. Chiang TI, Yu YH, Lin CH, Lane HY. Novel biomarkers of Alzheimer's disease: based upon N-methyl-D-aspartate receptor hypoactivation and oxidative stress. Clin Psychopharmacol Neurosci. 2021;19(3):423-433. doi: 10.9758/cpn.2021.19.3.423.
26. Galiniak S, Aebisher D, Bartusik-Aebisher D. Health benefits of resveratrol administration. Acta Biochim Pol. 2019;66(1):13-21. doi: 10.18388/abp.2018_2749.
27. Bertoldo A, Pizzol D, Yon DK, Callegari M, Gobbo V, Cuccurese P, et al. Resveratrol and female fertility: a systematic review. Int J Mol Sci. 2024;25(23):12792. doi: 10.3390/ijms252312792.
28. Shaito A, Posadino AM, Younes N, Hasan H, Halabi S, Alhababi D, et al. Potential adverse effects of resveratrol: a literature review. Int J Mol Sci. 2020;21(6):2084. doi: 10.3390/ijms21062084.

Received: 20.01.2026
Revised: 24.02.2026
Accepted: 26.02.2026
Published online first: 11.03.2026

Rocz Panstw Zakl Hig 2025;76(4):293-299
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/220202

ORIGINAL ARTICLE

SLEEP-RELATED BEHAVIOURS AND THEIR ASSOCIATIONS WITH OVERWEIGHT, OBESITY AND HYPERTENSION IN ADULTS

Jana Pastrnáková[1], Lucia Šubová[1], Martina Gažarová[1], Petra Lenártová[1], Magdalena Górnicka[2], Marta Habánová[1]

[1]Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovakia
[2]Institute of Human Nutrition Science, Department of Human Nutrition, Warsaw University of Life Science, Poland

ABSTRACT

Background. Sleep plays an important role in physiological regulation and has been increasingly recognised as a contributor to chronic non-communicable diseases, including obesity and hypertension.

Objective. This study examined the relationships between sleep duration, sleep-related problems, and selected health indicators, including BMI (body mass index) and hypertension, and evaluated the prevalence of sleep-related difficulties, stress, and night-shift work in adults.

Materials and Methods. A total of 260 respondents (125 men, aged 42.24 ± 12.35 years; BMI 27.19 ± 4.3 kg/m²; and 135 women, aged 47.83 ± 11.72 years; BMI 26.57 ± 6.54 kg/m²) completed a 14-item questionnaire assessing sleep duration and quality, sleep-related problems, night-time awakenings, fatigue, stress, night-shift work and self-reported chronic conditions, including physician-diagnosed hypertension.

Results. A significant association was found between insufficient sleep and overweight/obesity ($p = 0.003$), whereas no relationship was observed between sleep duration and hypertension ($p = 0.232$). Overall, 55% of respondents slept fewer than 7 hours, 35% slept 7-8 hours, and 10% slept more than 8 hours per night; overweight or obese individuals reported an average of 6.2 hours of sleep. Sleep problems were reported by 35% of participants, but showed no significant association with BMI. Stress was reported by 53% of respondents and was significantly associated with higher BMI ($p = 0.005$). Night-shift work was reported by 51.8% of participants and was significantly linked to overweight/obesity ($p = 0.039$). Hypertension was reported by 40.3% of respondents and was significantly associated with BMI ($p < 0.0001$), but not with sleep duration.

Conclusions. Insufficient sleep, stress, and night-shift work were associated with increased BMI, while no association was found between sleep duration and hypertension. These factors should be considered when addressing weight-related health risks.

Keywords: *sleep-related behaviours, body mass index, obesity, hypertension, non-communicable diseases*

INTRODUCTION

Obesity and hypertension are among the most important risk factors for cardiovascular diseases (CVDs) [1-4], which belong to the broader group of noncommunicable diseases (NCDs). NCDs represent a major global public health challenge, contributing substantially to morbidity and ranking among the leading causes of death and disability worldwide [5]. According to Unwin and Alberti [6], chronic NCDs account for nearly 60% of global mortality, with approximately 80% of NCD-related deaths occurring in low and middleincome countries. Cardiovascular diseases are the dominant contributor, responsible for almost 80% of all premature NCD deaths, underscoring their central role in the global NCD epidemic. Given the strong metabolic and cardiovascular consequences of obesity and hypertension, increasing attention has been directed toward additional modifiable factors that may influence these conditions, including sleep duration and sleep quality [7].

Obesity is also recognised as a significant risk factor for sleep disorders [8], and sleep disturbances have been shown to increase the risk of developing hypertension [9]. Sleep disorders are highly prevalent in the general population and are associated with substantial medical, psychological and social consequences [10]. Growing evidence further indicates

Corresponding author: Lucia Šubová, Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovakia; phone: +421910576729; email: xsuboval@uniag.sk

Publisher: National Institute of Public Health NIH - National Research Institute

that sleep disorders constitute an important and often under-recognized risk factor for major NCDs, including cardiovascular and metabolic diseases [11].

Insufficient or mistimed sleep perturbs multiple neuroendocrine pathways that regulate energy balance and cardiometabolic homeostasis. Experimental and clinical data show that curtailed or fragmented sleep can alter appetiteregulating hormones (e.g., leptin, ghrelin), impair insulin sensitivity, and shift energy intake toward caloriedense foods, thereby promoting positive energy balance and adiposity. These effects are mediated in part by activation of the hypothalamic-pituitary-adrenal (HPA) axis, changes in sympathetic tone and disruptions in glucose-insulin dynamics [12-14]. Together, these mechanisms provide biological plausibility for the observed links between short or irregular sleep and obesity, insulin resistance and cardiometabolic risk.

Healthy sleep is increasingly acknowledged as a key component of NCD prevention alongside nutrition and physical activity, yet it continues to receive insufficient attention in public health strategies [15]. Recent studies also demonstrate that poor sleep quality is strongly linked to chronic NCDs, with these associations partly mediated by mental-health factors such as anxiety and depression [16].

The International Classification of Sleep Disorders identifies more than 80 distinct sleep conditions, grouped into eight categories, including insomnia, sleepdisordered breathing (SDB) and sleep-related movement disorders [17]. Chronic sleep restriction has become increasingly common over recent decades, and both laboratory and epidemiological studies suggest that insufficient sleep contributes to the rising incidence of diabetes and obesity. Proposed mechanisms linking sleep restriction to metabolic dysfunction include impaired glucose metabolism, increased appetite and reduced energy expenditure [18].

Recent advances in sleep research further highlight the biological and clinical importance of sleep in maintaining metabolic and cardiovascular health [19]. Emerging technologies also show that physiological signals recorded during sleep can predict longterm risk for numerous health conditions, emphasising the diagnostic potential of sleep monitoring [20].

The aim of this study was to examine the associations between sleep duration, sleep-related problems and selected health indicators, including BMI and hypertension, and to assess the prevalence of sleep-related difficulties in an adult population.

MATERIAL AND METHODS

The study group consisted of 260 participants, including 125 men (42.24 ± 12.35 years; BMI 27.19 ± 4.3 kg/m²) and 135 women (47.83 ± 11.72 years; BMI 26.57 ± 6.54 kg/m²). Body weight was measured using a calibrated digital personal scale (Brutus Tanita HD 351; Harrmed Medica ČR, Tanita Europe, Hoofddorp, The Netherlands) with an accuracy of 0.1 kg. Body height was assessed using an ultrasonic height meter (Bodyson; ADE GmbH & Co., Hamburg, Germany). Body mass index (BMI) was calculated from the measured values (Table 1).

The average age of all respondents was 45.5 ± 12.44 years (range: 22-67 years). The mean height of the participants was 170.78 ± 8.66 cm, and the mean body weight was 78.37 ± 17.98 kg. The average BMI was 26.82 ± 5.68 kg/m², placing the mean respondent in the overweight category.

In addition to basic anthropometric characteristics, participants were classified by BMI category, as this

Table 1. Basic characteristics of the monitored population

Sex	Age (years) Mean ± SD	Height (cm) Mean ± SD	Weight (kg) Mean ± SD	BMI (kg/m²) Mean ± SD
Male ♂ (n = 125)	42.24 (± 12.35)	177.04 (± 6.96)	85.24 (± 14.77)	27.18 (± 4.30)
Female ♀ (n = 135)	47.83 (± 11.72)	166.31 (± 6.83)	73.46 (± 18.64)	26.57 (± 6.54)
Total (n = 260)	45.50 (± 11.72)	170.79 (± 8.66)	78.37 (± 17.98)	26.82 (± 5.68)

BMI – body mass index

Table 2. Characteristics of the study population according to BMI

Classification	BMI (kg/m²)	Sample size (n = 260)	Relative abundance (%)
Underweight	< 18.5	13	5
Normal weight	18.5-24.9	86	33
Overweight	25.0-29.9	86	33
Obesity I. degree	30.0-34.9	52	20
Obesity II. degree	35.0-39.9	18	7
Obesity III. degree	≥ 40	5	2

was one of the key variables assessed. The largest groups were respondents with normal weight (33%) and overweight (33%), while the smallest group was individuals with class III obesity (2%) (Table 2). BMI categories were determined according to the World Health Organisation (WHO) classification [21].

The participants administered a short, study-specific 14-item questionnaire designed to capture sleep-related behaviours and relevant cofactors. Items assessed: (i) sleep duration (hours/night; categorised as < 7 h, 7-8 h, > 8 h); (ii) difficulties falling asleep and/or maintaining sleep (yes/no); (iii) night-time awakenings (frequency categories); (iv) fatigue upon awakening and during the day (regular/occasional/none); (v) night-shift work (current or recent; yes/no); (vi) timing of the last meal before bedtime (≥ 2 h, last hour, immediately before bedtime, eating during the night); (vii) self-reported stress (work or personal; yes/no); and (viii) self-reported hypertension (physician diagnosis and/or antihypertensive medication; yes/no/unknown).

A total of 268 individuals completed the questionnaire; partially completed questionnaires were excluded. The final analytical sample consisted of 260 respondents who answered all items.

Statistical analysis

The questionnaire data were processed using Microsoft Office Excel 2010 (Los Angeles, CA, USA) and Statistica 12 (Dell Statistica, Tulsa, OK, USA). Statistical significance between the monitored categories was assessed using the *Chi*-square test, which evaluates the agreement between expected and observed frequencies. Results were considered statistically significant at the level of $p < 0.05$.

RESULTS

Results are presented for behavioural indicators (sleep duration, sleep-related problems, nocturnal awakenings, fatigue) and exposures (nightshift work, lastmeal timing, stress). Hypertension was selfreported.

Sleep duration

Among all respondents, the most commonly desired sleep duration was 7-8 hours (50%). A total of 27% preferred sleeping fewer than 7 hours, while 23% preferred sleeping more than 8 hours per night. When actual sleep duration was evaluated, 55% of respondents reported sleeping fewer than 7 hours per night, 35% reported sleeping 7-8 hours, and 10% reported sleeping more than 8 hours.

Table 4 presents the average sleep duration across BMI categories. Respondents with normal weight slept an average of 7.2 hours, whereas overweight or obese individuals averaged 6.2 hours per night. This difference was statistically significant ($p = 0.003$). Across all BMI categories, the overall mean sleep duration was 6.6 hours.

Difficulties falling asleep and sleep problems

In total, 29.4% of respondents reported difficulties falling asleep, and 35.1% reported sleep problems. The remaining 64.9% did not report sleep problems. No significant association was observed between BMI and the occurrence of sleep problems ($p = 0.410$).

Waking during sleep

Only 27.8% of respondents reported mostly uninterrupted sleep, while 72.2% experienced at

Table 3. The subjective need for sleep length and the actual length of sleep of the respondents

The subjective need for sleep	Absolute abundance (n)	Relative abundance (%)
Less than 7 hours	70	27
7-8 hours	130	50
More than 8 hours	60	23
Actual sleep length	Absolute abundance (n)	Relative abundance (%)
Less than 7 hours	143	55
7-8 hours	91	35
More than 8 hours	26	10

Table 4. Average length of sleep by weight category

Classification according to BMI	Average length of sleep (hours)	p-value*
Normal weight	7.2	NS
Overweight and obesity	6.2	0.003
All categories	6.6	NS

**Chi*-squared test; NS – not significant

least one night-time awakening (Table 5). The largest proportion (31.6%) reported two to three awakenings per night.

Fatigue after waking up/subsequently during the day

Regular fatigue after waking was reported by 35.1%, occasional fatigue by 53.2%, and no fatigue by 11.7%. Daytime fatigue was reported regularly by 36.9%, occasionally by 48.3%, and not at all by 14.8%.

Timing of the last meal before bedtime

A total of 30.2% of respondents did not eat for at least two hours before bedtime, 26.7% avoided eating during the last hour, 33.1% ate immediately before going to bed, and 10.3% ate during the night after waking. The association between BMI and lastmeal timing was statistically significant ($p = 0.020$).

Night-shift work

More than half of the respondents (51.8%) reported current or recent nightshift work. This variable was significantly associated with overweight and obesity ($p = 0.039$) (Table 8).

Stress

A total of 53.4% of respondents reported experiencing stress at work or in their personal lives, while 46.6% reported no stress. Although stress was not associated with sleep parameters, it was significantly associated with BMI ($p = 0.005$).

Hypertension

Hypertension was reported by 40.3% of respondents, 56.7% reported no hypertension, and 3.0% were unsure of their status. No significant association was found between sleep duration and hypertension ($p = 0.232$). However, BMI was significantly associated with hypertension ($p < 0.001$).

DISCUSSION

This study explored the relationships between multiple sleep-related behaviours and key health indicators, with a particular focus on overweight, obesity and hypertension. By examining sleep duration, sleep difficulties, night-time awakenings, fatigue, meal timing, nightshift work and stress, the findings provide insight into how different dimensions of sleep may interact with metabolic and cardiovascular risk factors in adults.

Table 5. Waking up during sleep (n = 260)

Sleep fragmentation – number of awakenings per night	
Usually don't wake up	27.8%
Once per night	27.4%
2-3 times per night	31.6%
More than 3 times per night	13.2%

Table 6. Fatigue after waking up, fatigue during the day (n = 260)

Possibilities	Fatigue after waking up (%)	Fatigue during the day (%)
Yes, regularly	35.1	36.9
Rarely	53.2	48.3
No	11.7	14.8

Table 7. The last-meal timing (n = 260)

Last meal before bedtime	
2 or more hours before bedtime	30.2%
1 hour before bedtime	26.4%
Eat right before going to sleep	33.1%
Eat at night after waking up from sleep	10.3%

Table 8. BMI and night-shift work (n = 260)

	Total (%)	Normal weight (%)	Overweight and obesity (%)	p-value*
Day-time work	48.2	25.8	48.3	NS
Night-shift work	51.8	51.7	74.2	0.039

**Chi*-squared test; NS – not significant

Although half of the respondents considered 7-8 hours of sleep ideal, 55% did not achieve this duration, indicating a discrepancy between perceived sleep needs and actual sleep behaviour. Such a mismatch may reflect lifestyle demands or insufficient sleep hygiene, highlighting the importance of promoting healthy sleep routines. This is consistent with European data showing that sleep disturbances remain prevalent, particularly in older adults, and are linked to increased morbidity and frailty [22]. Numerous studies further demonstrate that short sleep duration is associated with higher allcause mortality and adverse metabolic outcomes [23, 24].

Short sleep duration has also been widely associated with increased body weight and central adiposity [25, 26]. Evidence suggests a dose-response relationship, whereby the likelihood of obesity increases as sleep duration falls below 7 hours per night [27, 28]. Our findings are consistent with this trend, as insufficient sleep was significantly associated with overweight and obesity, whereas sleep problems such as difficulties falling or staying asleep did not show a significant relationship with BMI.

Sleep fragmentation, reflected in frequent nighttime awakenings, was common among respondents, with 72.2% reporting at least one awakening per night. Previous research indicates that disrupted sleep continuity contributes to poor sleep quality and is linked to metabolic dysfunction and chronic diseases [29]. Fatigue was also highly prevalent and aligns with evidence that both short sleep and fragmented sleep negatively affect daytime functioning and cognitive and physical performance [30-33].

More than half of the respondents (51.8%) reported current or recent nightshift work, which was significantly associated with overweight and obesity. This finding is supported by studies showing that shift work disrupts circadian rhythms and increases cardiometabolic risk [34-37]. Circadian misalignment associated with irregular work schedules is known to impair metabolic regulation, promote adiposity and elevate longterm health risks.

Stress was reported by 53.4% of participants and was significantly associated with BMI, suggesting that stress may contribute to weight gain through behavioural or physiological pathways. Although no association between stress and sleep parameters was found in this sample, previous research indicates that stress can disrupt sleep and eating patterns, which may, in turn, indirectly influence body weight.

Hypertension was reported by 40.3% of respondents. Although sleep duration was not associated with hypertension, BMI showed a strong and significant relationship with elevated blood pressure, which is consistent with wellestablished links between excess body weight and hypertension [38-40]. These results suggest that, within this population, weight status may play a more prominent role in hypertension risk than sleep duration alone.

Limitations of the study

This study has several limitations that should be taken into account when interpreting the findings. Its cross-sectional design enables the identification of associations between sleep-related behaviours, stress, BMI, and hypertension, but does not allow conclusions regarding causality or temporal direction. Information on sleep patterns, stress, and selected lifestyle factors was obtained through self-reported questionnaires, which may be subject to recall bias or subjective misestimation. The sample represents a specific adult population, which may limit the generalisability of the results to other groups. Furthermore, BMI was used as an indicator of body weight status, although it does not fully reflect differences in body composition. This crosssectional study used selfreported data for sleep, stress and hypertension and did not include validated diagnostic instruments for insomnia, anxiety or depression; therefore, results pertain to behavioural indicators and selfreports rather than clinical diagnoses.

CONCLUSION

This study showed that insufficient sleep duration, night shift work, and stress were significantly associated with higher BMI in adults. Respondents with overweight or obesity reported shorter sleep and a higher prevalence of stress and nightshift work, while neither sleep problems nor sleep duration were related to hypertension. Hypertension occurred more frequently among individuals with elevated BMI, confirming BMI as an important contributor to blood pressure, whereas no association was found between sleep duration and hypertension. These findings highlight the importance of considering sleep habits, occupational schedules and stress management when addressing excess body weight and related health risks.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding sources

The study was supported by the VEGA 1/0387/25 (50%) and KEGA 056UK-4/2025 (50%).

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Priviero F. Epigenetic modifications and fetal programming: Molecular mechanisms to control hypertension inheritance. Biochem Pharmacol. 2023;208:115412. doi: 10.1016/j.bcp.2023.115412.
2. Kjeldsen SE. Hypertension and cardiovascular risk: General aspects. Pharmacol Res. 2018;129:95-99. doi: 10.1016/j.phrs.2017.11.003.
3. Ortega FB, Lavie CJ, Blair SN. Obesity and cardiovascular disease. Circ Res. 2016;118(11):1752-1770. doi: 10.1161/CIRCRESAHA.115.306883.
4. Guo F, Garvey WT. Cardiometabolic disease risk in metabolically healthy and unhealthy obesity: Stability of metabolic health status in adults. Obesity (Silver Spring). 2016;24(2):516-525. doi: 10.1002/oby.21344.
5. Habib SH, Saha S. Burden of non-communicable disease: Global overview. Diabetes Metab Syndr. 2010;4(1):41-47. doi: 10.1016/j.dsx.2008.04.005.
6. Unwin N, Alberti KG. Chronic non-communicable diseases. Ann Trop Med Parasitol. 2006;100(5-6):455-464. doi: 10.1179/136485906X97453.
7. Direksunthorn T. Sleep and cardiometabolic health: A narrative review of epidemiological evidence, mechanisms, and interventions. Int J Gen Med. 2025;26(18):5831-5843. doi: 10.2147/IJGM.S563616.
8. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-867. doi: 10.1038/nature05485.
9. Di Murro A, Petramala L, Cotesta D, Zinnamosca L, Crescenzi E, Marinelli C, et al. Renin-angiotensin-aldosterone system in patients with sleep apnoea: Prevalence of primary aldosteronism. J Renin Angiotensin Aldosterone Syst. 2010;11(3):165-172. doi: 10.1177/1470320310366581.
10. Vgontzas AN, Kales A. Sleep and its disorders. Annu Rev Med. 1999;50:387-400. doi: 10.1146/annurev.med.50.1.387.
11. Barone MTU, de Castro Moreno CR, Micheletti Gomide Nogueira de Sá AC, Sady Prates EJ, Silveira J. Sleep disorders are an overlooked risk factor for non-communicable diseases. BMJ. 2023;383:2721. doi: 10.1136/bmj.p2721.
12. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med. 2004;141(11):846-50. doi: 10.7326/0003-4819-141-11-200412070-00008.
13. Hibi M, Kubota C, Mizuno T, Aritake S, Mitsui Y, Katashima M, Uchida S. Effect of shortened sleep on energy expenditure, core body temperature, and appetite: a human randomised crossover trial. Sci Rep. 2017;10(7):39640. doi: 10.1038/srep39640.
14. Liu PY. Rhythms in cortisol mediate sleep and circadian impacts on health, Sleep 2024; 47(9), zsae151. doi: 10.1093/sleep/zsae151.
15. Nunez C. Sleep health – The overlooked solution to combating non-communicable diseases. J mHealth. 2025. Available from: https://thejournalofmhealth.com/sleep-health-the-overlooked-solution-to-combating-non-communicable-diseases/.
16. de Menezes Júnior LAA, Almeida FM, Barbosa BCR, Machado-Coelho GLL, Meireles AL. Chronic non-communicable diseases and poor sleep quality during the COVID-19 pandemic: Mediation by anxiety and depression symptoms. Sleep Sci Pract. 2025;9:4. doi: 10.1186/s41606-024-00124-5.
17. American Academy of Sleep Medicine. International Classification of Sleep Disorders. 2nd ed. Westchester, IL; 2005.
18. Knutson KL, Spiegel K, Penev P, Van Cauter E. The metabolic consequences of sleep deprivation. Sleep Med Rev. 2007;11(3):163-178. doi: 10.1016/j.smrv.2007.01.002.
19. Dauvilliers Y. Advances in sleep research in 2024. Lancet Neurol. 2024;24(1):20-22. doi: 10.1016/S1474-4422(24)00486-1.
20. Stanford Medicine News Center. New AI model predicts disease risk while you sleep. Stanford Medicine; 2026. Available from: https://med.stanford.edu/news.html.
21. World Health Organization. A healthy lifestyle – WHO recommendations. 2010. Available from: https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations.
22. O'Donovan M, Crowley P, Flanagan E, O'Caoimh R. Sleep in Europe: Changes in prevalence of sleep disturbances in middle-aged and older Europeans. Age Ageing. 2024;53(4):afae178.314. doi: 10.1093/ageing/afae178.314.
23. Natal de Souza ÂM, de Souza Fernandes DP, Castro IS, Gróla FG, Ribeiro AQ. Sleep quality and duration and frailty in older adults: A systematic review. Front Public Health. 2025;13:1539849. doi: 10.3389/fpubh.2025.1539849.
24. Neikrug AB, Ancoli-Israel S. Sleep disorders in the older adult: A mini-review. Gerontology. 2010;56(2):181-189. doi: 10.1159/000236900.
25. Fu T, Guo R, Wang H, Yu S, Wu Y. The prevalence and risk factors of sleep disturbances in community-dwelling older adults: A systematic review and meta-analysis. Sleep Breath. 2025;29:110. doi: 10.1007/s11325-025-03267-6.
26. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Sleep duration and all-cause mortality: A systematic review and meta-analysis. Sleep. 2010;33(5):585-592. doi: 10.1093/sleep/33.5.585.
27. Ungvari Z, Fekete M, Varga P, Fekete JT, Lehoczki A, Buda A, et al. Imbalanced sleep increases mortality risk by 14–34%: A meta-analysis. GeroScience. 2025;47:4545-4566. doi: 10.1007/s11357-025-01592-y.
28. Gangwisch JE, Malaspina D, Boden-Albala B, Heymsfield SB. Inadequate sleep as a risk factor for obesity: Analyses of NHANES I. Sleep. 2005;28(10):1289-1296. doi: 10.1093/sleep/28.10.1289.
29. Hakim F, Wang Y, Carreras A, Hirotsu C, Zhang J, Peris E, et al. Chronic sleep fragmentation induces hypothalamic ER stress and leptin resistance. Sleep. 2015;38(1):31-40. doi: 10.5665/sleep.4320.

30. Rosenthal TC, Majeroni BA, Pretorius R, Malik K. Fatigue: An overview. Am Fam Physician. 2008;78(10):1173-1179.
31. Benkirane O, Simor P, Mairesse O, Peigneux P. Sleep fragmentation modulates neurophysiological correlates of cognitive fatigue. Clocks Sleep. 2024;6:602-618. doi: 10.3390/clockssleep6040041.
32. Goldman SE, Ancoli-Israel S, Boudreau R, Cauley JA, Hall M, Stone KL, et al. Sleep problems and daytime fatigue in older individuals. J Gerontol A Biol Sci Med Sci. 2008;63(10):1069-1075. doi: 10.1093/gerona/63.10.1069.
33. Ayas FY, Özcebe LH. Relationship between fatigue, sleep quality, and sleep deprivation. Sleep Breath. 2025;29:73. doi: 10.1007/s11325-024-03231-w.
34. Schettini MAS, Passos RFDN, Koike BDV. Shift work and metabolic syndrome updates. Sleep Sci. 2023;16(2):237-247. doi: 10.1055/s-0043-1770798.
35. Di Lorenzo L, De Pergola G, Zocchetti C, L'Abbate N, Basso A, Pannacciulli N, et al. Effect of shift work on BMI. Int J Obes. 2003;27(11):1353-1358. doi: 10.1038/sj.ijo.0802419.
36. Ramin C, Devore EE, Wang W, Pierre-Paul J, Wegrzyn LR, Schernhammer ES. Night shift work and chronic disease risk factors. Occup Environ Med. 2015;72(2):100-107. doi: 10.1136/oemed-2014-102292.
37. Sanford LD, Suchecki D, Meerlo P. Stress, arousal, and sleep. Curr Top Behav Neurosci. 2015;25:379-410. doi: 10.1007/7854_2014_314.
38. Nagai M, Kario K. Sleep disorder and hypertension. Nihon Rinsho. 2012;70(7):1188-1194. doi: 10.1038/jhh.2016.55.
39. Lin CL, Liu TC, Lin FH, Chung CH, Chien WC. Sleep disorders and hypertension in Taiwan. J Hum Hypertens. 2017;31(3):220-224. doi: 10.1038/jhh.2016.55.
40. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, et al. 2014 evidence-based guideline for the management of high blood pressure in adults. JAMA. 2014;311(5):507-520. doi: 10.1001/jama.2013.284427.

Received: 03.02.2026
Revised: 19.03.2026
Accepted: 31.03.2026
Published online first: 16.04.2026

Rocz Panstw Zakl Hig 2025;76(4):301-313
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/220380

ORIGINAL ARTICLE

HEALTHCARE PROFESSIONALS' KNOWLEDGE OF PROBIOTICS, PREBIOTICS, AND THE GUT MICROBIOTA – THE CITY OF KÉNITRA, MOROCCO: A PILOT STUDY

Hanane Lerhzouli[1], *Btihaj Al Ibrahmi*[1], *Samir Bikri*[2], *Soad Khal-Layoun*[1], *Abdellatif Bour*[1]

[1]Laboratory of Natural Resources and Sustainable Development, Team of Nutrition, Food and Health Sciences, Faculty of Sciences, Ibn Tofail University, Kénitra, Morocco
[2]Biology and Health Laboratory, Faculty of Sciences, Ibn Tofail University, Kénitra, Morocco

ABSTRACT

Background. In scientific literature, it is relatively rare to find information on healthcare professionals' current knowledge of probiotics, prebiotics, and gut microbiota.

Objective. The aim of our study was to assess healthcare professionals' knowledge of gut microbiota, probiotics, and prebiotics in the city of Kénitra, Morocco.

Materials and Methods. The data was collected via an online questionnaire, which we distributed through social media. A total of 143 healthcare professionals (78.3% women and 21.7% men) responded to this questionnaire. The questionnaire concerned knowledge of probiotics and prebiotics.

Results. Most respondents rated their knowledge of probiotics, prebiotics, and gut microbiota as average (40%) or poor (39%), while others rated their knowledge as good (11%) and only 2% had very good knowledge, with the remainder (8%) having no knowledge. The correct definition of probiotics chose 67.1% of respondents, broken down as follows: 80.4% of general practitioners, 76.9% of specialists, and 57% of nurses. *Lactobacillus acidophilus* (65%) and *Bifidobacterium bifidum* (50.3%) are the two species best known to respondents as probiotic strains. Furthermore, the most popular prebiotic is fructooligosaccharide (51%), followed by galactooligosaccharide (42.7%) then inulin (36.4%) and finally beta-glucan (14%). Among professionals 60.1% prescribed probiotics and/or prebiotics for diarrhea, followed by antibiotics (47.6%) then constipation (39.2%) and 21% of respondents recommended them for diabetes, 18.9% for obesity while only 3.5% used them for other pathologies.

Conclusion. This online survey revealed the current knowledge of healthcare professionals regarding probiotics, prebiotics and gut microbiota and highlights the importance of educating and training them through targeted learning programs.

Keywords: *probiotics, prebiotics, gut microbiota, healthcare professionals, knowledge, Morocco*

INTRODUCTION

The microorganisms colonizing the digestive tract were formerly called "intestinal flora", but in the early 1990s this term was replaced by intestinal microbiota after Carl Woese proposed a new classification of the kingdom of life [1]. Bacterial colonization of the gastrointestinal tract begins at birth and this colonization and the composition of the gut microbiome can be influenced by many criteria, such as the mode of delivery: in the case of a caesarean birth, the acquisition of the usual dominant bacterial species is delayed compared with a vaginal birth. Several studies have shown that breastfeeding can act as a vector for microorganisms from the mother to the newborn. Other factors can also be added; such as place of birth, diet and antibiotic therapy, which could play a role in disrupting bacterial colonization [2]. The intestinal microbiota is a highly complex ecosystem, consisting of around 10^{14} bacteria as well as other microorganisms such as viruses, archaea and fungi. *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* are the four dominant bacterial phyla of the intestinal microbiota, with 60% to 75% represented by *Firmicutes* and 30% to 40% by *Bacteroidetes* [3]. The taxon composition (genus and/or major phylogenetic groups) is the same for all individuals, but the diversity of species dominating the gut microbiota is unique and constitutes a specific fecal imprint for each individual [4]. The intestinal microbiota plays a crucial role in the recovery of energy from food, as bacteria degrade and ferment

Corresponding author: Btihaj Al Ibrahmi, Laboratory of Natural Resources and Sustainable Development, Team of Nutrition, Food and Health Sciences, Faculty of Sciences, Ibn Tofail University, Kénitra, Morocco; email: ibtihaje2178@gmail.com

Publisher: National Institute of Public Health NIH - National Research Institute

undigested food, providing low-molecular-weight molecules such as succinate, pyruvate and lactate, and short-chain fatty acids such as butyrate, propionate and acetate which are used by colonocytes [5], thus acting as a barrier against colonization by pathogenic bacteria and contributing to the development and maturation of the intestinal immune system [6].

The progress and evolution of techniques for analyzing the intestinal microbiota have led to the discovery of interesting relationships between microbiota-health-pathologies, linking an increasing number of pathologies to disturbances of the intestinal ecosystem. These relationships have opened the way to the potential interest of a preventive or therapeutic approach by modulating the microbiota and/or its functions with pro- or prebiotics [7]. At the beginning of the 20th century, Metchnikoff was the first to initiate work on the health benefits of lactic acid bacteria in fermented milks. His research has been described as groundbreaking in the field of probiotics. In 2001, the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) defined probiotics as "living microorganisms which, when ingested in adequate quantities, confer a health benefit on the host".

