ISSN 0035-7715
eISSN 2451-2311

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE

Quarterly
2024
Volume 75
Number 1 - MARCH

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, environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences

Available at http://wydawnictwa.pzh.gov.pl/roczniki_pzh

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 Education and Science (MEiN), CNKI Scholar

Full articles are freely accessible on the journal's website: http://wydawnictwa.pzh.gov.pl/roczniki_pzh/annals
The printed version of the journal is identical to the on-line version.

Editorial office:
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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 75 **2024** **Number 1**

Rocz Panstw Zakl Hig 2024;75(1):5-11
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0293

ORIGINAL ARTICLE

ASSOCIATION BETWEEN ADDED SUGAR CONSUMPTION IN FOODS AND BEVERAGES AND BODY MASS INDEX AMONG ADOLESCENTS IN UNIVERSITY SOUTHERN THAILAND: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background. Excessive consumption of added sugar is an essential contributing factor to weight gain in adolescence, leading to non-communicable diseases.

Objective. The aim of this study was to evaluate the added sugar consumption in foods and beverages and determine the association between free sugar consumption and BMI status.

Material and Methods. This cross-sectional study was conducted among 280 adolescents in university (18-22 years) recruited from undergraduate students at different schools. The information was acquired using a 24-hour dietary recall questionnaire. Adjusted binary logistic regression analysis was used to assess the associations between added sugar consumption in foods and beverages and nutritional status.

Results. Half of the participants had a BMI status in the normal range (51.8%). A large percentage of adolescents had eaten staple food only two times and did not have breakfast (49%). Additionally, most of the student did not eat a snack or drink beverages (57.7%). Consumption of vegetables, fruit, meat, and milk was higher in obese subjects than other groups. The results showed that adolescents consumed more added sugar (79.2%) than is recommended by the WHO. The majority of added sugar consumption were beverages (46.5%). The findings revealed that added sugar consumption among undergraduate students did not differ significantly depending on BMI.

Conclusion. This study indicated that added sugar consumption in university students exceeded the WHO recommendation, although there was no discernible difference in BMI status. The results would be useful for further study and may help dietitians provide appropriate nutrition education or campaigns to reduce added sugar consumption in Thai and Southeast Asia university students.

Key words: *added sugar consumption, adolescents in university, body mass index, BMI, food and beverages*

INTRODUCTION

The World Health Organization (WHO) reported that obesity has nearly tripled worldwide since 1975. In individuals 18 years and older, more than 1.9 billion are overweight, and over 650 million are obese. This is expected to increase further due to the fact that in 2019, 38 million children under the age of 5 were overweight or obese in 2020 [1]. This corresponds with sugar consumption statistics. Information on sugar worldwide shows that the global consumption of sugar amounted to 170.82 million metric tons in 2016 and has increased every year to an estimated 177.8 million metric tons by the end of 2021 [2]. Sugar consumption leads to further global health problems, most significantly an overweight and obese population, caused by an energy imbalance between calories consumed and calories expended. Consuming too much sugar more than necessary causes an increased risk of developing non-communicable diseases (NCDs) such as diabetes and cardiovascular diseases [3].

Sugars are divided into two categories: naturally occurring sugars and sugars that have been added.

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

Sugars are naturally occurring sugars that can be found in meals such as fruit (fructose) and milk (lactose). Sugars or caloric sweeteners that are added to foods or beverages during processing or preparation, such as sugar in coffee, are referred to as added sugars [4]. Added sugar consumption (ASC) is used to improve the flavor, color, texture, and shelf life of foods and beverages. This form of sugar, in essence, adds calories but has no nutritious value [5]. The recommendation from the WHO is to use ASC as a cut-off point for categorical data, i.e., the WHO recommends ASC less than or equal to 24 g per day or 5% of total energy intake [6]. In 2015, the average person in Thailand consumed approximately 26 teaspoons (104 g) of sugar per day as well as most people received their daily intake mainly from sweet beverages. This makes Thai people more likely to become obese; Thai rank second in ASEAN populations for obesity [7]. In 2016, a report on the situation of sugar consumption in Thailand, from the production Management Centre, Office of Cane and Sugar Board 2016, showed that the average Thai person consumed approximately 122 g of sugar per person per day. This is consistent with a report by the Global Agricultural Information Network (GAIN) 2017 that Thai people continue to consume more sugar every year [8]. In particularly, Thai people 18 years old older have a high body mass index (BMI). Due to decreasing industrial usage, Thai consumers' sugar consumption fell by 7% in the first three months of 2018 compared to the same period in 2017. To evade the new sugar tax, beverage producers began reformulating current goods to include more artificial sweeteners [9].

Thai people aged 18 years have more behavior and more options for eating a variety of foods. Adolescents have been influenced by social factors and ways of life, advances in technology, family life, and the environment. Thus, adolescents have changed eating behaviors. From a study on eating behavior related to food consumption in 2017, the results showed youth aged 15-24 years ate spicy, flavorless, and sweet dishes in a ratio 31.06%, 23.68%, and 19.95%, respectively [10]. Eating sweet foods can cause adverse effects on the body. Thai adolescents prefer to drink soft drinks rather than clean water. In addition, National Statistical Office Thailand found that the favorite beverage of adolescents (15-24 years) is a soft drink, and that the rate of sugar intake has increased almost three times. Overconsumption of sugar in the body is an important reason for changes in body mass index (BMI). Because of the rapid and complete absorption of simple sugars, excess sugar is converted to fat and stored in adipose tissue, especially in the waist and hip areas. This is a risk factor for high blood pressure, heart disease, stroke, and diabetes, as well as having psychological impacts [11].

Adolescents in university spent more than 8 hours attending lectures and completing reading material, leading to low physical activity. Together with home delivery service via online applications in Thailand, these factors may contribute to weight gain during a 4-year curriculum in universities. However, the pattern of sugar consumption and Nutritional status is less well documented in southern Thai university adolescents. Thus, this study aims to determine the amount of added sugar consumption from main meals and beverages using a 24-hour recall method. The results of this study may reflect the harmful effects of added sugar on human health.

MATERIAL AND METHODS

Design, setting, and participants

A cross-sectional design was conducted with enrolment of subjects based on academic year of southern Thai undergraduate students in Tha Sala district of Nakhon Si Thammarat province, Thailand (Latitude: 8° 38' 42.2'' N; Longitude: 99° 53' 47.6'' E). Undergraduate students aged 18-22 years from the different schools of the university with three semesters were recruited to be representative of undergraduate students because of a diversity of students, such as gender, race, religion, and beliefs. Excluded from the study were those aged outside 18-22 years, and individuals with potential memory loss, as participating in the interview relies on the respondent's memory. The G*Power 3.1 calculus program (www.gpower.hhu.de/en.html.3.11.61).21 was used to compute the overall sample size of students. The parameters were as follows: test family = exact; statistical test was correlation; a bivariate normal model was used; $\alpha = 0.05$; power = 0.8. The recommended sample size was 280 students, but it was increased by 10% to accommodate for lost samples. Participants were divided into four groups based on their BMI, which was calculated using self-reported height and body weight as weight in kilograms divided by the square of height in meters (kg/m^2), and categorized into four categories based on Asian-Pacific cut-off points underweight (<18.5 kg/m^2), normal weight (18.5–22. kg/m^2), overweight (23–24.9 kg/m^2), and obese (≥25 kg/m^2) [12,13]. Besides, self-reported height and weight were used to determine BMI.

Data collection

A 24-hour dietary recall questionnaire was used to collect information about ASC on a weekday from the Department of Health, Ministry of Public Health. Asking only on a weekday is appropriate to conduct the dietary recall where usually consumers, both in amount and in variety.

The data were collected by trained research assistants with a manual for data collection. Participants were asked about all their foods and beverages consumed over the previous 24 hours. During the face-to-face interviews, precise recipe components, food photographs, measuring spoons and cups, and calibrated digital food scales were utilized to collect as much information about the portion sizes of meals consumed as feasible.

The protocol was obtained from the Human Research Ethics Committee of Walailak University with a reference number of WUEC-19-173-01. Participants were educated briefly regarding the study protocols. Each participant received a Participant Information Sheet and an Informed Consent Sheet and was asked to read thoroughly before signing to prove their willingness to enroll. Their information was kept confidential. The researcher did not harm the participants and did not benefit from the study at all.

Added sugar determination

The amounts of added sugar consumption of foods and beverages was calculated from its composition using INMUCAL-Nutrients V.4.0 software Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand) and the data available from Online Thai Food Composition Database 2015 [14]. If this source did not have any food items or recipes, additional sugar was manually introduced based on the nutrition facts labels. The researchers double-checked all of the entries for accuracy.

Data analysis

The ASC results were statistically analyzed by statistical software (IBM SPSS Statistics for Windows Version 20.0, IBM Corp., Armonk, NY, USA). Descriptive statistics, one-way ANOVA, and multiple linear regressions were used for data analysis. Statistical significance of the difference between samples was set at a level of $p<0.05$.

RESULTS

The majority of the participants were female (81.4%), with an average age of 20±3 years. The subjects were generally normal weight (51.8%), followed by underweight (18.5%), obese (17.2%), and overweight (12.5%). The socioeconomic status, such as monthly stipend of study participants, was 4,200-4,800 Baht that influence food choice and BMI. On a daily basis, two-thirds of university students were reported to eat staple food only 2 times. Most of them never had breakfast (49%). Likewise, half of the students did not eat snacks or beverages (57.1%). On average, the university students consumed 4.2-4.6 servings of starchy foods, 1.0-2.6 servings of vegetables, 0.3-0.6 portion of fruit, 3.4-5.9 servings of meat, and 0.2-0.9 glassed of milk. The prevalence of vegetable, fruit, meat, and milk consumption was higher among obese students than among other weight status groups (Table 1).

Overweight university students who exceeded the WHO added sugar recommendation consumed higher quantities of added sugar compared with students in all other BMI categories. However, one-way ANOVA did not indicate significant differences in the quantity of consumption ($p\geq0.05$) across the added sugar groups. Moreover, a majority of the university students who exceeded the WHO recommendation (79%) consumed eight to twelve times more added sugar in foods and beverages than students who did not exceed the WHO added sugar recommendation. The major source of ASC in this study was found in beverages at 45-57 g (Table 2).

Binary logistic regression showed that weight status was not related to ASC in foods and beverages, and only beverages was significantly different among the underweight, overweight, and obese groups ($p\geq0.05$), as shown in Table 3. These variables were analyzed together by using multiple logistic regression analysis while controlling for other variables, such as age, sex, monthly spending, and the number of staple foods per day. Weight status was not related to ASC in foods and beverages of the student population, including underweight, overweight, and obese groups ($p\geq0.05$).

DISCUSSION

According to the Health Systems Research Institute, the prevalence of normal, underweight, overweight, and obese individuals was 55.5%, 19%, 16.1%, and 9.1%, respectively, among adolescents and young adults aged 15 to 29 years [15]. This study also found that the BMI of university students reached a normal BMI, and half of those were underweight, overweight, and obese. In addition, socioeconomic status, such as the monthly spending of study participants, did not differ significantly across the BMI groups nor did it influence food choice. Eating behavior is a key factor in one's health. Most Thai adolescents and youth did not eat breakfast (53.5%). The results of this research demonstrated that half of the university students skipped breakfast (49%). This was in accordance with a previous study showing that breakfast was the most skipped main meal by university students, followed by lunch or dinner/supper [16, 17]. The reason was that in Thai adolescents, three-fourths of males said it was due to lack of time, while one-half of females said the same. In addition, one-fourth of a females who skipped a meal stated the reason was to lose weight [10]. Moreover, Mahfouz et al. reported 83.3% of male and 95.1% of female university students had a habit

Table 1. Nutritional status according to demographic profile of participants

Demographic factor	BMI, kg/m^2			
	Underweight	Normal	Overweight	Obese
Age (Mean=20±2 years)				
Gender, n (%)				
Male	4 (1.4)	27 (9.6)	8 (2.9)	13 (4.7)
Female	48 (17.1)	118 (42.2)	27 (9.6)	35 (12.5)
Monthly stipend (Thai Baht)	4,200	4,800	4,600	4,700
Number of staple food per day, n (%)				
3 times	16 (5.7)	39 (13.9)	10 (3.6)	12 (4.3)
2 times				
Skip breakfast	24 (8.6)	72 (25.7)	17 (6.1)	24 (8.6)
Skip lunch	6 (2.1)	16 (5.7)	4 (1.4)	5 (1.8)
Skip dinner	2 (0.7)	5 (1.8)	1 (0.4)	2(0.7)
1 time	4 (1.4)	13 (4.6)	3 (1.1)	5(1.8)
Number of snack and beverage per day, n (%)				
3 times	5 (1.8)	16 (5.7)	4 (1.4)	5 (1.8)
2 times	9 (3.3)	27 (9.7)	7 (2.5)	9 (3.3)
1 time	9 (3.2)	19 (6.8)	4 (1.4)	6 (2.1)
Not	29 (10.4)	83 (29.6)	20 (7.1)	28 (10.0)
Food group consumption (serving/day)*, Mean±SD				
Starchy foods (rice-serving spoons)	4.4±1.2^a	4.2±1.9^a	4.6±2.2^a	4.2±1.7^a
Vegetable (rice-serving spoons)	1.4±0.9^{ab}	1.0±0.8^b	1.2±1.4^{ab}	2.6±0.9^a
Fruit (portions)	0.3±0.5^b	0.4±0.7^{ab}	0.6±0.9^b	0.6±0.7^{ab}
Meat (spoons)	3.4±1.7^b	3.4±1.7^b	5.5±2.2^a	5.9±2.9^a
Milk (glasses)	0.2±0.3^b	0.3±0.7^b	0.5±0.6^a	0.9±0.6^a
Added sugar consumption in foods and beverages, n (%)				
≤24 g/day	11 (3.9)	30 (10.7)	7 (2.5)	10 (3.6)
>24 g/day	39 (13.9)	116 (41.4)	28 (10.0)	39 (13.9)
Added sugar consumption in beverages, n (%)				
≤24 g/day	26 (9.3)	77 (27.5)	20 (7.1)	27 (9.6)
>24 g/day	24 (8.6)	69 (24.6)	15 (5.4)	22 (7.9)

*Mean difference was calculated using one-way ANOVA significant at $p<0.05$

^{a,b}Different alphabets in the same row for the same factor indicate significant differences (one-way ANOVA) at $p<0.05$

Table 2. Mean difference of added sugar consumption by nutritional status

Added sugar consumption	BMI, kg/m^2					
	Underweight	Normal	Overweight	Obese	F	p-value*
Foods and beverages, Mean±SD						
≤24 g/day	3.2±7.3	1.1±2.9	4.8±8.7	3.1±7.1	1.083	0.364
>24 g/day	51.5±26.6	45.4±16.4	52.6±31.0	50.6±22.7	1.494	0.217
Total	40.9±31.2	36.3±23.2	43.1±34.0	40.9±28.1	0.906	0.438
Beverages, Mean±SD						
≤24 g/day	0.8±4.2	2.5±5.8	0.6±2.5	2.4±6.2	1.225	0.303
>24 g/day	54.5±9.1	46.2±18.4	57.5±37.4	45.7±23.3	1.485	0.222
Total	26.6±33.8	23.2±25.6	24.9±37.4	21.8±27.0	0.271	0.846

*Mean difference was calculated using one-way ANOVA significant at $p<0.05$

Table 3. Binary analysis of added sugar consumption in foods and beverages and nutritional status

BMI, kg/m^2	Crude			Adjusted*		
	OR	95% CI	p-value	OR	95% CI	p-value
Added sugar consumption in foods and beverages						
Underweight	0.66	0.30-1.44	0.298	0.62	0.28-1.40	0.255
Normal	Reference	Reference	Reference	Reference	Reference	Reference
Overweight	0.50	0.21-1.16	0.108	0.50	0.21-1.20	0.122
Obese	0.53	0.25-1.16	0.112	0.47	0.21-1.06	0.071
Added sugar consumption in beverages						
Underweight	0.89	0.47-1.68	0.714	0.86	0.28-1.40	0.659
Normal	Reference	Reference	Reference	Reference	Reference	Reference
Overweight	0.72	0.34-1.52	0.386	0.76	0.21-1.20	0.489
Obese	0.68	0.35-1.33	0.262	0.67	0.21-1.06	0.256

*Adjusted for age, sex, monthly stipend, number of staple foods per day

of snacking throughout the day between meals, often consuming sugar-sweetened beverages [18].

Furthermore, in this study, the average consumption of all food categories among university students was found to be lower than the Department of Health, Ministry of Public Health, Thailand's recommendation. According to the energy need of 2,000 Kcal, Thai adolescents and young adults are advised to eat approximately 10 servings of rice, 5 servings of vegetables, 4 portions of fruit, 9 servings of meat, and 1 glass of milk a day [19]. In addition, vegetable and fruit consumption among university students is also the most important issue identified in this study. Similarly, Hakim et al. found that an inadequate amount of fruits lowers the recommendation in young urban Malaysian adults [20]. The reason for this finding was that university students changed their lifestyle, frequently consuming processed foods and ready-to-eat food.

A national survey reported the taste of the main dish that most adolescents often consume. Most had consumed spicy dishes (31.6%), followed by flavorless (24.1%), sweetness (20.3%), salty (13.7%), sour (7.6%), and other (2.7%). Consistent with what most youth have to consider before choosing the food is taste (19.9%). Additionally, the adolescents were more likely to add seasoning before eating or testing (64.8%) when compared with other ages. Sugar was added to 44.1% less food than fish sauce or soy sauce (63.8%) [10]. This study found the average consumption of added sugar in meals and beverages among university students exceeded the WHO recommendation in all weight categories. The major source of ASC found in this study was beverages, which accounted for 45-55% of total ASC or 80-100 g per person per day. Similarly, Promdee et al. showed that average sugar consumption in Thai undergraduate students was 68±38 g per day or 17 teaspoons of added sugar per day [21]. The results of this study demonstrated that the amount of added sugar consumed per day by southern Thai undergraduate students did not differ significantly between BMI categories. This was in line with a recent study that found no significant differences in added sugar intake among undergraduate students at a South African university based on their BMI [22].