This definition was subsequently reaffirmed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014. The ISAPP consensus document clarified that probiotics must be identified by strain and have validated effects, thereby distinguishing probiotic products from generic fermented foods [8].

The observed benefits of probiotics are regulation of intestinal transit, production of beneficial bacterial metabolites and competitive exclusion of pathogens. However, some strains may have specific effects such as vitamin synthesis, reinforcement of the intestinal barrier and an action on the metabolism of bile acids and/or certain enzymatic activities [9]. Some probiotics are able to increase lactose excretion in lactase-deficient adults, mainly due to the addition of bacterial-derived lactase intra luminally and some others can alleviate symptoms in patients suffering from irritable bowel syndrome [10]. Dietary carbohydrates that escape digestion in the upper digestive tract are substrates for colonic bacterial growth [11]; many dietary carbohydrates are classified as prebiotics. The FAO/WHO defines prebiotics as "a substrate that is selectively utilized by host microorganisms and confers a health benefit" [12]. This definition broadens the scope to include non-carbohydrate substances and sites in the body other than the gastrointestinal tract, while emphasizing the need for scientific evidence of beneficial effects. Prebiotics must meet certain criteria: 1) they must not be hydrolyzed either in the stomach or in the small intestine; 2) they must be selective for commensal colonic bacteria, promoting the growth and metabolism of organisms; 3) they must modify the composition of the microflora for a healthy composition; 4) they must induce beneficial effects in the host [13]. Several studies have demonstrated the efficacy of probiotics in the treatment of obesity and insulin resistance and type 2 diabetes, as well as gastrointestinal disorders and allergic conditions [14]. The limited number of studies that have been carried out to assess the perceptions and knowledge of healthcare providers have highlighted that healthcare professionals have limited knowledge of probiotics [15].

To date in Morocco there has been no study of healthcare professionals' perceived knowledge of intestinal microbiota, probiotics and prebiotics. The aim of this article was therefore to assess healthcare professionals' knowledge of intestinal microbiota, probiotics and prebiotics in the the city of Kénitra, Morocco.

MATERIAL AND METHODS

Study design and population

This pilot study was carried out among 143 healthcare professionals in the city of Kénitra over a three-month period from March 2025 to May 2025, aiming to assess their level of knowledge about gut microbiota, probiotics and prebiotics.

Data collection

We collected data using an online questionnaire that was distributed via social networks and sent to email addresses using snowball sampling. The survey questionnaire had two parts to collect the following data: 1 – epidemiological characteristics of respondents (age, type of profession, sector of activity), 2 – concerns various questions on the assessment of knowledge about intestinal microbiota, probiotics and prebiotics. The questions were modelled on those used in previous studies [16-18].

Participants were first asked to rate their knowledge of prebiotics and probiotics on a 5-point Likert scale, then asked questions about the definition of probiotics and prebiotics [19]. Participants were then asked to select the correct statements on intestinal microbiota and dietary fiber fermentation products, and were then asked whether they had ever prescribed probiotics and/or prebiotics to patients, and in which pathology. Finally, participants were asked to identify their sources of information on intestinal microbiota, probiotics and prebiotics.

Ethical considerations

The study protocol was previously approved by the ethics committee of provincial health and social protection delegation – Kénitra (N 1563/2025).

Statistical analysis

We collected all results online via Google Forms, and then data analysis was entered and carried out using SPSS version 25 software. Qualitative variables were expressed as percentages, while quantitative variables were presented as means and standard deviations. The Kruskal-Wallis test and the Mann-Whitney test were used to assess the total score for knowledge of probiotics, prebiotics and gut microbiota between the different groups of healthcare professionals.

The threshold of statistical significance was set at $p < 0.05$.

RESULTS

Sociodemographic characteristics of the study population

The sociodemographic characteristics of the study population are presented in Table 1. A total of 143 healthcare professionals from the city of Kénitra, Morocco completed the questionnaire. The mean age of respondents was 43.78 ± 12.25, ranging from 22 to 72 years. Women accounted for 78.3% of respondents, compared with 21.7% of men. Nurses were the main contributors to the study (55.2%), followed by general practitioners (GPs) (35.7%), while medical specialists and dieticians represented 9.1%. The percentage of healthcare professionals practicing in urban areas was 86.7%, compared with 13.3% in rural areas. The percentage of respondents working in the public sector was 82.5% vs. 17.5% in the private sector.

Table 1. Sociodemographic characteristics of the study population

Variable		Mean ± SD	Percentage
Age (years)		43.78 ± 12.25	-
Gender	Woman	-	78.3%
	Man	-	21.7%
Profession	General practitioner	-	35.7%
	Dietitian	-	9.1%
	Nurse	-	55.2%
Environment	Urban	-	86.7%
	Rural	-	13.3%
Sector	Public	-	82.5%
	Private	-	17.5%

Self-assessment of respondents' knowledge of probiotics, prebiotics and intestinal microbiota

Most respondents rated their knowledge as average (40%) or little knowledge (39%), others rated their knowledge as good (11%) and only 2% had very good knowledge, while the remainder (8%) had no knowledge at all. Healthcare professionals rated their knowledge with a median score of 3, and the self-assessment was statistically similar between the two genders of the professional ($p = 0.869$) (Figure 1).

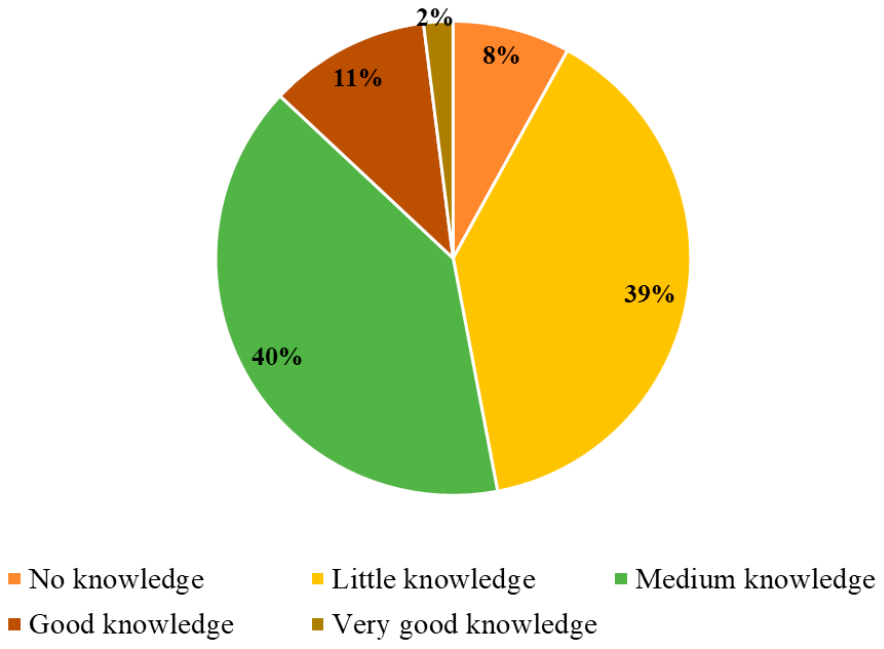


Figure 1. Self-evaluation of respondents' knowledge of probiotics, prebiotics and intestinal microbiota

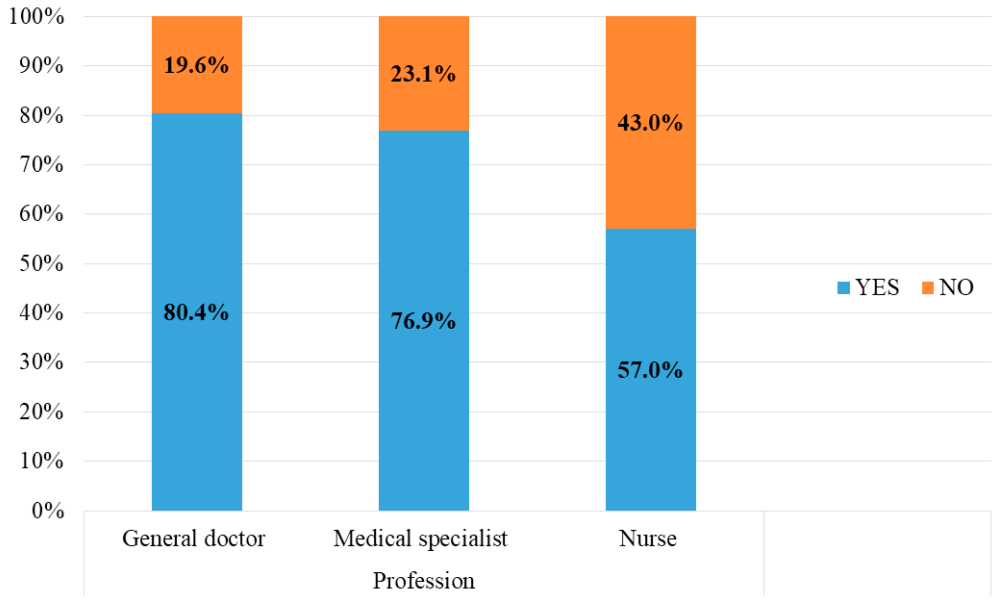


Figure 2. Knowledge of the correct definition of probiotics

Knowledge of the correct definition of probiotics

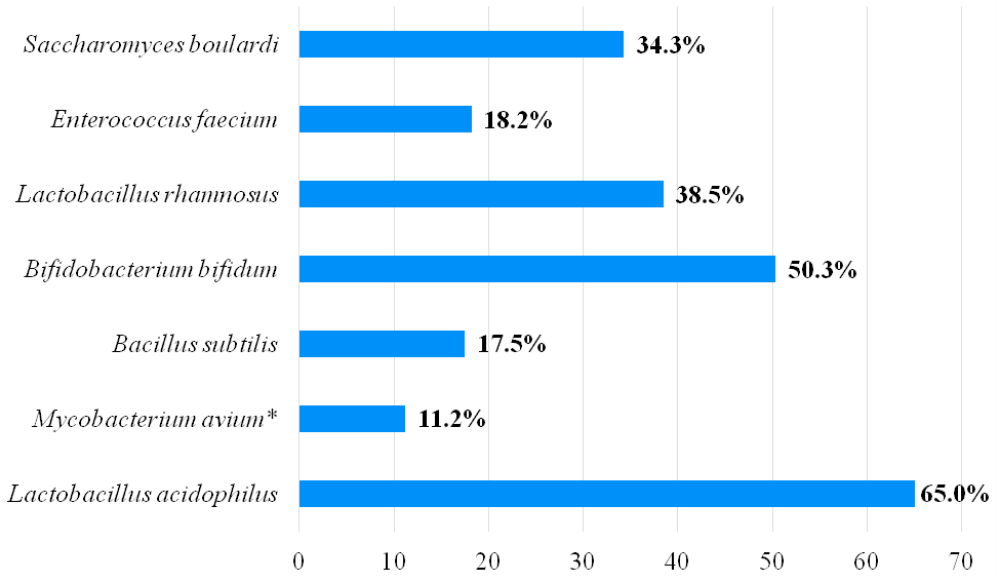
Figure 2 shows awareness of the correct definition of probiotics among different groups of healthcare professionals. Among respondents 67.1% chose the correct definition of probiotics, distributed as follows: 80.4% of general practitioners, 76.9% of specialists and 57% of nurses. There was a statistically significant relationship between profession and choice of the correct definition of probiotics ($p = 0.016$).

Respondents' knowledge of microbial species

Lactobacillus acidophilus (65%) and *Bifidobacterium bifidum* (50.3%) were the two species most familiar to respondents as probiotic strains, followed by *Lactobacillus rhamnosus* (38.5%), *Enterococcus faecium* (18.2%) and *Bacillus subtilis* (17.5%). Indeed, 11.2% of participants answered incorrectly, choosing *Mycobacterium avium* (Figure 3).

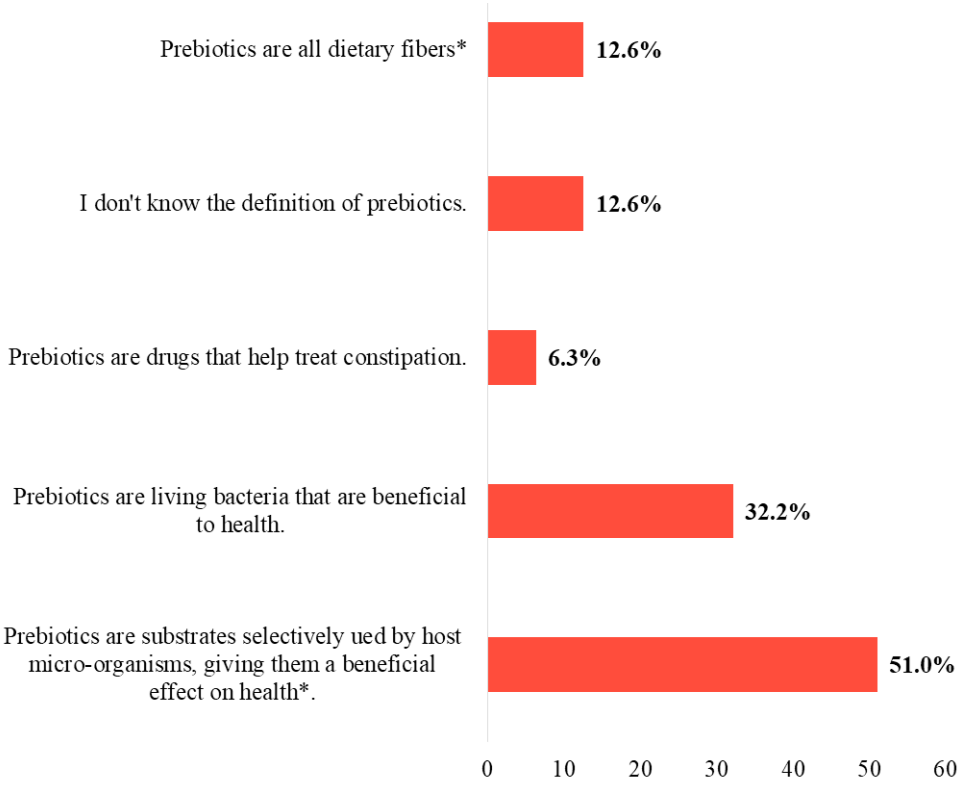
Respondents' knowledge of the correct definition of prebiotics

Respondents' knowledge of the correct definition of prebiotics is shown in Figure 4. The correct answers (answers 1 and 5) were chosen respectively by 51% and 12.6% of healthcare professionals, while 32.2%



*This species has no probiotic strains.

Figure 3. Respondents' knowledge of microbial species that possibly have probiotic strains

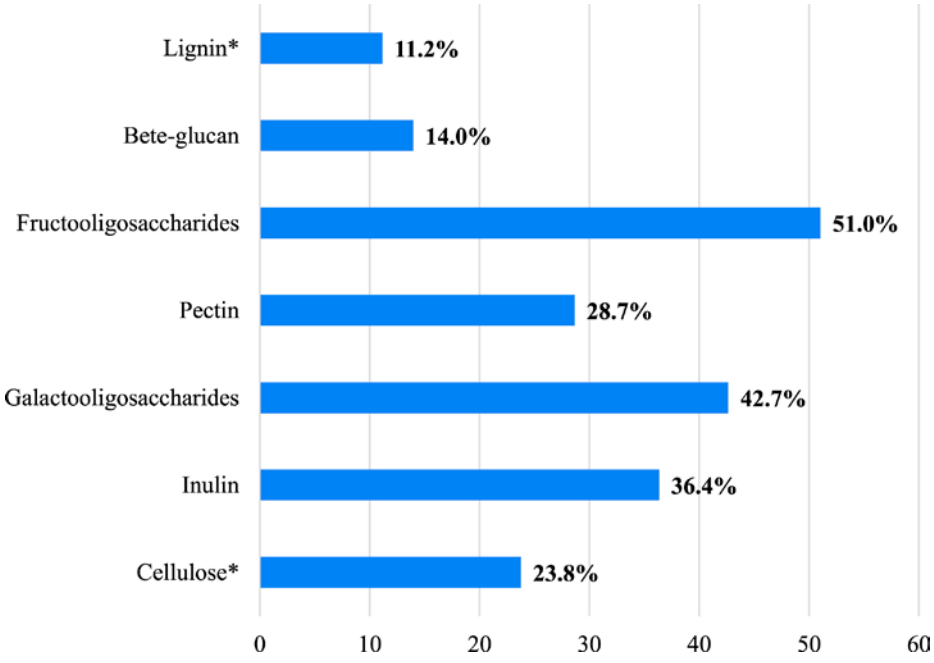


*These statements are correct.
Figure 4. Respondents' knowledge of the correct definition of prebiotics

of respondents ticked statement 4 which said that prebiotics are living bacteria, and 6.3% of professionals chose statement 3 ("Prebiotics are medicines that help treat constipation"). However, 12.6% did not know the definition of prebiotics. Among men, only 7.1% checked off the two correct choices of prebiotics, compared with 3.2% among women.

Respondents' knowledge of prebiotic types

Two of the options given were incorrect: lignin and cellulose; 23% of participants chose cellulose and 11.2% lignin. The most popular prebiotic was fructooligosaccharide (51%), followed by galactooligosaccharide (42.7%) then inulin (36.4%) and lastly beta-glucan (14%). Among women 4.5%



*These fibers are not prebiotics.
Figure 5. Respondents' knowledge of prebiotic types

ticked all the right answers, compared with 3.2% of men. Only 4.8% of urban health professionals ticked all the correct answers, versus 0% of rural professionals (Figure 5).

Respondents' knowledge of the intestinal microbiota

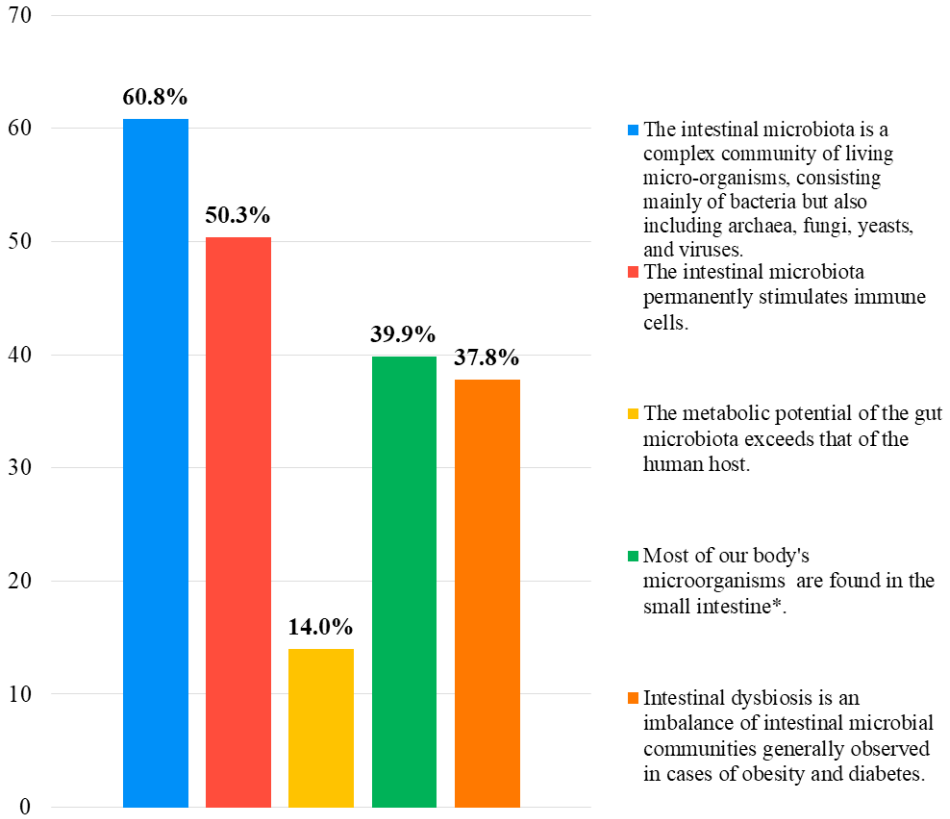
Only one of the five statements was false ("Most of our body's microorganisms are found in the small intestine") and 39.9% of participants ticked this statement as correct, while 60.8% ticked the answer "The intestinal microbiota is a complex community of living microorganisms, consisting mainly of bacteria but also archaea, fungi, yeasts and viruses", 50.3% chose the answer "The intestinal microbiota permanently stimulates immune cells", and 37.8% chose the answer "Intestinal dysbiosis is an imbalance of intestinal microbial communities generally observed in cases of obesity and diabetes" and only 14% of professionals ticked the answer "The metabolic potential of the intestinal microbiota exceeds that of the human host" (Figure 6). Among men, 6.5% selected all the correct answers, compared with 0% of women. The *Chi*-square test ($p = 0.007$) shows a statistically significant difference between men and women for this answer. Only 1.6% of urban professionals selected all the correct answers, compared with 0% of rural professionals.

Respondents' knowledge of the impact of diet on intestinal microbiota

The impact of diet on intestinal microbiota is shown in Figure 7. All choices are correct except the following: "Western diet and lifestyle are associated with a diverse gut microbiota". More than half of the participants (64.3%) selected the answer "The more diversified the diet, the more diversified the gut microbiota", 45.5% selected the answer "The adoption of different long-term diets results in different gut microbiota profiles", 24.5% ticked the answer "Dietary carbohydrates are the main drivers of gut microbiota characteristics", and 29.4% chose the answer "Dietary changes can lead to changes in gut microbiota within 24 hours"; and 26.6% of respondents chose the wrong answer. Among women, 3.6% ticked all the correct answers, compared with 6.5% of men.

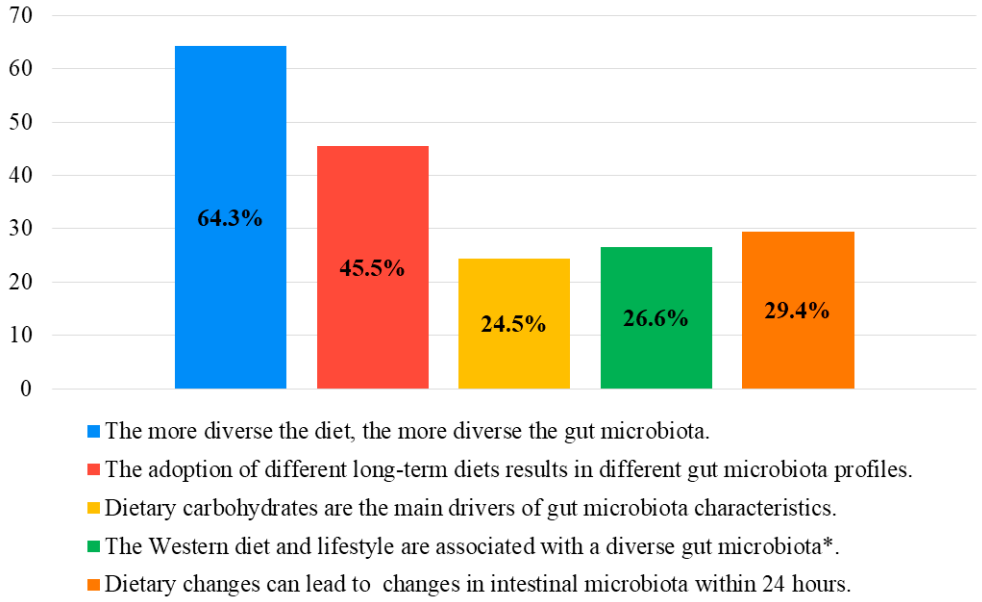
Respondents' knowledge of short-chain fatty acids

Respondents' knowledge of short-chain fatty acids is shown in figure 8. Approximately half (51%) chose acetate, 36.4% – propionate, 28% – butyrate, 26.6% – capric acid and only 11.9% – leucine. Among women, only 14.3% opted for all three correct answers and among men 12.9%. The percentage of urban professionals who ticked all the correct answers was 15.3%, compared with 5.3% in the rural group.



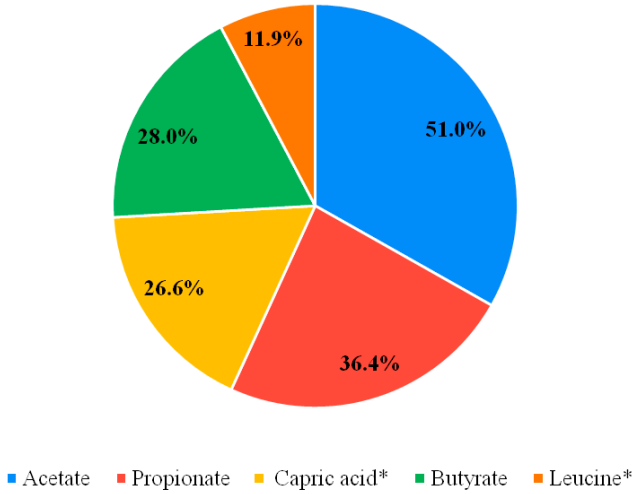
*This statement is false.

Figure 6. Respondents' knowledge of the intestinal microbiota



*This answer is false.

Figure 7. Respondents' knowledge of the impact of diet on intestinal microbiota



*These two chemical compounds are not short-chain fatty acids.

Figure 8. Respondents' knowledge of short-chain fatty acids

Respondents' knowledge of the role of short-chain fatty acids in glucose homeostasis

From the respondents' answers, we noted that 49% of healthcare professionals ticked the answer "By increasing intestinal hormone secretion and gluconeogenesis", 32.9% chose the answer "By providing energy to the colonocytes", and 28% opted for answer "By regulating the host's immune system" and only 25.9% chose answer "By reducing intestinal permeability" (Figure 9). The percentage of women who ticked all the correct answers was 3.6% vs. 6.5% in the men's group. The percentage of urban healthcare professionals who ticked all the correct answers was 4%, compared with 5.3% in rural areas.

Prescribing probiotics/prebiotics to patients

The prescribing of probiotics and/or prebiotics by respondents is shown in Figure 10. Most of them (79%) said "yes", against 21% who said "no".

Use of probiotics and/or prebiotics

Most of professionals (60.1%) prescribed probiotics and/or prebiotics for diarrhea, followed by antibiotics (47.6%) then constipation (39.2%) and 21% of respondents recommended them for diabetes, 18.9% for obesity, while only 3.5% used them for other pathologies (Figure 11).

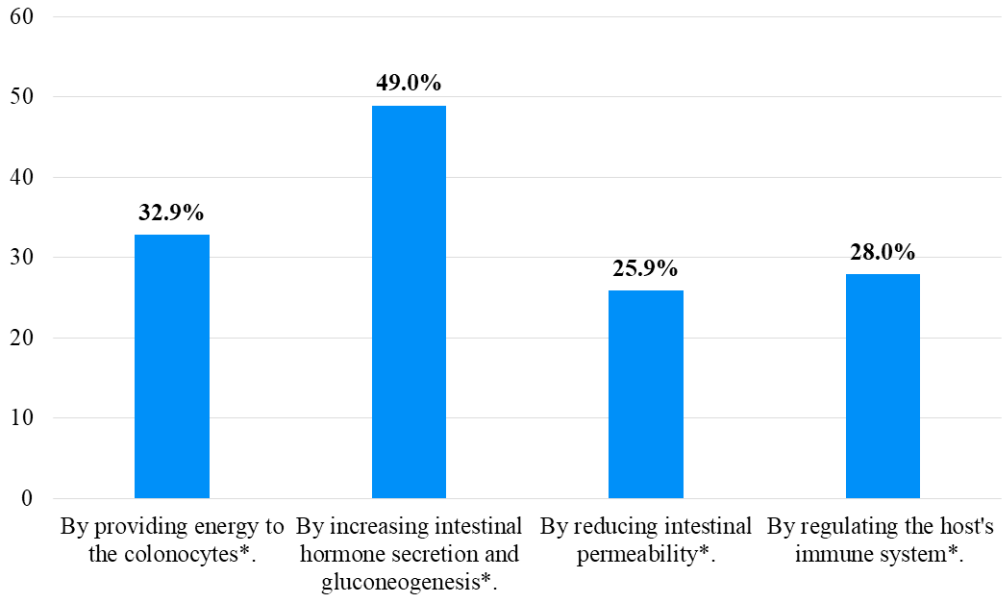
Source of knowledge about probiotics, prebiotics and intestinal microbiota

The purpose of this question is to find out what the participants' source of information is 65.7% of respondents rely on information from websites and 25.2% get their knowledge from university courses, while 21.7% rely on specialist journals (Figure 12).

Total respondent score calculated based on number of correct answers

A total respondent score was calculated based on the number of correct answers that were ticked out of all correct answers to eight questions (Q6 to Q13) on probiotics, prebiotics and the gut microbiota. The mean score was 40.60 reflecting a low score, with a high standard deviation (18.32) indicating variability between participants.

The total score obtained by the healthcare professionals was represented graphically by a moustache box; which revealed that the scores of general practitioners are homogeneous and higher



*These statements are correct.
Figure 9. Respondents' knowledge of the role of short-chain fatty acids in glucose homeostasis

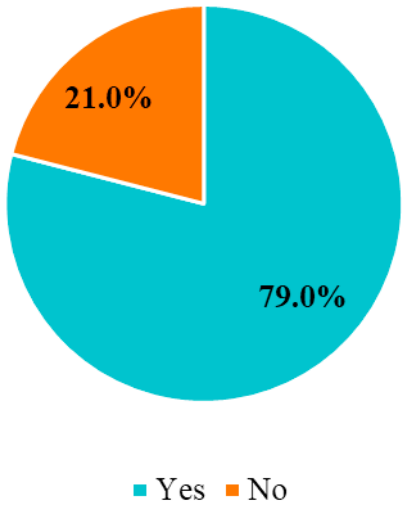


Figure 10. Respondents' use of probiotics and/or prebiotics

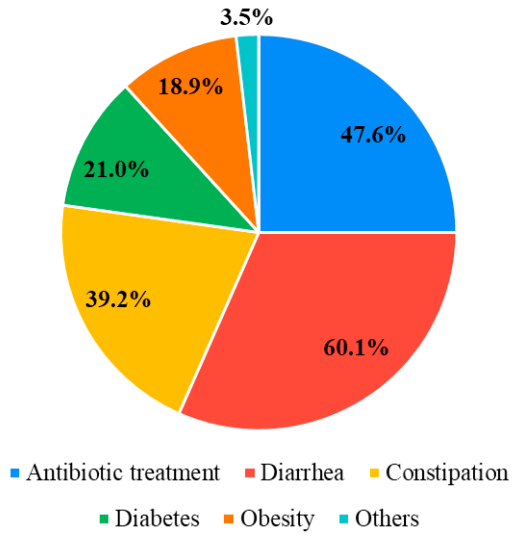


Figure 11. Distribution of pathologies for which respondents use probiotics and/or prebiotics

compared to specialist doctors and nurses. The box is stretched upwards in the nurses' category, attesting to an asymmetrical distribution, with extreme values in the same category reflecting a certain dispersion. The normality test (Kolmogorov-Smirnov and Shapiro-Wilk), as well as the Q-Qplot visualization, did not lead to the conclusion with certainty of the normality of distribution, so we opted for the non-parametric Kruskal-Wallis test to compare scores between professional categories. The Kruskal-Wallis test showed a statically significant difference in total score between the different groups of professionals ($p = 0.0$). This significant difference justifies pairwise comparisons. The Mann-Whitney test showed a significant difference in scores between general practitioners and nurses ($p = 0.0$) in favor of general practitioners, and scores differed significantly between specialist doctors and nurses ($p = 0.07$). There was no significant difference between GPs and specialists ($p = 0.565$) (Figure 13).

DISCUSSION

Various studies in different regions and professional categories have examined the perception and assessment of healthcare professionals' knowledge of intestinal microbiota, probiotics and prebiotics. These studies reveal the current state of knowledge, attitudes and practices of healthcare professionals, shedding light on gaps and strengths in their knowledge.