In addition, Gunes et al. also report freshman students in Turkey with a higher BMI (obese and overweight) consumed more foods and beverages than those with other nutritional statuses [23]. On the other hand, Piammongkol et al. found a lower consumption of added sugar level, in the third trimester, greater than 25 g but less than 50 g pregnant Thai-Muslim women in rural southern Thailand [24]. The explanation for this was because a variety of internal and external influences tend to impact university students' food and lifestyle patterns. As a result, it's critical to examine their nutritional consumption and establish the elements that impact their dietary and lifestyle behaviors in order to change these habits [25].

A previous report showed that increased taxes on sugary beverages result in lower consumption of these products [26]. During 2014 and 2015, a case study examining two years of implementation with a sugar-sweetened beverage tax in Mexico found an average reduction of 7.6% in purchases. Households with the fewest resources saw an 11.7% decline in purchases. The survey found a 2.1% rise in untaxed beverage sales, particularly bottled water [27]. Increasing the sugar tax in Thailand has also resulted in a lower sugar consumption rate of Thai people, which can be a guideline for the future prevention of NCDs in the Thai population.

The limitation of this study includes that this study used a single 24-hour dietary recall, which did not provide an accurate estimate of usual intake. Only undergraduate students from a single university

were included in this study; therefore, more research should be undertaken among students from several universities to provide a national perspective on university students' added sugar intake and its impact on their BMI. However, the consistency of the methodology, validated methods, and well-trained field workers used during the data collection and processing phases assured the validity of the data on dietary intake and anthropometric measures, which is an important strength of this study.

CONCLUSION

This study indicated that added sugar consumption in southern Thai university students exceeded the World Health Organization recommendation. Which, the majority source of added sugar consumption was a beverage. The results can use for further study, a reference in research on sugar consumption behavior, and help the dietitians to provide nutrition knowledge or dedicate the information with the general consumer for more understanding about added sugar consumption. That is consistent with the government has intended sugary tax to reduce its consumption among Thai people to prevent overweight, obesity, diet-related non-communicable diseases.

Authors' Note:
J.P, and R.Y. performed the study, analyzed the data, conducted the data interpretation, and drafted the manuscript. B.K, S.M, and W.T performed the study. J.P designed the study and reviewed the manuscript. All authors proofread and approved the final manuscript.

Acknowledgements
The authors would like to thank all of the subjects and enumerators for their participation in this study.

Conflict of interest
The authors declare no conflict of interest.

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Received: 19.01.2024
Accepted: 29.02.2024

Rocz Panstw Zakl Hig 2024;75(1):13-20
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0288

ORIGINAL ARTICLE

MICROBIOLOGICAL QUALITY OF GLUTEN-FREE MEALS, NATURALLY GLUTEN FREE FOODS, AND GLUTEN FREE-LABELLED PRODUCTS

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ABSTRACT

Background. The rising prevalence of gluten-related disorders such as celiac disease explains the increased consumption of gluten-free foods (GFF). However, these foods must be safe in terms of both gluten content and contamination by pathogenic microorganisms in order to avoid food poisoning.

Objective. The objective of this study was to assess the microbiological quality of gluten-free meals, naturally gluten free foods, and gluten free-labelled products.

Material and Methods. We collected 62 GFF samples including 20 meals (M-GF), 22 naturally gluten free (N-GFF) and 20 labelled (L-GFF) products, which were investigated for microbiological contamination according to Moroccan regulations guidelines, issued by the International Organization for Standardization (ISO). The analysis consisted of the detection of Salmonella and Listeria monocytogenes in each sample, and the quantification of the microbial load of the following six micro-organisms: total aerobic mesophilic flora, total coliforms, fecal coliforms, Staphylococcus aureus, Sulphite-Reducing Anaerobic, and yeasts and molds.

Results. A total of 372 analyses were carried out, showing a microbiological contamination rate of 5.1%. This contamination concerned N-GFF in 8.3% (predominantly with yeasts and molds), and meals prepared at home in 11.7 (predominantly with Staphylococcus aureus and coliforms). Only one case (0.8%) of contamination was observed in products labelled gluten-free and no contamination was noticed in meals prepared in food services. *Listeria monocytgenes* and *Salmonella* were not detected in any samples of food analyzed. These results indicate a good compliance of L-GFP and M-GF prepared in food services, while unsatisfactory quality was observed in N-GFF and M-GF prepared at home.

Conclusion. Therefore, rigorous hygienic practices and adequate corrective measures should be considered by celiac patients, especially regarding the N-GFF and M-GF prepared at home.

Key words: *Gluten-free foods; Microbiological quality; Contamination; Bacteria; Yeasts and Molds*

INTRODUCTION

Celiac disease (CD) is an autoimmune disease characterized by villous atrophy of the small intestinal mucosa, crypt hyperplasia and increased intraepithelial lymphocytes occurring in predisposed individuals [1]. Gluten remains the main factor involved in the development of this disease [2]. The prevalence of CD is generally estimated at 0.7 to 1.4% worldwide [3]. It can be asymptomatic, latent or silent and its

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

diagnosis is based on serological and biopsy tests [4]. The management of CD is based almost exclusively on gluten-free diet (GFD) [5]. This diet consists of regular consumption of naturally gluten-free foods, products labelled as "gluten-free" and/or gluten-free meals prepared at food services or at home. Good adherence of celiac patients to this diet requires the availability of gluten-free foods (GFF) with reasonable price and safe gluten content [6, 7]. In addition to the elimination of any source of gluten contamination, the safety of gluten-free foods requires the absence of pathogenic microorganisms. In fact, the latter contamination by pathogens can lead to further damage to the gut microbiota of celiac patients [8], and is mainly caused by bacteria, including *Enterobacteriaceae*, *Escherichia coli, Salmonella, Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum* and *perfringens, Yersinia enterocolytica, Campylob acter jejuni, Brucella, Shigella, Shigella, Vibrios* and *Bacillus cereus*. In Africa, *Enterobacteriaceae, Escherichia coli, Salmonella, Staphylococcus aureus* and *Listeria monocytogenes* are the most involved microorganisms in this contamination [9]. This contamination can be provoked by other micro-organisms such as fungi (moulds and yeasts), viruses and parasites. Hence, the study of microbiological quality of gluten-free foods will allow assessing their safety during all processes; from the harvesting of the raw material, manufacturing, transport, storage and handling to consumption. It will also help verify the effectiveness of preventive actions such as good hygiene practices (GHP) and good manufacturing practices (GMP) as well as the hazard analysis and critical control point system (HACCP).

The sanitary quality of food is assessed according to the type of micro-organisms. The presence of a single colony of virulent pathogens such as *Salmonella, Yersinia, Brucella, Listeria, salmonella* is sufficient to make it toxic and then non-compliant. Whereas less serious pathogens (aerobic mesophilic flora, sulphite-reducing anaerobes, *Staphylococci*, faecal or soil germs and yeasts and moulds) only become toxic when the number of colonies per g or per ml exceeds a toxicity threshold (S). The counting of bacteria in foods can be done by several methods such as filtration and liquid counting, but in practice, the most commonly used method is the solid medium count. This requires inoculating the microbial sample in bulk or on the surface of an agar medium, followed by the calculation of the number of colony forming units (CFU).

Due to high prevalence of CD in Morocco [10], the consumption of gluten-free foods especially naturally gluten-free foods (N-GFF) is considerably increasing. The consumption of naturally gluten-free foods such as pseudo cereals sold in bulk, is due to the low availability and the high prices of products labelled gluten-free (L-GFP) [11-12]. This may represent an additional risk of microbiological contamination of foods to be consumed. The objective of the present study was to investigate the microbiological quality of naturally gluten-free (N-GFF), labelled gluten-free (L-GFP) products and gluten-free meals (M-GF), prepared in food services or at home.

MATERIAL AND METHODS

Food samples

The study was carried out on 62 samples of gluten-free foods, which were divided into three groups. The first group included manufactured products labelled as follow: "gluten-free", "without gluten", "no gluten" and "zero gluten". The second group corresponded to naturally gluten-free foods, which do not contain wheat, rye and barley among their ingredients. Foods with labels containing terms such as 'made in a wheat processing plant', 'may contain wheat traces', 'wheat starch', 'hydrolyzed wheat proteins', 'malt extract', or 'malt extract aroma' were excluded from the study. Each group was composed of different food categories. The "Cereals/Pseudocereals GF" and "Dried vegetables GF" constituted the gluten-free labelled products (L-GFP). The "Cereals/ Pseudocereals" and "Dry Fruits/Dried vegetables" constituted N-GFF. The third group concerned "GF Meals" sold in food services or prepared at home (table 1). The food samples were collected in adherence with rigorous hygienic procedures.

Preparation of food samples

The preparation of foods consisted of diluting 25 g of the sample in 225 ml of previously sterilized buffered peptone water solution (10-1 dilution) in sterile bag. The bags were then shaken in a stomacher to ensure the dispersal of the germs. After what, decimal dilutions of samples were made from a stock solution.

Microbiological analysis

The microbiological analysis (detection and/or counting) of prepared foods steps were performed according to the recommendations of the National Office of Food Safety (ONSAA) and the National Institute of Standardization (IMANOR) [13], which comply with the standards settled by the International Organization for Standardization (ISO) and the French Association for Standardization (AFNOR).

Enumeration of total mesophilic germs (TAM)

The enumeration of the total aerobic mesophilic flora was carried out according to the Moroccan standard NM.08.0.121, based on ISO 4833-1 14]. One

Table 1. Categories of meals, naturally and labelled gluten-free analyzed

	Number sample	Select examples
Labelled gluten-free products	20	
Cereals/pseudo-cereals	10	Rice, Corn, Oat, Millet
Dried vegetables	10	Peas, Beans, Lentils, Haricot, Chickpeas, Soy
Naturally gluten-free foods	22	
Dry Fruits/ Dried vegetables	12	Cashew, Almond, Pistachio, Peanuts, Nut, Peas, Beans, Lentils, Haricot faba, Chickpeas, Soy
Cereals/Pseudo-cereals	10	Quinoa, Chia, Sesame, Rice, Flax seed, Corn, Oat, Rice, Corn, Ryegrass
Meals gluten-free	20	
Prepared in food services	10	prepared from cereals and pseudo-cereals (Bread, Cookies and Cakes)
Prepared at home	10	prepared from cereals and pseudo-cereals (Bread, Cookies and Cakes)
Overall	62	

ml of the stock solution and decimal dilutions were inoculated onto the PCA (Plate Count Agar) medium. The inoculum and the agar were mixed in a circular motion. Incubation was done at 30°C for 24 to 48 hours.

Enumeration of total coliforms and faecal coliforms

The enumeration of total coliforms and faecal coliforms was carried out according to NM 08.0.115 and ISO 4832 [15]. Using a sterile pipette, 1 ml of each decimal dilution was placed twice in two empty petri dishes prepared for this purpose and identified by sample type. Then, 10 to 15 ml of crystal violet and neutral red lactose agar (VRBL) was added to each petri dish, melted and cooled at 45±1°C. The inoculated plates were shaken to allow the inoculum to mix well with the agar. One set of plates was incubated at 30°C for 24 hours and furtherly used for total coliform counts. The other set of plates was incubated at 44°C for 24 hours and then used to enumerate faecal coliforms.

Search and count of sulphite-reducing anaerobes (Clostridium)

They are performed according to NM 08.0.125 from ISO 15213 [16]. 1 ml of the stock dilution and dilutions were inoculated into tubes containing 20 ml of molten Sulfite Polymyxin Sulfadiazine (SPS) agar medium and cooled to 45±1°C. The inoculum and the culture medium were mixed, without bubbling so as not to cause oxygenation of the medium, by rotating the wrist. Another layer of SPS was added to ensure a strict anaerobic medium. The tubes were incubated at 44°C for 24 h.

Staphylococcus aureus testing and counting

They were carried out according to NM 08.0.151 from ISO 6888 [17], following three steps. Firstly, isolation was done by spreading 0.1 ml of the stock solution on the surface of the Baird Parker medium in a homogeneous way, then incubating at 37°C for 24 to 48 hours. This was followed by enrichment, during which the black, shiny, convex colonies surrounded by a zone of clearing (suspect colonies) were inoculated into the BHI (Brain Heart Infusion) broth and incubated at 37°C for 24 hours. This was followed by confirmation, during which 0.3 ml of the broth culture is added to 0.3 ml of rabbit plasma and the tubes were incubated at 37°C for 24 hours. Coagulation of the plasma indicates the presence of *Staphylococcus aureus*.

Yeast and mould counts

It was done according to NM 08.0.123 from ISO 21527 [18]. It consists of spreading 0.1 ml of the stock solution and dilutions on the surface of Sabouraud media plates, then incubating at 37°C for 24 hours.

Search for Salmonella

It was carried out according to NM 08.0.116 from ISO 6579 [19] following four steps. First, pre-enrichment by incubating the stock solution for 18 h to 24 h at 36°C. Second, enrichment by adding 0.1 ml of the pre-enriched medium to tubes containing 10 ml of Vassiliadis Rappaport broth, followed by incubation at 42°C for 18-24 hours. This was followed by isolation by exhaustion on Hektoen medium with incubation at 37° for 24 hours. Salmonella appear as greenish or greenish colonies with a blackish centre. Suspect isolates were plated on nutrient slant agar for identification. Finally, biochemical identification through a classical gallery is performed by lactose fermentation, glucose, H2S and gas production, ONPG (ortho-Nitrophenyl-β-galactoside) test for β-galactosidase enzyme, oxidase test, and Urea-Indole test. In parallel, the biochemical identification was confirmed by the miniaturised "Api 20E" gallery consisting of 20 microtubes containing dehydrated substrates allowing 20 biochemical tests

(enzymatic or sugar fermentations) to be performed. After inoculation of the gallery, incubation was carried out at 37°C and visual reading begins after 24 hours.

Testing for Listeria monocytogenes

It was carried out according to NM 08.0.110 from ISO 11290 [20]. The pre-enrichment step was carried out by homogenising 25 g of the feed in 225 ml of Fraser demi by the stomacher and incubating for 24 hours at 37°C. Enrichment was achieved by inoculating 0.1 ml of the pre-enriched culture into 10 ml of selective Fraser broth and incubating at 37°C for 48 hours. Isolation is performed on Oxford medium and incubated at 37°C for 48 hours. Biochemical identification of the classical gallery was carried out using the haemolysis test based on the use of horse blood agar for 24 to 48 hours at 37°C. In parallel, biochemical identification was also carried out using an Api 20E gallery composed of 10 microtubes allowing 10 enzymatic tests to be performed.

In parallel, the suspect bacteria were identified using Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight (MALDI-TOF MS). This identification was based on the analysis of their constituent proteins. The identification was carried out from colonies obtained on different agar media. After depositing the colony to be identified in a thin layer, a mixture of water-acetonitrile-matrix allows the bacteria to burst and release the proteins. For bacteria with a wall that is more difficult to lyse (Gram-positive bacteria), a preliminary extraction with formic acid was done before the addition of the matrix. This technique was used mainly for *Staphylococcus aureus*, sulphite-reducing anaerobes and coliforms.

The reading and interpretation of the results were carried out according to the ministerial decree n°293-19 which sets the standards for the sanitary quality of each food category in Morocco (Decree n°293-19, 2019) fixing the NM.08.0.120 as the standard regulating the expression of the results [13]. The interpretation of the results was based on the use of three-class or two-class plan depending on the microorganism to be investigated. The latter was based on the setting of a limit m value above which the food is considered unacceptable (non-compliant) (m value > 0), and acceptable (compliant) when this m value is equal zero (m value < 0). The purpose was to confirm the presence or absence of serious pathogens (*Salmonella* and *Listeria monocytogenes*). The three-class plan was used to interpret the number of aerobic mesophilic flora (TAM), sulphite-reducing anaerobes (SRA), *staphylococci* (St), coliforms total(CT), faecal coliforms(FC), yeasts and moulds (Y&M). When the counted value (X) is between the lower limit (m) and the upper limit (M), foods are considered conform and acceptable but the microbiological quality is unsatisfactory. A value below m (X<m) indicates a satisfactory microbiological quality. The product is considered non-compliant (unacceptable) when the enumerated value exceeds M value.

Statistical analysis

Data were analyzed using SPSS Statistical Package software (SPSS version 25.0, SPSS Inc., Chicago, IL, ETATS-UNIS). The prevalence was calculated as a ratio between positive and total samples, and was given as percentage. The Chi-square test was used to assess the dependence between gluten-free food categories, and the difference was considered significant if p value <0.05.

RESULTS

In the 372 microbiological analyzes carried out on 62 gluten-free foods, the contamination rate was estimated at 5.1% (Figure 1). *Total aerobic mesophilic flora:* Regarding the number of TAM flora, none of gluten-free foods were considered microbiologically unacceptable, 77.4% were acceptable, and 22.6% of them were satisfactory.

Total coliforms & coliforms Faecal: Only 3.2 % and 6.4 % of gluten-free foods had a higher content of total coliforms and coliforms faecal respectively. The satisfactory quality in gluten-free foods was more noticeable regarding the number of faecal coliforms than total coliforms.

Sulphite-reducing anaerobes: The number of feeds with unacceptable quality did not exceed 1.6% for sulphite-reducing anaerobes. The majority of products were acceptable (83.9%), while the prevalence of satisfactory microbiological quality in investigated samples was 14.6%.

Staphylococcus aureus: The number of Staphylococcus aureus over the threshold was found in seven samples, causing 11.3% of unacceptable foods. Satisfactory quality was observed in 77.4% of samples, while 11.3% of them had an acceptable microbiological quality.

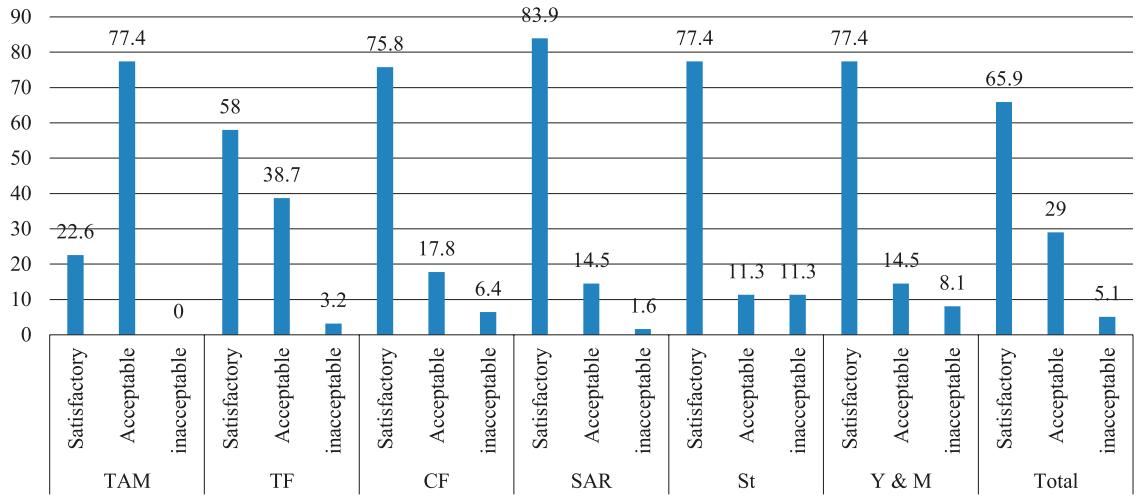
Yeasts and moulds: Regarding the number of yeasts and moulds, satisfactory, acceptable and unacceptable quality were noted in 77.4%, 14.5% and 8.1% respectively.

Furthermore, among the unacceptable microbiological quality foods analysed, none of them exceeded the toxic threshold (S=1000m).

Salmonella and Listeria monocytogenes detection: In all gluten-free foods analysed, neither Listeria monocytogenes nor Salmonella were detected.

Difference between L-GFP, N-GFF and meals-GF categories

Foods labelled gluten-free were the least contaminated compared to the other categories.



Legend: TAB: Total Aerobic Bacteria; TC: Total coliforms; FC: Faecal coliforms; St: *Staphylococci Aureus*; SAR: Sulphite-Reducing Anaerobic; Y & M: Yeasts and Molds.

Figure 1: Microbiological quality of microflora counted in gluten-free foods, expressed as a percentage

Table 2. Microbiological quality of gluten-free foods according to category of products and microorganism

Micro-organismes	Quality	L-GFP (%)	N-GFF (%)	Meals (%)		
				Prepared at home	Prepared in food service	Both-Meals
TAM	Satisfactory	35	9.1	10	40	25
	Acceptable	65	90.1	90	60	75
	Unacceptable	0	0	0	0	0
TF	Satisfactory	80	50	40	50	45
	Acceptable	20	54.5	50	50	50
	Unacceptable	0	4.5	10	0	5
FC	Satisfactory	90	77.3	40	80	60
	Acceptable	10	9.1	50	20	35
	Unacceptable	0	13.6	10	0	5
SAR	Satisfactory	95	72.7	80	90	85
	Acceptable	5	27.3	10	10	10
	Unacceptable	0	0	10	0	5
St	Satisfactory	100	86.4	30	60	45
	Acceptable	0	0	30	40	35
	Unacceptable	0	13.6	30	0	20
Y & M	Satisfactory	85	50	100	100	100
	Acceptable	10	31.8	0	0	0
	Unacceptable	5	18.2	0	0	0
Total	Satisfactory	80.8	57.6	50.0	70.0	60.0
	Acceptable	18.4	34.1	38.3	30.0	34.2
	Unacceptable	0.8	8.3	11.7	0.0	5.8

Legend: TAB: Total Aerobic Bacteria**; TC:** Total coliforms**; FC:** Faecal coliforms**; St:** *Staphylococci Aureus***; SAR:** Sulphite-Reducing Anaerobic; **Y & M:** Yeasts and Molds; **L-GFP:** Labelled gluten-free products**; N-GFF:** Naturally gluten-free foods**; M-GF :** Meals gluten-free.

Satisfactory quality**: X ≤ m;** Acceptable quality: **m ≤ X ≤ M;** Unacceptable quality (contaminated): **X ≥M With: m**: desired minimum threshold of contamination**, M:** maximum threshold of tolerable contamination; **X**=number of CFU/g in log

A Contamination by yeasts and moulds (Unacceptable quality) was noticed in 5% of L-GFP and in 18.2% of N-GFF. The latter category of foods was also contaminated by *Staphylococcus aureus*, faecal and total coliform in 13.6%, 13.6% and 5.4% of cases respectively. The gluten-free meals prepared at home were contaminated in prevalence of 11.7% mainly with *Staphylococcus aureus*. This food category was also contaminated by faecal coliforms, total coliforms and sulphite-reducing anaerobes. While, no contamination was observed in meals prepared in food services (Table 2).

DISCUSSION

There are many aspects related to the safety of gluten-free foods such as exact gluten content and contamination by physical or chemical substances [21, 22, 23]. Microbiological contamination of foods may be responsible for intestinal food poisoning in celiac patients whose intestinal villi are already damaged by atrophy [24]. Overall, among the sample analysed in our study, the majority of gluten-free foods displayed a satisfactory microbiological quality. These results are in accordance with those reported by similar studies conducted in Italy and Brazil [25, 26]. Contamination of gluten-free meals was particularly pronounced in home-prepared meals. It was mainly caused by *Staphylococcus aureus* and coliforms, which is probably due to poor hygienic conditions. Indeed, celiac patients give great importance to the gluten content in gluten-free foods and may neglect the contamination risk. No contamination of gluten-free meals prepared in food services was observed. This may reflect the importance that restaurants and bakeries place on microbiological safety during the preparation process of these foods. An Italian study conducted in a school catering facility reported similar findings [27]. Indeed, the non-detection of a serious microbiological risk in gluten-free and lactose-free foods prepared by the services of this school confirms the compliance with good hygienic practices following HACCP implementation [27].

Contamination of products labelled as "gluten-free" was almost absent and was noticed in less than 1% of the samples. This shows that hygienic practices have been followed during all the formulation processes of gluten-free products, in accordance to the HACCP system [28].

It was remarkable that naturally gluten-free foods were frequently contaminated with yeast and mould, which could be due to poor storage conditions. This can also be explained by the fact that these foods, dedicated mainly to patients on a gluten-free diet, are generally stored for a long time before being sold. Definitely, the longer the storage period of gluten-free foods, the more the load of yeasts and moulds increases [29]. In contrast, as a naturally gluten-free food, quinoa is generally free of microorganisms [25]. In the N-GFF of our study, coliform contamination was observed in 13.6%, which is probably related to improper handling during the processes of harvest, storage and sale.

As a serious health hazard, the presence of *Salmonella* and *Listeria monocytogenes* in gluten-free foods is alarming. In fact, *Salmonella* and *Listeria monocytogenes* are among the major causes of food-borne disease outbreaks [30]. Fortunately, no gluten-free foods have been contaminated with these dangerous bacteria. Similar findings were reported by studies conducted on L-GFP [25, 26]. Similarly, such contamination was absent in gluten-free meals prepared in food services as reported by Petruzzelli et al. [27].

At the limit of our knowledge, this study represents the first one carried out in Morocco and Africa, highlighting the importance of the microbiological safety of GFF.Our study focused on three food categories at once (N-GF, L-GFP and gluten-free meals). Nevertheless, as limitations, the sample size of foods analysed remains relatively small to draw definitive and others conclusion, especially about some more virulent food poisoning microorganisms (*Salmonella* and *Listeria Monocytogenes*). In addition, the unavailability of the *Bacillus cereus* specific agar medium in the context of this study, limited our ability to investigate the risks associated with the said organism. It is noteworthy to report, that the study *Bacillus cereus* is recommended by food regulatory organizations due to their frequent presence in foods [31].

CONCLUSION

The results of our study showed a high prevalence of contamination in naturally gluten-free foods (8.3%) and gluten-free meals prepared at home (11.7%), predominantly with yeasts and molds for the first category, and with *Staphylococcus aureus* and coliforms for the second category of foods. While no contamination was observed in gluten-free meals prepared in food services. We also noticed the absence of contamination with some pathogens like *Salmonella* and *Listeria monocytogenes*, known for their extreme virulence. Therefore, rigorous hygienic practices and adequate corrective measures should be considered by celiac patients, especially regarding the naturally gluten-free and meals gluten-free prepared at home.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Received: 23.11.2023
Revised: 23.01.2024
Accepted: 28.01.2024

Rocz Panstw Zakl Hig 2024;75(1):21-33
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0294

ORIGINAL ARTICLE

DETERMINATION OF VITAMIN C IN RAW FRUIT AND VEGETABLE HOMOGENATES: DIETARY EXPOSURE AND HEALTH EFFECTS OF EXCESS INTAKE IN ADULTS AND CHILDREN

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ABSTRACT

Objective. The aim of the study was to determine Vitamin C content in some fruits and vegetables (FAV) including apple, banana, orange, pineapple, watermelon, carrot and cucumber, sold in the local markets in Awka, Anambra State, Nigeria as well as Vitamin C content in two-component and three-component homogenates FAV. The work was also designed to investigate the dietary exposure and health effects of excess vitamin C intake in adults and children.

Material and methods. Vitamin C as total ascorbic acid (AA) after reduction of dehydroascorbic acid was analyzed using both titrimetric and spectrophotometric methods. The titrimetric method involved iodometric back-titration while the spectrophotometric method was done at an absorbance of 530 nm. The dietary exposure was evaluated as the total FAV intake multiplied by chemical concentration in the FAV whereas the health effect of excess vitamin C intake was conducted using the hazard quotient (HQ).

Results. The results revealed that Vitamin C for single fruits ranged from 11.76 - 41.17 mg/L for spectroscopic method and 16.9 – 31.84 mg/L for titrimetric method. Fruit homogenates showed Vitamin C concentrations of 14.70 – 220.58 mg/L and 17.23 – 209.09 mg/L for two-components homogenates: 29.41-132.35 mg/L and 31.05–113.10 mg/L for tri-components homogenates for spectrophotometric and titrimetric methods respectively. The results of dietary exposure and the health effects of excess vitamin C intake showed that children are more susceptible to health issues than adults in illnesses such as nausea, gastrointestinal pains, increased kidney stones and hyperactivity.

Conclusion. There is therefore the need for a national recommended dietary allowance for total ascorbic acid (AA) in FAV homogenates from a stakeholder point of view in Nigeria.

Key words: *Vitamin C, ascorbic acid, fruit, vegetables, FAV homogenates, food analysis, food composition, public health*

INTRODUCTION

Fruits and vegetables (FAV) are a rich source of vitamins and minerals that are beneficial and critical for human health in preventing diseases like diverticulosis, gastrointestinal health, urinary tract infections, cardiovascular, and cancer, as well as reducing inflammation, preventing cell senescence, controlling weight, reducing systolic and diastolic blood pressure, and improving vision [16, 49, 51]. Fruits and vegetables contain high levels of dietary fiber, especially in their seeds, skin, pods, peels, hull, husk, stem, etc. [42]. They have a host of biochemicals such as phytochemicals, minerals, vitamins, organometals, and inorganic metals amongst others in diverse concentrations that are absorbed from soil nutrients and biotransformation of cellular interactions from DNA matrix in addition to photons from ultraviolet and visible spectra of the sun. These biochemicals may be nutritive and non-nutritive which are/may be critical for normal body functioning as their consumption is crucial to micronutrient availability in the body. Their importance is further emphasized by the fact that the human body is unable to synthesize them in sufficient amounts to meet daily recommended allowances [57], even though required only in small amounts. Studies have reported that the consumption of at least 400 g of FAV per day is considered adequate and that a global decrease in FAV consumption is responsible for the increasing cases of cardiovascular diseases and cancer [52, 67].

One of the most abundant vitamins in FAV is ascorbic acid also known as vitamin C. Vitamin C content varies for different FAVs especially due to the growing conditions of the plant and their exposure to sunlight [8, 33]. Muhammad *et al.* [31] and Uğur *et al.* [59] reported in their works that vitamin C is

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

usually higher in young FAV, while Ferrari *et al.* [17] and Mieszczakowska-Frąc et al. [28] noted that ascorbic acid stability decreases with an increase in temperature and pH as evidenced by its loss during food heat processing. It is also established that ascorbic acid gradually decreases over certain storage periods [71].

Numerous known roles of vitamin C include its role as a cofactor in numerous enzymatic interactions involving crucial genetic processes, such as the regeneration of collagen-containing connective tissues during human wound healing [25, 30]. Additionally, vitamin C helps with the absorption of inorganic iron, lowers plasma cholesterol levels, inhibits the development of nitrosamines, and strengthens the immune system [44]. Another major important function of vitamin C is its antioxidant activity by reaction with singlet oxygen and other free radicals to reduce the risk of arteriosclerosis, which aids the body in preventing certain cancers, reduce oxidative damage including the oxidative modification of low-density lipoproteins which cause cardiovascular diseases, flu, muscular degeneration and cataract [63, 65]. Vitamin C also has an important role in the synthesis of protein as one of the amino acids used to build collagen-hydroxyproline is only synthesized on the availability of amino acids. Its deficiency notably leads to diminished collagen synthesis, which contributes to more severe symptoms of scurvy.

Laboratory studies have shown that ascorbic acid is capable of preventing the replication of HIV, with the ability to act as an excellent supplement for HIV patients [9]. Renker et al. [45] reported that consumption of Vitamin C at levels of 2 grams daily may help to control hepatitis, prevent flu and speed up recovery from influenza. Vitamin C also possibly decreases the incidence of urinary tract infection by increasing the acidity of urine, which makes it an inhospitable host for bacteria [6].

The consumption of Vitamin into the body may sometimes be challenging to people who are pharmacophobic but yet require it for proper body function. For this set of people, the consumption of fruit to supplement this, either singly or in blends becomes inevitable. Attempts had been made to determine the concentration of vitamin C in some selected FAV. Nonetheless, there is a lack of literature on dietary exposure and health effects of excess vitamin C intake in people. Therefore, the present study seeks to compare the concentration of vitamin C in some FAV and their homogenates using two analytical methods, as well as the dietary exposure and health effects of excess vitamin C intake in adults and children.

MATERIALS AND METHODS

Sample collection and preparation

Mature FAV: orange, apple, pineapple, carrot, watermelon, cucumber, and banana were randomly purchased from different sellers in a local market in Awka, Anambra State, Southeast region of Nigeria. The samples, thoroughly washed with distilled water before being chopped into 100 g each were weighed and the juice extracted using a juice extractor. The juices were filtered with muslin cloth to remove pulps, seeds, and other particles before being stored in labelled plastic bottles (n = 7) as A = apple (*Malus malus*); B = banana (*Musa paradisiaca*); C = carrot *(Daucus carota)*; D = cucumber (*Cucumus sativus*); E = orange *(Citrus sinensis)*; F = pineapple *(Ananas comosus)*; G = watermelon (C*itrullus lanatus).* Then, the juices were blended accordingly, and homogenized to produce the homogenates (n = 21) for two blends and (n = 15) for blends of three amounting to $N = \sum n = 43$ before further analyses were done. All the samples were analyzed in triplicate, and the concentrations of ascorbic acid were presented as the mean of the replicate values.

Preparation of stock and standard solution of ascorbic acid

Standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.01 g of the standard ascorbic acid (BASF) in a small amount of oxalic acid solution (0.5%) and then completed to 100 mL with the same solution to obtain a concentration of 100 µg/mL. A series of dilutions of 15, 20, 25, 30, and 35 µg/mL were prepared from stock ascorbic acid solution.

Preparation of standard calibration curve of ascorbic Acid

Standard calibration curve of ascorbic acid was established by graphing concentrations versus absorbance of ascorbic standard solutions by taking 5 mL of each standard solution and put in a test tube; then 0.5 mL of $KMnO_4$ (Spectrum Chemical Mfg.), solution was added. This solution was left to stand for 5 min. The absorbance of this standard solution was read at 530 nm against blank.

Vitamin C analysis by spectrophotometric method

10 mL of each sample was transferred into a 50 mL volumetric flask and 25 mL of oxalic acid (BASF) was added and gently mixed to get a homogenous mixture. It was then made up to mark with the same solution and the resulting solution was centrifuged for 15 min. Exactly 5 mL of each supernatant was transferred into a test tube, and 0.5 mL of $KMnO_4$ was added. The contents of each sample were thoroughly mixed and allowed to stand for 5 minutes before the

solutions were read at 530 nm against blank by UV-Vis spectrophotometer (Malvern Panalytical).

Calibration curve

The absorbances of the standard ascorbic acid were tabulated in Table 1 and used to determine the calibration curve by plotting the absorbance against concentration as shown in Figure 1.

Table 1. Concentration and absorbance of the standard

Concentration (µg/mL)	Absorbance
15	0.05
20	0.06
25	0.08
30	0.10
35	0.13

After determination of the ƛ of the coloured complex at 530 nm using 752N spectrophotometer, the absorbance of all the standards and concentration is known. The concentration of the vitamin C in the sample was calculated from the relation: y = 0.0034x .