This pilot study was conducted among 143 healthcare professionals in the city of Kénitra. Although the small number of respondents in our sample limits the generalizability of the results, it nevertheless allows us to identify significant

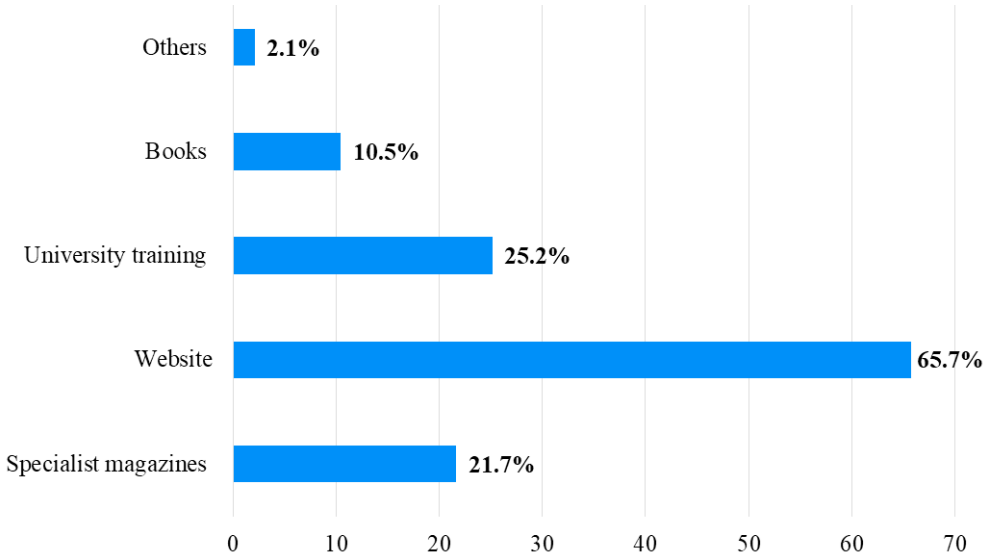


Figure 12. Respondents' sources of information on probiotics, prebiotics and gut microbiota

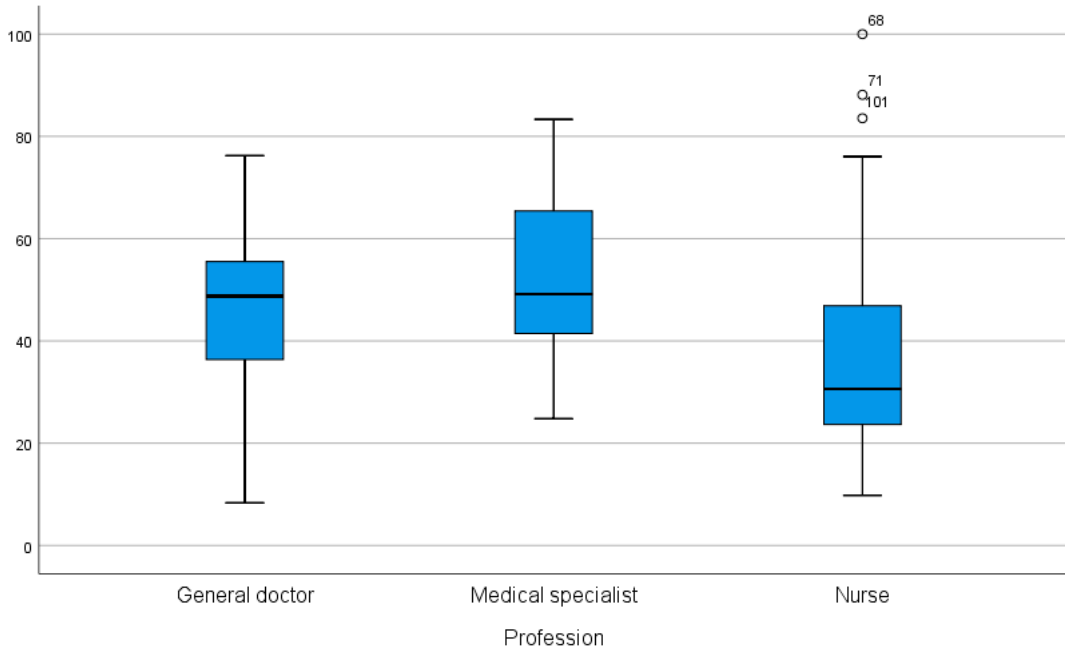


Figure 13. Total score of respondents

preliminary trends that warrant further investigation in future research. Our study showed that the average age of the participants was 43.78 ± 12.25 years. The majority of healthcare professionals rated their knowledge as either "average" or "limited". They rated their knowledge with a median score of 3, and the self-assessment was statistically similar between the two genders of the professional ($p = 0.869$). In the same vein, a study of European dieticians revealed that actual knowledge of probiotics and prebiotics was relatively low, while most participants perceived their knowledge as average to good. In addition, over 78% rated their knowledge of intestinal microbiota as average to good; however, actual knowledge of probiotics and prebiotics was low [17]. A survey of healthcare professionals in Pakistan revealed poor knowledge and practice of probiotics and prebiotics, with only 15.1% having good knowledge [20].

Our survey revealed that 67.1% of respondents chose the correct definition of probiotics and there was a statistically significant relationship between profession and choice of the correct definition of probiotics ($p = 0.016$). As many as 80.4% of general practitioners, 76.9% of specialist physicians and 57% of nurses chose the correct definition of probiotics. Moreover, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were the two species best known to respondents with probiotic strains, and a minority of participants chose *Enterococcus faecium* and *Bacillus subtilis*. In the same vein, a study carried

out in Mexico showed that the FAO definition of probiotics is recognized by 71% of gastroenterologists, and the majority of health professionals had gaps in detailed knowledge of probiotic strains [21]. Similarly, according to the results of a survey conducted in Iraq showed that 92.9% of respondents knew the definition of probiotics [22]. Related to this, a study conducted in Iraq highlighted good knowledge of probiotics among healthcare professionals such that 93.25% of participants were familiar with the term "probiotics" and 80.8% were able to accurately define probiotics [23]. Similarly, one study reported that the probiotic species *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Lactobacillus rhamnosus* were best identified (respectively 92%, 82% and 62%) by respondents as strain-containing probiotics, while *Enterococcus faecium* (29%), *Saccharomyces boulardi* (27%), *Escherichia coli* (25%), were the least recognized as containing probiotic strains, while a minority of participants (4%) incorrectly selected *Mycobacterium avium* which is not a probiotic strain [16].

It should be noted that *Enterococcus faecium* and *Bacillus subtilis* are the subject of scientific debate regarding whether they can be considered as a strains probiotics. Although they are widely used and recognized for their benefits, they do not enjoy the same automatic safety status as other more traditional strains [24]. These two microorganisms meet the WHO definition, and their beneficial effects include *Enterococcus faecium*: Widely used to treat diarrhea and balance microbiota, it produces bacteriocins that inhibit pathogens; *Bacillus subtilis*: Valued for its ultra-resistant spore form, it promotes the growth of good bacteria such as lactobacilli and stimulates intestinal immunity [25].

Unlike other bacterial strains considered probiotics, such as *Lactobacilli* and *Bifidobacteria*, *Enterococcus faecium* and *Bacillus subtilis* pose specific risks that require rigorous selection to verify their efficacy and safety. Concerns have been raised about the safety of certain strains of *Enterococcus faecium* as a probiotic dietary supplement due to this microbe's tendency to develop and acquire antimicrobial resistance to many commonly used antibiotics, particularly vancomycin and ampicillin [26]. Subsequently, several studies have suggested that these bacteria do not pose a risk to human health and may be considered promising candidates for future use as probiotics and bioprotective cultures in the food and/or feed industries [27]. Therefore, it cannot be said that they are not probiotics, but rather probiotics that should be used with caution or under certain conditions.

Our survey results showed that more than half of the respondents selected one of the correct answers regarding the definition of prebiotics, while a minority of participants chose the second correct answer and a small proportion of respondents stated that they did not know the definition of prebiotics. Similarly, this study showed that fructooligosaccharides and galactooligosccharides are the most popular prebiotics (51% vs. 42.7%) chosen by respondents. In the same perspective, a survey showed that 40% of dietitians participating in this study correctly defined prebiotics, thus 63% of respondents confirmed that not all dietary fibers are prebiotics and only 10% of participants responded appropriately that psyllium is not the best-known prebiotic; which highlighted a lack of awareness of the most studied prebiotics such as galactooligosaccharides and inulin-type fructans [17]. Another study by Oliver et al. [18] in 2014 highlighted a lack of familiarity with the term prebiotic, to the extent that only 22% of healthcare professionals were familiar with the word "prebiotic" and 78% of them managed to choose the correct definition of probiotics from the choices offered, so 17% got confused and gave the definition of probiotics to prebiotics. As well as health professionals when asked about the correct sources of prebiotics they recommend inappropriate sources and general healthy food groups (fruits, vegetables, grains) instead of prebiotic compounds such as inulin or fructooligosaccharides.

Today, there is confusion about the relationship between prebiotics and dietary fiber, specifically whether all prebiotics can be considered dietary fiber and vice versa. However, the Dietary Guidelines Advisory Committee (DGAC) in 2010 notes that not all dietary fiber is prebiotic, but all prebiotics are dietary fiber [28].

To date, our study was the first to assess healthcare professionals' knowledge of the gut microbiota and its relationship to diet. The results of our study on the definition of the gut microbiota showed that 39.9% of participants selected the incorrect statement, while varying proportions – ranging from 14% to 60.8% – of participants chose one of the correct statements regarding the definition of the gut microbiota. Furthermore, a small number of healthcare professionals mistakenly believed that a Western diet and lifestyle are associated with a diverse gut microbiota, while the correct statement chosen by the majority of the sample was "The more diverse the diet, the more diverse the gut microbiota". Mitsou et al. [17] in 2024 showed that 83% of participants reported a fair to good level of knowledge about the role of nutrition and diet as modulators of the gut microbiota. For all participants and dietitians, the median knowledge score on nutrition as a modulator of the gut microbiota was 8 out of 11 questions.

The results of our survey show that a small proportion of participants identified butyrate as a short-chain fatty acid, while acetate was recognized as a short-chain fatty acid by more than half of the

respondents and a small number of participants considered the branched-chain amino acid "leucine" to be a short-chain fatty acid. Short-chain fatty acids (SCFAs) are the primary products of dietary fiber fermentation by gut microbes; these SCFAs have beneficial effects on energy metabolism and glucose homeostasis. However, the percentage of correct answers among participants regarding the role of SCFAs in glucose homeostasis remained below 50%.

According to our findings, most healthcare professionals prescribe probiotics/prebiotics to patients to restore the balance of the gut microbiome, primarily to treat antibiotic-induced diarrhea and functional bowel disorders, as well as to strengthen immunity.

According to our findings, most healthcare professionals prescribe probiotics/prebiotics to patients, with the aim of restoring the balance of the gut microbiota, primarily to treat antibiotic-induced diarrhea and functional bowel disorders, as well as to strengthen immunity. These results concur with those of Abbas et al. [29] in 2024, who showed through a survey of pharmacists that 91.2% recognized the role of prebiotics in immune support and only 30% were aware of their cardiovascular benefits. Johnson et al. [30] showed a considerable difference in the recommendation of probiotics between healthcare professionals: 51.6% of nurses recommended them, compared with 91.2% of dieticians and 78% of GPs. According to the results of a study conducted in Mexico revealed that 56.5% of gastroenterologists prescribed probiotics to treat diseases and 39% of nutritionists prescribed them for health maintenance [21]. In parallel, a study conducted in Italy showed that gastroenterologists were more scientifically sound and followed guidelines when prescribing probiotics for conditions such as irritable bowel syndrome and inflammatory bowel disease [31]. Scientific research along the same lines showed that 99.7% of pharmacists and 97.7% of doctors prescribed probiotics for digestive ailments, and only 29.3% of pharmacists recommended them for genital-urinary problems, and 15.1% of doctors recommended them for dermatological symptoms [32].

The results obtained on respondents' knowledge of prebiotics, probiotics and the microbiota highlighted that respondents had a mean total score of 40.60 reflecting a low score, with a high standard deviation (18.32). This testifies to variability between participants, as well as the fact that the score of general practitioners is homogeneous and higher compared to specialist doctors and nurses, moreover nurses had an asymmetrical distribution of scores; with a statically significant difference in total score between the different groups of professionals. In Saudi Arabia, a study revealed that gastroenterologists' knowledge levels of probiotics and prebiotics are higher than dieticians', and 83.3% of gastroenterologists believe in their benefits for people with irritable bowel syndrome versus only 50% of dieticians [33], while general practitioners and dieticians generally report less in-depth knowledge of prebiotics and symbiotics [34-35]. In the same vein, another study showed that no significant differences were observed in knowledge between doctors, pharmacists and nutritionists [23].

According to our findings, participants' sources of information are quite varied, but most healthcare professionals rely on information from websites, while academic training and specialized journals serve as sources of information for only a small proportion of participants. In the United Arab Emirates, professional training and workshops determine pharmacists' knowledge of probiotics and prebiotics [29].

Recommendations can be made to strengthen healthcare professionals' knowledge through introducing modules on gut microbiota, probiotics, and prebiotics into the basic training curriculum for healthcare professionals, developing continuing education: organizing seminars and practical workshops for healthcare professionals and facilitating access to credible scientific resources.

CONCLUSION

In conclusion, this study highlighted the current knowledge of healthcare professionals in the Kénitra city about probiotics, prebiotics and the gut microbiota. Participants have gaps in definitions, knowledge of probiotics, prebiotics and gut microbiota, as well as probiotic strains and the role of prebiotic metabolites in maintaining and preventing host health. For this reason, further research is required to clarify and project the results obtained in the different regions of Morocco.

Acknowledgements

We would like to thank the participants from the bottom of our heart for their bravery and help.

Conflict of interest

The authors report there are no competing interests to declare.

REFERENCES

1. Axelos M, Brétillon L, Champomier-Vergès MC, Dequin S, Doré J, Régnier E, et al. Le Microbiote Intestinal notre nouvel allié santé. Ressources, la revue INRAE. 2022;2:46-65.
2. Leclerc M, Juste C, Blottière H , Doré J. Microbiote intestinal: un univers méconnu. Cahiers de Nutrition et de Diététique. 2007;42:22-27.

3. Landman C, Quévrain E. Le microbiote intestinal: description, rôle et implication physiopathologique [Gut microbiota: Description, role and pathophysiologic implications]. Rev Med Interne. 2016;37(6):418-423. doi: 10.1016/j.revmed.2015.12.012.
4. Doré J, Corthier G. Le microbiote intestinal humain [The human intestinal microbiota]. Gastroenterol Clin Biol. 2010;34 Suppl 1:S7-S15. doi: 10.1016/S0399-8320(10)70015-4.
5. Vily-petit J, Mithieux G. Le rôle de l'intestin et du microbiote dans les fonctions métaboliques. Médecine des Maladies Métaboliques. 2024;18(7):566-570.
6. Gérard P, Bernalier-Donadille A. Les fonctions majeures du microbiote intestinal. Cahiers de Nutrition et de Diététique. 2007;42:28-36.
7. Butel MJ. Les probiotiques et leur place en médecine humaine. Journal Des Anti-Infectieux. 2014;16(2):33-43.
8. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-14. doi: 10.1038/nrgastro.2014.66.
9. Joly F, Nuzzo A, Kapel N, Thomas M. Lien entre les probiotiques et le microbiote : vision du clinicien. Cahiers de Nutrition et de Dietetique. 2017;52:S5-S12.
10. Flourié B, Nancey S. Propriétés fonctionnelles des probiotiques. Cahiers de Nutrition et de Diététique. 2007;42:38-44.
11. Rycroft CE, Rastall RA, Gibson GR. The role of prebiotics in human gut microbiology. In: van Broekhoven A, Shapiro F, Anné J, editors. Novel Frontiers in the Production of Compounds for Biomedical Use. Focus on Biotechnology, Volume 1. New York, Boston, Dordrecht, London, Moscow: Kluwer Academic Publishers; 2002; p. 411-428. ISBN: 0-792-36747-2.
12. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14(8):491-502. doi: 10.1038/nrgastro.2017.75.
13. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics- a review. J Food Sci Technol. 2015;52(12):7577-7587. doi:10.1007/s13197-015-1921-1.
14. Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients. 2017;9(9):1021. doi: 10.3390/nu9091021.
15. Rani U, Ehrlich J, Fakhri G, Doklaijah M, Stewart T, Berry W, et al. On the Knowledge and Prescription of Probiotics by Pediatric Providers: A Cross-Sectional Study. Nutrients. 2025;17(6):963. doi: 10.3390/nu17060963.
16. Fijan S, Frauwallner A, Varga L, Langerholc T, Rogelj I, Lorber M, et al. Health Professionals' Knowledge of Probiotics: An International Survey. Int J Environ Res Public Health. 2019;16(17):3128. doi: 10.3390/ijerph16173128
17. Mitsou EK, Katsagoni CN, Janiszewska K. Knowledge of Dietitians on Gut Microbiota in Health-An Online Survey of the European Federation of the Associations of Dietitians (EFAD). Nutrients. 2024;16(5):621. doi: 10.3390/nu16050621.
18. Oliver L, Rasmussen H, Gregoire MB, Chen Y. Health care provider's knowledge, perceptions, and use of probiotics and prebiotics. Topics in Clinical Nutrition. 2014;29(2):139-149.
19. Joshi A, Kale S, Chandel S Pal DK. Likert scale: Explored and explained. Br J Appl Sci Technol. 2015;7(4):396-403. doi: 10.9734/BJAST/2015/14975.
20. Arshad MS, Saqlain M, Majeed A, Imran I, Saeed H, Saleem MU, et al. Cross-sectional study to assess the healthcare professionals' knowledge, attitude and practices about probiotics use in Pakistan. BMJ Open. 2021;11(7):e047494. doi: 10.1136/bmjopen-2020-047494.
21. Valdovinos-García LR, Abreu AT, Valdovinos-Díaz MA. Probiotic use in clinical practice: Results of a national survey of gastroenterologists and nutritionists. Uso de probióticos en la práctica clínica: resultados de una encuesta nacional a gastroenterólogos y nutriólogos. Rev Gastroenterol Mex (Engl Ed). 2019;84(3):303-309. doi: 10.1016/j.rgmx.2018.05.004.
22. Hashim ZA, Mohammed MH, Al-Zidan RN, Tawfeeq HR. Healthcare professionals' familiarity, perceptions, attitudes, and obstacles towards the use of prebiotics and probiotics. Acta Pharmaceutica Sciencia. 2025;63(2):352-366.
23. Soni R, Tank K, Jain N. Knowledge, attitude and practice of health professionals about probiotic use in Ahmedabad, India. Nutr Food Sci. 2018;48:125-35. doi: 10.1108/NFS-02-2017-0032.
24. Zommiti M, Cambronel M, Maillot O, Barreau M, Sebei K, Feuilloley M, et al. Evaluation of Probiotic Properties and Safety of *Enterococcus faecium* Isolated From Artisanal Tunisian Meat "Dried Ossban". Front. Microbiol. 2018;9:1685. doi: 10.3389/fmicb.2018.01685.
25. Lee MK, Jung HK, Kim DG, Park IS, Heo YL, Kang J, et al. Strain-Specific Effects of *Bacillus subtilis*, *Enterococcus faecium*, and *Pediococcus pentosaceus* Supplementation on Growth Performance, Immunity, and Disease Resistance in Olive Flounder (*Paralichthys olivaceus*). Fishes. 2025;10:465. doi: 10.3390/fishes 10090465.
26. Pi X, Teng W, Fei D, Zhao G, Liu W. Effects of Live Combined *Bacillus subtilis* and *Enterococcus faecium* on Gut Microbiota Composition in C57BL/6 Mice and in Humans. Front Cell Infect Microbiol. 2022;12:821662. doi: 10.3389/fcimb.2022.821662.
27. Wang X, Yang Y, Huycke MM. Risks associated with enterococci as probiotics. Food Res Int. 2020;129:108788. doi: 10.1016/j.foodres.2019.108788.
28. Slavin J. Fiber and prebiotics: mechanisms and health benefits. Nutrients. 2013;5:1417-1435. doi: 10.3390/nu5041417.
29. Abbas MO, Ahmed H, Hamid E, Padayachee D, Abdulbadia MT, Khalid S, et al. Pharmacists' Knowledge,

Perception, and Prescribing Practice of Probiotics in the UAE: A Cross-Sectional Study. Antibiotics (Basel). 2024;13(10):967. doi: 10.3390/antibiotics13100967.
30. Johnson N, Thomas L, Jordan D. Probiotics: assessing health professionals' knowledge and understanding. Gastrointest Nurs. 2016;14:26-33. doi: 10.12968/gasn.2016.14.1.26.
31. Marasco G, Bruni A, Nardone OM, Lopetuso LR. Insights into Probiotic Prescription among Gastroenterologists and Other Healthcare Professionals: Evidence from an Italian Survey. J Clin Med. 2024;13:4749. doi: 10.3390/jcm13164749.
32. Başar Güneş H, Bayraktar Ekincioğlu A, Karakan T, Demirkan K. Assessment of Knowledge and Attitudes of Physicians and Pharmacists on Probiotics: A Cross-Sectional Survey. Turk J Pharm Sci. 2024;21:36-41. doi: 10.4274/tjps.galenos.2023.36974.
33. Eid NM, Alsolami GA, Al-Nuafie HD, Malibari HW, Alsolami WD, Enani S. Assessment of Knowledge, Perception, and Practices Regarding Probiotics and Prebiotics Among Clinicians in Saudi Arabia: A Pilot Study. Cureus. 2024;16(1):e52080. doi: 10.7759/cureus.52080.
34. Zaid AN, Ali I, Jaradat N, Al Ramahi R, Hmeidat R, Nofal S, et al. Knowledge and awareness of Palestinian health care professionals towards probiotics, prebiotics and synbiotics: a cross-sectional study from Palestine. Journal of Biological Research. 2025;98:12742. doi: 10.4081/jbr.2025.12742.
35. Wilson Z, Whitehead K. A cross sectional survey to assess healthcare professionals' attitudes to and understanding of probiotics. Clin Nutr ESPEN. 2019;34:104-109. doi: 10.1016/j.clnesp.2019.08.004.

Received: 21.01.2026
Revised: 30.03.2026
Accepted: 07.04.2026
Published online first: 22.04.2026

Rocz Panstw Zakl Hig 2025;76(4):315-322
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/220379

ORIGINAL ARTICLE

ASSOCIATION OF SWEET TASTE PERCEPTION AND DIETARY HABITS WITH BODY MASS INDEX AMONG PUBLIC SCHOOL CHILDREN OF BELAGAVI DISTRICT, SOUTH INDIA

Roopali M. Sankeshwari, Anil V. Ankola, Siva Shankari, Yuvarani Kandasamy Parimala, Kavitha Ragu, Varkey Nadakkavukaran Santhosh

Department of Public Health Dentistry, KLE Vishwanath Katti Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi, India

ABSTRACT

Background. Healthy diet is significant for growth, development, and prevention from dietary related diseases in children and adults. Dietary imbalance can lead to many general and oral diseases and sugar rich diet can affect the teeth at an early age and continues towards adolescence causing several oral problems such as dental caries. Frequent consumption of a certain food causes changes in taste perception and leads to preference for that food. Taste perception may also be affected by several environmental, cultural, and genetic factors and has been found to be linked to oral health and body mass index (BMI).

Objective. This study evaluated the association between sweet taste perception, dietary habits, and body mass index among 13-15-year-old public school children in the Belagavi district.

Material and Methods. A descriptive cross-sectional study was conducted among 1300 school children aged 13-15 years. Data were collected using a self-designed and validated questionnaire to assess dietary habits. Sweet taste perception was evaluated using sweet taste threshold (TT) and sweet taste preference (TP). Based on the sweet taste perception scores, participants were categorized into low, medium, and high groups. Body mass index was calculated using standard criteria. Descriptive statistics, Spearman's correlation test and ANOVA were performed. Statistical significance was considered for p-value ≤ 0.05.

Results. Of the 1300 participants, 625 (48.1%) were males and 675 (51.9%) were females. A majority of the students demonstrated a low sweet taste threshold (78.7%), while 66% exhibited a medium level of sweet taste preference. More than half of the participants (57.8%) were underweight. A statistically significant association was observed between BMI and sweet taste preference ($p < 0.05$).

Conclusions. Sweet taste preference and dietary habits were significantly associated with BMI among adolescents (sugar preference and sugar exposure were positively correlated with BMI, while nutrient score was negatively correlated). These findings suggest that taste perception and dietary behaviours may play an important role in the nutritional status of school-going children.

Keywords: *school children, dietary habits, BMI, sweet taste perception, India*

INTRODUCTION

A healthy diet is significant for growth, development, and prevention of dietary related diseases both in children and adults. Among the five different basic taste perceptions like sweet, sour, bitter, salty and savory/umami, sweet taste is often linked with impacts on health and more so on oral health. Sugar, vital source of energy, is also delicious and enjoyable, making it easy to satiate hunger. Its sweet taste encourages people, especially children, to consume more of it. Flavor is the primary dimension by which young children determine food acceptance [1]. It is assumed that children's preferences for sweets influence the choice of high-calorie foods. Frequent consumption of a certain food causes changes in taste perception and leads to preference for that food. Taste perception, in turn, may also be affected by several environmental, cultural, and genetic factors and has been found to be linked to oral health and body mass index (BMI). Sweet taste perception is a genetically driven feature that can influence a person's preference for sweet foods, which in turn can affect their body mass index and overall dietary intake [2].

Corresponding author: Roopali M. Sankeshwari, Professor and Head of Department of Public Health Dentistry, KLE Vishwanath Katti Institute of Dental Sciences, KLE Academy of Higher Education and Research, JNMC Campus, Nehru nagar, Belagavi, India; email: drroopali@kledental-bgm.edu.in; phone: 09844837197

Publisher: National Institute of Public Health NIH - National Research Institute

A balanced meal is necessary for the proper growth and development of children and helps to tackle nutritional problems in this age group. However, studies reveal that many children do not eat enough fruits, vegetables, seafood, fiber, and other essential nutrients, leading to potential nutritional deficiencies [3-5]. Nutritional inequalities in India are already well characterized across caste, economic position, gender and place of residence by a single axis [6]. This can be attributed to poor living standards, food insecurity, and inadequate dietary practices among individuals living in poverty. Poverty exacerbates the risk of malnutrition, making those affected more vulnerable due to inadequate living conditions, food insecurity, and poor dietary practices.

Today's children are exposed to social media in wide various ways and become easily fascinated by the advertisements of colorful, unhealthy and cariogenic foods. There is an increasing concern that the intake of free sugar, particularly in the form of sugar-sweetened beverages, contributes to overall energy intake and may reduce the consumption of foods that provide more nutritionally adequate calories [7]. This can lead to an unhealthy diet, weight gain, and an increased risk of developing non communicable diseases (NCD). The World Health Organization (WHO) recommends a reduced intake of free sugars throughout the lifetime. For both adults and children, WHO strongly recommends reducing free sugar intake to less than 10% of total energy intake [7]. A recent Lancet EATS commission recommends that daily intake of food from all the seven good groups is essential for appropriate growth and development of children [8].

There is a scarcity of literature about the association of sweet taste perception, its impact on daily nutrient intake and BMI, especially among school children and hence, the present study has been attempted with an aim to fill this lacuna.

The aim of this study is to investigate the relationship between sweet taste perception, dietary habits, and BMI among children attending public schools.

MATERIAL AND METHODS

Design and method

This cross-sectional study was conducted among children studying in public schools across Belagavi district from November 2023 to March 2024. Belagavi is the largest district in Karnataka which has 15 subdivisions and approximately 1.3 million children belong to the age group of 12-15 years.

Ethical considerations

Ethical approval was obtained from the Institutional Research and Ethics Committee (Ref. No: 165). Prior to the commencement of the study, required permissions from school authorities and parental consent were obtained.

Data collection

Participants were selected based on specific inclusion and exclusion criteria. The study included children aged 13-15 years who were willing to provide informed consent when studying at public schools. Children with special healthcare needs or underlying medical conditions were excluded. The sample size was calculated based on a similar cross-sectional study [9] on school children aged 13-15 years, which found a prevalence rate of 25.9% for taste preference (TP) at a concentration of 12.84 g/L. Taking these findings into account and considering a 10% attrition rate, the sample size was calculated using the formula: $n = z^2 \cdot p \cdot q / d^2$, (where p = prevalence of taste perception (25.9% in this case) [11], q = 74.1 (1-p), d = allowable error (2.5) and z = 1.96. Required sample size was 1228 which was rounded off to 1300 with a 10% allowable error.

A two-stage random sampling technique was employed in this study. Five subdivisions were randomly chosen from the 15 subdivisions in Belagavi district, followed by the random selection of four schools from each chosen subdivision using the lottery method. From each subdivision, 260 children meeting the inclusion criteria were chosen, resulting in a total sample size of 1300 students.

Study questionnaire consisted of four sections: socio-demographic details, BMI recording, sweet taste perception and 3 days diet diary. Students were instructed to write the details of their dietary intake for 3 days including one weekend. They were reinforced to write the details of all the foods and beverages consumed by them, including in-between meal sweets/snacks. They were advised to mention the approximate portion size of the food consumed.

Pilot study: A pilot study was conducted on 130 participants belonging to a similar age group, after the questionnaire was finalized, to test its comprehensibility and feasibility. Subtle changes were made in the questionnaire to reduce any ambiguity.

BMI was calculated as per Quetelets formula [10], using a portable digital weighing machine and a measuring tape. Repeated checks were conducted to ensure the accuracy and reliability of the measurements obtained. BMI-for-age Z-scores were obtained using the WHO Anthro software, with the World Health Organization (2020) standards applied to categorize BMI.

For children over five years old, underweight was defined as a Z-score below -2, overweight was classified as a Z-score above +1 but less than or equal to +2, and obesity was defined as a Z-score above +2 as per WHO recommendation [11].

Analytical-grade sucrose (Sigma Aldrich) and sterile distilled water were used to prepare sugar solutions under sterile conditions. Fresh solutions were prepared for each visit.

The sugar exposure score was calculated based on the intake of liquid, solid, sticky, and slowly dissolving foods, as recorded in a three-day dietary record. It was categorized as excellent if the score was 5 or less, good if it was 10, and in the watch-out zone if it was 15 or more.

The nutrient score was assessed based on data extracted from 3 days dietary records. All the food items consumed were divided into four food groups:

- bread/cereals,
- milk/milk products,
- meat/poultry products,
- vegetables and fruits.

From the dietary records, the food or mixed food dishes were classified into one or more of the appropriate food groups. For each serving of these foods listed in the food intake record, a check was placed in the appropriate food group block.

The number of checks were added and multiplied by the number shown:

- milk group – multiplied by 8, highest possible score – 24,
- meat/poultry group – multiplied by 8, highest possible score – 24,
- vegetables and fruits group: vitamin A – multiplied by 6, highest possible score – 6, vitamin C – multiplied by 6 and highest possible score – 6, others – multiplied by 6 highest possible score – 12,
- bread and cereal group – multiplied by 6 highest possible score – 24.

All the points were added and the total was the score for four food group scores. Average of three days score was calculated and food score was interpreted as follows:

- 72 to 96 – excellent,
- 64 to 72 – adequate,
- 56 to 64 – barely adequate,
- 56 or less – not adequate.

The examiners were standardized and calibrated by a panel of experts before the start of the study to ensure consistent examination and uniform interpretation of the data recorded.

Procedure

The purpose of the study was explained in detail to the participants, and their informed consent and assent forms were obtained. A team of three examiners collected demographic details from the participants, and then proceeded to assess sweet taste threshold (STT), sweet taste preference (STP), and dietary intake using a dietary record. Sucorse solution which was used to assess STT, STP were prepared as per the modified technique prescribed by Furquim et al. [12]. The children were presented with ten sucrose solutions ranging in concentration from 1.63 g/L (0.0047 M/L) to 834.56 g/L (2.40 M/L), with each step doubling the concentration in grams per litre. A dropper was used to administer the 5 mL of solutions, and between tastings, the children rinsed their mouths with filtered water. The assessment of STT involved identifying the lowest concentration at which the participants could detect the presence of sucrose and differentiate it from water. STP which is the solution chosen by participants based on the level of sweetness they preferred in a drink. Based on their STT and STP levels, the children were categorized as "low" if their preferred solution was within the range of 1.63-12.84 g/L, "medium" if within 25.67-102.69 g/L, and "high" if within 205.38-834.56 g/L. Dietary information was collected using a three-day diet record to estimate sugar intake and food group scores.

Anthropometric measurements: Height of participants was recorded using digital measuring scale (model: Seca 213 portable Stadiometer height-rod). The weight was noted using an electronic calibrated scale, and rounded off to the nearest kilograms.

Statistical analysis

Obtained data was entered into Microsoft Excel and subjected to statistical analysis using software package IBM SPSS Statistics Version 21. Descriptive statistics, Spearman's correlation test and ANOVA were performed to determine association between the BMI Z-score, as the dependent variable, and various predictors. Statistical significance was considered for p-value of ≤ 0.05.

RESULTS

Socio-demographic characteristics of the participants

Of the total 1300 school children, 48.1% were males with mean age of the participants being 14.53 ± 0.67 years. Majority (58%) of children belonged to the upper-lower socio-economic class as per Kuppuswamy's Classification [13]. In terms of BMI, majority (58.8%) of participants were underweight, whereas 8.1% were overweight (Table 1). School children exhibited lower BMI Z-scores (skewed left) compared to the WHO standard reference (Figure 1).

Sugar exposure, nutrient score, sugar threshold and sugar preference

Majority of participants had an excellent sugar exposure score (54.5%) and low sugar threshold (78.6%). When sugar preference was recorded, approximately (66.1%) had medium sugar preference. However, nutrient scores recorded among the

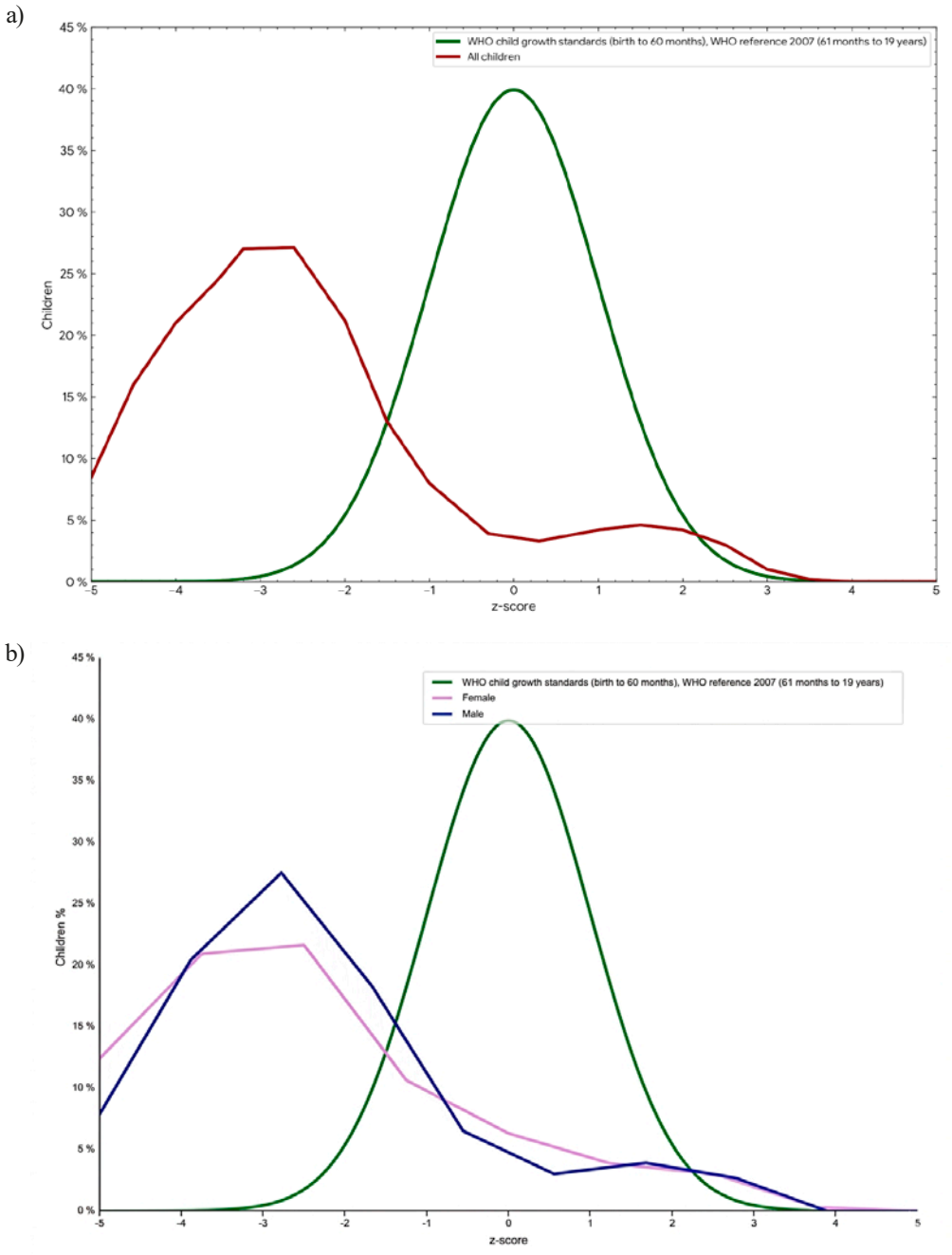


Figure 1. Kernel density plots of BMI-for-age Z-scores versus WHO 2007 reference (61 months-19 years): a) all children (n = 1300); b) males (n = 625) and females (n = 675)

participants revealed that majority had inadequate nutrition (Table 1).

When sugar exposure was analyzed, it was found to be higher among females, obese participants BMI Z-score > +2·SD and those from lower socio-economic status. Similar findings were obtained for both the sugar threshold and preference scores. However, both the scores were higher among males unlike sugar exposure scores. Nutrient scores on the other hand were higher among males, healthy individuals, and those from upper socio-economic status (Table 2).

Among the study parameters, only BMI showed a statistically significant difference with all the variables such as nutrient score, sugar exposure, threshold, and preference scores, whereas gender showed statistically significant difference only in nutrient score ($p \leq 0.05$) (Table 2).

Relationship between BMI and various study variables

Table 3 depicts the correlation between BMI and study variables such as sugar exposure, nutrient score, sugar threshold, and sugar preference. Sugar threshold, sugar preference and sugar exposure showed positive linear correlations with BMI ($p \leq 0.05$). However, there was a negative correlation observed between nutrient score and BMI ($p \leq 0.05$).

Table 1. Distribution of study variables among participants

Characteristics	N (%)
Age	
Mean ± SD	14.53 ± 0.67
13 years	132 (10.2%)
14 years	349 (26.8%)
15 years	819 (63.0%)
Gender	
Male	625 (48.1%)
Female	675 (51.9%)
Socio-economic status	
Upper class	3 (0.2%)
Upper middle class	67 (5.2%)
Lower middle class	426 (32.8%)
Upper lower class	754 (58.0%)
Lower class	49 (3.8%)
Body mass index	
Underweight	764 (58.8%)
Healthy	249 (19.2%)
Overweight	105 (8.1%)
Obese	181 (13.9%)

Characteristics	N (%)
Sugar exposure	
Excellent	709 (54.5%)
Good	568 (43.7%)
Watch out zone	23 (1.8%)
Sugar threshold	
Low threshold	1022 (78.6%)
Medium threshold	277 (21.3%)
High Threshold	0 (0.0%)
Sugar preference	
Low threshold	102 (7.8%)
Medium threshold	859 (66.1%)
High threshold	338 (26.0%)
Nutrient score	
Not adequate	1175 (90.4%)
Barely adequate	79 (6.1%)
Adequate	16 (1.2%)
Excellent	30 (2.3%)

SD – standard deviation

Table 2. Distribution of sugar exposure, nutrient score, sugar threshold and sugar preference among various demographics and BMI

Parameters	Sugar exposure Mean ± SD	Nutrient score Mean ± SD	Sugar threshold Mean ± SD	Sugar preference Mean ± SD
Gender				
Male	5.31 ± 3.46	41.81 ± 14.76	3.66 ± 1.11	6.74 ± 1.52
Female	5.76 ± 3.81	37.25 ± 12.29	3.63 ± 1.06	6.30 ± 1.55
t value[a]	-2.25	6.05	0.52	5.19
p-value	0.082	< 0.001*	0.365	0.992
Socio-economic status				
Upper	3.30 ± 2.21	47.10 ± 13.71	3.33 ± 1.15	5.00 ± 1.73
Upper middle	5.53 ± 3.38	41.91 ± 15.19	3.29 ± 1.21	6.46 ± 1.51
Lower middle	5.63 ± 3.69	39.52 ± 13.78	3.66 ± 1.07	6.47 ± 1.53
Upper lower	5.47 ± 3.63	39.43 ± 13.47	3.68 ± 1.07	6.54 ± 1.57
Lower	6.04 ± 4.02	35.24 ± 14.51	3.51 ± 1.08	6.53 ± 1.45
F value[b]	0.65	1.93	2.23	0.87
p-value	0.627	0.103	0.064	0.483
Body mass index				
Underweight	5.29 ± 3.44	40.23 ± 13.83	3.55 ± 1.08	6.42 ± 1.56
Healthy	5.69 ± 3.97	40.39 ± 13.86	3.79 ± 0.96	6.37 ± 1.44
Overweight	6.03 ± 3.78	39.01 ± 12.76	3.53 ± 1.19	6.65 ± 1.73
Obese	6.11 ± 3.89	34.61 ± 12.64	3.93 ± 1.13	7.01 ± 1.46
F value[b]	3.48	9.20	8.38	7.99
p-value	0.015*	< 0.001*	< 0.001*	< 0.001*

[a]Unpaired t test; [b]ANOVA test; * $p \leq 0.05$ is considered statistically significant.

Table 3. Correlation of BMI with various study variables

Parameters	Body mass index (BMI)	
	r	p-value
Sugar exposure[a]	0.072	0.010*
Nutrient score[a]	-0.141	< 0.001*
Sugar threshold[b]	0.187	< 0.001*
Sugar preference[b]	0.109	< 0.001*

[a]Pearson's correlation coefficient; [b]Spearman correlation coefficient; *p-value ≤ 0.05 is considered as statistically significant.

Table 4 summarizes the multiple regression analysis models performed using BMI as the dependent variable and the predictors: age, gender, socio-economic status, sugar threshold, sugar preference, sugar exposure, and nutrient score. This analysis revealed that, except for gender, all other predictors significantly influenced BMI ($p \leq 0.05$), and the dependence on these predictors is found to be 13.1%.

DISCUSSION

Educating children in adopting healthy food choices can pave a way in developing individuals who are health conscious and would preferably adopt a healthy life style.

Taste is an important factor that significantly influences food choices, preferences, and intake in young children. It has been proven in previous studies that children aged 11 to 13 years possess varied basic taste perceptions which could influence their eating behaviour [14]. Among the different tastes, sweetness plays a major role in the selection of food [15]. However, there is an individual difference in this taste preference which could be a result of genetics or by early exposures [1]. Children's preference for sweets and related sugar consumption might be due to exposure to sugary foods, and their growth maturity in addition to parental influence [16]

WHO stated that an unhealthy diet can pave way for various non-communicable diseases such as dental caries, obesity and overweight [17]. Children are at a higher risk for unhealthy dietary behaviours with their preferences for food selection based on high-calorie intake, especially sweets. Recently Central Board of Secondary Education in India has taken up the issue of 'sugar consumption' seriously and has come up with 'Sugar Boards' in schools to educate students about the risks of excessive sugar intake.

Body mass index is an important parameter to monitor growth of a child and has been reported equivocally in the literature. In a study by Ashi et al. authors noticed that most of their study participants in Saudi Arabia belonged to 'normal' category [11], contrary to this, in the present study, 58% of the students were in the underweight category. This prevalence rate is higher compared to 28% reported for Karnataka as per Comprehensive National Nutritional Survey (CNSS) 2019 [18]. The type of diet, religion, area of residences, purchasing power of the family, food adequacy, maternal literacy rate may be some of the factors which could have an indirect impact on the BMI of the children. However as per CNSS report, it was interesting to note, that, as the demands of the growth spurt decreased, boys started to gain weight and the prevalence of low BMI slowly declined to ~15% by age 19 [18].

In the current study, 90.4% of participant's nutrient score was 'not adequate'. This was very high compared to the result of the CNSS report, which stated that, 28% of the adolescents had inadequate nutrient score. The disparity could be explained on the basis of calculation of food group score. In the current study we have employed the food group score calculation as prescribed by Nizel and Pappas [19], whereas CNSS has employed an ingenious method – children were assessed for consumption of seven food groups during the previous day; grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A-rich

Table 4. Multiple linear regression analysis of BMI with other variables

Parameters	Coefficient r	SE	t	95% CI	p-value	Adjusted R^2
Dependent variable: BMI						
Constant	-	4.189	-4.563	-27.333 – -10.898	0.001*	0.131
Age	0.195	0.259	7.440	1.421 – 2.439	0.001*	
Gender	0.005	0.411	-0.178	-0.880 – 0.734	0.859	
Socio-economic status	0.213	0.303	8.197	1.890 – 3.080	0.001*	
Sugar threshold	0.067	0.192	2.462	0.096 – 0.848	0.014*	
Sugar preference	0.080	0.136	2.921	0.130 – 0.662	0.004*	
Sugar exposure	0.070	0.055	2.677	0.039 – 0.256	0.008*	
Nutrient score	0.118	0.015	-4.411	-0.095 – -0.037	0.001*	

SE – standard error; CI – confidence interval; *$p \leq 0.05$ is considered as statistically significant.

fruits and vegetables; and other fruits and vegetables [18]. Only frequency was assessed in CNSS, unlike the current study, which calculated food score for each child. Majority of the participants in the current study did not consume eggs/meat, milk and milk products or fruits; unfortunately, they had easy access to junk foods which had zero nutritional value.

Several studies have tried assessing sweet taste perception and obesity with contrasting results suggesting diversity in the relationship between them [20, 21]. Previous studies have reported that taste perception is more distinct in normal weight than in obese children stating that a higher sweet threshold was noted among obese children [22]. A study conducted on representational sample from Europe concluded that taste threshold is related to the weight of an individual and the taste sensitivity increases with increasing age [23].

The influence of sweet taste perception on dietary intake in the context of dental caries and BMI was assessed among the Saudi Arabian population [11]. There was an absence of any significant relationship between dietary patterns and BMI in their study which was in contrast to the study by Washi and Ageib which concluded that being overweight and the number of meals intake in children have a significant relation [24]. In the present study, sugar reference scores were directly related to the BMI status of the children with the highest score for overweight children and vice versa, thereby revealing a positive correlation between BMI and sugar preference.

Gender differences in sweet taste perception have been documented by studies that stated that poor dietary habits are linked to food choice, with taste being an aspect considered by females more than males [25]. It was interesting to note that in the current study boys had more preference for sugar than girls and this was statistically significant. Though exact scientific cause responsible for this finding is obscure it may be related to physiological reasons. Children may be less sensitive to certain tastes, and may require higher concentration of sweetness to get the same sensation as adults. Increased preference for sugar may also be due to increased calorific requirements or hormonal changes that take place during puberty, particularly with secretion of leptin and insulin which are known to decrease sweet taste preference [16].

Limitations of the study

The present study has a few limitations that need to be addressed for implication in a public health perspective. Sweet taste perception and dietary habits with BMI were assessed in this present study.

Most children followed the instructions for entering dietary records, but personal variations in presentation and reporting along with individual variability in taste threshold can possibly cause a bias which was inherent to the study. Social desirability bias cannot be completely ruled out.

Strengths of the study

The current study was conducted on a wide representational sample. The study provided an uncommon opportunity to sensitize and create awareness towards sugar intake and general health.

CONCLUSION

This study reveals there was a significant association observed between sweet threshold, sweet preference, and gender with BMI. A high prevalence of underweight participants was observed, with sugar exposure and preferences positively correlating with BMI, especially among those from lower socio-economic backgrounds. Nutrient scores were generally low, particularly among those with lower BMI, emphasizing the need for targeted nutritional interventions to support healthier growth in underprivileged groups.

Future recommendations

Children and teachers need to be aware of the association between sweet taste and general health. Educating young minds on making healthy choices can be a key component in fostering "healthier individuals" of tomorrow. Longterm follow-up studies assessing sugar intake and its impact on growth and development of general health could be taken up on a representative sample.

Public health significance

It was shocking to note that 90.3% of student's nutritional score was "not adequate," whereas 57.8% were underweight. This is an alarming number given the fact that there are many flagship nutritional programs ongoing in Karnataka state, like mid-day meal scheme and Kshera Bhagy program to name a few. The prevalence of undernutrition in school age children remains a considerable public health problem in South Indian part of the country. Long term follow-up studies can give more insight into effect of diet on overall growth and development of the children as well as its impact on the development or progression of oral diseases.

Acknowledgements

We thank Dr. Vinuta Hampiholi, for careful proof reading of the manuscript. We are grateful to all the study participants and their parents for their support and cooperation.

Funding

This work was supported by the KAHER Faculty grant (grant number 22-23/D.10042314).

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Mennella JA, Pepino MY, Reed DR. Genetic and environmental determinants of bitter perception and sweet preferences. Pediatrics. 2005;115(2):e216-e222. doi: 10.1542/PEDS.2004-1582.
2. Takeichi H, Taniguchi H, Fukinbara M, Tanaka N, Shikanai S, Sarukura N, et al. Sugar intakes from snacks and beverages in Japanese children. J Nutr Sci Vitaminol (Tokyo). 2012;58(2):113-7. doi: 10.3177/jnsv.58.113.
3. Awasthi S, Kumar D, Dixit S, Mahdi AA, Gupta B, Agarwal GG, et al. Association of dietary intake with micronutrient deficiency in Indian school children: a cross-sectional study. J Nur Sci. 2023;12:e104. doi: 10.1017/JNS.2023.83.
4. Awasthi S, Kumar D, Mahdi AA, Agarwal GG, Pandey AK, Parveen H, et al. Prevalence of specific micronutrient deficiencies in urban school going children and adolescence of India: A multicenter cross-sectional study. PLoS One. 2022;17(5):e0267003. doi: 10.1371/journal.pone.0267003.
5. Vecchio MG, Paramesh EC, Paramesh H, Loganes C, Ballali S, Gafare CE, et al. Types of food and nutrient intake in India: a literature review. Indian J Pediatr. 2014;81 Suppl 1:17-22. doi: 10.1007/s12098-014-1465-9.
6. Kochupurackal SU, Channa Basappa Y, Vazhamplackal SJ, Srinivas PN. An intersectional analysis of the composite index of anthropometric failures in India. Int J Equity Health. 2021;20(1):1-11. doi: 10.1186/S12939-021-01499-Y/FIGURES/3.
7. World Health Organization. Guideline: sugars intake for adults and children. Geneva: World Health Organization; 2015.
8. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet. 2019;393(10170):447-92. doi: 10.1016/S0140-6736(18)31788-4.
9. Ashi H, Campus G, Bertéus Forslund H, Hafiz W, Ahmed N, Lingström P. The Influence of Sweet Taste Perception on Dietary Intake in Relation to Dental Caries and BMI in Saudi Arabian Schoolchildren. Int J Dent. 2017;2017:4262053. doi: 10.1155/2017/4262053.
10. Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr Today. 2015;50(3):117-128. doi: 10.1097/NT.0000000000000092.
11. World Health Organization. Body mass index-for-age (BMI-for-age) [Internet]. Geneva: World Health Organization; 2020 [cited 2024 May 25]. Available from: https://www.who.int/toolkits/child-growth-standards/standards/body-mass-index-for-age-bmi-for-age.
12. Furquim TR, Poli-Frederico RC, Maciel SM, Gonini-Júnior A, Walter LR. Sensitivity to bitter and sweet taste perception in schoolchildren and their relation to dental caries. Oral Heal Prev Dent. 2010;8(3):253-259.
13. Kumar N, Gupta N, Kishore J. Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012. Indian J Public Health 2012;56(1):103-4. doi: 10.4103/0019-557X.96988.
14. Ervina E, Berget I, Nilsen A, Almli VL. The ability of 10–11-year-old children to identify basic tastes and their liking towards unfamiliar foods. Food Qual Prefer 2020;83:103929. doi: 10.1016/j.foodqual.2020.103929.
15. Birch LL. Development of food preferences. Annu Rev Nutr. 1999;19:41-62. doi: 10.1146/annurev.nutr.19.1.41
16. Coldwell SE, Oswald TK, Reed DR. A marker of growth differs between adolescents with high vs. low sugar preference. Physiol Behav. 2009;96(4-5):574-80. doi: 10.1016/j.physbeh.2008.12.010.
17. Sommer I, Nußbaumer-Streit B, Gartlehner G. WHO guideline on carbohydrate intake for adults and children Gesundheitswesen. 2024;86(12):798-802. doi: 10.1055/a-2364-2388.
18. Ministry of Health and Family Welfare (MoHFW), Government of India, UNICEF and Population Council. Comprehensive National Nutrition Survey (CNNS) National Report. New Delhi; 2019.
19. Nizel AE, Papas AS. Nutrition in clinical dentistry. Philadelphia: Saunders; 1989.
20. Bartoshuk LM, Duffy VB, Hayes JE, Moskowitz HR, Snyder DJ. Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. Philos Trans R Soc Lond B Biol Sci. 2006;361(1471):1137-1148. doi: 10.1098/rstb.2006.1853.
21. Donaldson LF, Bennett L, Baic S, Melichar JK. Taste and weight: Is there a link? Am J Clin Nutr. 2009;90(3):800S-803S. doi: 10.3945/ajcn.2009.27462Q.
22. Overberg J, Hummel T, Krude H, Wiegand S. Differences in taste sensitivity between obese and non-obese children and adolescents. Arch Dis Child. 2012;97(12):1048-52. doi: 10.1136/archdischild-2011-301189.
23. Jilani H, Intemann T, Buchecker K, Charalambos H, Gianfagna F, De Henauw S, et al. Correlates of bitter, sweet, salty and umami taste sensitivity in European children: Role of sex, age and weight status - The IDEFICS study. Appetite. 2022;175:106088. doi: 10.1016/j.appet.2022.106088.
24. Washi SA, Ageib MB. Poor diet quality and food habits are related to impaired nutritional status in 13- to 18-year-old adolescents in Jeddah. Nutr Res. 2010;30(8):527-534. doi: 10.1016/j.nutres.2010.07.002.
25. Kourouniotis S, Keast RSJ, Riddell LJ, Lacy K, Thorpe MG, Cicerale S. The importance of taste on dietary choice, behaviour and intake in a group of young adults. Appetite. 2016;103:1-7. doi: 10.1016/j.appet.2016.03.015.

Received: 17.01.2026
Revised: 01.04.2026
Accepted: 07.04.2026
Published online first: 28.04.2026

Rocz Panstw Zakl Hig 2025;76(4):323-333
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/220651

ORIGINAL ARTICLE

HEALTH STATUS, RISK FACTORS AND EFFECTS OF OLIVE LEAF EXTRACT INFUSION (*OLEA EUROPAEA* L.) ON BLOOD PRESSURE IN A HYPERTENSIVE POPULATION

Fouad Affane[1,2], *Zakaria Meskini*[3], *Ali Araf*[3], *Zouheyr Hadri*[1], *Hiba Naas*[1], *Djamila Yssaad*[4]

[1]Department of Nutritional Sciences, Laboratory of Environment, Natural Plant Substances and Food Technology, Faculty of Natural and Life Sciences, Ahmed Zabana University of Relizane, Algeria
[2]Department of Biology, Laboratory of Clinical and Metabolic Nutrition (LCMN), Faculty of Natural and Life Sciences, Oran 1-Ahmed Ben Bella University, Oran, Algeria
[3]Department of Agricultural Sciences, Laboratory of Environment, Natural Plant Substances and Food Technology, Faculty of Natural and Life Sciences, Ahmed Zabana University of Relizane, Algeria
[4]Department of Biological Sciences, Laboratory of Environment, Natural Plant Substances and Food Technology, Faculty of Natural and Life Sciences, Ahmed Zabana University of Relizane, Algeria

ABSTRACT

Background. Hypertension is a major global public health concern and a leading contributor to cardiovascular morbidity and mortality. Despite pharmacological treatment, blood pressure (BP) control remains inadequate in many patients, highlighting the need for complementary, non-pharmacological approaches.

Objective. The aim of the study was to evaluate the effect of olive leaf infusion consumption on systolic (SBP) and diastolic (DBP) in hypertensive adults and to explore associated cardiometabolic and lifestyle factors.

Material and Methods. A preliminary dietary survey was conducted to assess the daily salt intake of hypertensive patients recruited at the Mohammed Boudiaf Public Hospital Relizane, Algeria. Only patients with a comparable daily salt intake (6 to 8 g/day) (220 patients) were selected to participate in a six-week prospective observational study, after being divided into two groups: a control group A (n = 110) and an experimental group B (n = 110) consuming an olive leaf infusion twice a day at fixed times before meals (once in the morning and once in the evening), in addition to the same diet. BP was measured weekly. Anthropometric, clinical, and lifestyle data were collected through structured interviews. Temporal changes in BP were analyzed using repeated-measures ANOVA with Greenhouse-Geisser correction, and correlation analyses were performed to assess associations between variables. Baseline characteristics, including age and hypertension severity, were comparable between groups.

Results. Olive leaf infusion consumption was associated with a significant reduction in SBP and DBP from the second week onward. Repeated measures ANOVA demonstrated a significant main effect of time on SBP ($p < 0.001$) and a significant time and group interaction ($p < 0.001$). DBP showed similar effects (time and group interaction, $p < 0.001$), with BP reductions plateauing after week four. Correlation analyses identified significant associations between BP, salt intake, obesity indices, and sleep disturbances. Short-term consumption of olive leaf infusion was associated with clinically meaningful reductions in SBP and DBP in hypertensive adults.

Conclusion. These findings support olive leaf infusion as a potential complementary, non-pharmacological strategy within integrated hypertension management. Standardized phytochemical characterization is needed to confirm efficacy and guide clinical implementation.

Keywords: *health status, risk factors, olive leaf, Olea europaea L., blood pressure, hypertension*

INTRODUCTION

High blood pressure (BP) constitutes a major global public health challenge. It rarely occurs alone and is a leading contributor to cardiovascular morbidity and mortality, including myocardial infarction, chronic kidney disease, and stroke. A continuous and linear relationship exists between BP levels and cardiovascular risk, irrespective of age [1-3].

Corresponding author: Fouad Affane, Department of Nutritional Sciences, Laboratory of Environment, Natural Plant Substances and Food Technology, Faculty of Natural and Life Sciences, Ahmed Zabana University of Relizane, Bourmadia City, 48000, Relizane, Algeria; email: affanefouad@gmail.com; phone: +213(0)5-51-95-68-24

Publisher: National Institute of Public Health NIH - National Research Institute

In 2024, hypertension affected approximately 1.4 billion adults aged between 30 to 79 years worldwide, representing nearly 33% of this age group. Projections indicate that this number will reach 1.56 billion individuals by the end of 2025, with an estimated 7.5 million deaths annually, accounting for approximately 12.8% of global mortality [3]. The burden of hypertension has more than doubled since 1990, when approximately 650 million adults were affected, largely due to population growth and population ageing [3].

In Algeria, hypertension represents a significant public health concern. In 2024, the prevalence among adults aged between 30 to 79 years was estimated at 7.7 million individuals (17% of the population), of whom approximately 6.4 million had uncontrolled BP, highlighting substantial gaps in diagnosis, treatment, and effective disease management [3].

Hypertension is clinically defined as a systolic BP ≥ 140 mmHg and/or a diastolic BP ≥ 90 mmHg, measured on at least two separate occasions on different days, or by the current use of antihypertensive medication [3]. The development of hypertension is multifactorial and insidious, often progressing asymptomatically. It results from complex interactions between non-modifiable factors, such as genetic predisposition, sex, age, and family history. And modifiable environmental, lifestyle factors and eating habit who can be influenced by multiple factors [4]. Including overweight and obesity, diabetes mellitus, physical inactivity, psychosocial stress, and dietary habits, particularly excessive sodium intake [5].

The management of hypertension encompasses both pharmacological and non-pharmacological approaches. Lifestyle modification and dietary interventions include sodium restriction through a low-sodium (hypo-sodium) diet (< 5 g/day), increased consumption of vegetables, fresh fruits, fish, nuts, and unsaturated fatty acids (olive oil), low consumption of red meat, and consumption of low-fat dairy products, are recommended as the first-line strategy and offers several advantages in BP control and cardiovascular risk reduction [6-9].

However, lifestyle interventions alone are frequently insufficient, necessitating long-term pharmacological treatment. Indeed, antihypertensive drugs are essential for treating hypertension, although their use can be costly and are often associated with adverse effects, which may contribute to poor treatment adherence, patient dissatisfaction, and suboptimal BP control [10]. Consequently, the use of foods from natural sources, such as marines or plants by-products [11-16], could prove beneficial when combined with pharmacological treatment to counteract the adverse effects of this medication. Moreover, the World Health Organization encourages research into alternative and complementary strategies for hypertension management, including the integration of traditional and herbal medicine practices [17].

Among phytotherapeutic approaches, the olive tree (*Olea europaea* L.) has attracted growing scientific interest. Widely cultivated in the Mediterranean region, the olive tree holds considerable economic, cultural, and social importance [18]. Its by-products, particularly olive leaves, have long been used in traditional medicine in European and Mediterranean countries such as Greece, Spain, Italy, France, Turkey, Morocco, and Tunisia [19-21]. Their chemical composition varies according to many factors such as olive variety, climatic conditions, tree age, and wood content [22]. Olive leaves contain variable levels of organic matter (76.4-92.7 g/100 g dry matter), low levels of crude protein (6.31-10.9 g/100 g dry matter), a significant amount of amino acids (89.9 g/100 g total nitrogen), and the nitrogen attached to the cell walls is high but variable (49.2 and 35.4 g/100 g total nitrogen). Crude fat content also varies in olive leaves (2.28-9.57 g/100 g dry matter) [22]. Mannitol and glucose are the two most abundant soluble carbohydrates in olive leaves, contributing 27.1-30.8%, with mannitol levels fluctuating from season to season [22]. Moreover, olive leaves have been shown to be rich in certain bioactive compounds, notably a wide variety of phenolic compounds, such as secoiridoids (oleuropein, ligstroside, dimethyloleuropein), hydroxytyrosol, tyrosol, caffeic and ferulic acids, and flavonoids (apigenin, luteolin, luteolin-7-*O*-glucoside, etc.) [23]. Furthermore, a recent study compared the chemical composition of certain Algerian and Tunisian varieties of olive leaves (*Olea europaea* L.) by identifying nineteen compounds in extracts by HPLC (high-performance liquid chromatography), with oleuropein being the predominant compound, followed by hydroxytyrosol and verbacoside [24].

Olive leaf extracts are recognized for their nutritional, hypolipidemic, antioxidant, and anti-inflammatory properties and have been employed in the management of several chronic conditions, including hypertension [25-27]. They are commonly consumed as herbal infusions or as powdered dietary supplements [20, 26, 28].

To date, clinical evidence from Algeria regarding the effects of olive leaf infusion on blood pressure in hypertensive patients remains scarce. Therefore, the present study aims, first, to assess cardiometabolic comorbidities, particularly overweight and obesity commonly associated with hypertension, and second, to evaluate the effects of a six-week intervention with olive leaf infusion on blood pressure parameters in a randomly selected sample of hypertensive patients recruited from Mohamed Boudiaf Public Hospital in the Wilaya of Relizane.