Equation was extracted from the calibration curve in Figure 1 where x is the concentration of the vitamin C in the sample and y is the absorbance of the sample at 530 nm.

added. Each was titrated with 0.015 M IO_3 (BASF) till a blue-black end point was reached. Concentration in mg/L of ascorbic acid in the samples was calculated as a function of iodine used up.

Dietary exposure of Vitamin C

The dietary exposure of Vitamin C was conducted according to method used by Kim *et al.,* (2015). The authors suggested that dietary exposure is the sum of food intake (consumption rate) with chemical concentration in FAV sources (vitamin C from spectrophotometric method) and is given as:

$$\textit{Dietary exposure} = \sum_{i=1}^{n} (\textit{Food intake} \times \textit{Chemical concentration in FAV})$$

Where:
Food intake = daily consumption rate using on-the-spot assessment from market survey (67, 68]
Chemical concentration = spectroscopic result of FAV

The food intake (FI) was determined from market survey conducted in Eke-Awka, Anambra State, Southeastern Nigeria using purchasing strength, taste, availability, seasonal variation, frequency, mixing period, preparation style and farming style across different locations, which was made to develop the

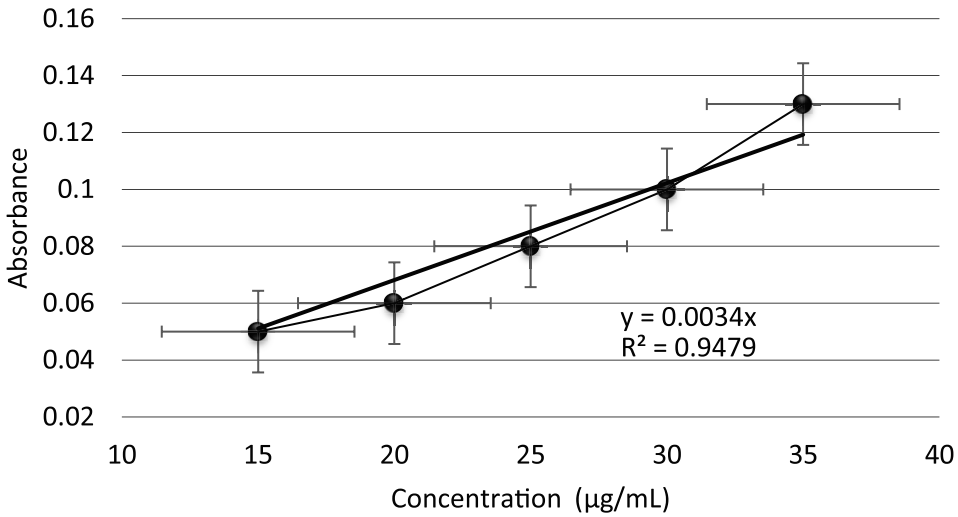

Figure 1. Standard calibration curve of vitamin C

Vitamin C analysis by titration method

A 5 mL aliquot of each sample was extracted with 20 mL of oxalic acid, followed by centrifugation at 1800 rpm for 10 mins. The supernatant was decanted and made up to 200 mL mark in a volumetric flask before 1 mL of each sample was put in 200 mL flask and made up to mark with distilled water. 10 mL of each was measured in a conical flask and then 5 mL of 0.2 M KI, 2.5 mL of 1.0 M HCl and a few drops of starch indicator (Spectrum Chemical Mfg.) solution

daily consumption rate (DCR) [7, 21, 22, 26]. The dietary intake was evaluated with 20% mid limit and 95% upper limit to minimize errors potential from on-the-spot assessment conducted from uncertainty [64].

Although for health concerns, there is a need to evaluate with body weight and reference guideline for adverse health intake quotient, which is given as hazard quotient (HQ) [37, 38, 40, 41] as shown in equation below.

$$Hazard\ quotient = \frac{Dietary\ exposure}{body\ weight \times References\ guideline}$$

Where:
Body weight: 80 kg (adult, 16 – 30 years), 15 kg (children, 0 – 15) from site specific assessment conducted on 100 random respondents
Reference guideline = Recommended dietary allowance (RDA) [32].

Results obtain with the spectrophotometric method were used to calculate dietary exposure because we assumed this method is more specific and sensitive than the titrimetric method.

Statistical analysis

Statistical analyses were done using Microsoft Excel version 2010. Variations were considered significant at $p<0.05$ and results are presented as mean.

RESULTS AND DISCUSSION

Concentration matrix using the two analytical methods

Tables 2, 3 and 4 depict the concentration of vitamin C for spectrophotometric and titrimetric methods in FAV, two-component and tri-component homogenates respectively.

Orange and pineapple showed the highest vitamin C contents for both methods as seen in Table 2, while cucumber showed the least availability of vitamin C in both methods. The vitamin C content reported by Mohammed et al. [29] and Isam et al. [23] for watermelon, orange, cucumber, apple, and banana are lower compared to the present study, likely because of the experimental methods and the geographical area of sampling of the fruits. Also, the variation in results for this study and other reports can be explained based on climatic conditions as light and temperature are factors that affect vitamin C contents in fruits and vegetables and have been reported to affect the chemical composition of horticultural crops. The use of nitrogen fertilizers has also been fingered as a potential alternative to plant nutrients [50, 54].

Table 3 shows the results of vitamin C in samples of two-component homogenates FAV, revealing that the highest concentration of vitamin C was recorded in orange mixtures such as orange-pineapple, orange-apple and orange-watermelon. Citrus fruits have a high vitamin C content, which was enhanced when combined with other fruits that have comparable ascorbic acid concentrations. Research has demonstrated that vitamin C is the main antioxidant found in citrus fruits [69]. The lowest vitamin C content was found mostly in apple mixtures, excluding those containing orange and pineapple. Even though apples are not a significant source of vitamin C [62], the majority of commercially available apple juices are fortified to contain one or more reference dietary intake daily values, although ascorbic acid in apples degrades very quickly [58]. Nonetheless, two-component homogenate FAVs showed improved vitamin C content compared to single FAV juices.

In the tri-component homogenate FAVs shown in Table 4, highest vitamin C content was seen in cucumber-orange-pineapple and orange-pineapple-watermelon mixtures, corroborating the position of orange and pineapple as high vitamin C fruits. The lowest vitamin C content was recorded in carrot-cucumber-watermelon and apple-banana-cucumber mixtures. Again, as in two-component homogenate FAVs, vitamin C was higher than in tri-component homogenate FAVs than single fruit/vegetable. The results show that homogenate FAV extracts could contribute substantially to the 45 mg WHO [68] daily recommended dietary allowance of vitamin C. The results obtained are in agreement with the results reported by Awsi and Er-Dorcus [5], where maximum vitamin C concentration was recorded in pineapple juice blended with orange and carrot juice. Jain and Khurdiya [24] also reported that when Indian gooseberry juice was blended with other fruit juices, their vitamin C content was boosted.

Yoshizaki *et al.* [70] noted that mixed fruit juices have epidemiological advantages such as in reducing

Table 2. Concentration of FAV (mg/100g)

Sample codes	Sample	Spectroscopic Method (530nm)	Titrimetric Method
A	Apple	26.47	26.58
B	Banana	20.58	31.41
C	Carrot	17.64	29.64
D	Cucumber	11.76	16.79
E	Orange	41.17	32.80
F	Pineapple	38.23	31.84
G	Watermelon	11.76	26.74

Table 3. Concentration of two-component homogenates (mg/100 g)

Sample	Two blends	Spectroscopic Method (530nm)	Titrimetric Method
AB	Apple + Banana	38.23	35.64
AC	Apple + Carrot	47.05	37.54
AD	Apple + Cucumber	35.29	40.71
AE	Apple + Orange	161.78	155.20
AF	Apple + Pineapple	70.58	71.60
AG	Apple + Watermelon	35.29	34.01
BC	Banana + Carrot	35.29	38.02
BD	Banana + Cucumber	38.23	43.72
BE	Banana + Orange	50.00	48.24
BF	Banana + Pineapple	97.05	99.78
BG	Banana + Watermelon	44.11	42.21
CD	Carrot + Cucumber	14.70	17.23
CE	Carrot + Orange	47.05	41.82
CF	Carrot + Pineapple	50.00	43.24
CG	Carrot + Watermelon	58.82	41.66
DE	Cucumber + Orange	35.29	32.16
DF	Cucumber + Pineapple	155.88	160.78
DG	Cucumber +Watermelon	29.44	28.96
EF	Orange + Pineapple	220.58	209.09
EG	Orange + Watermelon	135.35	136.38
FG	Pineapple + Watermelon	79.41	68.39

Table 4. Concentration of tri-component homogenates (mg/100 g)

Sample codes	Tri-component homogenates	Spectroscopic Method (530nm)	Titrimetric Method
ABC	Apple + Banana + Carrot	64.70	63.36
ABD	Apple + Banana + Cucumber	55.88	54.69
ABE	Apple +Banana + Orange	76.47	66.53
ABF	Apple + Banana + Pineapple	70.58	65.89
ABG	Apple + Banana + Watermelon	61.76	57.73
BCD	Banana + Carrot + Cucumber	32.35	34.37
BCE	Banana + Carrot + Orange	88.23	75.08
BCF	Banana + Carrot + Pineapple	82.35	71.28
BCG	Banana + Carrot + Watermelon	44.11	32.63
CDE	Carrot + Cucumber + Orange	61.76	65.26
CDF	Carrot + Cucumber + Pineapple	29.41	31.05
CDG	Carrot + Cucumber + Watermelon	50.00	51.32
DEF	Cucumber + Orange + Pineapple	132.35	112.60
DEG	Cucumber + Orange + Watermelon	70.58	75.08
EFG	Orange + Pineapple + Watermelon	105.88	113.10

the risk of some cancers, metabolic disorders, cardiovascular disease and stroke. Rossi et al. [46] further noted that they may contribute significantly in increasing protective serum antioxidants. Nonetheless, mixed fruit juices do not automatically translate to improved vitamin C and mineral concentration, but may be based on FAV type. This can be seen in the USDA (SR21) database where black currant, which as a single fruit, on its own contains more vitamin C and minerals than any group of mixed fruits [12].

The efficiency of the method used in ascertaining concentration for vitamin C cannot be attributed to any single method as it varied indiscriminately. Nonetheless, the spectrophotometric method seemed to give a more stable result. Isam et al. [23] in a comparative study noted that there was no significant difference between the two methods, but the spectrophotometric method has been favoured over the titrimetric method. However, these discrepancies may be due to titration errors and the difficulty in ascertaining the end-point where the extracts are coloured, especially the reddish-purplish colours. For the iodometric titration method which is based on an oxidation-reduction reaction, there may be other factors such as the presence of other reducing substances besides ascorbic acid in the foods. Holloway et al. [20] noted that molecules like phenols, sulphydryls, and triose reductones; and ferrous, cuprous, or sulphite ions can reduce the dye giving rise to high and false titration results. However, Nweze et al. [34] also noted that interferences may be overcome by pH adjustment to reduce the speed of the reaction, such that most interfering materials react very much slower than ascorbate.

Comparison of the two methods

The concentrations of Vitamin C for both spectroscopic method and titration method of determination showed good efficiency of quantification, as spectrophotometric method was influenced by sensitivity and selectivity across all samples and respective blends in comparison with titrimetric method. Several researchers have focused on different methods of determining ascorbic acid (vitamin C): El Shara and Mussa [15] and Adebayo [3] analyzed for Vitamin C using UV-Vis spectrophotometer and titration in vegetables and fruits. They revealed that UV-Vis method provides higher sensitivity in ascorbic acid concentration than titration, as they affirmed that the concertation matrix is dependent on different factors such as geography and location, variety in species, harvest period, temperature, storage duration and temperature, handling and preconditioning (ripening stage), which can invariably lead to contradictory results as seen in diverse researches [18, 56].

Hagos et al. [18] assessed for ascorbic acid (Vitamin C) using ATR-FTIR (Attenuated total reflectance – Fourier transformed infrared spectroscopy and UV-Vis methods in aqueous extract of pumpkin, as UV-Vis method showed high accuracy, sensitivity and precision as well as fast determination than ATR-FTIR for quantitative and qualitative processes.

Sharma et al., [48] assessed vitamin C concentration in commercial fruit juice and fresh fruits sold in Nepal using titration, thin-layer chromatography and UV-Vis, as they observed that UV-Vis was better than the other two methods.

Several other researchers [1, 2, 4, 11, 36, 47, 56, 73] have utilized different analytical methods such as chromatography (solid, liquid, gas), titrimetric, voltammetry, fluorometry, potentiometry, UV-Vis, FTIR, capillary electrophoresis and reverse phase for determination of ascorbic acid (Vitamin C) from fresh fruits, vegetables and processed/extract fruit juices; as UV-Vis has been assessed to meet good specificity, sensitivity, rapid, accurate and simple in operation. Although the analytical methods have diverse extraction procedures, and analytical technique and experimental variation differences, there is a close relationship in terms of linearity, validity, accuracy and reproductivity across their respective analytical results as Abe-Matsumoto et al. [1] stated that "*LOQ (limit of quantification) and LOD (limit of detection) are not critical for choosing best method*" but reliability and sensitivity are critical in vitamin C studies.

Dietary exposure

The dietary exposure to Vitamin C was conducted for fruits/vegetables and FAV homogenates to estimate the likely exposure level of food chemicals for a population group in addition to associated adverse or health risks associated with continual exposure to extreme concentration [27, 53]. The daily consumption rate using on-the-spot assessment from a market survey [67, 68] is shown in Table 5.

Health effect of excess vitamin C intake

According to the National Institute of Health [32], vitamin C has minimal toxicity and does not portend any serious adverse health conditions, while it is critical to note that 70 – 90% of vitamin C is absorbed in cells, tissues, and plasma from moderate intake of 30 – 180 mg/day and less than 50% unabsorbed that are not metabolized are excreted as urine or sweat as salt. The commonest illnesses associated with vitamin C non-metabolism are nausea, abdominal spasms, gastrointestinal disturbances, and diarrhoea [21, 22, 60].

Hazard quotient (HQ) was evaluated using the recommended dietary allowance (RDA) of 45 mg/day [68] for children and adults in addition to lower and upper limits of 20% and 95%, which shows that in Figure 2 and Table 6 using HQ reference of 1.0 to cause adverse health effects implies that children at 95% tolerance were above one for age group between 0.-15 years might experience increase in carbon dioxide (CO_2) in the intestine from oxidized (dehydroascorbic acid) form of unabsorbed vitamin C in high dose causing nausea [72].

There are several side effects associated with large doses (exposure) of vitamin C when it exceeds RDAs for diverse age groups, as it is known that fruits/vegetables have different concentrations, which

Table 5. Consumption rate of analyzed fruit/vegetable and FAV homogenates in (g/day) using on-the-spot assessment

Sample codes	Sample	On-the-spot assessment
A	Apple	0.6
B	Banana	25.0
C	Carrot	0.13
D	Cucumber	6.5
E	Orange	5.7
F	Pineapple	3.5
G	Watermelon	5.2
AB	Apple + Banana	21.6
AC	Apple + Carrot	1.7
AD	Apple + Cucumber	2.5
AE	Apple + Orange	2.1
AF	Apple + Pineapple	1.9
AG	Apple + Watermelon	2.3
BC	Banana + Carrot	12.2
BD	Banana + Cucumber	20.1
BE	Banana + Orange	19.1
BF	Banana + Pineapple	22.1
BG	Banana + Watermelon	18.5
CD	Carrot + Cucumber	1.4
CE	Carrot + Orange	2.4
CF	Carrot + Pineapple	4.1
CG	Carrot + Watermelon	2.5
DE	Cucumber + Orange	5.0
DF	Cucumber + Pineapple	4.6
DG	Cucumber +Watermelon	3.0
EF	Orange + Pineapple	6.1
EG	Orange + Watermelon	6.0
FG	Pineapple + Watermelon	3.9
ABC	Apple + Banana + Carrot	12.9
ABD	Apple + Banana + Cucumber	10.1
ABE	Apple +Banana + Orange	21.9
ABF	Apple + Banana + Pineapple	17.0
ABG	Apple + Banana + Watermelon	11.4
BCD	Banana + Carrot + Cucumber	9.2
BCE	Banana + Carrot + Orange	11.0
BCF	Banana + Carrot + Pineapple	12.9
BCG	Banana + Carrot + Watermelon	20.0
CDE	Carrot + Cucumber + Orange	3.3
CDF	Carrot + Cucumber + Pineapple	4.9
CDG	Carrot + Cucumber + Watermelon	2.0
DEF	Cucumber + Orange + Pineapple	2.4
DEG	Cucumber + Orange + Watermelon	1.8
EFG	Orange + Pineapple + Watermelon	7.1