MATERIAL AND METHODS

Study design

This study was carried out during a six-week period in February and March 2025. A structured questionnaire was sent to a randomly selected sample of hypertension patients recruited at Mohammed Boudiaf Public Hospital, located in the Wilaya of Relizane, Algeria. All participants were informed of the study's aims and methods, and their informed consent was obtained.

Eligibility criteria

The inclusion criteria were as follows: adults aged 18 years and older, regardless of sex or ethnicity, with elevated blood pressure (≥ 140/90 mmHg; classified as hypertension according to the World Health Organization [6]. Patients already receiving at least one antihypertensive medication (prescribed by their doctors), such as calcium antagonists, beta-blockers, angiotensin II receptor antagonists (also known as sartans), and diuretics, were also included. Patients with other complications associated with high blood pressure were also taking other medications, including for the treatment of type II diabetes (hypoglycemic sulfonamides (gliclazide, glimepiride) and GLP-1 receptor antagonists (aGLP-1)), type I diabetes (insulin therapy: rapid-acting insulins and long-acting or slow-acting insulins), hypercholesterolemia (statins and inhibitors of intestinal cholesterol absorption), etc.

To assure the research population's reliability and homogeneity, a preliminary dietary survey was undertaken to assess daily salt intake among hypertension patients admitted to hospital. Patients having a salt consumption of less than 6 g/day or more than 8 g/day were excluded from the research. Only patients with a moderate and steady daily salt consumption of 6 to 8 g were eligible for participation.

Participant characteristics and group allocation

A total of 220 hypertension individuals who met the inclusion criteria were included. Participants varied in age range from 18 to 94 years and included both sexes. The study population was divided randomly into two groups of equal size (n = 110 each), with age and gender distributions matched. The control group (A), participants had a normosodic diet and without olive leaf infusion consumption. Experimental group (B) following a normosodic diet and with olive leaf infusion consumption twice daily, once in the morning and once in the evening with meals, throughout a six-week period.

Olive leaf infusion preparation protocol

Throughout the experiment, between February and March 2025, olive leaves (*Olea europaea* L.) were collected from a local farm located in the Olive Groves region (a geographically identified cultivation area under this name, located in the immediate vicinity of the city of Relizane and the Bourmadia area). They were brought to the experimental site to be rinsed with clean water (to remove all impurities) and spread out on grids (to allow homogeneous air circulation) and turned regularly, thus allowing drying at an ambient temperature (approximately 25°C) and protected from light (to prevent oxidation of sensitive compounds) according to the standards of the European Pharmacopoeia 9.0 (PhEur, 2017) [29]. There is no fixed minimum time in days for drying; the leaves are considered dry when they become brittle and break easily under the pressure of the fingers, they are then mechanically ground into fine pieces [29], and weighed in small quantities of 5 g per dose [20, 30], and placed in small opaque plastic bags and vacuum-sealed and stored in a dry place at room temperature (between 15°C and 25°C) and away from light to be ready for use [29]. During each day of the six weeks of the experiment, each patient prepared two infusions of olive leaves (*Olea europaea* L.) per day, which they consumed at fixed times before meals, one in the morning and one in the evening [19, 30]. They brought drinking water to a boil and poured 150 mL into a glass containing a 5 g dose of olive leaves (prepared for them beforehand), let it steep for 10 minutes, then strained it through a sieve and consumed it [20, 30].

Data collection and assessment methods

A structured questionnaire was developed and pre-tested prior to implementation to ensure clarity, relevance, and comprehensiveness. The bioethics committee of the University Ahmed Zabana of Relizane gave its approval under reference (01/E.C.A.E/F.N.L.S/U.A.Z.R/2025). Data were collected through face-to-face interviews conducted by teachers specializing in the field of nutrition and health, in a private setting at Mohammed Boudiaf Public Hospital. Each interview lasted approximately 30 minutes.

The questionnaire was divided into three main sections:

- General health status and socioeconomic characteristics: this section collected information on demographic and socioeconomic variables, as well as anthropometric and health-related parameters by studying the various complications related to high blood pressure, including: Overweight and obesity (grade I and grade II obesity), type I and type II diabetes, cardiovascular diseases (left ventricular hypertrophy, heart failure and myocardial infarction), renal problems (renal lithiasis (or kidney stones), benign nephroangiosclerosis and chronic renal failure), cholesterol (high LDL), thyroid problems (hyperthyroidism), colon problems

(irritable bowel syndrome), and other diseases (e.g., osteoarthritis, gout). We measured the body weight (kg) [using Beurer MS 01: a mechanical scale with a capacity ranging from 1 kg to 120 kg and a finer graduation of 100 g], the height (m) [using Seca 213: a stadiometer with a measuring range of 20 cm to 205 cm and a graduation of 1 mm], the waist circumference and the hip circumference (cm) [using Seca 201: an ergonomic perimeter measuring tape that allows measurement from 0 to 205 cm, graduated every 1 mm and with an accuracy of 5 mm], of each patient at the beginning of the experiment to calculate their body mass index [BMI (kg/m^2) = weight (kg)/height $(m)^2$], their waist-to-hip ratio [WHR = waist circumference (cm)/hip circumference (cm)], and their body fat index [BFI (%) = (1.20 x BMI) + (0.23 x age) – (10.8 x sex (males = 1, females = 0)) – 5.4] according to Deurenberg et al. (1991) [31].

- Assessment of dietary salt intake: assessment of sodium consumption, data were compiled and analyzed using Microsoft Excel to estimate daily salt consumption levels and potential excessive intake according to Monntoya et al. (2019) [7].
- Blood pressure measurement: On the first day of each subsequent week of the experiment, i.e. days 1, 7, 14, 21, 28, 35 and 42, patients take their blood pressure at home using an oscillometric device or a calibrated manual sphygmomanometer, following the various recommendations of the ESC guidelines 2024 [8], that they were taught, as follows: Two consecutive measurements are taken in the morning (1 to 2 minutes apart) at the same time, immediately after waking, on fasting, and before taking any medication, and two consecutive measurements are taken in the evening (1 to 2 minutes apart) at the same time, before bedtime and after dinner. At the end of each weekly measurement period, the average of all daily values is calculated for each patient.

Statistical analysis

Data were organized in Microsoft Excel for initial processing. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed for normality using the Shapiro-Wilk test. To evaluate within-subject changes in BP over time, repeated-measures analysis of variance (ANOVA) was applied. The assumption of sphericity was tested using Mauchly's test; when violated, the Greenhouse-Geisser correction was employed. Between-subject factors included sex, age category, and treatment group, and interaction effects were examined to identify differential responses between groups.

Associations between continuous and categorical variables were assessed using correlation coefficient (ρ). All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

RESULTS

Description of the study population

Baseline characteristics of the study population are summarized in Table 1**.** The mean age of participants in the control group A was 56.7 ± 17.6 years**,** while experimental group B was 58.6 ± 14.5 years. In both groups, adults aged between 40 to 65 years constituted the predominant age category, accounting for 52.7% and 59.1% in groups A and B respectively.

Regarding socio-professional status, most female participants in both groups were housewives (40.9% in group A and 49.1% in group B). In total, 30% of group A had liberal professions, whereas retired represented 30% of group B. Physical activity was very reduced, reported by 19.1% of participants in group A and 10% in group B (Table 1). Regarding physical activity, 31.8% of men (28 out of 88) reported engaging in regular sport, compared to only 3% of women (4 out of 132).

Regarding hypertension length, approximately 8.2% of participants in group A and 9.1% in group B had been recently diagnosed with high blood pressure. A duration of less than five years was reported by 25.4% of group A and 50.9% of group B, whereas durations exceeding five years were reported by 30.9% and 25.5% of participants in group A and B, respectively. In addition, the durations exceeding ten years were reported by 30.9% and 43.6% of participants in group A and group B, respectively.

At baseline (day 1), mean systolic and diastolic BP values were 168.8 ± 11.3 mmHg and 103.0 ± 10.4 mmHg in group A, and 171.2 ± 7.4 mmHg and 99.9 ± 7.6 mmHg in group B. All participants were receiving at least one antihypertensive medication, administered as monotherapy, for the management of hypertension and associated conditions.

Estimated daily salt intake at baseline was comparable between groups, with values of 6.8 ± 0.8 g/day in group A and 6.5 ± 0.7 g/day in group B. The mean reported sleep duration was 5.8 ± 1.2 hours in group A and 8.2 ± 1 hours in group B. Sleep disturbances were more frequently reported in group A (65.4%) compared with group B (25.4%). Lifestyle-related variables differed significantly between groups. Sleep duration showed a significant difference ($p < 0.001$), with shorter sleep durations (3.5-7 h) predominating in group A and longer durations (7-9 h) in group B.

Anthropometric measurements of the study population

Participants in the control group A had a mean body weight of 82.9 ± 15.8 kg, a mean body mass index (BMI) of 28.9 ± 5.5 kg/m², a waist-to-hip ratio (WHR) of 0.8 ± 0.1, and a body fat index (BFI) of 40.2 ± 8.4%. In comparison, participants experimental group B exhibited lower mean anthropometric values, with a body weight of 75.1 ± 11.8 kg, BMI of 26.9 ± 4.1 kg/m², WHR of 0.9 ± 0.1, and BFI of 37.9 ± 6.6%.

Table 1. Study population description

Variables	Control group A	Experimental group B
Average age (years)	56.74 ± 17.6	58.64 ± 14.5
Age group classification (%)		
Young adults [18-40 years]	17.3	10
Adults [41-65 years]	52.7	59.1
Seniors [> 65 years]	30	30.9
Socio-professional category (%)		
Civil servant/liberal profession	30	15.5
Unemployed/job seeker	7.3	5.5
Retired	21.8	30
Housewife	40.9	49.1
Lifestyle		
Physical activity	19.1%	10%
Mean sleep duration	5.8 ± 1.2 h	8.2 ± 1 h
Daily salt intake	6.8 ± 0.8 g/day	6.5 ± 0.7 g/day
Anthropometrics		
Mean body weight	82.9 ± 15.8 kg	75.1 ± 11.8 kg
Body mass index (BMI)	28.9 ± 5.5 kg/m^2	26.9 ± 4.1 kg/m^2
Body fat index (BFI)	40.2 ± 8.4%	37.9 ± 6.6%

Prevalence of chronic diseases associated with hypertension:

The prevalence of comorbid conditions associated with hypertension is summarized in Table 2. The majority of participants reported at least one additional chronic condition, affecting 95.4% of individuals in group A and 83.6% in group B. Overweight (with a BMI ≥ 25 and < 30) was the most frequently reported condition (33.6% in group A and 38.2% in group B). Followed by grade I obesity (with a BMI ≥ 30 and < 35) (29.1% in group A and 26.6% in group B). Grade II obesity (with a BMI ≥ 35 and < 40) was significantly more prevalent in group A (12.7%) than in group B (1.8%) ($p = 0.002$). While hypercholesterolemia (17.3%) and renal disorders (10.9%; $p < 0.001$) were observed only in group A. Cardiovascular disease differed significantly between groups ($p = 0.019$), group B had a high prevalence (13.6%) compared to group A (4.6%).

Correlation analysis (Figure 1) showed that age was positively associated with hypertension duration ($r = 0.501$, $p < 0.001$), BFI ($r = 0.309$, $p < 0.001$), and sleep disorders ($r = 0.341$, $p < 0.001$).

Correlations confirmed strong interrelationships among anthropometric indices, while SBP and DBP showed no significant association with adiposity measures. Physical activity was inversely associated with adiposity indicators, and sleep duration was

Table 2. Overview of other chronic diseases associated with hypertension

Variables	Group A	Group B	χ^2 value (df)	p-value
Other chronic diseases (%)				
Yes	95.5	83.6		
No	4.6	16.4		
Hypotension	0	4.6	5.116 (1)	0.024
Overweight	33.6	38.9		
Grade I obesity	29.1	23.6		
Grade II obesity	12.7	1.8	9.706 (1)	0.002
Type 1 diabetes	33.6	30		
Type 2 diabetes	0	3.6	4.074 (1)	0.044
CVD	4.6	13.6	5.500 (1)	0.019
Renal problems	10.9	0	12.692 (1)	< 0.001
Cholesterol	17.3	0	20.796 (1)	< 0.001
Thyroid	1.8	13.6	10.774 (1)	0.001
Colon	40.9	0	56.571 (1)	< 0.001
Others	23.6	11.8	5.267 (1)	0.022

	Gender	Age	BP Period	Hours of sleep	Sleep disorders	SBP (Day 1)	DBP (Day 1)	BMI	Job	Weight	Size	Waist_size	HIP	WHR	Sport
Gender	1														
Age	0.062	1													
BP Period	-0.025	0.515	1												
Hours of sleep	-0.088	-0.003	-0.296	1											
Sleep disorders	0.056	0.331	0.449	-0.42	1										
SBP (Day 1)	0.046	0.062	0.034	0.026	-0.072	1									
DBP (Day 1)	-0.037	-0.025	0.048	-0.104	0.037	0.456	1								
BMI	-0.246	-0.123	0.007	-0.124	-0.087	0.064	0.085	1							
Job	-0.538	0.403	0.205	0.219	0.106	-0.003	-0.036	0.1	1						
Weight	0.205	-0.091	0.014	-0.186	-0.018	0.076	0.097	0.803	-0.142	1					
Size	0.729	0.033	0.007	-0.112	0.092	0.019	0.019	-0.283	-0.392	0.333	1				
Waist_size	0.15	-0.051	0.142	-0.419	0.203	-0.001	0.092	0.571	-0.138	0.703	0.233	1			
HIP	0.146	-0.01	0.21	-0.471	0.271	-0.029	0.073	0.463	-0.111	0.618	0.272	0.944	1		
WHR	0.079	-0.094	-0.154	0.075	-0.168	0.061	0.044	0.382	-0.098	0.36	-0.031	0.313	-0.01	1	
Sport	-0.4	0.206	0.065	0.174	-0.115	0.097	-0.037	0.161	0.464	-0.024	-0.289	-0.137	-0.141	-0.02	1

Figure 1. Association between anthropometric measurements

negatively correlated with sleep disorders and hypertension duration ($p < 0.05$).

Age was strongly associated with hypertension duration ($r = 0.52$, $p < 0.001$) and sleep disorders ($r = 0.45$, $p < 0.001$). Adiposity indicators were highly interrelated, with BMI showing strong positive correlations with waist and hip circumferences ($p < 0.001$).

Evolution of blood pressure

The temporal evolution of systolic and diastolic BP over the six-week period is illustrated in Figures 2 and 3. In group B, a progressive reduction in both SBP and DBP was observed from the second week (day 14) onward. Compared with group A, reductions of 8.1% (SBP) and 10.5% (DBP) were observed on day 14. On day 28, SBP and DBP decreased by 12.5% and 18.8%, respectively, in group B compared with group A. By the end of the intervention (day 42), reductions reached 17.2% for SBP and 22.7% for DBP relative to the control group.

Systolic Blood Pressure

Mauchly's test indicated a violation of the sphericity assumption for the within-subjects factor day ($W = 0.701$, χ^2 (20) = 70.40, $p < 0.001$); therefore, the Greenhouse-Geisser correction ($\varepsilon = 0.890$) was applied. A repeated-measures ANOVA revealed a significant main effect of time on systolic BP (F (5.34, 1067.53) = 109.02, $p < 0.001$, partial $\eta^2 = 0.353$). A significant interaction between time and treatment group was also observed (F (5.34, 1067.53) = 36.41, $p < 0.001$, partial $\eta^2 = 0.154$), indicating differential BP changes over time between groups. However, no

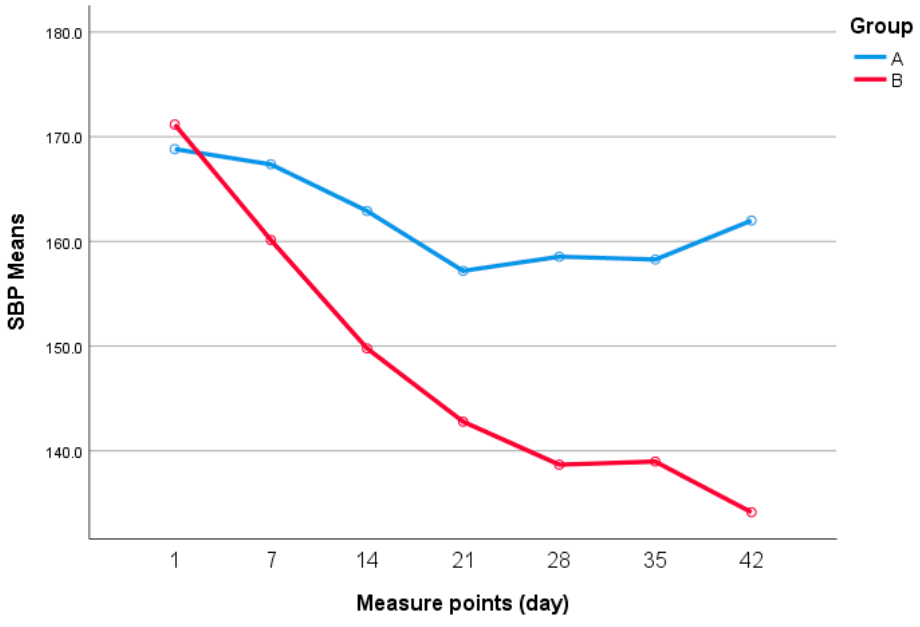


Figure 2. Systolic blood pressure evolution

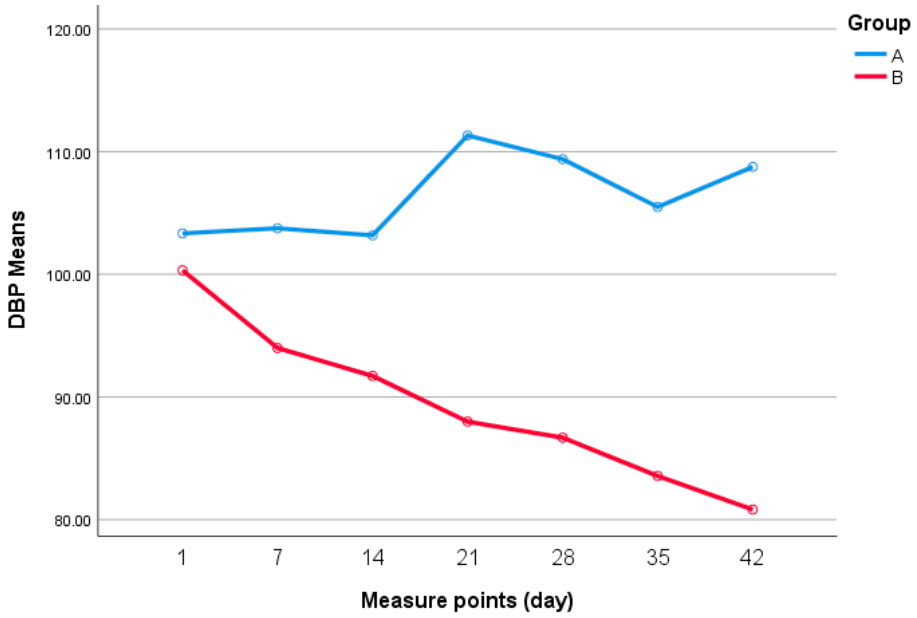


Figure 3. Diastolic blood pressure evolution

statistical significance was observed between time and sex interaction or time and age interaction.

Bonferroni-adjusted post hoc comparisons showed that SBP at day 1 was significantly higher than at all subsequent time points ($p < 0.001$). The largest reductions occurred between day 1 and day 28 (mean difference = 23.1 mmHg) and day 1 and day 21 (20.9 mmHg). No significant differences were observed among days 21-42, indicating a plateau effect.

Diastolic blood pressure

For DBP, Mauchly's test again indicated a violation of sphericity ($W = 0.619$, χ^2 (20) = 94.87, $p < 0.001$), and the Greenhouse-Geisser correction was applied ($\varepsilon = 0.861$). A significant main effect of time was observed (F (5.16, 1032.64) = 10.22, $p < 0.001$, partial $\eta^2 = 0.049$), with DBP decreasing progressively from day 1 (M = 101.83 mmHg) to day 42 (M = 94.79 mmHg). Pairwise comparisons confirmed that DBP values on days 35 and 42 were significantly lower than those on days 1-14 ($p < 0.05$).

A significant main effect of treatment group was also identified, with olive leaf infusion consumers showing lower mean DBP values (M = 89.3 ± 0.8) compared with non-consumers (M = 106.5 ± 0.7; $p < 0.001$). Neither sex nor age range significantly influenced DBP trajectories over time ($p > 0.05$).

DISCUSSION

The present study provides a comprehensive clinical and lifestyle characterization of hypertensive adults and demonstrates a significant blood pressure (BP) lowering effect associated with short term consumption of olive leaf infusion. The predominance of adults aged over 40 years in our sample aligns with epidemiological data reported by the World Health Organization, which indicates that hypertension affects most individuals between 30 and 79 years of age worldwide [3]. This age distribution reflects the cumulative impact of metabolic, behavioural, and vascular risk factors that increase with advancing age [3].

Women were more frequently represented among hypertensive participants, particularly within the housewife category. This finding is consistent with previous reports indicating higher healthcare utilization among women, which may contribute to increased diagnosis rates [32]. Additionally, sociocultural factors [33], lower levels of structured physical activity, and hormonal influences particularly those related to pregnancy and menopause may contribute to hypertension risk among women [34]. Comparable sex distributions have been reported by González et al. (2024) [35], who observed a predominance of female hypertensive patients (66.3%) and identified sedentary lifestyle and family history as major contributing factors.

A substantial proportion of participants had a long-standing history of hypertension, with nearly one-third diagnosed for more than five years and a notable subgroup exceeding ten years of disease duration. All participants reported current antihypertensive pharmacotherapy, often combined with non-pharmacological measures. These findings contrast with those of Guillén et al. (2025) [36], who reported suboptimal treatment adherence in a comparable population, highlighting regional and contextual

differences in disease management. The frequent use of combined pharmacological and lifestyle interventions observed in our cohort is consistent with reports among older hypertensive populations [33, 35] and reflects current guideline-based recommendations [8].

Comorbidity burden was high, with most participants presenting at least one chronic condition associated with hypertension. Overweight and obesity were the most prevalent comorbidities, followed by type 2 diabetes, dyslipidemia, and cardiovascular disease. These observations are consistent with extensive evidence identifying excess adiposity as a major modifiable risk factor for hypertension. The strong interrelationships observed between body mass index, central adiposity, and metabolic disorders reinforce the central role of obesity in hypertension pathophysiology) [37]. Data from the Framingham Heart Study further support these findings, demonstrating a markedly increased risk of hypertension with increasing BMI and estimating that a substantial proportion of hypertension cases are attributable to excess body weight [36].

Beyond BMI, central adiposity indicators such as waist-to-hip ratio have been shown to better predict cardiovascular and mortality risk. Malik and Adoubi (2019) [39] reported nearly a twofold increase in hypertension prevalence among individuals with abdominal obesity, underscoring the clinical relevance of fat distribution. In addition, hypertension frequently coexisted with diabetes, dyslipidemia, renal dysfunction, and cardiovascular disease in our population, consistent with previous epidemiological studies [40]. Sleep disorders were also common and showed significant associations with hypertension duration and adiposity, supporting emerging evidence that sleep disturbances constitute an independent risk factor for elevated BP [41].

A key finding of this study is the significant and progressive reduction in systolic and diastolic BP among participants consuming olive leaf infusion over six weeks. BP reductions became apparent from the second week of intervention and were sustained through the end of the study, with a clear divergence from the control group. These results are consistent with previous interventional studies demonstrating antihypertensive effects of olive leaf preparations [26, 28]. Basuny et al. (2020) [20] reported clinically meaningful reductions in BP after eight weeks of olive leaf tea consumption, with more pronounced effects observed in hypertensive individuals compared with normotensive controls.

Olive leaf has long been used in traditional Mediterranean medicine for cardiovascular conditions, and its antihypertensive properties are increasingly supported by clinical evidence [26, 28]. Proposed mechanisms include enhanced diuresis, vasodilation, and modulation of vascular tone [26, 28]. Experimental and clinical studies have shown that aqueous olive leaf extracts exert antihypertensive effects comparable to conventional treatments, particularly when used as adjunctive therapy [26, 27].

The biological activity of olive leaf is largely attributed to its rich content of polyphenolic compounds, notably oleuropein and hydroxytyrosol, which possess potent antioxidants and anti-inflammatory properties [25]. These compounds may improve endothelial function, reduce oxidative stress, and attenuate low-grade inflammation, a key mechanism implicated in hypertension [26]. Olive leaves also contain flavonoids, triterpenoids (including oleanolic, ursolic, and maslinic acids), minerals, and squalene, which may contribute synergistically to BP regulation through mechanisms that extend beyond nitric oxide-mediated vasodilation [27]. Notably, olive leaf extracts exhibit exceptionally high antioxidant capacity, exceeding that of green tea and vitamin C, which may further support vascular protection [27].

At the end of our experiment, our patients reported experiencing no adverse effects after consuming the olive leaf infusion. A systematic review and meta-analysis have demonstrated that consuming olive leaf (*Olea europaea* L.) extract poses no risk to hypertensive patients [42, 28]. Moreover, a recent study by Basuny et al. (2020) [20] showed that consuming a daily dose of 10 g of olive leaf (*Olea europaea* L.) infusion for 8 weeks in both hypertensive and healthy patients resulted in no toxicity or other adverse health effects. Even better, this infusion improves their liver functions by decreasing ALT (alanine aminotransferase) and AST (aspartate aminotransferase) (markers of liver inflammation), on the one hand, and on the other hand, this infusion improves the renal functions of these patients by a significant decrease in serum creatinine and urea levels [20].

CONCLUSION

This study provides evidence that short term consumption of olive leaf infusion is associated with a significant reduction in systolic and diastolic blood pressure in hypertensive adults. These findings support the relevance of integrated hypertension management strategies that combine pharmacological treatment with lifestyle and dietary interventions, including the use of natural, non-invasive plant-based products. Although exploration in nature, the observed blood pressure lowering effects were consistent, clinically meaningful, and concordant with existing experimental and clinical evidence, reinforcing the biological plausibility of olive leaf derived bioactive compounds in cardiovascular regulation. Olive leaf infusion therefore appears to represent a promising

complementary, non-pharmacological approach for supporting blood pressure control in hypertensive populations. The present findings provide a strong rationale for further investigation. Standardized phytochemical characterization is needed to confirm efficacy, establish dose response relationships, and clarify the role of olive leaf-based formulations in the development of functional foods and evidence-based adjunct therapies for hypertension prevention and management.

Conflict of interest

The authors declare that they have no conflicts of interest.

Informed consent

The study was conducted in accordance with the ethical principles outlined in the World Medical Association's Declaration of Helsinki (Sections 25-32; WMA, 2024). Participation was entirely voluntary, and informed consent was obtained from all respondents before completing the enrolment in the study. Participants were clearly informed about the study objectives, data confidentiality, and their right to withdraw at any time without providing a reason.

REFERENCES

1. Stevens SL, Wood S, Koshiros C, Law K, Glasziou P, Stevens RJ, et al. Blood pressure variability and cardiovascular disease: systematic review and meta-analysis. BMJ. 2016;354:i4098. doi: 10.1136/bmj.i4098.
2. Seidu S, Lawson CA, Kunutsor SK, Khunti K, Rosano GMC. Blood pressure levels and adverse cardiovascular outcomes in heart failure: A systematic review and meta-analysis. Eur J Heart Fail. 2024;26(5):1111-1124. doi: 10.1002/ejhf.3108.
3. World Health Organization. Global report on hypertension 2025: high stakes: turning evidence into action [Internet]. Geneva: World Health Organization; 2025 [cited 2025 Sep 23]. Available from: https://www.who.int/publications/i/item/9789240115569.
4. Sood M, Shende R., Saha S, Eruva V. Systematic Review Article: Study of Risk Factors for Hypertension. Eur J Cardiovasc Med. 2024;14(6):19-22.
5. Benenson I, Waldron FA, Jadotte YT, Dreker MP, Holly C. Risk factors for hypertensive crisis in adult patients: a systematic review. JBI Evid Synth. 2021;19(6):1292-1327. doi: 10.11124/JBIES-20-00243.
6. World Health Organization. Hypertension [Internet]. Geneva: World Health Organization; 2025 [cited 2025 Sep 25]. Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension.
7. Monntoya L, Ducher M, Florens N, Fauvel J-P. Évaluation de l'autoquestionnaire ExSel® pour dépister une consommation excessive de sel chez les patients consultant en néphrologie [Evaluation of the ExSel® self-questionnaire to screen for an excess salt intake in patients followed in a nephrology consultation]. Ann Cardiol Angeiol (Paris). 2019;68(4):275-278. doi: 10.1016/j.ancard.2019.07.001 [in French].
8. McEvoy JW, McCarthy CP, Bruno RM, Brouwers S, Canavan MD, Ceconi C, et al. 2024 ESC guidelines for the management of elevated blood pressure and hypertension. Eur Heart J. 2024;45(38):3912-4018. doi: 10.1093/eurheartj/ehae178.
9. Chaiwong S, Yeesa S, Somporn R, Senrit S, Chamnannao S, Sudjarawu S, et al. The knowledge of dietary sodium, sodium consumptive behavior, sodium in food, and urinary sodium of hypertensive patients in Thailand. Rocz Panstw Zakl Hig. 2025;76(2):103-113. doi: 10.32394/rpzh/207107.
10. Margolis KL, Crain AL, Pawloski PA, Ziegenfuss JY, Trower NK, Bergdall AR, et al. Blood pressure medication side effect symptoms and patient treatment satisfaction and adherence. J Am Board Fam Med. 2025;38(2):312-29. doi: 10.3122/jabfm.2024.240288R1.
11. Affane F, Boukhari Benahmed Daidj N, Louala S, Munezero AN, Lamri-Senhadji MY. Effets d'un régime obésogène enrichi en coproduits de sardine sur les facteurs de risque pro-athérogènes, chez le rat Wistar [Effects of an obesogenic diet enriched in sardine by-products on pro-atherogenic markers in Wistar rats]. Ann Cardiol Angeiol (Paris). 2016;65(3):214-218. doi: 10.1016/j.ancard.2016.04.011 [in French].
12. Affane F, Louala S, El Imane Harrat N, Bensalah F, Chekkal H, Allaoui A, et al. Hypolipidemic, antioxidant and antiatherogenic property of sardine by-products proteins in high-fat diet induced obese rats. Life Sci. 2018;(15):199:16-22. doi: 10.1016/j.lfs.2018.03.001.
13. Affane F, Bensalah F, Harrat NI, Chekkal H, Louala S, Lamri-Senhadji MY. Les protéines purifiées à partir de sardines améliorent les chiffres tensionnels, l'équilibre glycémique, les voies métaboliques anti-athérogènes et la capacité anti-oxydante, chez le rat obèse [Sardine purified proteins improve blood pressure, glycemic control, anti-atherogenic metabolic pathways and antioxidant capacity in obese rats]. Ann Cardiol Angeiol (Paris). 2018;67(3):154-160. doi: 10.1016/j.ancard.2018.04.007 [in French].
14. Harrat NEI, Louala S, Bensalah F, Affane F, Chekkal H, Lamri-Senhadji M. Anti-hypertensive, anti-diabetic, hypocholesterolemic and antioxidant properties of prickly pear nopalitos in type 2 diabetic rats fed a high-fat diet. Nutr Food Sci. 2019;49(3): 476-490. doi: 10.1108/NFS-06-2018-0169.
15. Chekkal H, Harrat NEI, Affane F, Bensalah F, Louala, S, Lamri-Senhadji M. Cactus young cladodes improves unbalanced glycemic control, dyslipidemia, prooxidant/antioxidant stress biomarkers and stimulate lecithin-cholesterol acyltransferase and paraoxonase activities in young rats after cafeteria diet exposure. Nutr Food Sci. 2020;50(2):288-302. doi: 10.1108/NFS-01-2019-0025.
16. Chekkal H, Harrat NEI, Bensalah F, Affane F, Louala, S, Lamri-Senhadji M. The cactus cladode (*Opuntia ficus indica*) modulates uricemia, improves endothelial dysfunction and corrects oxidative

damage in rats fed a cafeteria diet. World J Sci Technol Sustain Dev. 2020;17(4):355-365. doi: 10.1108/WJSTSD-05-2019-0023.
17. World Health Organization. Global action plan for the prevention and control of noncommunicable diseases 2013-2020 [Internet]. Geneva: World Health Organization; 2013. [cited 2013 Nov 14]. Available from: https://www.who.int/publications/i/item/9789241506236.
18. Şahin S, Bilgin M. Olive tree (*Olea europaea* L.) leaf as a waste by-product of table olive and olive oil industry: a review. J Sci Food Agric. 2018;98(4):1271-1279. doi: 10.1002/jsfa.8619.
19. Arab K, Bouchenak O, Yahiaoui K. Évaluation de l'activité biologique des feuilles de l'olivier sauvage et cultivé. Afrique Science: Revue Internationale Des Sciences Et Technologie. 2015;9(3),159-166. Available from: https://www.ajol.info/index.php/afsci/article/view/112228.
20. Basuny AM, Hussein RRS, Mohamed MR, Mohamed A, Ali SAG. Evaluating the safety and efficacy of the traditional use of olive leaves decoction as antihypertensive agent in elderly people. Plant Arch. 2020;20(2)8111-8120. Available from: http://www.plantarchives.org/20-2/8111-8120%20(6901).pdf.
21. El SN, Karakaya S. Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. Nutr Rev. 2009;67(11):632-638. doi: 10.1111/j.1753-4887.2009.00248.x.
22. Rahmanian N, Jafari SM, Wani TA. A comprehensive review: Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. Trends Food Sci Technol. 2015;42(2):150-172. doi: 10.1016/j.tifs.2014.12.009.
23. Quirantes-Piné R, Lozano-Sánchez J, Herrero M, Ibáñez E, Segura-Carretero A, Fernández-Gutiérrez A. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. Phytochem Anal. 2013;24(3):213-23. doi: 10.1002/pca.2401.
24. Khelouf I, Karoui IJ, Lakoud A, Hammami M, Abderrabba M. Comparative chemical composition and antioxidant activity of olive leaves *Olea europaea* L. of Tunisian and Algerian varieties. Heliyon. 2023;9(12):e22217. doi: 10.1016/j.heliyon.2023.e22217.
25. Romani A, Mulas S, Heimler D. Polyphenols and secoiridoids in raw material (*Olea europaea* L. leaves) and commercial food supplements. Eur Food Res Technol. 2017;243(3):429-35. doi: 10.1007/s00217-016-2756-3.
26. Hermans MP, Lempereur P, Salembier J-P, Maes N, Albert A, Jansen O, et al. Supplementation Effect of a Combination of Olive (*Olea europea* L.) Leaf and Fruit Extracts in the Clinical Management of Hypertension and Metabolic Syndrome. Antioxidants (Basel). 2020;9(9):872.1-15. doi: 10.3390/antiox9090872.
27. de Oliveira NM, Machado J, Chéu MH, Lopes L, Barroso MF, Silva A, et al. Potential therapeutic properties of *Olea europaea* leaves from selected cultivars based on their mineral and organic profiles. Pharmaceuticals. 2024;17(3):274.1-31. doi: 10.3390/ph17030274.
28. Lachovicz R, Ferro-Lebres V, Almeida-de-Souza J, Pereira JA. Efficacy of Olive Leaf Extract in Improving Blood Pressure in Pre-Hypertensive and Hypertensive Individuals: A Systematic Review and Meta-Analysis. Phytother Res. 2025;39(6):2863-2874. doi: 10.1002/ptr.8509.
29. PhEur (European Pharmacopoeia). Olive Leaf Dry Extract. Monograph (MG) 2313, 9th edition. Council of Europe (COE) - European directorate for Quality of Medicines. Strasbourg, France; 2017.
30. European Medicines Agency. Committee on Herbal Medicinal Products (HMPC). European Union herbal monograph on *Olea europaea* L., folium [Internet]. Amsterdam: EMA; 2017 [cited 2026 March 29]. Available from: https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-olea-europaea-l-folium-first-version_en.pdf.
31. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Br J Nutr. 1991;65(2):105-14. doi: 10.1079/bjn19910073.
32. Keil J, Brendler V, Sachse C, Zülke A, Zeynalova S, Engel C, et al. Gender-Specific Differences in the Utilization of Health Care Services in an Urban Population Sample. Gesundheitswesen. 2020;82(3):17-23. doi: 10.1055/a-0820-3584.
33. Moustakim R, Mziwira M, El-Ayachi M, Belahsen R. Sociodemographic, nutritional and health status factors associated with adherence to Mediterranean diet in an agricultural Moroccan adult's population. Rocz Panstw Zakl Hig. 2023;74(2):167-175. doi: 10.32394/rpzh.2023.0256.
34. Espeche W, Minetto J, Cerri G, Reitovich L, Leiva Sisnieguez CE, Todoroff J, et al. A cohort study evaluating preeclampsia risk-based cut-offs for ambulatory blood pressure monitoring in high-risk pregnant women. Time for a change? Hypertens Res. 2025;48(12):3268-3277. doi: 10.1038/s41440-025-02416-4.
35. González DS, Ortega MRO, Linares LF, González DM, Sánchez DMS. Characterization of arterial hypertension in older adults. Health Leadersh Qual Life. 2024;3(520):1. Available from: https://dialnet.unirioja.es/servlet/articulo?codigo=9958752.
36. Guillén GJF, Matías JLR, Mazón MYM, López EV. Knowledge of arterial hypertension (aht) and adherence to treatment in adults. Rev Gest Soc Ambient. 2025;19(7):1-11. doi: 10.24857/rgsa.v19n7-006.
37. Shariq OA, McKenzie TJ. Obesity-related hypertension: a review of pathophysiology, management, and the role of metabolic surgery. Gland Surg. 2020;9(1):80-93. doi: 10.21037/gs.2019.12.03.
38. Andersson C, Nayor M, Tsao CW, Levy D, Vasan RS. Framingham Heart Study: JACC Focus Seminar, 1/8. J Am Coll Cardiol. 2021;77(21):2680-2692. doi: 10.1016/j.jacc.2021.01.059.
39. Malik KS, Adoubi KA. Obésité, hypertension artérielle et niveau d'activité physique dans une population

noire africaine [Obesity, high blood pressure and physical activity levels in a black African population]. Ann Cardiol Angeiol (Paris). 2019;68(3):133-138. doi: 10.1016/j.ancard.2018.08.017 [in French].
40. Wang Z, Ma L, Liu M, Fan J, Hu S. Writing Committee of the Report on Cardiovascular Health and Diseases in China. Summary of the 2022 Report on Cardiovascular Health and Diseases in China. Chin Med J (Engl). 2023;136(24):2899-2908. doi: 10.1097/CM9.0000000000002927.
41. Qiu B, Wang Q, Ling Z, Wen S, Yao Q, Xiao Y, et al. Physical Activity, sleep disorders and hypertension: observational and Mendelian randomization analyses. Blood Press. 2025;34(1):2569387.1-12. doi: 10.1080/08037051.2025.2569387.
42. Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T, et al. Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: comparison with Captopril. Phytomedicine. 2011;18(4):251-8. doi: 10.1016/j.phymed.2010.08.016.

Received: 31.12.2025
Revised: 30.03.2026
Accepted: 14.04.2026
Published online first: 30.04.2026

Rocz Panstw Zakl Hig 2025;76(4):335-342
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/220854

ORIGINAL ARTICLE

PROXIMATE AND MINERAL COMPOSITION, BIOLOGICAL PROPERTIES, AND ACUTE TOXICITY OF *ARISARUM VULGARE* O. TARG. TOZZ., A TRADITIONAL WILD FAMINE FOOD PLANT IN MOROCCO

Abdelghani Aboukhalaf[1,2], *Houda El Yacoubi*[1], *Atmane Rochdi*[1]

[1]Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kénitra, Morocco
[2]Research Unit on Nutrition & Food Sciences, Laboratory of Anthropogenetic, Biotechnologies and Health, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco

ABSTRACT

Background. During famine periods in Morocco, *Arisarum vulgare* O. Targ. Tozz was traditionally consumed in rural areas and contributed to population survival. Despite its historical importance, limited research exists on its nutritional composition, bioactive properties and safety.

Objective. The current study aimed to evaluate the proximate and mineral compositions, phytochemical profile and acute toxicity of *A. vulgare* tubers.

Material and Methods. Nutritional composition was determined using standard food analysis methods, whereas polyphenol and flavonoid contents and antioxidant activity were assessed using colorimetric assays. Antimicrobial activity was evaluated by disc diffusion and broth microdilution methods, and safety was assessed by performing acute toxicity.

Results. The finding revealed that this plant is rich in carbohydrates but low in protein and fat, while mineral and phenolic contents were at moderate levels. Biological evaluation of the extracts showed remarkable antioxidant activity and wide antibacterial and antifungal effects. The aqueous extract obtained by traditional decoction showed no acute toxicity with LD50 higher than 5000 mg/kg, which suggests that the traditional preparation method plays an important role in reducing the toxicity of *A. vulgare*.

Conclusions. The results highlight the nutritional value of *A. vulgare* tubers and their content in bioactive compounds showing antioxidant and antimicrobial activities.

Keywords: *Arisarum vulgare O. Targ. Tozz, famine food, nutritional profile, antioxidant activity, antimicrobial activity, toxicity*

INTRODUCTION

Morocco has experienced several historical periods of famine and food scarcity, particularly during the second half of the twentieth century, which profoundly affected rural populations [1]. During these crises, limited access to staple foods such as cereals and potatoes forced communities to rely on alternative food resources as emergency food sources, making traditional knowledge of local flora essential for survival [2, 3]. Wild edible plants (WEPs) have long served as a safety net for poor rural communities, especially in remote areas, where they were mainly used as subsistence or famine foods [3]. Ethnobotanical surveys conducted in different regions of Morocco have documented a rich diversity of WEPs traditionally used during periods of food shortage, highlighting their importance in local food systems and cultural heritage [4-7].

Among these species *Arisarum vulgare* O. Targ. Tozz (Araceae) is a tuberous wild plant that has been traditionally consumed during famine periods [8]. In Morocco, this species was locally known by the vernacular name ‘‘*Irni*’’, a term closely associated with years of hardship and food deprivation. The tubers were used as a famine food and incorporated into various traditional dishes like vegetable preparations and staple foods such as couscous and bread, often after specific processing methods intended to reduce their inherent toxicity, notably

Corresponding author: Abdelghani Aboukhalaf, Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, 14000 Kénitra, Morocco; email: aboukhalaf.a@ucd.ac.ma

Publisher: National Institute of Public Health NIH - National Research Institute

prolonged cooking (up to 24 h) [2, 5]. Despite its reputation as a potentially toxic plant, its use during food shortage underscores its historical role as an emergency resource when few alternatives were available. *A. vulgare* contains alkaloids such as irniine and bgugaine, which are associated with significant toxicity. These compounds have been shown to induce hepatotoxicity, and DNA damage in liver cell models, which may explain the toxic effects observed after consuming the tubers [8, 9]. The accumulation of toxic secondary metabolites in plants is widely recognized as an adaptive defense strategy against both biotic and abiotic stress factors, and such compounds are often produced as part of complex chemical defense systems that enhance plant survival under adverse environmental conditions [10]. Beyond its use as a subsistence food, *A. vulgare* has also been traditionally used in folk medicine for the management of various ailments, including infections, pain, headaches, digestive disorders, skin conditions, respiratory complaints, wounds, and burns, as well as for the treatment of cancer in traditional practices [8, 11, 12].

To date, comprehensive scientific data addressing its nutritional value biochemical composition, and toxicological profile are poorly documented. The objective of the present study was therefore to provide an integrated evaluation of *A. vulgare* tubers by investigating their nutritional composition, mineral content, phytochemical profile, antioxidant and antimicrobial activities, as well as their acute toxicity. This study aims not only to assess its traditional use as a famine food but also to explore its potential as a supplementary food resource in local diet, provided that is safety is ensured.

MATERIAL AND METHODS

Sample collection and preparation

Fresh tubers of *A. vulgare* were collected in March 2025 from Sidi Bennour, located in central Morocco. About 20 tubers from different plants were collected, randomly selected, and combined to obtain a representative sample for analysis. After collection, the samples were rinsed with distilled water and air dried in a shaded area. The dried plant material was then milled into a fine powder using a laboratory mill and sieved to obtain a uniform particle size. The resulting powder was extracted by maceration in methanol for 24 h, as this solvent is effective in extracting a wide range of bioactive compounds related to antioxidant and antimicrobial activities [13]. In parallel, an aqueous decoction was prepared for the acute toxicity test to reflect the traditional way the plant is used and to better represent how it is consumed. The extracts were subsequently filtered through Whatman filter paper (No. 1), and the filtrates were concentrated under reduced pressure using a rotary evaporator at 55°C. The dried extracts were stored at 4°C until further use.

Animals

Acute toxicity was evaluated using healthy, non-pregnant young female Wistar rats aged 8-12 weeks (170-180 g). A total of 15 rats were used and divided into three groups (n = 5 per group): a control group and two treated groups receiving doses of 2000 and 5000 mg/kg of aqueous extract, respectively. The animals were housed in standard cages and acclimatized for one week under controlled environmental conditions (23 ± 2°C; 12 hours light/dark cycle) prior to the initiation of treatment. Food and water were provided *ad libitum* throughout the acclimatization period. All animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by local institutional animal ethics committee (Approval No: 07/2025/NRSDL, approved in July 2025).

Determination of nutritional composition

The proximate composition of *A. vulgare*, including moisture, ash, crude protein, fat and carbohydrate contents, was determined following previously described standard methods. Moisture and ash contents were determined by oven-drying and muffle furnace methods [15], respectively, while crude protein was quantified using the Kjeldahl method [16]. Total fat content was assessed by Soxhlet extraction technique using hexane as solvent [15]. Carbohydrate content was calculated by difference according to the equation: Carbohydrates (%) = 100% - (moisture + ash + fat + crude protein).

Mineral element composition was determined using inductively coupled plasma-atomic emission spectrometry (ISP-AES; Jobin Yvon, Ultima 2) equipped with an axial viewing plasma, following the method described by [16]. Major elements (K, Ca, Mg, Na, and S) and trace elements (Fe, Zn, Cu, and Mn) were quantified.

Total phenolic content (TPC)

TPC was determined using Folin-Ciocalteu method as described by Kim et al. [17]. Briefly, 100 µL of methanolic extract was mixed with 1 mL of diluted Folin-Ciocalteu reagent (1:10, v/v). After 5 min, 1 mL of Na_2CO_3 solution (7%) was added, followed by immediate dilution with 0.4 mL of distilled water. The mixture was incubated for 1.5 h in the dark, and absorbance was measured at 760 nm using a spectrophotometer. TPC was calculated from a gallic acid calibration curve and expressed as gallic

acid equivalents per gram of extracts (mg GAE/g extract).

Total flavonoid content (TFC)

TFC of the tuber methanolic extract of *A. vulgare* was determined using aluminum chloride colorimetric method [18]. Briefly, 400 µL of extract was mixed with 120 µL of 5% $NaNO_2$. After 5 min, 120 µL of $AlCl_3$ (10%) was added, followed by the addition of 800 µL of 1M NaOH after a further 6 min. Absorbance was measured at 510 nm using a spectrophotometer. All analyses were performed in triplicate, and TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE·g^{-1} extract).

***In vitro* antioxidant evaluation**

Antioxidant activity was evaluated using the 2,2-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay as described by Ksouri et al. [19]. Methanolic extract at various concentrations (1-1000 µg/mL) were combined with an equal volume methanolic DPPH solution (0.004%, w/v). The mixtures were kept incubated for 30 min at room temperature in the dark, and absorbance recorded at 517 nm. All measurements were carried out in triplicate, and results were expressed as IC50 values (µg/mL), defined as the concentration required to scavenge 50% of the initial DPPH radicals. Ascorbic acid was used as a reference standard.

Antimicrobial activity

Microorganism strains

A total of six bacterial strains, comprising three Gram-positive bacteria and three Gram-negative bacteria, as well as two fungal strains, were used in this study. The strains were obtained from the Institute Pasteur Collection (CIP) and the American Type Culture Collection (ATCC). The tested microorganisms included *Bacillus subtilis* (ATCC66331), *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC19433), *Citrobacter freundii* (ATCC8090), *Escherichia coli* (CIP54127), *Pseudomonas* sp., *Cryptococcus neoformans* (CIP 960) and *Candida albicans* (48.72).

Disk diffusion assay

The antimicrobial potential of the extract was evaluated by the disc diffusion assay, following the procedure reported by Aboukhalaf et al. [20].

Minimum inhibitory concentration (MIC) determination

The MIC of the *A. vulgare* extract was determined using a resazurin-based microdilution method, according to Lahlou et al. [21].

Minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) determinations

Wells showing no visible growth after MIC determination were subcultured to recovery media for MBC and MFC assessment and incubated under appropriate conditions. Absence of growth indicated bactericidal or fungicidal activity. MBC/MIC and MFC/MIC ratios were calculated with values ≤ 4 considered bactericidal/ fungicidal and values > 4 considered bacteriostatic/fungistatic [21].

Acute toxicity study

Acute oral toxicity study of the aqueous decoction extract of *A. vulgare* was evaluated in accordance with Organization for Economic Co-operation and Development (OECD) guideline 425 at limit doses of 2000 and 5000 mg/kg body weight [22].

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Differences among samples were analysed using one-way analysis of variance (ANOVA) at a 95% confidence level. Data normality was checked prior to analysis. Values were considered statistically significant at $p < 0.05$.

RESULTS

Nutritional composition

The proximate composition of *A. vulgare* tubers is presented in Table 1. The moisture content was

Table 1. Nutritional composition of *A. vulgare*

Proximate (%)	DW	FW
Moisture	8.35 ± 0.55	78.13 ± 0.17
Ash	6.98 ± 0.76	1.53 ± 0.16
Proteins	5.54 ± 0.32	1.21 ± 0.07
Fat	1.07 ± 0.30	0.23 ± 0.07
Carbohydrates	78.06 ±1.89	17.07 ± 0.41
Mineral composition (mg/100 g)	DW	FW
Mg	16.62 ± 0.31	3.63 ± 0.07
Ca	95.18 ± 0.85	20.82 ± 0.19
P	19.97 ± 0.14	4.37 ± 0.03
K	53.12 ± 0.32	11.62 ± 0.07
Na	43.97 ± 0.27	9.61 ± 0.06
Zn	0.235 ± 0.001	0.051 ± 0.0002
Cu	0.043 ± 0.0002	0.009 ± 0.00004
Fe	4.040 ± 0.014	0.88 ± 0.003
Mn	0.161 ± 0.003	0.035 ± 0.001

Data are expressed as mean ± standard deviation (SD); (n = 3); on dry weight (DW) and fresh weight (FW) basis.

relatively low (8.35%). Carbohydrates were the predominant component of *A. vulgare* (78.06%), followed by ash (6.98%), protein (5.54%), and fat (1.07%). Mineral analysis revealed appreciable levels of macroelements (Table 1), including calcium (20.82 mg/100 g fresh weight (FW)), potassium (11.62 mg/100 g FW), sodium (9.61 mg/100 g FW), phosphorus (4.37 mg/100 g FW), and magnesium (3.63 mg/100 g FW). Among microelements, iron content reached 40.88 mg/100 g FW, while zinc and manganese were present in moderate amounts and copper in lower concentrations.

Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extract are presented in Table 2. The extract showed a TPC of 29.76 mg GAE/g extract and a TFC of 0.69 mg GAE/g extract.

Antioxidant capacity

The antioxidant capacity of the *A. vulgare* tubers extract, along with standard antioxidant (ascorbic acid), was evaluated using the DPPH radical scavenging assay through the determination of IC50 values. Lower IC50 values indicate stronger antioxidant activity. Table 2 presents the obtained results. As shown, the studied extract exhibited strong free radical scavenging activity, with a low value (230.19 µg/mL). The IC50 value is further lower for ascorbic acid (8.15 µg/mL) used as positive control.

Antimicrobial activity

The antimicrobial activity of *A. vulgare* was evaluated against a panel of pathogenic microorganisms using inhibition zone measurements, MIC and MBC/MFC assays (Table 3 and 4). The extract exhibited strong inhibitory effects against all tested Gram-

Table 2. Total phenol and flavonoid contents and antioxidant activity of methanolic extract of *A. vulgare*

	Total phenolic Content mg GAE/g	Total flavonoids Content mg QE/g	Antioxidant activity (IC50 µg/mL)	
			Extract	Ascorbic acid
Extract	29.76 ± 1.93	0.69 ± 0.55	230.19 ± 0.73	8.15 ± 0.01

Data are expressed as mean ± standard deviation (SD); (n = 3).

Table 3. Inhibition zone diameter (mm) of *A. vulgare* methanolic extract in the disk diffusion method

	Disk inhibitory zone (mm)		
Microorganisms	*A. vulgare* extract	Ampicillin	Econazole
S. aureus	22 ± 0.32	28 ± 0.06	-
E. faecalis	22 ± 0.97	29 ± 0.04	-
Bacillus sp.	26 ± 0.76	28 ± 0.06	-
C. freundii	20 ± 0.44	26 ± 0.08	-
E. coli	24 ± 0.22	28 ± 0.13	-
Pseudomonas sp.	17 ± 0.64	23 ± 0.25	-
C. albicans	13 ± 1.33	-	22 ± 0.2
C. neoformans	-	19 ± 0.2	24 ± 0.2

Data values are presented as mean ± standard deviation (SD); (n = 3); - = no activity or not determined.

Table 4. Antimicrobial parameters (MIC, MBC, MFC) of the tested extract

Strains	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio	MFC (mg/mL)	MFC/MIC ratio	Type of activity
S. aureus	0.097	0.097	1	-	-	Bactericidal
E. faecalis	0.097	3.125	32	-	-	Bacteriostatic
Bacillus sp.	0.78	3.125	4	-	-	Bactericidal
C. freundii	0.194	3.125	16	-	-	Bacteriostatic
E. coli	1.56	6.25	4	-	-	Bactericidal
Pseudomonas sp.	0.194	3.125	16	-	-	Bacteriostatic
C. albicans	-	-	-	-	-	-
C. neoformans	6.25	-	-	12.5	2	Fungicidal

- = not determined.

positive and Gram-negative bacteria and yeasts tested, with inhibition zone diameters ranging from 13 to 26 mm. In contrast, no activity was detected against the yeast *C. albicans*. The tested extract exhibited a maximum zone of inhibition against *Bacillus* sp., while a minimum zone of inhibition was observed against *C. neoformans*.

The antimicrobial efficacy of the extract was further confirmed by MIC determination (Table 4), with MIC values ranging from 0.097 to 6.25 mg/mL. The highest MIC value was observed for *S. aureus* and *E. faecalis* (0.097 mg/ml).

Furthermore, the extract exhibited bactericidal activity against *S. aureus*, *E. coli* and *Bacillus* sp., as well as fungicidal activity against *C. neoformans*, as indicated by MBC/MIC and MFC/MIC ratios ≤ 4. In contrast, bacteriostatic effects were observed against *E. faecalis*, *C. freundii* and *Pseudomonas* sp., with MBC/MIC ratios > 4.

Acute toxicity

The acute toxicity of *A. vulgare* water decoction was assessed at doses of 2000 and 5000 mg/kg body weight. Throughout the 15 day observation period, no mortality or treatment related clinical signs of toxicity were recorded, in particular, symptoms commonly associated with acute toxicity, including drowsiness, edema, loss of reflexes, diarrhea, tremors, excessive urination, or salivation, were not observed in treated animals.

Furthermore, no statistically significant differences ($p > 0.05$) were noted between the control and treated groups in terms of body weight evolution (Figure 1), food intake, or water consumption (Table 5) during the experimental period.

DISCUSSION

Nutritional composition

To the best of our knowledge, this is the first study reported the nutritional composition of *A. vulgare*. The low moisture content observed is favorable for storage stability and limits microbial spoilage. Carbohydrates are among the main nutrients in the human diet and their levels in plants are an important indicator of nutritional and caloric value [23]. In the present study, the high carbohydrate content indicates that this species can serve as a source of energy. Although is nutritional contribution depends on its inclusion in a diversified diet.

The ash content was higher than that reported for several wild edible tuberous plants traditionally

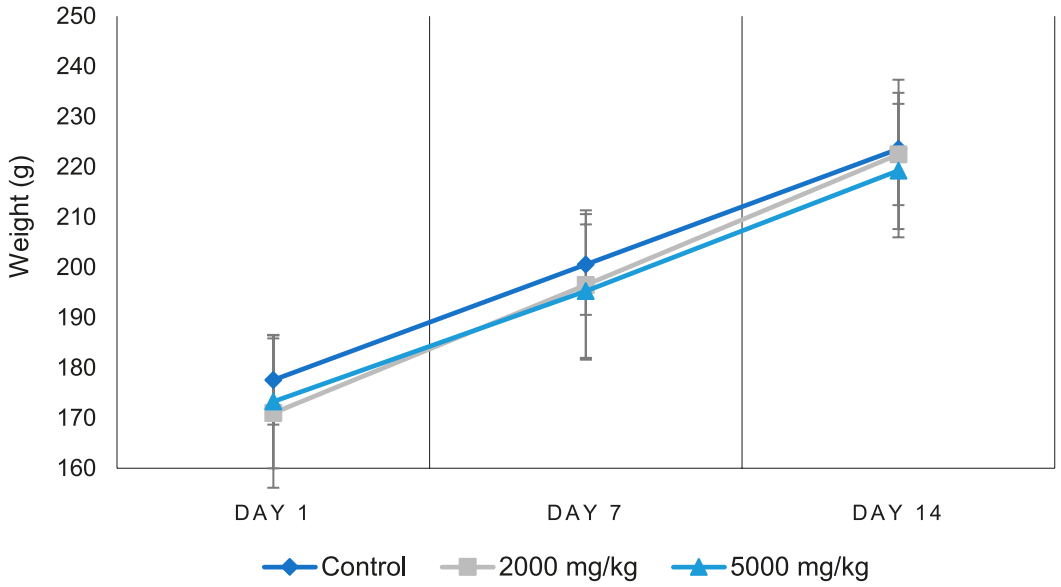


Figure 1. Body changes in rats treated with *A. vulgare* aqueous extract during the acute toxicity study (mean ± SD, n = 5). No significant differences versus control ($p > 0.05$).

Table 5. Effect of *A. vulgare* extract on food intake (g) and water intake (mL) in experimental rats during acute toxicity study

	Groups							
	Food intake (g)				Water intake (mL)			
	Control	2000 mg/kg	5000 mg/kg	p-value	Control	2000 mg/kg	5000 mg/kg	p-value
A. vulgare	47.15 ± 2.06[a]	46.96 ± 1.96[a]	46.72 ± 2.18[a]	> 0.05	47.22 ± 2.43[a]	47.76 ± 1.36[a]	46.54 ± 2.56[a]	> 0.05

Data are presented as mean ± standard deviation (SD); (n = 5 per group); [a] = no significant differences versus control.

consumed in Lokop forest, (East Aceh, Indonesia) [24], suggesting a higher mineral contribution. The protein content (5.54%) exceeded previously reported values for similar wild tubers (1.72-4.27%) [24], although still lower than that of protein-rich leafy vegetables, this protein level remains nutritionally relevant. The low fat content of this species (1.07%) suggests that *A. vulgare* can be classified as a low-fat food. However, its potential health effects depend on dietary patterns and nutritional balance.

Calcium is an essential mineral vital for bone and tooth development, muscle function, nerve transmission, and detoxification processes [25]. The calcium content of *A. vulgare* (20.82 mg/100 g FW) is comparable to that of commonly consumed tuber vegetables in Morocco such as sweet potato (22 mg/100 g FW), carrot (30 mg/100 g FW) and, turnip (33 mg/100 g FW), although higher than potato (8 mg/100 g FW) [26]. Potassium content was higher than sodium, which results in a Na/K ratio below 1. As an indicator of cardiovascular health, a low Na/K ratio is generally associated with a reduced risk of hypertension and related metabolic disorders [27]. Therefore, the low Na/K ratio observed enhances the dietary value of *A. vulgare* tubers and supports their inclusion in mineral balanced diets.

Iron is required for haemoglobin formation and oxygen transport [28]. The high Fe content compared to conventional tuberous and root vegetables such as sweet potato (0.57-0.73 mg/100 g FW) [29], carrot (0.30 mg/100 g FW), beetroot (0.8 mg/100 g FW) and radish (0.34 mg/100 g FW) [30] highlights the potential of *A. vulgare* as a source of this essential micronutrient. Zinc and manganese, which are involved in immune function, antioxidant defense, and enzymatic activity [28], were present in moderate amounts, while copper, essential for iron metabolism and redox processes, was found at lower levels. These levels suggest a limited to moderate contribution to daily micronutrient requirements. Overall, these results suggest that *A. vulgare* can contribute to mineral intake. Nevertheless, its contribution varies among minerals and may also be affected by traditional processing methods such as prolonged cooking.

Total phenolic and flavonoid contents

Polyphenols are considered essential functional components of the human diet. They are classified into several groups, among which flavonoids, represent the largest and most widespread family [31]. In the current study, the moderate total phenolic content and low flavonoid content suggest that phenolic acids and other non-flavonoid phenolics may constitute the major contributors of total phenolic of the extract. The obtained TPC and TFC values were found to be lower than the values reported for the tubers of the same species by Bouafia et al. [11], who recorded 176.00 ± 1.08 mg GAE/g of dry extract for TPC and 81.21 ± 1.24 mg QE/g of dry extract for TFC. In contrast, Kadri et al. [14] reported lower total phenolic contents in the hydromethanolic extract of *A. vulgare* seeds, with value of 12 mg GAE/g of extract, which is lower than the value obtained in the present study. However, the same study reported a higher TFC (3.4 mg QE/g extract) than the value recorded in the present finding. Such variations in TPC and TFC contents may be attributed to differences in climatic conditions, seasonal factors, geographical location, plant part used, harvest time, and extraction methods.

Antioxidant activity

The antioxidant activity observed in this study indicates that the extract possesses a noticeable free radical scavenging capacity. This activity was higher than that reported by Messaoudi et al. [32], but lower than the activity observed by Bouafia et al. [11] for the ethanolic and aqueous extracts of the same plant parts. Such variability in antioxidant activity may be attributed to differences in climatic and environmental conditions. The antioxidant capacity of the extracts of the examined species in DPPH assay is likely related to the presence of specific phenolic compounds, such as rutin, hesperidin, isoquercitrin, catechin, chlorogenic acid, syringic acid, salicylic acid, kaempferol, and luteolin, which are well known for their strong antioxidant properties [11].

Antimicrobial activity

The results of the present study indicate that *A. vulgare* exhibited a broad-spectrum antimicrobial activity associated with stronger effects against Gram-positive bacteria than Gram-negative bacteria. This difference may be explained by structural differences in bacterial cell walls. These findings are consistent with previous findings by Bouafia et al. [11], who demonstrated that the ethanolic extract of *A. vulgare* was effective against *S. aureus*, *E. coli*, *Enterococcus faecium*, and *Salmonella typhimurium*, with the highest inhibition zone observed against *E. faecium*. Similarly, Messaoudi et al. [32] reported that the methanolic extract exhibited antimicrobial activity against *Klebsiella pneumoniae*, *S. aureus* and *C. albicans*.