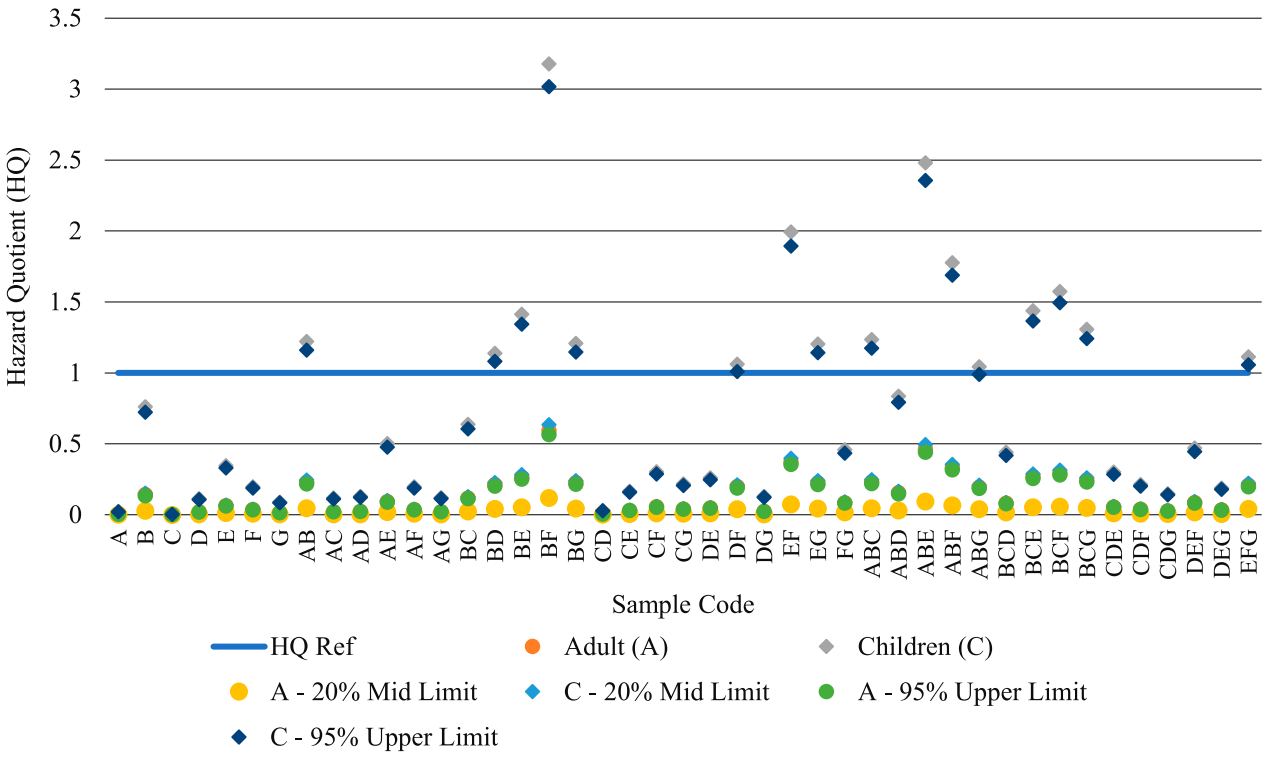

Figure 2. Hazard quotient of vitamin C in fruits/vegetables and FAV homogenates

has been reported to ischemic stroke, cause enamel erosion during chewing, increase in renal stones and allergic response [14, 22, 55, 60]. Although diarrhea and abdominal pain may occur from excretion of unmetabolized vitamin C for a few periods of 1 – 2 weeks, it can be managed by reducing FAV intake or dividing the cumulative amount or frequency of intake into multiple doses in minimized forms [13, 19, 72].

Vitamin C has been associated with iron (Fe)-induced oxidative stress from oxidable health metals such as lead and mercury that are known toxins causing blood related illness such as leukaemia [35, 39, 72]. An extensive study conducted by Pullar *et al.,* [43] has shown that high dose of vitamin C can cause elevated mood in male tertiary students in New Zealand, as conflicting analogies stated that there might be slight to strong correlation of vitamin C in high dose to cause depression [7, 66].

Therefore, it is advantageous to state that this study was conducted as a dose-response-effect relationship quotient to assess vitamin C intake from fruits/vegetables and FAV homogenates in both adults and children. Hence, from Figure 2, children are relatively at risk from immense exposure. There might be issue of uncertainty across the utilization of on-the-spot assessment conducted for dietary intake of these fruits in addition to overestimation or underestimation of results in the study, as it is a known fact that vitamin C has immense functionality to improve human health, quality of life and treat illnesses/diseases in addition to other essential elements and chemicals that are critical for dietary input from different race, locality and food style that might have increase or decrease vitamin C concentration [10, 61, 67].

CONCLUSION

The study has shown that FAV homogenate juices, especially those containing orange and pineapple, showed higher improvements in Vitamin-C content compared to single fruit/vegetable juices, and can contribute substantially to daily recommended dietary allowance. The two methods (spectrophotometry and titration) analyzed indicated that the concentration of vitamin C in single fruit/vegetable juice ranged between 11.76 – 41.17 mg/100g and 16.79 – 32.80 mg/100 g; while two-component FAV homogenates ranged between 14.70 – 220.58 mg/100g and 17.23 – 209.09 mg/100 g; and tri-component FAV homogenates ranged between 29.41 – 132.35 mg/100g and 31.05 – 113.10 mg/100g respectively. There was little or no observed difference between spectrophotometric and the titrimetric method. However, the spectrophotometric method is favourable and satisfactory for vitamin C determination because of its sensitivity, ease and minimal errors. The dietary exposure and health effect of vitamin C excess intake showed that children are susceptible to have health concerns such as nausea, gastrointestinal pains, increase renal stone and excessive. With the improved vitamin-C content available in FAV homogenates, it

Table 6. Hazard Quotient of Vitamin C in FAV and FAV homogenates

Sample codes	Adult (A)	Children (C)	A - 20% Mid Limit	C - 20% Mid Limit	A - 95% Upper Limit	C - 95% Upper Limit	HQ Reference
A	0.004	0.024	9E-04	0.005	0.004	0.022	1
B	0.143	0.762	0.029	0.152	0.136	0.724	1
C	6E-04	0.003	1E-04	7E-04	6E-04	0.003	1
D	0.021	0.113	0.004	0.023	0.02	0.108	1
E	0.065	0.348	0.013	0.07	0.062	0.33	1
F	0.037	0.198	0.007	0.04	0.035	0.188	1
G	0.017	0.091	0.003	0.018	0.016	0.086	1
AB	0.229	1.223	0.046	0.245	0.218	1.162	1
AC	0.022	0.118	0.004	0.024	0.021	0.113	1
AD	0.025	0.131	0.005	0.026	0.023	0.124	1
AE	0.094	0.503	0.019	0.101	0.09	0.478	1
AF	0.037	0.199	0.007	0.04	0.035	0.189	1
AG	0.023	0.12	0.005	0.024	0.021	0.114	1
BC	0.12	0.638	0.024	0.128	0.114	0.606	1
BD	0.213	1.138	0.043	0.228	0.203	1.081	1
BE	0.265	1.415	0.053	0.283	0.252	1.344	1
BF	0.596	3.177	0.119	0.635	0.566	3.019	1
BG	0.227	1.209	0.045	0.242	0.215	1.148	1
CD	0.006	0.03	0.001	0.006	0.005	0.029	1
CE	0.031	0.167	0.006	0.033	0.03	0.159	1
CF	0.057	0.304	0.011	0.061	0.054	0.289	1
CG	0.041	0.218	0.008	0.044	0.039	0.207	1
DE	0.049	0.261	0.01	0.052	0.047	0.248	1
DF	0.199	1.062	0.04	0.212	0.189	1.009	1
DG	0.025	0.131	0.005	0.026	0.023	0.124	1
EF	0.374	1.993	0.075	0.399	0.355	1.894	1
EG	0.226	1.203	0.045	0.241	0.214	1.143	1
FG	0.086	0.459	0.017	0.092	0.082	0.436	1
ABC	0.232	1.236	0.046	0.247	0.22	1.175	1
ABD	0.157	0.836	0.031	0.167	0.149	0.794	1
ABE	0.465	2.481	0.093	0.496	0.442	2.357	1
ABF	0.333	1.778	0.067	0.356	0.317	1.689	1
ABG	0.196	1.043	0.039	0.209	0.186	0.991	1
BCD	0.083	0.441	0.017	0.088	0.079	0.419	1
BCE	0.27	1.438	0.054	0.288	0.256	1.366	1
BCF	0.295	1.574	0.059	0.315	0.28	1.495	1
BCG	0.245	1.307	0.049	0.261	0.233	1.242	1
CDE	0.057	0.302	0.011	0.06	0.054	0.287	1
CDF	0.04	0.213	0.008	0.043	0.038	0.203	1
CDG	0.028	0.148	0.006	0.03	0.026	0.141	1
DEF	0.088	0.471	0.018	0.094	0.084	0.447	1
DEG	0.035	0.188	0.007	0.038	0.034	0.179	1
EFG	0.209	1.114	0.042	0.223	0.198	1.058	1

will be an important source of the nutrient for this class of individuals. Therefore, there is need to develop a national recommended dietary intake in Nigeria to assist all value chain (medical, industrial players, ministries, department, and agencies of government) to be equipped with information as regards essential nutrients.

Data availability statement: All data and materials are available from Authors, on reasonable request.

Disclosure statement conflict of interest

No potential conflict of interest was reported by the authors.

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Received: 02.10.2023
Accepted: 08.03.2024

Rocz Panstw Zakl Hig 2024;75(1):35-44
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0290

ORIGINAL ARTICLE

KNOWLEDGE AND EATING HABITS REGARDING FUNCTIONAL FOOD AMONG ADULTS

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ABSTRACT

Background. Functional food is a key element in the prevention and treatment of many diseases. The ingredients it contains, such as phytosterols that lower cholesterol, also have a preventive effect on type 2 diabetes, atherosclerosis and heart attack. Phenolic compounds have antioxidant, anti-inflammatory and antiviral properties. Xylo-oligosaccharides control insulin levels, and fibre lowers blood pressure, potentially reducing insulin resistance. These beneficial properties mean that there is an increasing interest in this kind of food.

Objective. The aim of the study was to assess the state of knowledge and behaviour regarding functional food among adults and to answer the question whether there are differences between the state of knowledge and behaviour of women and men.

Material and methods. The survey was conducted among 301 people, including 181 women and 120 men. The research tool was an original survey questionnaire.

Results. The definition of functional food is known to 42.5% of people (47.5% of women and 35% of men), while the definition of prebiotic is known to 41.9% of people (43.1% of women and 40.0% of men). For 56.2% of respondents, the factor encouraging the consumption of functional food was a healthy lifestyle, and for 54.7% of them, the product composition was the main purchase criterion. Among functional products, cereals or muesli were most often consumed for breakfast by 35% of men and 55.8% of women, 42.5% of men and 33.7% of women were eaten oils for lunch. For dinner they most often consumed fruit teas, herbal teas, herbal mixtures, this answer was given by 25.8% of men and 29.8% of women.

Conclusions. Knowledge of functional foods is unsatisfactory, and no differences in the knowledge of women and men have been observed. Consumption of functional food is generally low, and no differences in consumption have been observed between women and men.

Key words: *functional food, eating habits, nutritional knowledge, adults*

INTRODUCTION

Functional food is this kind of food that has a proved beneficial effect on functioning of the body by reducing the risk of certain diseases, improving well-being and, consequently, increasing the quality of life of people who consume it [24]. These foods may have a beneficial effect on both one and several body functions. In addition to the ingredients normally included in its composition, it also contains additional substances or an increased concentration of one of the ingredients originally included in its composition [24].

Nowadays, there is an increasing interest in maintaining a good quality of life, and one of the methods is taking care of health. This has led to an increase in consumer interest in functional foods. Its attractiveness arises from the possibility of application by various consumer groups, such as pregnant women, infants, children, school youth, elderly individuals, and athletes. Additionally, its preventive and therapeutic properties in the context of many diseases are appealing. [11]. The presence of phytosterols in its composition lowers cholesterol levels, preventing atherosclerosis and type 2 diabetes. [17,19]. The phenolic compounds contained in it have antioxidant, anti-inflammatory, antibacterial, antiviral and antithrombotic properties [14]. Functional food plays a crucial role in the prevention of cardiovascular diseases, protection of the central and peripheral nervous systems, increase in the number of T

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

lymphocytes, and improvement of immune function. [16, 26, 27].

Additionally, it exhibits anticancer properties [9]. Polyphenols in food lower blood pressure, improve endothelial function, normalize lipid profile, and minimize the risk of cardiovascular diseases [22]. They improve the condition of blood vessels and the circulatory system by factors vasoprotective [1]. They also protect the heart during chemotherapy [6]. Resveratrol and hydroxytyrosol inhibit platelet aggregation and reduce venous inflammation [22]. Xylooligosaccharides, as components of functional food, regulate insulin secretion without increasing the glucose level in the blood, thus preventing the occurrence of insulin resistance and diabetes [20]. They exhibit prebiotic properties, supporting immunity [20]. Dietary fiber present in functional products lowers blood pressure and may contribute to reducing insulin resistance, which is a cause of arterial hypertension [12, 18]. Continuous technological progress and a regularly increasing assortment on the food market encourage consumers to increase their knowledge in this area. Thanks to this, more and more people are aware of the health benefits of choosing functional products. Due to their versatile applications and increasing interest in them, an assessment of knowledge and behaviors related to their consumption has been undertaken. The study was conducted among employees in the food industry, considering the fact that they are responsible for its production. Their awareness and behaviors in this regard are therefore significant.

The aim of the study was to assess the state of knowledge and eating habits related to functional food among adults and to answer the question whether there are differences between the state of knowledge and habits among women and men.

MATERIAL AND METHODS

301 people took part in the study, including 181 women and 120 men, average age - 39.9 years. The survey was conducted at the turn of July and August 2021 among employees of food industry plants in Katowice. The inclusion criterion was employment in departments directly involved in food production and conscious consent to participate in the study, while the exclusion criteria were employment in administrative departments and lack of consent to participate in the study. All study participants expressed their consent to the use of its results.

The research tool was an original anonymous survey questionnaire consisting of 25 questions, including both single and multiple-choice questions. The first part, referred to as the specification, included questions about gender, year of birth, education, place of residence, number of people in the household, type of occupation and department of employment, as well as the weight and height of the respondents.

The questions included in the pivotal part covered knowledge about functional food and its consumption, knowledge of understanding of definition of functional food, recognizing correct statements about it, sources of information on functional products, and understanding the definition of prebiotics and their role. Participants were also asked to self-assess their knowledge of functional food. Consumption-related questions verified information such as consumption frequency, factors influencing its consumption, place of purchase, and types of functional products consumed.

Body weight and height were used to assess participants' BMI, following WHO criteria: underweight <18.5, normal weight – 18.5-24.99, overweight 25-29.99 and obesity ≥30 [23].

Statistical analysis was performed based on procedures available in the Statistica v. 13.3 program (StatSoft Inc., Tulsa, OK, USA). The statistical significance of differences between the frequencies of qualitative variables was assessed based on the results of the Pearson *Chi*-square test, the Yates correction test and the Fisher exact test. The correlation analysis was performed based on the Φ-Yule and V-Cramer contingency coefficient. The interpretation of the results was based on the criterion of statistical significance $p<0.05$.

RESULTS

301 people took part in the study, including 60.1% (N=181) women and 39.9% (N=120) men. The largest group were people aged 41-50, 32.6% (N=98), with secondary education, 37.9% (N=114), living in the city, 88.0% (N=265), living in multi-person households and having normal body weight, 47.2% (N=142).

Knowledge of functional food

The answers to the question about the functional food definition, statements about it, and sources where to look for information on its daily consumption in the study group in general and taking account of gender are presented in Figures 1, 2 and 3.

When asked about the definition of functional food, respondents most often indicated the correct answer and noted that it is food that has a documented beneficial impact on health, much bigger than that resulting from the presence of nutrients considered essential. This answer was given by 42.5% of people, including more women (47.5%) than men (35.0%) (Figure 1). When asked which statement related to functional food, the respondents indicated that it is food used in the daily diet that has an additional

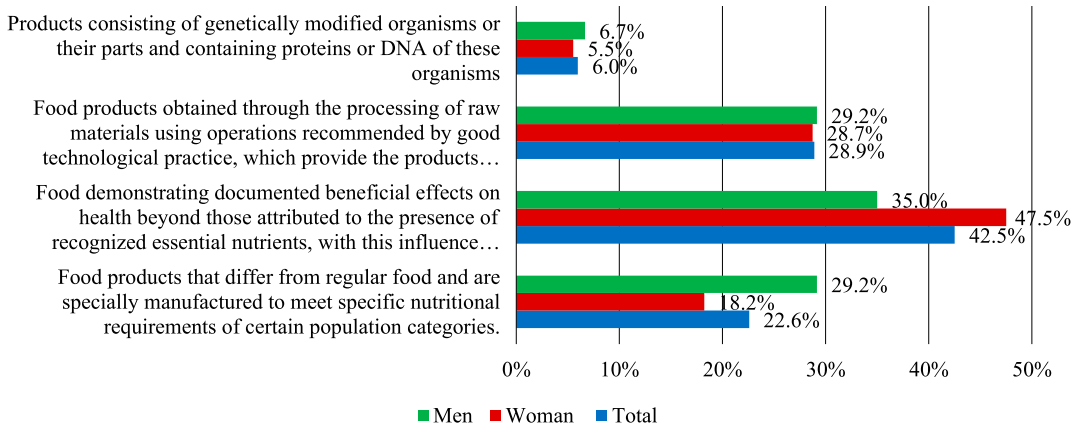

Figure 1. Knowledge of the definition of functional food, in total and by gender (N=301)