The low MIC values observed in the present study, particularly for *S. aureus* and *E. faecalis*, further support the strong antimicrobial potential of the extract. These results are consistent with the findings presented by Aydin et al. [12], who observed inhibitory effects of *A. vulgare* extracts against *E. coli* and *S. aureus*.

Additionally, the bactericidal and fungicidal effects observed against several strains, as indicated by MBC/

MIC and MFC/MIC ≤ 4, highlight the effectiveness of the extract in inhibiting microbial growth. However, the bacteriostatic effects observed for some strains suggest a variable response depending on the microorganism.

The antimicrobial properties of *A. vulgare* may be attributed to its content of bioactive compounds, particularly alkaloids such as bgugaine and irniine, which have been previously reported to play a role in pathogen control [33].

Acute toxicity

The absence of mortality and clinical signs of toxicity suggest that the aqueous decoction of *A. vulgare* did not induce acute toxicity at the tested doses. No significant differences in body weight, food intake, or water consumption were observed, supporting its safety under the experimental conditions. Although *A. vulgare* is traditionally considered toxic, its consumption has been reported during periods of famine, which suggests that traditional preparation methods such as aqueous decoction may reduce its toxicity.

CONCLUSION

This study provides insight into the nutritional and biological characteristics and acute toxicity of *A. vulgare* tubers traditionally used as a subsistence food during periods of food scarcity. Proximate analysis indicated that the tubers are predominantly composed of carbohydrates, which highlights their role as a source of energy rather than a complete nutritional food. Protein and fat were relatively low, while mineral analysis revealed moderate levels of essential elements, particularly calcium and iron. Phytochemical analysis showed moderate amounts of phenolic compounds and flavonoids, and the methanolic extract exhibited antioxidant and antimicrobial activities. Acute toxicity assessment did not reveal adverse effects at the tested doses, suggesting a favorable short-term safety profile.

Therefore, *A. vulgare* should be regarded mainly as an emergency or subsistence food providing energy and certain micronutrients rather than a rich source of nutrients. Their traditional use during famine periods may be explained by their availability and caloric contribution. Further studies including chronic toxicity and biochemical and histopathological evaluations, are required to ensure their long term safety for human consumption.

Declaration of competing interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Thomson M. A Morbid Affair: Epidemics and Famine in Morocco, 1860–1888. J Inst Afr Stud. 2019;2:86-98. doi: 10.31132/2412-5717-2019-47-2-86-99.
2. Ghanimi R, Ouhammou A, Ahouach A, Cherkaoui M. Ethnobotanical study on wild edible plants traditionally used by Messiwa people, Morocco. J Ethnobiol Ethnomed. 2022;18(1):16. doi: 10.1186/s13002-022-00500-4.
3. Nassif F, Tanji A. Revisiting Famine Plants And Their Role In Fighting Hunger And Saving Lives In Morocco. Life Sci Leafl. 2013;43:29-56.
4. Essaih S, Sahel K, Aboukhalaf A, Chamlal H, Elbiyad J, Atouife S, et al. Biodiversity of wild edible-medicinal and cosmetic plants with traditional uses in the Moroccan province of Taounat in the Rif montain. Rev Esp Antrop Fís. 2024;49:07-35.
5. Aboukhalaf A, Tbatou M, Kalili A, Naciri K, Moujabbir S, Sahel K, et al. Traditional knowledge and use of wild edible plants in Sidi Bennour region (Central Morocco). Ethnobot Res Appl. 2022;23(11):265-273. doi: 10.32859/era.23.11.1-18.
6. Tbatou M. Belahyan A, Fagroud M, Belahsen R. Wild Edible Plants Traditionally Used In The Rural Area Of El Jadida (Center Of Morocco)-Assessing Traditional Knowledge Erosion. Life Sci Leafl. 2016;78:30-51.
7. Nassif F, Tanji A. Gathered food plants in Morocco the long-forgotten species in ethnobotanical research. Life Sci Leafl. 2013;37:17-54.
8. Rakba N, Melhaoui A, Loyer P, Delcros JG, Morel I, Lescoat G. Bgugaine, a pyrrolidine alkaloid from *Arisarum vulgare*, is a strong hepatotoxin in rat and human liver cell cultures. Toxicol Lett. 1999;104(3):239-248. doi: 10.1016/S0378-4274(98)00375-0.
9. Rakba N, Melhaoui A, Rissel M, Morel I, Loyer P, Lescoat G. Irniine, a pyrrolidine alkaloid, isolated from *Arisarum vulgare* can induce apoptosis and/or necrosis in rat hepatocyte cultures. Toxicon. 2000;38(10):1389-1402. doi: 10.1016/S0041-0101(99)00232-9.
10. Ozay C, Kılıncarslan O, Mammadov R. Interactions between heavy metals and glucosinolates as defense mechanisms in Brassicaceae. Turk J Sci Rev. 2016:9:12-22. Available on: https://dergipark.org.tr/en/pub/derleme/article/389389.
11. Bouafia Z, Boudjelal A, Bouaziz-Terrachet S, Bouhenna MM, Bouchahdane S, Boulila A, et al. *Arisarum vulgare*: Bridging Tradition and Science Through Phytochemical Characterization and Exploring Therapeutic Potential via In vitro, In vivo, and In silico Biological Activities. Chemistry Select. 2024;9(38):e202402754. doi: 10.1002/slct.202402754.
12. Aydın Ç, Özay C, Düşen O, Mammadov R, Orhan F. Total Phenolics, Antioxidant, Antibacterial and Cytotoxic Activity Studies of Ethanolic Extracts *Arisarum vulgare* O. Targ. Tozz. and *Dracunculus vulgaris* Schott. Int J Second Metab. 2017;4(2):114-122. doi: 10.21448/ijsm.307944.
13. Mehmood A, Murtaza G. Phenolic contents, antimicrobial and antioxidant activity of *Olea*

ferruginea Royle (Oleaceae). BMC Complement Altern Med. 2018;18(1):173. doi: 10.1186/s12906-018-2239-0.
14. Kadri H, Djilani SE, Djilani A. Phytochemical constituents, antioxidant activity, total phenolic and flavonoid contents of *Arisarum vulgare* seeds. Acta Sci Pol Technol Aliment. 2013;2(2): 169-173.
15. Ibourki M, Azouguigh F, Jadouali SM, Sakar EH, Bijla L, Majourhat K, et al. Physical fruit traits, nutritional composition, and seed oil fatty acids profiling in the main date palm (*Phoenix dactylifera* L.) varieties grown in Morocco. J Food Qual. 2021;2021(1):5138043. doi: 10.1155/2021/5138043.
16. Ait Bouzid H, Bijla L, Ibourki M, Asbbane A, Sakar EH, Koubachi J, et al. Nutritional potential of Ziziphus lotus (L.) lam. Almonds as compared to some oilseeds from Morocco: Evidence from proximate composition, mineral profiling, and oil physicochemical traits. J Food Qual. 2024(1):6685429. doi: 10.1155/2024/6685429.
17. Kim SJ, Min SC, Shin HJ, Lee YJ, Cho AR, Kim SY, et al. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. Meat Sci. 2013;93(3):715-722. doi: 10.1016/j.meatsci.2012.11.029.
18. Aboukhalaf A, Lahlou Y, Kalili A, Rocha JM, Belahsen R. Enrichment of bread with powder from the aerial parts of purslane: effects on mineral content, phenolic compounds, antioxidant activity, sensory properties, and nutritional enhancement. Croat J Food Sci Technol. 2025;17(2):1-12. doi: 10.17508/CJFST.2025.17.2.02.
19. Ksouri A, Dob T, Belkebir A, Krimat S, Chelghoum C. Chemical composition and antioxidant activity of the essential oil and the methanol extract of Algerian wild carrot *Daucus carota* L. ssp. *carota*. (L.) Thell. J Mater Environ Sci. 2015;6(3):784-791.
20. Aboukhalaf A, Abdessadek J, Lahlou Y, Ikhiar N, Essaih S, Elbiyad J, et al. Assessment of phenolic and flavonoid contents, antioxidant and antimicrobial activities of Moroccan propolis. Rocz Panstw Zakl Hig. 2024;75(1):75-81. doi: 10.32394/rpzh.2024.0292.
21. Lahlou Y, El Amraoui B, El Wahidi M, Moujabbir S, Aboukhalaf A, Bamhaoud T. Evaluation of the anti-*Candida albicans* and anti-*Cryptococcus neoformans* activity of the essential oils of three Moroccan medicinal plants. OP Conf Ser: Earth Environ Sci. 2022;1090(1):012026. doi: 10.1088/1755-1315/1090/1/012026.
22. OECD. Test No. 425. Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals. Section 4. Paris: Éditions OCD; 2008. doi: 10.1787/9789264071049-en.
23. Ludwig DS, Hu FB, Tappy L, Brand-Miller J. Dietary carbohydrates: role of quality and quantity in chronic disease. BMJ. 2018;361:k2340. doi: 10.1136/bmj.k2340.
24. Suwardi AB, Indriaty NZ, Navia ZI. Nutritional evaluation of some wild edible tuberous plants as an alternative foods. Innovare J Food Sci. 2018;6(2):9-12.
25. Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW, et al. Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes. Ann N Y Acad Sci. 2022;1512(1):10-28. doi: 10.1111/nyas.14758.
26. USDA (United States Department of Agriculture). FoodData Central. [cited 2026 Mar 8]. Available from: https://fdc.nal.usda.gov/.
27. Baek SY, Kim HK. Association of dietary sodium-to-potassium ratio with cardiometabolic risk factors in Korean adults: Findings from the Korean National Health and Nutrition Examination Survey. Nutrients. 2023;15(14):3134. doi: 10.3390/nu15143134.
28. Razzaque MS, Wimalawansa SJ. Minerals and Human Health: From Deficiency to Toxicity. Nutrients. 2025;17(3):454. doi: 10.3390/nu17030454.
29. Sanoussi AF, Adjatin A, Dansi A, Adebowale A, Sanni LO, Sanni A. Mineral composition of ten elites sweet potato (*Ipomoea Batatas* [L] Lam) landraces of Benin. Int J Curr Microbiol Appl Sci.2016;5(1):103-115. doi: 10.20546/ijcmas.2016.501.00.
30. Knez E, Kadac-Czapska K, Dmochowska-Ślęzak K, Grembecka M. Root Vegetables-Composition, Health Effects, and Contaminants. Int J Environ Res Public Health. 2022;19(23):15531. doi: 10.3390/ijerph192315531.
31. Rana A, Samtiya M, Dhewa T, Mishra V, Aluko RE. Health benefits of polyphenols: A concise review. J Food Biochem. 2022;46(10):e14264. doi: 10.1111/jfbc.14264.
32. Messaoudi Y, Feknous N, Badouna B, Feknous S, Sekiou O, Bouziane I, et al. Antioxidant, Antibacterial and Anti-Inflammatory Activities of *Arisarum vulgare* Extracts on Wistar Rats Asthma Model. Lat Am J Pharm. 2025;44(3):298-312.
33. Melhaoui A, Mallea M, Jossang A, Bodo B. Antibiotic and antifungal pyrrolidine alkaloids, from *Arisarum vulgare*. Nat Prod Lett. 1993; 2(3):237-242. doi: 10.1080/10575639308043815.

Received: 23.01.2026
Revised: 18.04.2026
Accepted: 21.04.2026
Published online first: 05.05.2026

Rocz Panstw Zakl Hig 2025;76(4):343-351
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/221408

ORIGINAL ARTICLE

ADHERENCE TO THE MEDITERRANEAN DIET AMONG ADOLESCENTS: A COMPARISON OF TWO VERSIONS OF KIDMED

Imane Haddou, Halima Daif, Halima Belaoufi, Ghizlane Benaddi, Mohammed Elayachi, Omar El Hiba, Rekia Belahsen

Laboratory of Anthropogenetics, Biotechnologies and Health, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco

ABSTRACT

Background. Although the Mediterranean diet (MD) is recognized for its protective effect against cardiometabolic risk, adherence to this diet tends to decrease among adolescents. The KIDMED index used to assess adherence to MD in children and adolescents has not undergone a formal revision since its initial publication in 2004.

Objective. To assess adherence to the MD using both the original (2004) and updated (2019) versions of KIDMED, in the same sample of adolescents.

Methods. A cross-sectional study was conducted with 129 adolescents. Anthropometric measurements included body weight and height to calculate the body mass index (BMI) and BMI-z-score, and waist circumference (WC) were taken, and physical activity levels were assessed using the short version of the IPAQ questionnaire. Adherence to the MD was assessed using the original version (OV, 2004) and updated versions (UV, 2019) of KIDMED.

Results. Adherence to MD was generally moderate, with a mean score of 5.88 ± 3.22 according to the (OV) and 5.61 ± 3.05 according to the (UV). The latter led to an increase in the proportion of adolescents with low adherence (30.2% vs. 22.5%) and a decrease in those classified as having good adherence (25.6% vs. 28.7%). Agreement between the two classifications was moderate. Significant differences were observed for several items, including fruit consumption, breakfast quality, frequenting fast-food restaurants, and consumption of sugary products.

Conclusion. The UV of KIDMED in light of current nutritional recommendations, leads to a more demanding assessment of eating behaviors and better identification of at-risk dietary patterns in adolescents, without altering the OV structure.

Keywords: *adolecents, Mediterranean diet, KIDMED, revision, physical activity, anthropometry, ultra-processed foods*

INTRODUCTION

Adolescence is a pivotal period in the life cycle, marked by significant biological, psychological, and behavioral changes that have a lasting impact on health in adulthood [1]. During this phase, adopting healthy eating habits and a balanced lifestyle plays a crucial role in preventing non-communicable diseases in adulthood, including obesity, type 2 diabetes, and cardiovascular diseases [1, 2]. However, in many countries, particularly those undergoing nutritional transition, adolescent behaviors are increasingly characterized by sedentary lifestyles and high consumption of energy-dense foods, sugars, saturated fats, and ultra-processed products [3, 4]. The globally applicable healthy eating model proposed in 2019 by the EAT-Lancet Commission is largely based on the Mediterranean diet [5], thus reinforcing the international promotion of this dietary model as one of the most beneficial for health [6, 7].

In children and adolescents, good adherence to this dietary model is associated with a more favorable weight and better nutritional balance [8, 9]. However, several studies conducted with children and adolescents have reported a gradual decline in adherence to this diet, observed recently even in Mediterranean countries, linked to increasing sedentary lifestyles and the adoption of Western-style eating habits. A systematic review has indeed shown that adherence to this diet is often low among young people, suggesting an erosion of traditional eating habits in this age group [10]. Other recent studies have also confirmed a low prevalence of good adherence, interpreted as a transition to a Westernized diet [11]. Furthermore, the literature indicates the coexistence of less active lifestyles and Western eating habits with

Corresponding author: Rekia Belahsen, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, Boulevard des Facultés, 24000, El Jadida, Morocco; email: rbelahsen@yahoo.com; phone: +212664971616

Publisher: National Institute of Public Health NIH - National Research Institute

lower adherence to the Mediterranean diet among adolescents [12].

The KIDMED questionnaire, developed in 2004 by Serra-Majem et al. [13] to assess adherence to the Mediterranean diet among young people, is one of the most widely used tools in pediatric nutritional epidemiology. It allows for the assessment, through a series of simple questions, of the extent to which children's and adolescents' eating habits conform to the principles of the Mediterranean diet. However, the nutritional context in which the KIDMED was designed, characterized by a more limited availability of ultra-processed foods and less exposure to industrial products, differs significantly from that observed today [13, 14].

Although several authors have proposed adaptations to the KIDMED test to better reflect the evolution of nutritional recommendations and the adolescent food environment, it has not undergone any official revision since its initial publication in 2004 [13]. Recent recommendations address aspects not included in the original version of KIDMED, including carbohydrate quality, limiting free sugars, and the importance of reducing consumption of ultra-processed foods [4, 15].

The overestimation of dietary quality that can result from the use of certain traditional indicators of adherence to the Mediterranean diet, particularly among children and adolescents, has been highlighted by several authors [15]. In fact, this overestimation is associated with the fact that food processing and the overall nutritional quality of the diet are insufficiently considered. Other authors have also emphasized the need to integrate more qualitative dimensions of the Mediterranean model and to take into account its evolution in contemporary dietary contexts [16].

Indeed, a positive association between adherence to the Mediterranean diet, physical activity levels, and body composition has been reported in young people by numerous studies. Other studies have highlighted the link between physical fitness and physical activity and better adherence to the Mediterranean diet among adolescents [17], as well as a correlation between Mediterranean-style eating behaviors and better levels of physical fitness and well-being in children and adolescents [18, 19]. Similarly, a literature review generally supports positive associations between adherence to the Mediterranean diet and healthy lifestyle habits, including physical activity [20].

This study aims to examine the extent to which an updated interpretation of the KIDMED, based on a more rigorous reading aligned with contemporary nutritional recommendations while retaining the conceptual structure and items of the original questionnaire, is relevant and can influence the assessment of adherence to the Mediterranean diet among adolescents. Furthermore, this updated version of the KIDMED (2019) is not a new validated instrument but rather a methodological reinterpretation of the original KIDMED, offered solely for comparative purposes to assess the impact of this update on the classification of eating behaviors [15]. In this context, this study aims to compare classifications of adherence to the Mediterranean diet using the original KIDMED questionnaire published in 2004, and its updated version published in 2019, applied to the same group of adolescents, in order to analyze the methodological impact of the updated interpretation on the assessment of eating behaviors.

MATERIAL AND METHODS

Study population

This cross-sectional study was conducted during the 2024/2025 school year with 129 adolescents aged 14 to 18 years, enrolled in a public secondary school in the city of El Jadida, Morocco. The study population included boys and girls recruited from the selected school after obtaining the necessary authorizations from the relevant educational authorities. Adolescents enrolled at the school, who agreed to participate, and who were present at the time of data collection were included in the study. However, students who were absent during data collection, those following special diets, those with chronic illnesses, and those whose questionnaires were incomplete or did not allow for an assessment of adherence to the Mediterranean diet were excluded from the study.

Assessment of adherence to the Mediterranean diet

Adherence to the Mediterranean diet was assessed using the KIDMED questionnaire, developed by Serra-Majem et al. (2004) for children and adolescents [13]. This tool, widely used in studies of young populations, assesses both adherence to the Mediterranean diet and certain general eating habits, such as skipping breakfast or consuming fast food. It comprises 16 closed-ended questions, answered with yes or no. Items with a positive connotation are scored +1, while items with a negative connotation are scored -1, resulting in a total score ranging from -4 to +12. According to initial recommendations, this score allows participants to be classified into three levels of adherence: low (≤ 3), medium (4 to 7), and high (≥ 8).

Dietary data were collected using a questionnaire administered to adolescents during the survey. Based on the responses obtained, KIDMED scores were calculated according to both the original 2004 version and the updated 2019 version, allowing both versions to be applied to the same sample of adolescents. This approach made it possible to examine the extent to which the changes introduced in the updated version

Table 1. Main differences between the original version (2004) and the updated version (2019) of the KIDMED questionnaire

Domain/ element concerned	KIDMED 2004	KIDMED 2019 update	Relevance for the comparison performed
Daily fruit consumption	Included fruit or natural fruit juice in the first item	Proposes removing the choice: «or natural fruit juice» and only keep whole fruit	Makes the updated version stricter and may lower the score where juice was previously counted as equivalent to whole fruit
Pasta/rice consumption	Positive criterion based on frequent consumption of pasta or rice	Adds the requirement that the pasta/rice be whole grain	Introduces a quality criterion, not just frequency
Consuming cereals or cereal products at breakfast	Positive article for the consumption of cereals or cereal products at breakfast	Specifies that they must be whole grain cereals/whole grains at breakfast	The assessment of breakfast is strengthened by distinguishing between refined and whole grains
Skipping breakfast	The initial wording «does not eat breakfast»	Has been reformulated as «skips breakfast»	This clarifies the wording and makes it easier to interpret
Purpose of the revision	Original 16-item instrument designed to raise awareness among young people about the benefits of the Mediterranean diet	Targeted revision of certain items, without a complete overhaul of the tool	This allows the reader to understand that the comparison between the versions is based primarily on a limited number of modified items

This table is compiled based on Serra-Majem et al. (2004) [13] and Altavilla and Caballero-Pérez (2019) [15].

could influence the assessment and classification of adherence to the Mediterranean diet.

To facilitate understanding of the comparison between the two versions of the KIDMED index, Table 1 summarizes the main differences between the original KIDMED questionnaire (2004) and its updated version (2019), compiled based on the findings of Serra-Majem et al. [13] and Altavilla and Caballero-Pérez [15].

In the present study, both versions were applied to the same sample of adolescents to examine the potential impact of the changes introduced on the assessment and classification of adherence to the Mediterranean diet.

Anthropometric measurements

Anthropometric variables were measured for each adolescent according to the World Health Organization (WHO) standards and procedures [21, 22]. Body weight was recorded in lightly dressed, barefoot participants using a calibrated electronic scale with an accuracy of 0.1 kg, in accordance with international recommendations for nutritional surveys in children and adolescents. Height was measured using a wall-mounted height chart with an accuracy of 0.1 cm. Adolescents were standing barefoot with legs straight, arms relaxed at their sides, heels together, and their head positioned in the Frankfort plane and in contact with the vertical support, according to standardized anthropometric protocols [21]. Body mass index (BMI), also known as the Quetelet index, was calculated by dividing body weight (kg) by height squared (m^2), in accordance with international definitions of general obesity [21]. To account for age and sex, age-specific BMI z-scores were calculated from WHO references using WHO AnthroPlus software (version 1.0.4), a tool recommended for assessing growth and weight status in children and adolescents worldwide [24].

Waist circumference was measured standing with a non-stretch measuring tape placed without compression midway between the last rib and the anterior superior iliac crest, on the mid-axillary line, at the end of a normal expiration, in accordance with WHO recommendations for assessing central adiposity [22]. This measure is recognized as a relevant indicator of cardiometabolic risk in adolescents [22, 23].

Physical activity level assessment

Physical activity (PA) levels were assessed in the participating adolescents using the short version of the IPAQ questionnaire [25], which provides information on physical activities performed in the last seven days. According to the IPAQ protocol classification criteria, participants were divided into three PA levels: low, moderate, and high [26, 27].

Anthropometric and physical activity data were recorded for the purpose of describing the study sample and were not the primary methodological element in the comparison between the two versions of the KIDMED.

The level of physical activity (PA) in the participating adolescents was assessed using a short

version of the IPAQ questionnaire [25], which provides information on physical activity performed in the last seven days.

Statistical analyses

Descriptive statistics were used to analyze the data on sociodemographic and anthropometric characteristics, as well as the physical activity level of the adolescents included in the study. Quantitative variables were expressed as mean ± standard deviation (SD), while qualitative variables were presented as counts and percentages.

Means KIDMED scores obtained with the 2004 and 2019 versions, administered to the same sample of adolescents, were compared using a paired-data test. Depending on the data distribution, either a paired Student's t-test or the Wilcoxon signed-rank test was used. Item-by-item comparisons between the two versions of the KIDMED questionnaire were performed using McNemar's test for paired data.

The consistency between the classifications obtained with the two versions of the KIDMED questionnaire was assessed using Cohen's *kappa* coefficient. The interpretation of *kappa* values was performed according to the classification proposed by Landis and Koch [28], with weak agreement for values < 0.20, acceptable agreement between 0.21 and 0.40, moderate agreement between 0.41 and 0.60, substantial agreement between 0.61 and 0.80, and near-perfect agreement above 0.80. The comparison of Mediterranean diet adherence categories obtained from the 2004 and 2019 versions of the KIDMED questionnaire was examined using the Stuart-Maxwell test for paired multinomial data. The threshold for statistical significance was set at $p < 0.05$.

Ethical considerations

The study was conducted after obtaining authorization from the relevant educational authorities, namely the Regional Academy of Education and the school administration. The study was conducted in accordance with the ethical principles outlined in the World Medical Association's Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants [29] . Participation was voluntary. Before data collection, the adolescents and their parents or legal guardians were informed of the study's objectives and procedures, as well as their right to withdraw at any time. All participants gave their oral consent before being included in the sample. The anonymity and confidentiality of the information collected were strictly guaranteed throughout the study.

RESULTS

General characteristics of the adolescents studied

The participants' sociodemographic and anthropometric characteristics, as well as the level of physical activity are presented in Table 2. The adolescents included in the study have a mean age of 15.3 ± 1.8 years, the majority were girls representing 66.7% of the sample. Their mean body mass index (BMI) was 21.9 ± 3.6 kg/m²; the mean BMI z-score was 0.32 ± 0.98, and mean waist circumference was 74.8 ± 10.1 cm. Regarding physical activity level, 44.2% of adolescents reported a low level, 40.3% a moderate level, and 15.5% a high level.

Adherence to the Mediterranean diet

The distribution of adolescents according to their level of adherence to the Mediterranean diet, assessed using both the original (2004) and recent (2019) versions of KIDMED, is presented in Table 3.

Table 2. Sociodemographic and anthropometric characteristics and level of physical activity

Variables, total (N = 129)	Values mean ± SD or n (%)
Age (yrs), mean ± SD	15.3 ± 1.8
Sex, n (%)	
Girls	86 (66.7)
Boys	43 (33.3)
BMI (kg/m²), mean ± SD	21.9 ± 3.6
BMI z-score, mean ± SD	0.32 ± 0.98
Waist circumference (cm), mean ± SD	74.8 ± 10.1
Physical acrivity level, n (%)	
Low	57 (44.2)
Moderate	52 (40.3)
High	20 (15.5)

BMI – body mass index; SD – standard deviation.

Table 3. Mean KIDMED scores distribution of Mediterranean diet adherence levels according to the 2004 and 2019 versions of the KIDMED questionnaire

Versions of KIDMED	KIDMED score mean ± SD	Adherence (%)		
		Low	Medium	High
Original version (2004)	5.88 ± 3.22	22.5	48.8	28.7
Updated version (2019)	5.61 ± 3.05	30.2	44.2	25.6

SD – standard deviation; Adherence categories: ≤ 3 = low adherence; 4-7 = medium adherence; ≥ 8 = high adherence.

As shown in Table 3, the mean KIDMED adherence scores were 5.88 ± 3.22 using the original 2004 version and 5.61 ± 3.05 with the updated 2019 version, corresponding in both cases to moderate adherence to the Mediterranean diet. The adherence categories were defined as follows: low (≤ 3), medium (4-7), and high (≥ 8). This table shows that, compared to the original 2004 version of KIDMED, the recent version leads to an increase in the proportion of adolescents classified as having low adherence (30.2% vs. 22.5%) and slight decreases in the proportions of adolescents classified as having good adherence (25.6% vs. 28.7%) and medium adherence (44.2% vs. 48.8%).

Table 4 presents the cross-classification of adherence levels to the Mediterranean diet obtained with the two versions, 2004 and 2019, of the KIDMED questionnaire administered to the same sample of adolescents. In total, 83 out of 129 adolescents (64.3%) were classified in the same adherence category by both versions, while 46 (35.7%) were reclassified into a different category. The observed reclassifications mainly involved shifts between adjacent categories. The Stuart-Maxwell test did not show a statistically significant difference between the marginal distributions of the two versions ($\chi^2 = 4.744$; df = 2; p = 0.093). However, Cohen›s *kappa* coefficient showed moderate agreement between the two classifications (*kappa* = 0.455), according to the interpretation of Landis and Koch.

Comparison of responses to questions from the two versions of the KIDMED questionnaire

Table 5 presents the item-by-item comparison of positive responses obtained with the 2004 and 2019 versions of the KIDMED questionnaire. The table shows statistically significant differences for several

Table 4. Cross-tabulation of adherence levels between the two versions of the KIDMED in the same sample of adolescents

KIDMED 2004 / KIDMED 2019	Low	Medium	High	Total
Low	19	9	1	29
Medium	16	35	6	57
High	4	10	29	43
Total	39	54	36	129

The values correspond to the number of adolescents. The diagonal cells represent the concordance of classifications according to the 2004 and 2019 versions of the KIDMED questionnaire. Agreement between classifications was assessed using Cohen's *kappa* coefficient (*kappa* = 0.455), indicating moderate agreement. Differences between marginal distributions were examined using the Stuart-Maxwell test for paired multinomial data ($\chi^2 = 4.744$; df = 2; p = 0.093).

Table 5. Item-by-item comparison of responses to the original (2004) and updated (2019) versions of the KIDMED questionnaire in the sample of adolescents studied

Questions	KIDMED 2004 n (%)	KIDMED 2019 n (%)	p-value
Eat a fruit or fruit juice every day	78 (60.5)	52 (40.3)	0.001**
Eat a second fruit every day	34 (26.4)	28 (21.7)	0.18
Eat raw vegetables (salad) or cooked once a day	92 (71.3)	90 (69.8)	0.64
Eat raw or cooked vegetables more than once a day	41 (31.8)	39 (30.2)	0.72
Eat fish regularly (≥ 2-3 times/week)	38 (29.5)	35 (27.1)	0.48
Eat at least once a week in a fast-food restaurant	44 (34.1)	61 (47.3)	0.03*
Eat dried vegetables (legumes) more than once a week	85 (65.9)	83 (64.3)	0.71
Eat pasta or rice at least 5 times a week	74 (57.4)	70 (54.3)	0.39
Eat cereals and their derivatives for breakfast	101 (78.3)	83 (64.3)	0.002**
Eat dried fruits (nuts) regularly	69 (53.5)	76 (58.9)	0.21
Eat olive oil in your home regularly	110 (85.3)	108 (83.7)	0.62
Skip breakfast	25 (19.4)	39 (30.2)	0.01*
Eat milk and dairy products for breakfast	72 (55.8)	69 (53.5)	0.58
Eat industrial pastries for breakfast	38 (29.5)	52 (40.3)	0.04*
Eat 2 yogurts or 40 g cheese every day	21 (16.3)	19 (14.7)	0.63
Eat sweets, chocolates, candies several times a day	46 (35.7)	58 (45.0)	0.03*

n (%) – number (percentage) of positive responses; The comparisons between the 2004 and 2019 KIDMED versions were performed using McNemar's test for paired data; * $p < 0.05$; ** $p < 0.01$.

items. The proportion of positive responses was significantly lower with the 2019 version for the item relating to daily consumption of fruit or fruit juice (40.3% vs. 60.5%, p = 0.001) as well as for the item concerning the consumption of cereals and cereal products at breakfast (64.3% vs. 78.3%, p = 0.002). Conversely, significantly higher proportions were observed with the 2019 version for eating at a fast-food restaurant at least once a week (47.3% vs. 34.1%, p = 0.030), skipping breakfast (30.2% vs. 19.4%, p = 0.010), consuming processed pastries for breakfast (40.3% vs. 29.5%, p = 0.040), and consuming sweets or candy several times a day (45.0% vs. 35.7%, p = 0.030). No statistically significant differences were observed for the other items (p > 0.05).

DISCUSSION

This study compared adolescents' adherence to the Mediterranean diet, as assessed by two versions of the KIDMED questionnaire: the original 2004 version and the more recent 2019 version. The results show that the updated 2019 version of KIDMED provides a more rigorous, demanding, and discriminating assessment of dietary behaviors, particularly regarding food quality and meal structure (breakfast). Data from the assessment using both versions of KIDMED indicate the stable maintenance of traditional components of the Mediterranean diet, such as the consumption of vegetables, fish, legumes, and olive oil, while deviations mainly concerned dietary behaviors whose nutritional importance has been reassessed in recent years.

The present data revealed an overall moderate adherence to the Mediterranean diet among the adolescents studied. This finding is frequently reported in the literature on adolescent populations, particularly in similar contexts characterized by a nutritional transition or a globalization of eating habits [30, 31]. This moderate adherence is jointly associated with insufficient physical activity levels in nearly half of the high school students participating in this study, a situation also widely documented as a major determinant of the risk of overweight and altered metabolic profile in adolescents [32]. The coexistence of low physical activity levels and heterogeneous anthropometric indicators may reflect the existence of subgroups with unfavorable cardiometabolic profiles, including among adolescents who do not necessarily present with marked excess weight, a phenomenon described in epidemiological studies of young people [20, 33].

Moderate adherence to the Mediterranean diet, as assessed in this study using the KIDMED index, appears consistent with an unfavorable behavioral profile, as the protective benefits of this dietary pattern are closely linked to its integration into an overall active lifestyle [34]. Furthermore, the marked heterogeneity of dietary behaviors, already reported among adolescents, is reflected in the high dispersion of KIDMED scores observed in the study sample, suggesting that the combination of poor diet quality and low levels of physical activity in some individuals may increase the risk of central adiposity and metabolic imbalance [6, 20, 35].

The average adherence scores obtained with both versions of KIDMED remained in the moderate adherence category. However, the slightly lower average score observed with the 2019 updated version, along with the higher proportion of adolescents classified in the low adherence category, suggests that this version allows for a more demanding and nuanced assessment of adherence to the Mediterranean diet and adolescents' eating behaviors. This observed result is consistent with findings in the literature reporting an overestimation of dietary quality as assessed by traditional indices of adherence to the Mediterranean diet when these indices do not sufficiently consider food quality and its degree of processing [36, 37]. Based on these findings, it therefore appears that the updated version of the KIDMED is better aligned with contemporary nutritional recommendations, which emphasize carbohydrate quality, the consumption of minimally processed foods, and the qualitative structuring of meals, in accordance with the principles of the Dietary Guidelines for Americans 2020-2025 [38]. Furthermore, a comparison between the original KIDMED (2004) and the updated KIDMED (2019) reveals differences in the assessment of adherence levels. The increase in the proportion of adolescents classified as having low adherence and the decrease in those classified as having good adherence in the updated version suggest a reclassification of certain profiles previously considered favorable, a shift already anticipated by methodological critiques of traditional indices [36, 37]. This reclassification appears consistent with the integration of qualitative criteria more aligned with current nutritional recommendations, particularly regarding the limitation of ultra-processed foods and added sugars [37].

Indeed, the KIDMED, initially developed by Serra-Majem et al. (2004), was designed in a food environment where the availability and consumption of ultra-processed foods were significantly more limited than they are today [13]. This increasingly rapid evolution of food systems over the past few decades necessitates updating the interpretation of assessment tools to better reflect contemporary dietary practices, particularly among children and adolescents [16]. The differences observed in this study therefore do not reflect a real decline in dietary habits, but rather a more demanding and realistic assessment.

Analysis of the classification agreement between the original and updated versions of the KIDMED, applied to the same adolescents, reveals moderate overall concordance, a level of agreement expected when two tools or two versions assess the same profile with different levels of rigor [38]. The observed disagreements mainly concern reclassifications between adjacent categories, which is generally interpreted as a refinement of profile discrimination rather than a methodological inconsistency [12, 39].

Furthermore, the analysis of classification agreement between the two versions reveals moderate overall concordance, with reclassifications mostly observed between adjacent categories. This partial concordance suggests that, although both versions share a common conceptual basis, they are not strictly interchangeable. This interpretation is consistent with recent literature highlighting that adherence to the Mediterranean diet in adolescents is a multidimensional construct influenced by both dietary quality and overall lifestyle patterns, requiring increasingly refined assessment tools to accurately capture these complexities [12]. The updated version thus appears as a coherent methodological evolution, allowing for better discrimination of dietary profiles, particularly among adolescents with intermediate adherence, a key population for nutritional prevention.

Item-by-item analysis of the questionnaire shows that the differences between the 2004 version of KIDMED and the updated 2019 version mainly concern dietary behaviors whose nutritional importance has been reassessed over the past two decades, including fruit consumption, breakfast quality, frequenting fast-food restaurants, and consumption of sugary products [40]. Conversely, the stability observed for the consumption of vegetables, fish, legumes, and olive oil confirms that these components remain robust and widely accepted pillars of the Mediterranean diet, regardless of changes in the assessment criteria [13, 34]. The particularly sharp decrease in reported daily consumption of "fruit or fruit juice" as assessed with the updated version reflects a stricter distinction between whole fruit and juice, now recommended due to the high free sugar content of juice and its low satiating effect [41, 42]. Similarly, the differences observed in the consumption of cereals and processed pastries at breakfast reflect a qualitative reassessment of this meal, the composition of which is now recognized as crucial for overall dietary quality in adolescents [32, 40]. The increased proportion of adolescents identified as regularly frequenting fast-food restaurants and frequently consuming sweets with the updated version of KIDMED suggests improved detection of risky eating behaviors, consistent with data showing that these practices are major markers of unbalanced diets and are associated with an increased risk of obesity and metabolic syndrome in young people [20, 37]. This increased sensitivity of the updated version appears to align with criticisms leveled against older tools and supports the fact that they are often less sensitive to the impact of ultra-processed foods on overall diet quality [19, 37]. These results suggest that, overall, the updated version of KIDMED (2019) does not challenge the conceptual foundations of the original questionnaire but rather refines its assessment by incorporating qualitative criteria that are now central to nutritional recommendations [15, 37]. This evolution improves the differentiation of risky eating behaviors among adolescents and reinforces the value of the KIDMED index as an assessment tool adapted to contemporary food environments [15].

In light of the reported data, this study enabled a comparative analysis of adolescent adherence to the Mediterranean diet using the original version of the KIDMED questionnaire and an updated version based on current nutritional recommendations. These guidelines encourage increased consumption of fruits, vegetables, legumes and whole grains while limiting free sugars, highly processed foods and excess salt and saturated fat [38], and with documented benefits of a Mediterranean diet rich in unprocessed foods on cardiometabolic health and the prevention of chronic diseases.

CONCLUSION

The comparative approach used in this study to assess adherence to the Mediterranean diet among adolescents, using the original version of the KIDMED questionnaire and an updated version, applied to the same sample of adolescents, allowed for the analysis of differences in overall scores, classifications by level of adherence, and variations in responses to questionnaires observed item by item. This provided an integrated view of the methodological effects related to updating the KIDMED questionnaire.

The results show that the recent version of KIDMED leads to a more demanding assessment of dietary behaviors, as it results in an increase in the proportion of adolescents classified as having low adherence and a decrease in those classified as having good adherence compared to the original version. This shift reflects a more nuanced and realistic interpretation, taking into account the quality of food consumed, meal structure, and the consumption of ultra-processed products, rather than an actual deterioration in eating habits. The main value of this work lies in its methodological contribution, demonstrating that an updated interpretation of a widely used tool like the KIDMED can improve the relevance of nutritional assessment without calling into question the foundations of the original questionnaire. This approach addresses

concerns expressed in the literature regarding the need to adapt dietary assessment tools to evolving food environments and nutritional recommendations, particularly for children and adolescents.

These results also highlight the public health importance of using tools that can accurately identify risky eating behaviors in order to better target preventive interventions. Updating the KIDMED could thus provide valuable support for monitoring adolescents' eating habits and for evaluating nutrition education programs aimed at promoting a healthy lifestyle.

Acknowledgments

The authors express their sincere gratitude to all the adolescents who participated in this study, as well as to their parents or legal guardians for their cooperation and consent.

Thanks are also extended to the school administrators and teaching staff for facilitating the smooth data collection process.

Conflicts of interest

The authors declare that they have no conflicts of interest related to this study.

REFERENCES

1. World Health Organization. Health for the world's adolescents: a second chance in the second decade. Geneva: WHO; 2014.
2. Moreno Aznar LA, Vidal Carou MDC, López Sobaler AM, Varela Moreiras G, Moreno Villares JM. Role of breakfast and its quality in the health of children and adolescents in Spain. Nutr Hosp. 2021;38(2):396-409. doi: 10.20960/nh.03398.
3. Food and Agriculture Organization of the United Nations, World Health Organization. Sustainable healthy diets: guiding principles. Rome: FAO; 2019.
4. World Health Organization. Report of the Commission on Ending Childhood Obesity. Geneva: WHO; 2016.
5. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet. 2019;393(10170):447-492. doi: 10.1016/S0140-6736(18)31788-4.
6. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr. 2010;92(5):1189-1196. doi: 10.3945/ajcn.2010.29673.
7. Trichopoulou A, Vasilopoulou E. Mediterranean diet and longevity. Br J Nutr. 2000;84(Suppl 2):S205-S209. doi: 10.1079/09658219738855.
8. González-Domínguez Á, Domínguez-Riscart J, Millán-Martínez M, Lechuga-Sancho AM, González-Domínguez R. Exploring the association between circulating trace elements, metabolic risk factors, and adherence to a Mediterranean diet among children and adolescents with obesity. Front Public Health. 2023;10:1016819. doi: 10.3389/fpubh.2022.1016819.
9. Rosi A, Scazzina F, Giampieri F, Álvarez-Córdova L, Abdelkarim O, Ammar A, et al. Lifestyle factors associated with children's and adolescents' adherence to the Mediterranean diet: the DELICIOUS project. Nutrients. 2025;17(1):26. doi: 10.3390/nu17010026.
10. Iaccarino Idelson P, Scalfi L, Valerio G. Adherence to the Mediterranean diet in children and adolescents: a systematic review. Nutr Metab Cardiovasc Dis. 2017;27. doi: 10.1016/j.numecd.2017.01.002.
11. Belaoufi H, El-Jamal S, Sahel K, Aboukhalaf A, Friki F, Chamlal H, et al. Adherence to Mediterranean diet in Moroccan school-age adolescents: sociodemographic, socioeconomic and lifestyle determinants. Rocz Panstw Zakl Hig. 2024;75(3):261-273. doi: 10.32394/rpzh/194469.
12. Masini A, Dallolio L, Sanmarchi F, Lovecchio F, Falato M, Longobucco Y, et al. Adherence to the Mediterranean diet in children and adolescents and association with multiple outcomes: an umbrella review. Healthcare. 2024;12(4):449. doi: 10.3390/healthcare12040449.
13. Serra-Majem L, Ribas L, Ngo J, Ortega RM, García A, Pérez-Rodrigo C, et al. Food, youth and the Mediterranean diet in Spain: development of KIDMED index. Public Health Nutr. 2004;7(7):931-935. doi: 10.1079/phn2004556.
14. Singh GM, Micha R, Khatibzadeh S, Shi P, Lim S, Andrews KG, et al. Global, regional, and national consumption of sugar-sweetened beverages, fruit juices, and milk: a systematic assessment. PLoS One. 2015;10(8):e0124845. doi: 10.1371/journal.pone.0124845.
15. Altavilla C, Caballero-Pérez P. An update of the KIDMED questionnaire, a Mediterranean diet quality index in children and adolescents. Public Health Nutr. 2019;22(14):2543-2547. doi: 10.1017/S1368980019001058.
16. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today: science and cultural updates. Public Health Nutr. 2011;14(12A):2274-2284. doi: 10.1017/S1368980011002515.
17. Evaristo OS, Moreira C, Lopes L, Abreu S, Agostinis-Sobrinho C, Oliveira-Santos J, et al. Associations between physical fitness and adherence to the Mediterranean diet with health-related quality of life in adolescents. Eur J Public Health. 2018;28(4):631-635. doi: 10.1093/eurpub/cky043.
18. Chacón-Cuberos R, Zurita-Ortega F, Martínez-Martínez A, Olmedo-Moreno EM, Castro-Sánchez M. Adherence to the Mediterranean diet is related to healthy habits, learning processes, and academic achievement in adolescents: a cross-sectional study. Nutrients. 2018;10(11):1566. doi: 10.3390/nu10111566.
19. Muros JJ, Cofre-Bolados C, Arriscado D, Zurita F, Knox E. Mediterranean diet adherence is associated

with lifestyle, physical fitness, and mental wellness among 10-year-olds in Chile. Nutrition. 2017;35:87-92. doi: 10.1016/j.nut.2016.11.002.
20. García-Hermoso A, Ezzatvar Y, López-Gil JF, Ramírez-Vélez R, Olloquequi J, Izquierdo M. Is adherence to the Mediterranean diet associated with healthy habits and physical fitness? A systematic review and meta-analysis including 565421 youths. Br J Nutr. 2022;128(7):1433-1444. doi: 10.1017/S0007114520004894.
21. World Health Organization. Growth reference data for 5-19 years. Geneva: WHO; 2007.
22. World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation. Geneva: WHO; 2011.
23. Ashwell M, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity. Int J Food Sci Nutr. 2005;56(5):303-307. doi: 10.1080/09637480500195066.
24. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ. 2007;85(9):660-667. doi: 10.2471/blt.07.043497.
25. Hagströmer M, Bergman P, De Bourdeaudhuij I, Ortega FB, Ruiz JR, Manios Y, et al. Concurrent validity of a modified version of the IPAQ-A in European adolescents: the HELENA study. Int J Obes (Lond). 2008;32(Suppl 5):S42-S48. doi: 10.1038/ijo.2008.182.
26. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-1395. doi: 10.1249/01.MSS.0000078924.61453.FB.
27. IPAQ Research Committee. Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ). 2005.
28. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-174.
29. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Participants. JAMA. 2025;333(1):71-74. doi: 10.1001/jama.2024.21972.
30. Grosso G, Galvano F. Mediterranean diet adherence in children and adolescents in southern European countries. NFS J. 2016;3:13-19. doi: 10.1016/j.nfs.2016.02.004.
31. Intorre F, Foddai MS, Venneria E. Mediterranean diet adherence in adolescents of different cultures and geographical proveniences: a pilot study. Adolescents. 2022;2(2):336-349. doi: 10.3390/adolescents2020026.
32. Moreno LA, Gottrand F, Huybrechts I, Ruiz JR, González-Gross M, De Henauw S, et al. Nutrition and lifestyle in European adolescents: the HELENA study. Adv Nutr. 2014;5(5):615S-623S. doi: 10.3945/an.113.005678.
33. Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents. Lancet Child Adolesc Health. 2020;4(1):23-35. doi: 10.1016/S2352-4642(19)30323-2.
34. Trichopoulou A, Martínez-González MA, Tong TY, Forouhi NG, Khandelwal S, Prabhakaran D, et al. Definitions and potential health benefits of the Mediterranean diet. BMC Med. 2014;12:112. doi: 10.1186/1741-7015-12-112.
35. Vélez-Alcázar AE, García-Roca JA, Vaquero-Cristóbal R. Adherence to the Mediterranean diet and its influence on anthropometric and fitness variables in high-level adolescent athletes. Nutrients. 2024;16(5):624. doi: 10.3390/nu16050624.
36. Martínez-González MA, Hershey MS, Zazpe I, Trichopoulou A. Transferability of the Mediterranean diet to non-Mediterranean countries. Nutrients. 2017;9(11):1226. doi: 10.3390/nu9111226.
37. Monteiro CA, Cannon G, Moubarac JC, Levy RB, Louzada MLC, Jaime PC. The UN decade of nutrition, the NOVA food classification and the trouble with ultra-processing. Public Health Nutr. 2018;21(1):5-17. doi: 10.1017/S1368980017000234.
38. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary Guidelines for Americans 2020–2025. 9th ed. Washington (DC): U.S. Government Publishing Office; 2020.
39. Streiner DL, Norman GR. Health measurement scales: a practical guide to their development and use. 4th ed. Oxford: Oxford University Press; 2008.
40. O'Neil CE, Byrd-Bredbenner C, Hayes D, Jana L, Klinger SE, Stephenson-Martin S. The role of breakfast in health: definition and criteria for a quality breakfast. J Acad Nutr Diet. 2014;114(12 Suppl):S8-S26. doi: 10.1016/j.jand.2014.08.022.
41. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality: a systematic review and dose-response meta-analysis of prospective studies. Int J Epidemiol. 2017;46(3):1029-1056. doi: 10.1093/ije/dyw319.
42. World Health Organization. Guideline: sugars intake for adults and children. Geneva: WHO; 2015.

Received: 27.03.2026
Revised: 27.04.2026
Accepted: 04.05.2026
Published online first: 12.05.2026

Rocz Panstw Zakl Hig 2025;76(4):353
https://roczniki.pzh.gov.pl/ https://doi.org/10.32394/rpzh/219142

ERRATUM

ERRATUM: CORRECTIONS OF AFFILIATIONS: LONGITUDINAL GROWTH TRAJECTORIES OF PRETERM INFANTS WITH AND WITHOUT INTRAUTERINE GROWTH RESTRICTION UP TO 24 MONTHS OF CORRECTED AGE: THE INFLUENCE OF EARLY FEEDING PATTERNS

Nouhayla Bouali[1,2], Khalid El Kari[3], Fatima Zahra Laamiri[4], Ilham Elouardighi[5], Lamyae Elyazigi[5], Imane Zizi[5], Redouane Belouali[1], Hassan Aguenaou[3], Amina Barkat[5,] and Mohamed Khalis[1,2,6,*]*

[1]Mohammed VI International School of Public Health, Mohammed VI University of Sciences and Health (UM6SS), Casablanca, Morocco
[2]Department of Public Health and Clinical Research, Mohammed VI Center for Research and Innovation, Rabat, Morocco
[3]Joint Research Unit on Nutrition and Food, RDC-Nutrition AFRA/IAEA, Ibn Tofail University-CNESTEN, Rabat-Kénitra, Morocco
[4]Hassan First University of Settat, Higher Institute of Health Sciences, Laboratory of Health Sciences and Technologies, Settat, Morocco
[5]Research Team on Health and Nutrition of Mother and Child, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco
[6]Higher Institute of Nursing Professions and Health Techniques, Rabat, Ministry of Health and Social Protection, Rabat, Morocco

In the paper 'Longitudinal growth trajectories of preterm infants with and without intrauterine growth restriction up to 24 months of corrected age: the influence of early feeding patterns' [1] the affiliations were published incorrectly. The errors concerned affiliations 1 and 2, while affiliation 6 (for Mohamed Khalis) was omitted. The authors apologize for any inconvenience that it may have caused.

The affiliations should be corrected as follows:

Before:

[1]Public Health, Mohammed VI International School of Public Health, Mohammed VI University of Sciences and Health, Casablanca, Morocco

[2]Public Health, Department of Public Health and Clinical Research, Mohammed VI Center for Research and Innovation, Rabat, Morocco

[5]Research Team on Maternal and Child Health and Nutrition, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco

After:

[1]Mohammed VI International School of Public Health, Mohammed VI University of Sciences and Health (UM6SS), Casablanca, Morocco

[2]Department of Public Health and Clinical Research, Mohammed VI Center for Research and Innovation, Rabat, Morocco

[5]Research Team on Health and Nutrition of Mother and Child, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco

[6]Higher Institute of Nursing Professions and Health Techniques, Rabat, Ministry of Health and Social Protection, Rabat, Morocco

REFERENCES

1. Bouali N, El Kari K, Laamiri FZ, Elouardighi I, Elyazigi L, Zizi I, et al. Longitudinal growth trajectories of preterm infants with and without intrauterine growth restriction up to 24 months of corrected age: the influence of early feeding patterns. Rocz Panstw Zakl Hig. 2025;76(3):259-271. doi: 10.32394/rpzh/217603.

*Amina Barkat and Mohamed Khalis contributed equally to this work

Publisher: National Institute of Public Health NIH - National Research Institute

Rocz Panstw Zakl Hig 2025;76(3):354-356

INSTRUCTION FOR AUTHORS

Scope of the Journal

The journal Roczniki Państwowego Zakładu Higieny - Annals of the National Institute of Hygiene is the peer-reviewed scientific journal that publishes original research articles, reviews, short communications and letters to the Editor.
The journal is devoted to research studies on food and water safety, nutrition, dietetics, environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences.

General rules for manuscript submission

All manuscripts in English should be submitted to the Editorial Office exclusively online through the editorial system (https://www.editorialsystem.com/rpzh/). Submissions by e-mail will not be accepted.

1. Submitted manuscripts must be an original work, not have been previously published nor be under consideration for publication in another journal.
2. Only high scientific quality articles in English complying with the scope of the journal will be considered for publication.
3. The journal requires that authors provide an ORCID IDs when submitting a manuscript for publication.
4. If the article is accepted for publication, the Corresponding Author will receive 'License to Publish Statement' document via the editorial system. The signed document should be submitted to the Editorial Office. Without the submission of the signed document, it will not be possible to publish the article in a journal.
5. The Editorial Office reserves the right to make editorial corrections to the manuscript that do not influence the substantive content of the article, without prior notification to the author.

Manuscript arrangement

Manuscripts submitted for publication should be prepared in doc, docx, rtf formats using Microsoft Word Times New Roman 12 font and 1.5 space between lines on A4 format.
Manuscript should include: (1) title page, (2) text of the manuscript, (3) Tables, (4) Figures, (5) Appendices. A complete manuscript should not exceed 16 pages, including text, tables, figures and references.

Abstract should not exceed more than 300 words. The original research article should consist of the following sections: Background, Objective, Material and Methods, Results, Discussion and Conclusions. The abstract of a review article does not need to be divided into sections.

Key words. 5-7 words or short phrases according to the MeSH (Medical Subject Headings) catalogue available at www.nlm.nih.gov/mesh/meshhome.html.

Original research article should be divided into the sections: Introduction/Background, Material and Methods, Results, Discussion, Conclusions, Acknowledgements, Conflict of interest and References.

Review article should include: Introduction/Background, Conclusions, Acknowledgements, Conflict of interest and References. The remaining section titles depend on the topic of the article.
Introduction/Background should contain the scientific rationale and the aim of the study or in the case of a review the purpose of the article. Only references directly related to the paper should be cited.

Material and Methods should provide detailed information on the subject of the study, methods, reagents, apparatus and techniques used in sufficiently exhaustive way to enable readers to repeat the experiments or observations. For generally known methods references should be given together with name of the methods or statistical analysis used in the study. For new or substantially modified methods detailed descriptions are to be added. In the case of experimental studies on laboratory animals, the information should be provided

on the approval by a local Ethics Commission. In the case of clinical studies in humans that they have been performed with the ethical standards according to the Helsinki Declaration.

Results should be presented in a logical sequence in the text, the same applies to the tables and figures. The data from the tables and figures should not be repeated in the text, where only the most important observations from the studies are to be summarized.

Discussion should emphasize the new and important aspects of the results and a comprehensive interpretation of the results obtained against the background of results obtained by other authors. Quotations should be restricted to those with immediate relevance to the author's findings.

Conclusions should be stated in points or descriptively and should be logically connected with the aims stated in the introduction. Statements and conclusions not derived from own observations should be avoided. If a hypothesis is proposed it must be stated clearly.

Acknowledgements. One or more statements should specify: (1) persons who contributed substantially to the study but cannot be regarded as authors, such as technical assistants, statisticians, data collectors etc. Their assistance should be acknowledged for the sake of transparency. It must be clear that they are not responsible for the final version of the article. The consent of all the persons named in the acknowledgements must be obtained; (2) all sources of financial and material support, which should specify the nature of the support. The recommended form is: "This work was supported by …….: (name of the organization, project number etc.)".

Conflict of interest. Information regarding conflict of interest should be disclosed. If there is no conflict, authors should declare that.

References should be presented according to the Vancouver referencing style. References should be cited in the order in which they first appear in the text. If the same publication is cited again the same number should be given. The journal title should be abbreviated in accordance with MEDLINE. Each position in the list of references should start from the new line and contain: consecutive number, author's (authors') surname(s) and initials of name(s), full title of the paper, official journal title abbreviation (according to the List of Journals Indexed in Index Medicus), year, volume, number, the first and the last page number. If the cited article or book has a DOI (Digital Object Identifier), it should be included in the references. The titles of the cited papers in other language than English should be translated into English. In the text of the manuscript only the number referring to the cited publication listed in references should be given in square brackets. The list of references should include only those that are cited in the text of the manuscript.
The journal Roczniki Państwowego Zakładu Higieny – Annals of the National Institute of Hygiene should be cited as: Rocz Panstw Zakl Hig.

The examples of reference style are given below:

Journal article with 1-6 authors:

1. Mertens E, Colizzi C, Peñalvo JL. Ultra-processed food consumption in adults across Europe. Eur J Nutr. 2022;61(3):1521-1539. doi: 10.1007/s00394-021-02733-7.

Journal article with more than 6 authors:

2. Osei-Kwasi HA, Boateng D, Danquah I, Holdsworth M, Mejean C, Terragni L, et al. Acculturation and Food Intake Among Ghanaian Migrants in Europe: Findings From the RODAM Study. J Nutr Educ Behav. 2020;52(2):114-125. doi: 10.1016/j.jneb.2019.09.004.

Book:

3. Kerner S, Chou C, Warmind M. Commensality: From Everyday Food to Feast. London: Bloomsbury Publishing PLC; 2015. ISBN 9780857857361.

Book chapter:

4. Lucas BL, Feucht SA. Nutrition in childhood. In: Mahan LK, Escott-Stump S, editors. Krause's Food & Nutrition Therapy. 12th ed. St. Louis, MO: Saunders Elsevier; 2008. p. 222–245. ISBN 9780808923787.

Internet source:

5. World Health Organization. GHE: Life expectancy and healthy life expectancy [Internet]. Geneva: World Health Organization; 2024. [cited 2024 Jan 19] Available from: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-life-expectancy-and-healthy-life-expectancy.

Legislative acts:

6. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance). OJ L 354, 31.12.2008, p. 16–33. Available from: http://data.europa.eu/eli/reg/2008/1333/oj.

Tables should be prepared in separate file(s) in doc, docx, rtf, odt formats and numbered using Arabic numerals. The title should be place directly above each table. Tables should always be cited in the text in consecutive numerical order. Each column in tables should have a brief heading, more extensive explanation should be given below the table, if necessary. The number of tables should be limited to the necessary minimum.

Figures and photographs should be prepared in separate file(s) in jpg, gif, tif, png, docx formats and numbered in Arabic numerals according to the sequence of their appearance in the text. The titles should be placed below the figures. Photographs must be of high quality.

Tables, figures or photographs should be cited in the text in consecutive numerical order: (Table 1), (Figure 1), (Photo 1).

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

Peer-review process

- Roczniki Państwowego Zakładu Higieny – Annals of the National Institute of Hygiene uses the double blind peer-review model in the process of reviewing manuscripts. It means that authors and reviewers remain anonymous and don't know each other's identities.
- The manuscripts submitted for publication undergo in the Editorial Office the initial evaluation in terms of compliance of the subject matter with the profile and formal requirements of the journal.
- The manuscripts that do not meet the journal's requirements are not qualified for the further publishing process, and the authors are informed. Manuscripts that meets these requirements and receives a positive initial evaluation is directed for peer-review evaluation to at least two independent reviewers, experts in the field of the subject matter of the manuscript.
- The reviewers must ensure independence of evaluation and no conflict of interest. They should be affiliated in different institution than the authors.
- The review form is available to reviewers after logging into the Editorial System.
- The review is in writing form and contains the reviewers' final conclusions as to whether the article should be published in its current form, after minor corrections, after major changes, or not it should be published.
- The Editor-in-Chief, on the basis of the reviews received, decides whether the manuscript will be accepted for publication, sent back to the corresponding author for correction, or rejected. The author receives a written review and information on the status of the submitted manuscript and its qualification for publication.
- The author should respond to all comments and remarks made by reviewers and submit a corrected version of the manuscript. This version of manuscript is re-checked before the Editor-in-Chief makes the final decision on acceptance it for publication.

REVIEWERS

Rocz Panstw Zakl Hig Vol 76, 2025

The Editors would like to thank all the Reviewers for their invaluable contribution to the review of manuscripts submitted for publication in the journal Roczniki Państwowego Zakładu Higieny (Annals of the National Institute of Hygiene). Without your efforts and cooperation, it would not be possible to maintain the high quality of the journal.

Bayındır-Gümüş Aylin – Department of Nutrition and Dietetics, Faculty of Health Sciences, Kırıkkale University, Kırıkkale, Turkey
Bełtowski Jerzy – Faculty of Medical Sciences, Medical University of Lublin, Poland
Bobiński Rafał – Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, University of Bielsko-Biala, Poland
Bobrowska-Korczak Barbara – Department of Toxicology and Food Science, Faculty of Pharmacy, Medical University of Warsaw, Poland
Bzikowska-Jura Agnieszka – Department of Clinical Dietetics, Medical University of Warsaw, Poland
Drywień Małgorzata – Institute of Human Nutrition Sciences, Warsaw University of Life Sciences, Poland
Gajda-Wyrębek Joanna – National Institute of Public Health NIH – National Research Institute, Warsaw, Poland
Gažarová Martina – Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Slovak University of Agriculture, Nitra, Slovak Republic
Górnicka Magdalena – Institute of Human Nutrition Sciences, Warsaw University of Life Sciences, Poland
Grajek Mateusz – Department of Public Health, Faculty of Public Health in Bytom, Silesian Medical University in Katowice, Poland
Gruszka Wojciech – Department of Biological Sciences, Faculty of Physical Culture in Gorzów Wielkopolski, Poznan University of Physical Education, Poland
Hnać Aneta – Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland
Jeruszka-Bielak Marta – Institute of Human Nutrition Sciences, Warsaw University of Life Sciences, Poland
Kawalec Anna Maria – Department of Paediatric Nephrology, Faculty of Medicine, Wroclaw Medical University, Poland
Kiciak Agata – Department of Food Technology and Quality Evaluation, School of Public Health in Bytom, Medical University of Silesia in Katowice, Poland
Kieliszek Marek – Department of Food Biotechnology and Microbiology, Institute of Food Sciences, Warsaw University of Life Sciences, Poland
Kilanowicz-Sapota Anna – Department of Toxicology, Faculty of Pharmacy, Medical University of Lodz, Poland
Kiss Anna – Chair and Department of Pharmaceutical Biology, Medical University of Warsaw, Poland
Kociszewska-Najman Bożena – Department of Neonatology and Rare Diseases, Medical University of Warsaw, Poland
Kolarzyk Emilia – Higher School of Health Promotion, Kraków, Poland
Kopiczko Anna – Department of Human Biology, Józef Piłsudski University of Physical Education in Warsaw, Poland
Krata Agnieszka – Institute of Applied Radiation Chemistry, Faculty of Chemistry, University of Lodz, Poland
Łagowska Karolina – Department of Human Nutrition and Dietetics, Poznań University of Life Sciences, Poland
Liber Anna – Department of Nutrition, Institute of Mother and Child, Warsaw, Poland
Lorková Marta – MKP Medical Center, Nitra, Slovak Republic
Ludwicki Jan K. – National Institute of Public Health NIH – National Research Institute, Warsaw, Poland
Maćkiw Elżbieta – National Institute of Public Health NIH – National Research Institute, Warsaw, Poland
Malczyk Ewa – Faculty of Health Science and Physical Education, University of Applied Science in Nysa, Poland
Marek Kardas – Department of Food Technology and Quality Evaluation, The School of Health Sciences in Bytom, Medical University of Silesia in Katowice, Poland
Miranowicz-Dzierżawska Katarzyna – Central Institute for Labour Protection – National Research Institute, Warsaw, Poland
Moskwa Justyna – Department of Bromatology, Faculty of Pharmacy with the Division of Laboratory Medicine, Medical University of Białystok, Poland
Özay Cennet – Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Izmir Katip Celebi University, Türkiye

ARTICLES PUBLISHED IN 'ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY' VOLUME 76, 2025

Open access:
https://roczniki.pzh.gov.pl/

Number 1, Vol. 76, 2025

Number 2, Vol. 76, 2025

Number 3, Vol. 76, 2025

Number 4, Vol. 76, 2025

AUTHORS' INDEX

Volume 76, 2025

SUBSCRIPTION

Journal Roczniki Państwowego Zakładu Higieny - Annals of the National Institute of Hygiene
may be ordered through the Library
of the National Institute of Public Health NIH - National Research Institute
24 Chocimska street, 00-791 Warsaw, Poland
Phone: +48 22 54 21 262 or +48 22 54 21 264
e-mail: biblioteka@pzh.gov.pl

The year 2025 is the last year of subscription to the journal.
Starting with the 1/2026 issue, the journal will not be published in print.
It will be published only in the online version.

Indeks 37468

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
[ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE]

Volume 76 **2025** **Number 4**