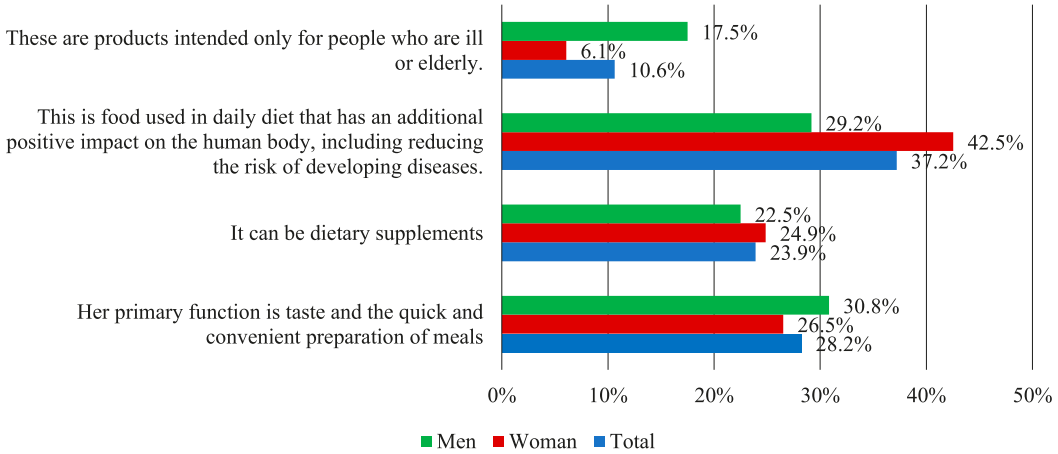

Figure 2. Responses to the question which statement pertains to functional food, total and by gender (N=301)

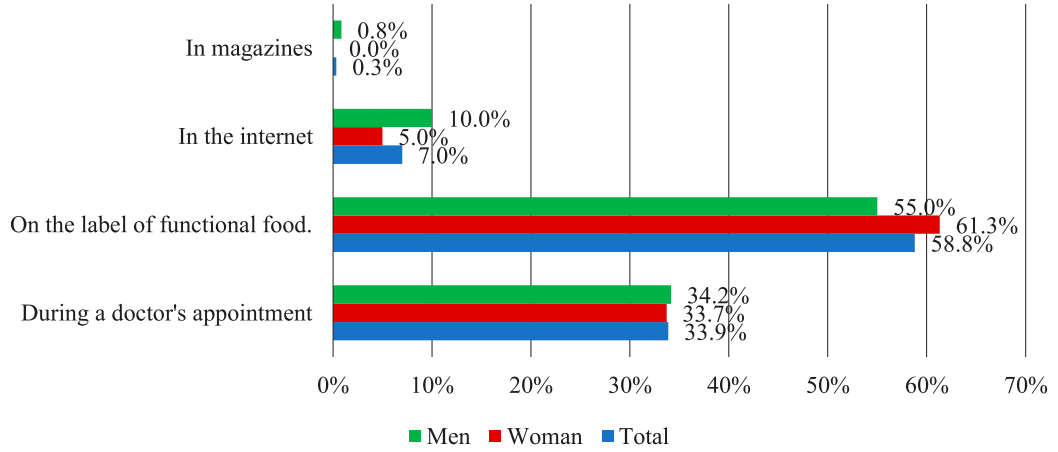

Figure 3. Responses to the question where to look for recommendations for daily consumption of functional food, total and be gender (N=301)

positive effect on the human body, moreover, it reduces the risk of disease development. This answer was given by 37.2% of people, including more women (42.5%) than men (29.2%) (Figure 2). When asked where to look first for recommendations on the daily consumption of functional food, the respondents most often indicated that this information should be included in a functional food label; this answer was given by 58.8% of people, including more women (61.3%) than men (55.0%) (Figure 3).

Answers to questions about the definition of prebiotics and their functions in functional foods in the studied group in general and taking account of gender are presented in Figures 4 and 5.

In the question about the definition of a prebiotic, respondents most often indicated that they are

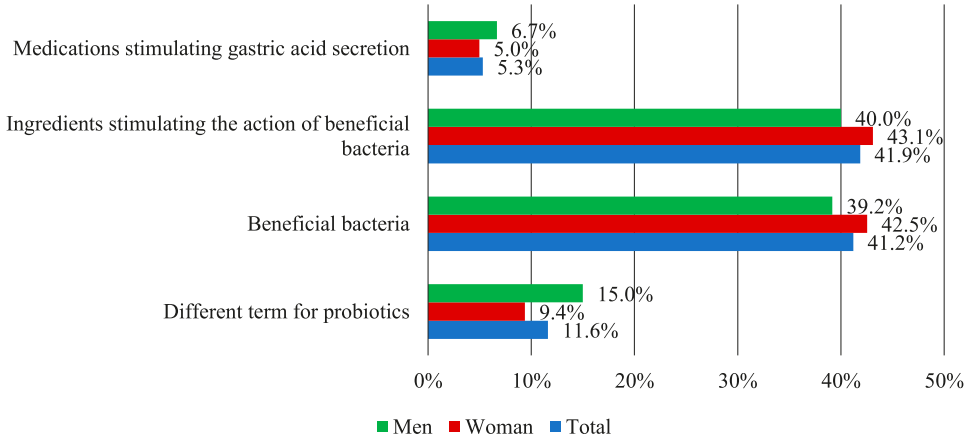

Figure 4. Knowledge of the definition of prebiotics, total and by gender (N=301)

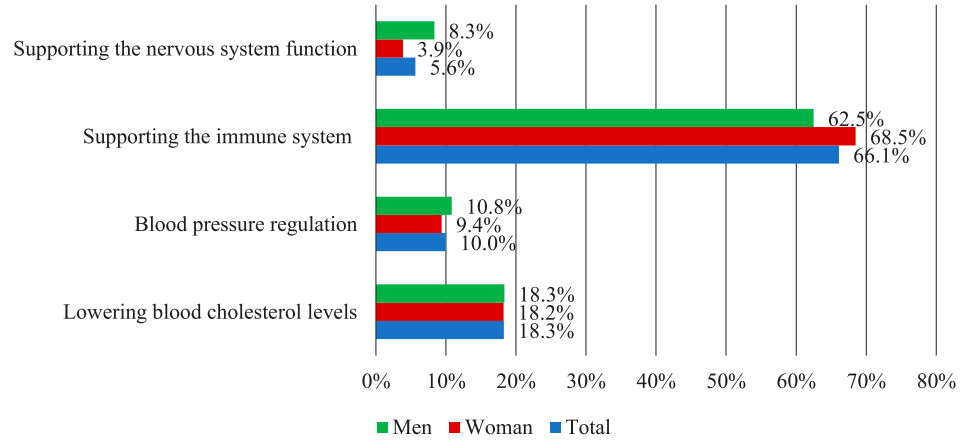

Figure 5. Knowledge of the role of probiotics in functional food, total and by gender (N=301)

ingredients that stimulate the action of health-promoting bacteria; this answer was given by 41.9% of people, including slightly more women (43.1%) than men (40.0%) (Figure 4). The surveyed people most often pointed out that the role of probiotics is to support the human immune system; this answer was given by 66.1% of the respondents, including more women (68.5%) than men (62.5%) (Figure 5).

When asked how the surveyed people assessed their knowledge of functional food, 44,5% marked a unsatisfactory level, more men (50.8%) than women (40.3%) indicated such a response. An analysis of gender differences in the level of knowledge regarding functional foods showed a higher level of knowledge among women compared to men, but these differences are not statistically significant (p=0.14).

Habits related to functional food

The answers to the question about the frequency of consumption of functional food and the health factors encouraging its consumption in the study group in general and by gender are presented in Table 2. The respondents most often indicated that they did not consume functional food (33.2%), this answer was given by more men (40.0%) than women (28.7%). The analysis mostly did not reveal statistically significant gender differences in the frequency of consumption of functional products; only the frequency of functional product consumption (daily or several times a week) was slightly (p=0.07) higher among women (Table 2). To the question about factors encouraging food consumption, 203 individuals responded, and among the factors, the most frequently indicated was leading a healthy lifestyle (56.2%). This response was more commonly chosen by men (59.7%) than women (54.2%). No significant differences were observed in the frequency of indicating individual health factors that encourage the consumption of functional products (Table 2).

The answers to the question about features, apart from a price, taken into account when choosing functional food and about a place of purchase of functional food, in the surveyed group in total and taking into account gender, are presented in Table 3.

The surveyed people indicated that the ingredients of the product is a feature they take into account when choosing the food; this answer was given by 54.7% of people, more women (59.5%) than men (45.8%).

Table 1. Gender-based differences in functional food knowledge (N=301)

	Total	Women	Men	Test; p Correlation coefficients
	N(%)	N(%)	N(%)	
Level of knowledge of functional food				
Unsatisfactory	134 (44.5)	73 (40.3)	61 (50.8)	Fi=5.46 p=0.14 V_c=0.13
Satisfactory	89 (29.6)	53 (29.3)	36 (30.0)	
Good	72 (23.9)	51 (28.2)	21 (17.5)	
Very good	6 (2.0)	4 (2.2)	2 (1.7)	

N – number of observations; Fi – Fisher's exact test; p – level of statistical significance; Vc – V Cramer's contingency coefficient

Table 2. Functional food consumption frequency (N=301) Health factors encouraging functional food consumption (N=203)

		Total	Woman	Men	Test; p Correlation coefficient
		N (%)	N (%)	N (%)	
Frequency of consumption of functional products					
Daily		14 (4.7%)	9 (5.0%)	5 (4.2%)	χ_y^2 = 10.07 p=0.07 V_c=0.18
Several times a week		57 (18.9%)	40 (22.1%)	17 (14.2%)	
Several times a month		60 (19.9%)	39 (21.5%)	21 (17.5%)	
Once a month		22 (7.3%)	9 (5.0%)	13 (10.8%)	
Less than once a month		48 (16.0%)	32 (17.7%)	16 (13.3%)	
Not at all		100 (33.2%)	52 (28.7%)	48 (40.0%)	
Health factors encouraging the consumption of functional food (max 3 answers)					
Possible answers			Total (N=203)	Woman (n=131)	Men (n=72)
The presence of own or family members' chronic diseases			41 (20.2%)	26 (19.9%)	15 (20.8%)
Food allergies or intolerances to certain food ingredients in oneself or family members			49 (24.1%)	31 (23.7%)	18 (25.0%)
Preventive action against chronic diseases			84 (41.4)	57 (43.5%)	27 (37.5%)
Weight loss			81 (39.9%)	46 (35.1%)	35 (48.6%)
Leading a healthy lifestyle without any specific reasons.			114 (56.2%)	71 (54.2%)	43 (59.7%)
Other, e.g. the desire to improve physical performance			3 (1.5%)	2 (1.5%)	1 (1.4%)
Most frequently indicated health factors					
Prevention against chronic diseases	No	119 (58.6%)	74 (56.5%)	45 (62.5%)	χ^2=0.69 p=0.41 Φ =0.06
	Yes	84 (41.4%)	57 (43.5%)	27 (37.5%)	
Weight loss	No	122 (60.1%)	85 (64.9%)	37 (51.4%)	χ^2=3.53 p=0.06 Φ =0.13
	Yes	81 (39.9%)	46 (35.1%)	35 (48.6%)	
Healthy lifestyle	No	89 (43.8%)	60 (45.8%)	29 (40.3%)	χ^2=0.58 p=0.45 Φ =0.05
	Yes	114 (56.2%)	71 (54.2%)	43 (59.7%)	

N - number of observations; χ_y^2 – *Chi* square test with Yates correction; χ^2 – *Chi* square test result; p – level of statistical significance; Vc – V Cramer's contingency coefficient; Φ-Yule'a – Mean Square Contingency Coefficient

The analysis did not reveal statistically significant differences between genders in terms of the frequency of indicating individual features of functional products taken into account when purchasing them. When purchasing functional food, a similar percentage of women and men took into account a product content, information about health benefits and a recommendation from friends.

To the question of where they purchase functional food, respondents most frequently indicated supermarkets or shopping centers (54.0%), with more men (59.1%) than women (51.2%) providing such responses (Table 3).

The surveyed people indicated that they most often eat cereals or muesli for breakfast (47.5%), this answer was given by more women (55.8%) than men (35.0%) and vegetable oils for dinner (37.2%), this answer was given by more men (42.5%) than women (33.7%). The respondents most often consume fruit, herbal teas and herbal mixtures for dinner (28.2%), a comparable percentage of women (29.8%) and men (25.8%), and chocolate between main meals (31 %), this answer was given by more women (31.5%) than men (30.3%).

Statistically significant differences were observed in the frequency of consumption for breakfast cereals and muesli ($p<0.001$) as well as yogurts ($p=0.01$). A similar, significant relationship was noted for the frequency of yogurt consumption ($p=0.03$) and fruit, herbal, and herbal blend teas ($p=0.03$) as snacks between meals. For each of the mentioned products, the frequency of consumption was higher in the female group (Table 4).

Table 3. Gender-based analysis of determinants and purchase locations (N=203)

Criteria apart from price that guide you when choosing functional food (max 3 answers)					
-		Total (N=203) N (%)	Woman (N=131) N (%)	Men (N=72) N (%)	
Product composition		111 (54.7)	78 (59.5)	33 (45.8)	
Taste and smell		78 (38.4)	45 (34.4)	33 (45.8)	
Info on the health benefits of product		88 (43.6)	60 (45.8)	28 (39.4)	
Content of additional nutrients		57 (28.1)	37 (28.2)	20 (27.8)	
Recommendations of friends		81 (40.1)	49 (37.7)	32 (44.4)	
Product brand		15 (7.4)	7 (5.3)	8 (11.1)	
Nice packaging		14 (7.0)	7 (5.4)	7 (9.9)	
Advertising and marketing		8 (3.9)	7 (5.3)	1 (1.4)	
Other e.g. fashion		3 (1.5)	2 (1.5)	1 (1.4)	
		Total	Woman	Men	Test; p Correlation coefficient
		N (%)	N (%)	N (%)	
Most frequently indicated characteristics of products					
Product composition	No	92 (45.3)	53 (40.5)	39 (54.2)	χ^2=3.52 p=0.06 Φ =0.13
	Yes	111 (54.7)	78 (59.5)	33 (45.8)	
Information about the health benefits of specific products	No	114 (56.4)	71 (54.2)	43 (60.6)	χ^2=0.76 p=0.38 Φ =0.06
	Yes	88 (43.6)	60 (45.8)	28 (39.4)	
Recommendations of friends	No	121 (59.9)	81 (62.3)	40 (55.6)	χ^2=0.88 p=0.35 *Φ* =0.07
	Yes	81 (40.1)	49 (37.7)	32 (44.4)	
Place where you most often buy functional food					
Small shops near the place of residence		56 (27.7)	43 (32.8)	13 (18.3)	χ_y^2 =5.59 p=0.13 V_c=0.16
Supermarkets or shopping centers		109 (54.0)	67 (51.2)	42 (59.1)	
Stores specializing in functional food		18 (8.9)	11 (8.4)	7 (9.9)	
Online stores specializing in functional food		19 (9.4)	10 (7.6)	9 (12.7)	

N - number of observations; χ^2 - Chi square test result; χ_y^2 - Chi square test with Yates correction; p - level of statistical significance; Vc - V Cramer's contingency coefficient; Φ-Yule'a - Mean Square Contingency Coefficient

Table 4. Common functional product consumption across meals (N=203)

		Total	Woman	Men	Test; p Correlation coefficient
		N (%)	N (%)	N (%)	
Most commonly consumed functional products for breakfast					
Cereals and muesli	No	158 (52.5)	80 (44.2)	78 (65.0)	χ^2=12.52 p<0.001 Φ =0.20
	Yes	143 (47.5)	101 (55.8)	42 (35.0)	
Yogurts	No	167 (55.5)	90 (49.7)	77 (64.2)	χ^2=6.09 p=0.01 Φ =0.14
	Yes	134 (44.5)	91 (50.3)	43 (35.8)	
Crispbread and waffles	No	216 (71.8)	133 (73.5)	83 (69.2)	χ^2=0.66 p=0.42 Φ =0.05
	Yes	85 (28.2)	48 (26.5)	37 (30.8)	
Most commonly consumed functional products for lunch					
Low-fat enriched cheeses, curds and kefir	No	273 (90.7)	166 (91.7)	107 (89.2)	χ^2=0.55 p=0.46 Φ =0.04
	Yes	28 (9.3)	15 (8.3)	13 (10.8)	
Juices, nectars,and multivitamin fruit drinks	No	249 (82.7)	148 (81.8)	101 (84.2)	χ^2=0.29 p=0.59 Φ =0.03
	Yes	52 (17.3)	33 (18.2)	19 (15.8)	
Vegetable oils	No	189 (62.8)	120 (66.3)	69 (57.5)	χ^2=2.39 p=0.12 *Φ* =0.09
	Yes	112 (37.2)	61 (33.7)	51 (42.5)	
Most commonly consumed functional products for dinner					
Crispbread and waffles	No	230 (76.4)	141 (77.9)	89 (74.2)	χ^2=0.56 p=0.46 Φ =0.04
	Yes	71 (23.6)	40 (22.1)	31 (25.8)	
Fruit teas, herbal teas, herb blends	No	216 (71.8)	127 (70.2)	89 (74.2)	χ^2=0.57 p=0.45 Φ =0.04
	Yes	85 (28.2)	54 (29.8)	31 (25.8)	
Low-fat enriched cheeses. curds, and kefir	No	244 (81.1)	142 (78.5)	102 (85.0)	χ^2=2.01 p=0.16 *Φ* =0.08
	Yes	57 (18.9)	39 (21.5)	18 (15.0)	
Most commonly consumed functional products between main meals					
Yogurts	No	228 (75.7)	129 (71.3)	99 (82.5)	χ^2=4.95 p=0.03 Φ =0.13
	Yes	73 (24.3)	52 (28.7)	21 (17.5)	
Fruit teas, herbal teas, herb blends	No	218 (72.4)	123 (68.0)	95 (79.2)	χ^2=4.54 p=0.03 Φ =0.12
	Yes	83 (27.6)	58 (32.0)	25 (20.8)	
Chocolate	No	207 (69.0)	124 (68.5)	83 (69.7)	χ^2=0.05 p=0.82 *Φ* =0.01
	Yes	93 (31.0)	57 (31.5)	36 (30.3)	

N - number of observations; χ^2 - *Chi* square test result; p - level of statistical significance; Φ-Yule'a - Mean Square Contingency Coefficient

DISCUSSION

Nowadays, there is an increase in consumer's awareness of health issues. The consequence of which is an increased interest in functional food, which helps to meet this task, both in terms of improving health as well as improving the quality and length of life. The analysis of my own research results showed that 42.5% of the respondents pointed to the correct answer defining functional food as food having a documented beneficial effect on health beyond that resulting from the presence of nutrients considered essential. In turn, in a study conducted by Demir Hüly among students of nutrition and dietetics, 46.3% of

fourth-year students associate functional food with functional products and 43.9% with food products that have a beneficial effect on the body. However, in the first-year group, 25.6% associate it with food products that have a beneficial effect on the body, 23.0% with functional products, and 41% did not answer this question [10]. Meanwhile, in the study by Balogh et al. [5] conducted among 168 consumers, 91.7% of them indicated the correct definition of functional food as one that has detectable positive effects on one or more important body functions. However, in the first-year group, 25.6% associate it with food products that have a beneficial effect on the body, 23.0% with functional products, and 41% did not answer this question [10]. Meanwhile, in a study by Balogh et al. conducted among 168 consumers, 91.7% of them indicated the correct definition of functional food as one that has detectable positive effects on one or more important body functions [5].

The results of our own research showed that 37.2% of people indicated that functional food is one that, when used in the daily diet, has an additional positive effect on the human body, including reducing the risk of disease development. This is consistent with the results of the research by the Interactive Market Research Institute, conducted among 1,000 people over 15 years of age, where a similar answer was given by 50% of respondents, characterizing functional food as used in a daily diet and having an additional positive effect on the human body, including reducing the risk of developing a disease. [21]. Author's research shows that the largest percentage of respondents (43.2%) rate their knowledge of functional food as sufficient. In turn, the results of the study by Zabrocki and Suszek show that 50.7% of the people participating in the study describe their level of knowledge as rather small and fragmentary [28]. As the results of our own research show, 41.9% respondents most often indicated the correct definition of a prebiotic. Students of the Medical University of Wrocław demonstrated greater knowledge in this area, with 85% correctly defining prebiotics [13] Also in the study by Turgul Ersak et al. conducted among obstetricians, 61.8% of them working for less than 12 years and 60% of them working for 12 years or more were able to define prebiotics correctly as compounds that stimulate the growth and activity of beneficial microorganisms [25]. In our own study, 66.1% of respondents declared that the main function of probiotics is to support the human immune system. As a comparison, in the study by Jamy-Kmiecik et al., 52.4% of people gave correct answers, stating that probiotics have an immunological and digestive protective function [13]. However, in the study by Babin et al. conducted among students and lecturers of Sechenov University in Moscow, it was shown that the surveyed people knew that probiotics have a beneficial effect on digestion (98% of lecturers and 95% of students), were aware of the role of probiotics in the functioning of the immune system (93% of lecturers and 85% of students), in the fight against obesity (72% of lecturers and 66% of students) and in the prevention of respiratory (76% of lecturers and 56% of students) and urinary tract infections (65% of lecturers and 63% of students). However, they are less likely to associate the impact of probiotics on heart health (52% of lecturers and 56% of students) [4]. According to the study by Betz et al. conducted among patients from hospitals in Chicago, 60% of those who knew the correct definition of probiotics believed that they had a beneficial effect on heart [7].

The author's study shows that most people do not consume functional food. However, taking into account the fact that only 42.5% of respondents are able to indicate the correct definition of functional food, its consumption at least several times a month by 43.5% of all the respondents can be considered sufficient. This result contradicts the findings of a study conducted by Akhter and Dil Bahadur, where 37% of the participants indicated that they consume functional food daily

According to the author's research, the factor encouraging people to consume functional foods is the desire to lead a healthy lifestyle (56.2% of responses) and to take preventive measures against chronic diseases (41.4% of responses). In turn, in the study by Krupa and Dec, the most frequently mentioned factors were health care, affordable price and taste; such answers were given by 51% and 39% of people, respectively [15]. Altun et al. conducted a study on a group of 550 dietitians who graduated from the Faculty of Nutrition and Dietetics of universities in Turkey. As the authors showed, during the COVID-19 pandemic, 38.5% of them consumed functional food to maintain intestinal health, 36.8% wanted to lead a healthy lifestyle and 34.9% to protect themselves against COVID-19 [3].

Our own research indicates that, when choosing functional products, the composition of the product is of the utmost importance, in addition to price - this answer was given by 54.7% of respondents. Similar results were obtained in research by the Market Research Institute, where the same answer was given by 49% of respondents [21]. According to our own research, the majority of individuals obtain information about functional food from labels on the packaging of such food (58.8%). Quantitative research by Krupa and Dec conducted among customers of grocery stores also shows that the most frequently indicated source of information about the biological value of these products are the labels on the packaging [15]. As our own research shows, 54.0% of individuals most commonly purchase functional food in supermarkets

or shopping centers. However, in a study by Deborah et al. conducted among a group of 427 Italian consumers, as many as 90% indicated supermarkets as the place of purchase for this type of food [8].

Our own research, similar to a study conducted by the Interactive Market Research Institute, indicates variations in the consumption of functional products depending on the type of meal. Both studies show that the most frequently consumed products for breakfast are cereals or muesli. For lunch, people most often choose vegetable oils, juices, nectars and multivitamin drinks. However, for dinner, crispbread and wafers are preferred, and between main meals, chocolate, juices, nectars, multivitamin drinks, and yogurts are reached for [21]. In contrast, in the study by Krupa and Dec, fruit and vegetable preparations, probiotic yoghurts, cereals and bakery products were indicated as the most commonly consumed products. However, in this study the consumption of these products was not divided in relation to meals [15].

CONCLUSIONS

Knowledge of functional foods is unsatisfactory, and no differences in the knowledge of women and men have been observed.

The consumption of functional food is generally low, and no differences in its consumption have been observed between women and men.

It seems important to continue spreading nutritional education regarding the role of functional food in both prevention and treatment of many diseases.

Conflict of interest

The authors declare no conflict of interest.

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Received: 06.11.2023
Revised: 25.01.2024
Accepted: 29.01.2024

Rocz Panstw Zakl Hig 2024;75(1):45-58
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0296

ORIGINAL ARTICLE

ADHERENCE TO THE MEDITERRANEAN DIET IN TWO MOROCCAN POPULATIONS LIVING AT DIFFERENT DISTANCES FROM THE MEDITERRANEAN SEA

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ABSTRACT

Background. There is growing strong scientific evidence over the past few decades that the Mediterranean diet (MD) has protective effects on cardiometabolic health.

Objective. This study aimed to assess MD adherence and its association with sociodemographic and lifestyle factors among women living in two Moroccan provinces, El Jadida and Tetouan, located at different distances from the Mediterranean Sea.

Material and methods. It is a cross-sectional study involved 355 subjects of which 55.8% reside in the province of El Jadida, and data on socio-demographic characteristics, lifestyle, cardiovascular risks, medical history and of food frequency consumption were collected. Compliance with the MD was assessed with a simplified MD adherence score based on the weekly frequency of consumption of eight food groups.

Results. The overall mean Simplified Mediterranean Diet Score was 4.37 ± 1.47 with inadequate compliance in 55.2% of the sample. No significant association was found between adherence to MD and geographic, socio-demographic, lifestyle or the major cardiovascular risk factors. However, the participants do not comply with half of the recommendations based on the Mediterranean diet pyramid. The lowest level of compliance was observed for olive oil, followed by sweets, eggs, potatoes, fruits, red meat, vegetables, legumes, olives, nuts and seeds. The increased contribution of sugars, dairy products and meat to the overall food intake is significant in the category with high adherence to MD.

Conclusion. The study data indicate that Mediterranean Diet is far from being a global pattern in this Moroccan population. The study draws attention to the need for a promoting intervention to maintain this pattern as the original diet in the region.

Key words: *Mediterranean diet, adherence to Mediterranean diet, Mediterranean diet score, distance to Mediterranean sea, lifestyle factors, women, Morocco*

INTRODUCTION

The Mediterranean diet (MD) is known as a protective dietary pattern against diseases including cardiovascular disease, diabetes, metabolic syndrome, obesity, cancer and other chronic non-communicable diseases [1] in populations living around the Mediterranean basin but also elsewhere. Many studies have attributed this effect to the high consumption of plant foods and the low consumption of foods of animal origin [2, 3, 4, 5, 6, 7, 8]. However, this dietary model refers to the traditional food habits of the countries populations around the Mediterranean [9], with benefits on health but also on food and nutritional security, on the environment and biodiversity protection [10], making of this model a sustainable diet [11]. It consists of the use of olive oil as the main source of fat; a high consumption of vegetables, legumes, a moderate

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

consumption of fish; fruits, cereals, nuts, eggs, dairy products, white meat, wine; and low consumption of red meat, cold cuts, potatoes and sweets, all combined with regular physical activity [12]. There is not a single model but rather several MDs since each country in the Mediterranean area has its own gastronomic customs influenced by particular socio-cultural, religious and economic factors [13]. Indeed, wine, beer and pork are excluded from the diet of Muslim countries, whereas they are widely consumed in European Mediterranean countries [14, 15]. In addition, large variations in intake and a westernization of dietary habits resulting in a decrease in adherence to MD have also been reported in Mediterranean and non-Mediterranean countries [16, 17]. Morocco, a middle-income country in North Africa, is located on the southwest coast of the Mediterranean Sea and is divided into 12 regions with an estimated population of 36 million in 2020. The official languages are Arabic and Berber but French and Spanish are also spoken there because of a previous history occupation. The majority of the population is of the Muslim faith [18, 19]. Like several developing and Mediterranean countries, Morocco is experiencing the different facets of a global transition, including a demographic, epidemiological and nutritional transition [20]. The Moroccan society has experienced remarkable socioeconomic disparities and stable economic growth over the past decades [21]. The lifestyle and dietary habits of the population have also changed [22] as a result of population growth and increasing urbanization with almost 63% of the population living in urban environment [23], economic development, globalization and increased production and food industry. These changes led to a deviation from MD in relationship with the nutritional transition and the increasing adoption of a pattern marked by sedentary behavior and a shift from consumption of traditional foods rich in whole grains, vegetables and fruits and poor in fat, to that of foods high in refined cereals, sugar, fat and animal products [11]. The origin of nutritional transition have been associated to economic development parallel to concomitant lifestyle changes including food, but also socioeconomic and demographic factors that influence the eating habits of Moroccan populations.

The present comparative study therefore aimed to identify the sociodemographic and lifestyle factors, associated with adherence to MD, in Moroccan women living in two provinces El Jadida and Tetouan located at different distances from the Mediterranean Sea.

MATERIAL AND METHODS

Study participants and study area

The cross-sectional study was carried out in 2016 in two provinces, El Jadida and Tetouan, located at different distances from the Mediterranean Sea. Tetouan is located on the Mediterranean coast and El Jadida is 466 km away from it. The study involved a population of women aged 18 and over. Pregnant and breastfeeding women, as well as physically and mentally handicapped subjects were excluded from the study. The study was conducted in accordance with the Declaration of Helsinki and the study protocol was accepted by the Moroccan dedicated authority of the Ministry of Health. All participants were informed about the study objectives and that they can leave the survey at any time if they wish, they also gave their consent before taking part in the survey.

The province of El Jadida, included in the greater Casablanca-Settat Region and located on the Moroccan Atlantic coast, is one of the richest regions, due to its climate diversity, its geographical position and its agricultural activity (Figure 1). According to the General Population Census of 2014 [24], the total population of the province of El Jadida is 786,716, 40% in urban and 60% in rural areas.

Figure 1. MAP showing the geographical location of provinces El Jadida and Tetouan

The province of Tetouan, part of the Greater Region of Tangier-Tetouan, is located in the extreme northwest of the Kingdom, in the eastern part of the Tingitan peninsula and the Rif mountains. Located at the junction of two seas (the Mediterranean and the Atlantic Ocean) and only at 14 kilometers from the European continent, the province is bounded by the Strait of Gibraltar and the Mediterranean Sea to the north and by the Atlantic Ocean to the west. Its position on two coastal facades and the presence of large reliefs and medium heights, the biogeographical context

of the Tangier-Tetouan Region gives it a temperate Mediterranean climate with oceanic influence. The region is one of the most fertile areas of Morocco, with a rainfall rate between 1000 and 1800 mm/year and high biodiversity. The total population of the province of Tetouan is 549,769 inhabitants, with 72% in urban and 28% in rural areas [25].

Data on medical history sociodemographic characteristics and lifestyle factors

Data were collected using a validated questionnaire completed face-to-face by trained interviewers. The information collected concerned medical history (hypertension, diabetes, etc.), socio-demographic characteristics (age, place of residence (urban or rural), level of education, marital status, profession, housing category, monthly family income) and household characteristics (type of dwelling, and household size). Age was recorded and classified into three categories (<35; 35-47 and ≥47) based on the distribution of the sample in tertiles. Educational attainment was classified into three categories: illiterate (0 years), ≤6 years of schooling or informal education and >6 years of schooling (secondary, university). Marital status was divided into two classes: married and unmarried (single or divorced and widowed), the profession in two groups: with employment (active or student) and without paid employment (unemployed and housewife). Housing has been grouped into 4 classes: traditional housing (working-class district), luxurious (villa), modern (apartment) and poor housing (including slums, bedrooms), the family in composite or nuclear type, the family income was classified according to the categories established by HCP in low income (< 3000 MAD) corresponding to the minimum wage in Morocco (International Labor Organization, 2020), Medium (3000 to 4999 MAD) and High (5000≥MAD), the equivalent exchange rate is: 1 MAD = 0.095 Euro).

Data on lifestyle were collected; they included the tobacco consumption and the level of physical activity. Tobacco use is categorized into "smokers" (current daily and occasional smokers), "ex-smokers" (if they have quit smoking for more than 3 months at the time of the survey) and "Never" (they had never smoked in their life). Physical activity assessed by a questionnaire included moderate and vigorous activities performed at work and in leisure during a usual week of the previous month. Physical activity was then defined according to WHO guidelines recommending the practice of at least 30 minutes of regular, moderate or vigorous physical activity on most days (including household activities) [26].

Anthropometric measurements

All anthropometric data was recorded. Height (± 0.5 cm) and weight (± 0.5 kg) were measured and the body mass index (BMI) is calculated by dividing the body weight (in kg) by the square of the height (in m) and the WHO thresholds for overweight (BMI ≥25) and obesity (BMI ≥30) were used to define general obesity (World Health Organization, 2000).

Blood pressure was measured in women in a seated position using a mercury sphygmomanometer after a rest period of at least 10 min. Two groups of women are established according to their BP, the group with and the group without high BP. Elevated BP is defined by the Adult Treatment Panel III criteria as systolic blood pressure ≥ 130 mmHg and/or diastolic BP ≥ 85 mm Hg or on medication for hypertension [27].

Simplified Mediterranean dietary score

In this study, the evaluation of the degree of adherence to MD was carried out using a simplified Mediterranean diet score (MDS) [28], constructed following an adaptation of the MDS, proposed by *Trichopoulou* et al. [8, 29]. The score calculation is based on the self-reported frequency of weekly intake of each food group, with the exception of fruit, where the total weekly intake was calculated by multiplying the number of days of intake per week by the number of servings consumed per day, this score includes eight components (vegetables, legumes, fruits, cereals, olive oil, fish, meat and dairy products). To calculate the total frequency of each component, the frequency of the elements that belong to it was added, dairy products (milk, yogurt and cheese), cereals (bread, cereals, potatoes, rice, pasta and couscous) and meat (red meat, white meat and processed meat). As the ratio of monounsaturated to saturated fatty acids could not be calculated for fat intake, the olive oil intake was considered as the main dietary source of monounsaturated fatty acids in Morocco, in the absence of pork consumption for religious reasons [28]. A value of 0 or 1 was assigned to each of the components using the gender-specific median of the sample as the threshold. For beneficial components (vegetables, legumes, fruits, grains, and fish), people with intakes below the median were assigned a value of 0, and people with intakes at or above the median were assigned a value of 1. People who consumed the olive oil for seasoning or for cooking were given a value of 1 and non-consumers are given value of 0. For the presumed harmful components (meat and dairy), people with consumption below the median were assigned a value of 1, and people with consumption at or above the median were assigned a value of 0. Thus, people who consumed olive oil for seasoning or cooking were given a value of 1 and 0 for non-consumers.

Given that alcohol consumption is prohibited for religious reasons, and therefore not usual or probably under-reported in the Moroccan population, in particular women, this component was not taken into account in the calculation of the simplified MD score. Thus, the simplified total score of the MD ranging from 0 (minimum adhesion) to 8 (maximum adhesion), makes it possible to classify the participants into two groups according to the level of their adhesion to the MD, "low" adhesion to the MD (0 to 4 points), and "high" adherence to the MD (5 to 8 points).

Statistical analysis

Food frequency consumption and sociodemographic characteristics of the sample were described according to high (simplified MeDi score 5–8) vs low (simplified MeDi score 0–4) MeDi adherence. A descriptive analysis was conducted to compute medians and means with standard deviation (SD) for quantitative variables and frequencies (%) for qualitative variables. Student's t-test was used for comparison of the means and the chi-square tests for comparison of proportions between the two groups. The level of significance was established at $p < 0.05$. All analyses were performed with the use of Statistical Package for the Social Sciences SPSS (version 23).

RESULTS

A number of 355 out of 500 women recruited in the two provinces studied, participated in the survey. Individuals not included did not complete all of the survey data. The final sample with complete food data included thus 198 (55.8%) women from El Jadida and 157 (44.2%) from Tetouan. The women average age was 41.57 ± 12.91 years with a difference between the two provinces (43.75 years± 13.09 in El Jadida against 38.8 years± 12.16 in Tetouan).

In the overall sample, the prevalence of obesity was 32.1%, that of diabetes 12.1% and that of hypertension was 41.1% with higher prevalence in El Jadida than in Tetouan respectively for obesity (43.4% vs 17.8%; $p<0.001$) and diabetes (17.7% vs 5.1%; $p<0.001$).

Table 1 presents data on the socio-demographic and lifestyle characteristics according to the region of the population under study. The table shows significant differences between the populations in the two provinces, for household size, number of children, type of dwelling, physical activity, age groups, income average and occupation of the head of household, place of residence and for the type of family.

Table 1. The population sociodemographic and lifestyle characteristics according to the region

	Total % (n=355)	El Jadida (n = 198)	Tetouan (n = 157)	P-value
Area of residence				
Urban	38.3 (136)	29.8 (59)	49 (77)	0.000
Rural	61.7 (219)	70.2 (139)	51 (80)	
Age groups (years)				
≤35	32.1 (114)	26.3 (52)	39.5 (62)	0.006
35–47	31.5 (112)	30.8 (61)	32.5 (51)	
≥47	36.3 (129)	42.9 (85)	28 (44)	
Marital status				
Married	81.7 (290)	79.8 (158)	84.1 (132)	0.185
Not Married	18.3 (65)	20.2 (40)	15.9 (25)	
Education				
Illeterate	44.5 (158)	48.5 (96)	39.5 (62)	0.1
< 6 years	30.7 (109)	30.8 (61)	30.6 (48)	
≥ 6 years	24.8 (88)	20.7 (41)	29.9 (47)	
Average income (MAD)				
< 3000 MAD	60.2 (213)	63.6 (126)	55.8 (87)	0.004
3000 - 5000	22.3 (79)	24.7 (49)	19.2 (30)	
≥ 5000	17.5 (62)	11.6 (23)	25 (39)	
Household head occupation				
Unemployed	79.4 (282)	85.4 (169)	72 (113)	0.002
Employed	20.6 (73)	14.6 (29)	28 (44)	

Household size				
<4 peoples	17.5 (62)	17.7 (35)	17.2 (27)	0.04
4 to 6 people	57.2 (203)	52 (103)	63.7 (100)	
>6 peoples	25.4 (90)	30.3(60)	19.1 (30)	
Family type				
Composite	63.9 (227)	55.6 (110)	74.5 (117)	0.000
Nuclear	36.1 (128)	44.4 (88)	25.5 (40)	
Number of children/woman				
No children	13.5 (48)	10.6 (21)	17.2 (27)	0.022
1 to 2 children	30.1 (107)	26.8 (53)	34.4 (54)	
3 and more	56.3 (200)	57 (124)	55.6 (76)	
Housing				
Poor housing	38.3 (136)	42.9 (85)	32.5 (51)	0.031
Modern	11.8 (42)	8.1 (16)	16.6 (26)	
Luxurious	2.8 (10)	3.5 (7)	1.9 (3)	
Traditional housing	47 (167)	45.5 (90)	49 (77)	
Smoking				
Current and ex-smokers	1.7 (6)	2.5 (5)	0.6 (1)	0.171
Never smoke	98.3 (349)	97.5 (193)	99.4 (156)	
Physical activity (≥ 30 min/day)				
Yes	86.8 (308)	90.4 (179)	82.2 (129)	0.017
No	13.82(47)	9.6 (19)	17.8 (28)	

Chi^2 test (percent (number)), statistically significant differences are defined as $P< 0.05$.

The distribution according to place of residence reveals that globally the female population was predominantly rural (61.7%) representing 70.2% in El Jadida against 51% in Tetouan. The proportion of women in the youngest age group was higher (39.5%) in the province of Tetouan and that of the oldest age group was the most present in El Jadida (42.9%).

In addition, the population as a whole (60.2%) had the lowest monthly income, the majority of which (63.6%) was in El Jadida. Furthermore, 85.3% of the sample lived in popular or precarious housing. The proportion of people considered to be physically active was 86.8%, women who were non-smokers or did not consume tobacco were the most represented (98.3%) and 63.9% of women belonged to composite families, mainly in the province of Tetouan with 56.3% including 3 or more children.

Consumption of major foods and food groups by region is shown in Table 2. The data in the table reveals significant differences in dietary intakes of different food groups. The women of El Jadida had a higher frequency of weekly consumption of meat (red, white and processed meat), potatoes (mean = 5.44 frequency), vegetables (mean = 6.96 frequency), cereals (mean = 5.05 daily frequency), herbs, spices, garlic and onions (mean = 6.67 frequency) and drinks (mean = 5.43 daily frequency) than those in Tetouan. On the other hand, women in Tetouan consumed more legumes (mean = 3.59 weekly frequency), eggs (mean = 3.29 weekly frequency) and more olive oil ((mean = 3.29 weekly frequency)= 0.90 daily frequency). Olive oil was the main source of fat for cooking or seasoning (86.2%). However, no difference was found for other foods (dairy products, sweets, fruits, olives, nuts, seeds and fish).

Compliance with the guide or the Mediterranean diet pyramid' recommendations, shows that the diet of the study women was not compliant for all types of food groups and that, the diet of at least half of these women, was inconsistent for red meat, legumes, potatoes, eggs, sweets, olives, nuts and seeds, vegetables, fruits, and olive oil.

Figure 2 presents the distribution of the studied women sample, according to adherence to the MD and Figure 3 shows the distribution of the scores of adherence to this diet. In both provinces, more than half of the women (55.2%) had high adherence to MD (against 44.8% with low adherence) (Figure 2), but no significant difference is reported between the two regions. The results also show a bell-shaped distribution of the different adhesion scores between 0 and 8, as illustrated in Figure 3.

The study sample sociodemographic and lifestyle characteristics are presented in Table 3. The results show that there is no significant association of sociodemographic characteristics, neither lifestyle

Table 2. The mean values of usual consumption of major foods or food groups according to the region among the population studied

Dietary variable	Servings*	Total (N)	Consumption (%)	Recommendation (%)	EL JADIDA	TETOUAN	P-value
Weekly							
Red meat	<2	3.64 ± 2.73	95.8	32.1	4.31 ± 2.99	2.79 ± 2.08	0.000
White meat	2	2.94 ± 2.69	92.4	52.7	3.71 ± 3.02	2.97 ± 1.79	0.000
Processed meat	≤1	0.66 ± 0.07	77.2	82.8	0.86 ± 0.1	0.42 ± 0.08	0.002
Legumes	≥2	2.7 ± 0.22	94.4	48.7	1.99 ± 0.1	3.59 ± 0.47	0.000
Potatoes	≤3	5.11 ± 3.26	92.4	29.3	5.44 ± 3.52	4.70 ± 2.85	0.034
Fish	≥2	3.04 ± 2.78	95.8	56.1	3.07 ± 2.66	3.01 ± 2.94	0.838
Eggs	2-4	2.8 ± 2.74	82.8	23.3	2.68 ± 2.66	3.29 ± 2.82	0.035
Sweets	≤2	17.27 ± 11.85	94.9	7.0	17.97 ± 12.38	16.38 ± 11.13	0.211
Daily							
Dairy products	2	1.8 ± 1.32	94.6	62.8	1.68 ± 1.33	1.94 ± 1.31	0.076
Olives, nuts, seeds	1–2	1.03± 0.83	94.6	36.4	0.97 ± 0.71	1.12 ± 0.96	0.099
Herbs, spices, garlic, onions	-	6.09 ± 5.81	97.2	-	6.67 ± 5.42	5.35 ± 2.49	0.033
Water and herbal infusions	-	4.78 ± 3.87	94.6	-	5.43 ± 4.16	3.95 ± 3.32	0.000
Every Main Meal							
Vegetables	≥2	6.39 ± 2.39	97.2	44.8	6.96 ± 2.97	5.67 ± 2.81	0.000
Fruits	1–2	4.15 ± 3.37	97.2	29.5	3.84 ± 3.03	4.53 ± 3.73	0.056
Cereals	1–2	4.65 ± 1.74	98	64.2	5.05 ± 1.6	4.15 ± 1.78	0.000
Olive oil	1	0.77 ± 0.62	86.2	2.0	0.67 ± 0.52	0.90 ± 0.71	0.000

Recommendations based on the Mediterranean diet pyramid and other studies (Bach-Faig et al., 2011b; Sofi et al., 2013). EMM: Every Main Meal. Dairy products (milk, yogurt, and cheese), cereals (bread, cereals, rice, pasta, and couscous), Potatoes (potato, sweet potato), sweets (Sugar, jelly, candies, pastries, and sweetened fruit juices), white meat (poultry and turkeys) and red meat (veal, lamb, camel, and goat). Variables are presented as mean (standard deviation). Statistically significant differences are *defined as $P < 0.05$.

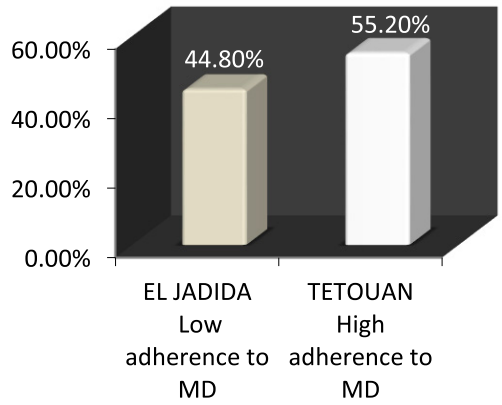

Figure2. Distribution of adherence to Mediterranean Diet

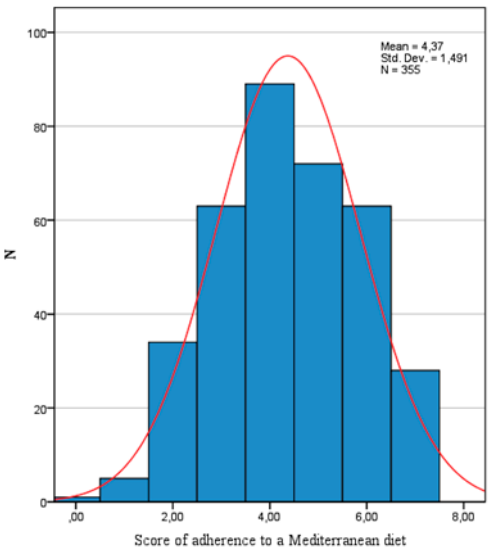

Figure3. Levels of adherence to a Mediterranean Diet

Table 3. The sociodemographic and lifestyle characteristics and cardiovascular risk factors according to the adherence to MD

	Total (n = 355)	Low 1–4 (n = 196)	High 5–8 (n = 159)	P-value
Area of residence				
Urban	38.3 (136)	37.8 (74)	39 (62)	0.448
Rural	61.7 (219)	62.2 (122)	61 (97)	
Age groups (years)				
≤35	32.1 (114)	32.7 (64)	31.4 (50)	
35–47	31.5 (112)	29.6 (58)	34 (54)	0.666
≥47	36.3 (129)	37.8 (74)	34.6 (55)	
Marital status				
Married	81.7 (290)	78.6 (154)	85.5 (136)	0.06
Not Married	18.3 (65)	21.4 (42)	14.5 (23)	
Education				
Illeterate	44.5 (158)	43.9 (86)	45.3 (72)	0.915
< 6 years	30.7 (109)	31.6 (62)	29.6 (47)	
≥ 6 years	24.8 (88)	24.5 (48)	25.2 (40)	
Average family income (MAD)				
< 3000 MAD	60.2 (213)	64.3 (126)	55.1 (87)	0.176
3000 - 4999	22.3 (79)	20.9 (41)	24.1 (38)	
≥ 5000	17.5 (62)	14.8 (29)	20.9 (33)	
Household head occupation				
Unemployed	79.4 (282)	82.1 (161)	76.1 (121)	0.102
Employed	20.6 (73)	17.9 (35)	24.3 (38)	
Household size				
<4 people	17.5 (62)	<ee	15.7 (25)	0.534
4 to 6 people	57.2 (203)	54.6 (107)	60.4 (96)	
>6 people	25.4 (90)	26.5 (52)	23.9 (38)	
Family type				
Composite family	63.9 (227)	61.2 (120)	67.3 (107)	0.141
Nuclear family	36.1 (128)	38.8 (76)	32.7 (52)	

Number of children				
No children	13.5 (48)	15.3 (30)	11.3 (18)	0.498
1 to 2 children	30.1 (107)	28.6 (56)	32.1 (51)	
3 and more	56.3 (200)	56.1 (110)	56.6 (90)	
Housing				
Poor housing	38.3 (136)	34.7 (68)	42.8 (68)	
Modern	11.8 (42)	13.8 (27)	9.4 (15)	0.278
Luxurious	2.8 (10)	3.6 (7)	1.9 (3)	
Traditional housing	47 (167)	48 (94)	45.9 (73)	
Smoking				
Current and ex-smokers	1.7 (6)	2.6 (5)	0.6 (1)	0.164
Never smoker	98.3 (349)	97.4 (191)	99.4 (158)	
Physical activity (≥ 30 min/day)				
Yes	86.8 (308)	87.8 (172)	85.5 (136)	0.323
No	13.2(47)	12.2 (24)	14.5 (23)	
Class BMI (Kg/m2)				
< 25	26.8 (95)	27.6 (54)	25.8 (41)	
25 – 29.9	41.1 (146)	44.9 (88)	36.5 (58)	0.108
≥ 30	32.1 (114)	27.6 (54)	37.7 (60)	
Hypertension				
No	58.9 (209)	56.6 (111)	61.6 (98)	
Yes	41.1 (146)	43.4 (85)	38.4 (61)	0.199
Diabetes				
No	87.9 (312)	86.7 (170)	89.3 (142)	0.284
Yes	12.1 (43)	13.3 (26)	10.7 (17)	

BMI: body mass Index.chi^2 test (percent (number)), statistically significant differences are defined as P< 0.05

nor the other cardiovascular risk factors studied with the adhesion to MD. Comparative analysis showed no statistically significant difference of major cardiovascular risk factors, including obesity, overweight (p=0.108), diastolic and systolic hypertension (p=0.199), and diabetes (p=0.284) between the two population groups with inadequate or adequate levels of adherence to the Mediterranean diet; while a difference but not statistically significant was found for heart rate. The same result is found for both regions analyzed independently.

Table 4 presents the results concerning the distribution of the simplified MD score according to compliance with adherence to the Mediterranean diet (low or high compliance). The simplified Mediterranean diet score value was on average 4.37±1.49 in the general study population, with no significant difference between the two regions. However, a significant difference between both low and high compliance groups was found at the level of each region (P < 0.000). In accordance with the objective, the consumption of vegetables, cereals, fruits, legumes, fish and olive oil were significantly increased with the increased adherence to the MD and were lower with lower adherence to MD in both regions (P<0.0001). However, the consumption of dairy products and meat were not significantly different among the women of the two categories of adherence to the DM or between the two regions of residence (P =0.091 and P =0.928 successively). This relationship of food groups to categories of adherence to the MD remains the same in the whole population for the dairy products (P =0.1) and meat (P = 0.136) consumption.

DISCUSSION

This work examined the influence of the geographical situation on the degree of adherence to Mediterranean diet, in women from two Moroccan regions at different distances to the Mediterranean basin. The study women population were from two provinces, Tetouan in the Tangier-Tetouan-Al Hoceima Region to the north near the Mediterranean Sea and El Jadida in the Casablanca-Settat region, located in the center on the Atlantic coast, both distant of 486 Km. To meet the targeted objectives, food intake and adherence to MD as well as compliance

Table 4. Distribution of Simplified Mediterranean Dietary Score (SMDS) and dietary intake of food groups according to the categories of adherence to Mediterranean diet and region of residence, n = 355

	EL JADIDA (n = 198)				**TETOUAN (n = 157)**			
	All	Low adherence 1–4 (n = 97)	High adherence 5–8(n = 101)	*P-value*	All	Low adherence 1–4 (n = 81)	High adherence 5–8 (n = 76)	*P-value*
SMDS	4.37 ± 1.47	3.33 ± 0.97	5.68 ± 0.79	0.000	4.36 ± 1.51	3.22 ± 0.87	5.74 ± 0.8	0.000
Food groups								
Dairy products[(a)]	1.68± 1.33	1.58 ± 1.21	1.81 ± 1.46	0.220	1.94 ± 1.31	1.77 ± 1.16	2.13 ± 1.45	0.091
Vegetables[(a)]	6.96 ± 2.97	5.74 ± 2.27	8.48 ± 3.05	0.000	5.67 ± 2.81	4.37 ± 2.55	7.24 ± 2.27	0.000
Cereals[(a)]	5.05 ± 1.6	4.5 ± 1.64	5.73 ± 1.26	0.000	4.15 ± 1.78	3.63 ± 1.83	4.78 ± 1.51	0.000
Fruits[(a)]	3.84 ± 3.03	2.95 ± 2.65	4.98 ± 3.13	0.000	4.53 ± 3.73	3.03 ± 2.66	6.35 ± 4.04	0.000
Meat [(b)]	8.89 ± 5.32	8.25 ± 4.82	9.69 ± 5.82	0.059	5.19 ± 3.63	5.16 ± 3.87	5.22 ± 3.35	0.928
Legume[s(b)]	1.99 ± 1.48	1.5 ± 1.37	2.61 ± 1.39	0.000	3.59 ± 0.47	2.45 ± 0.32	4.98 ± 0.96	0.008
Fish[(b)]	3.07 ± 2.66	2.05 ± 1.79	4.34 ± 3.01	0.000	3.01 ± 2.94	1.79 ± 1.62	4.48 ± 3.33	0.000
Olive oil (%)	86.9	82.2	92.3	0.029	85.4	71.9	100	0.000

(a)Frequency of consumption per day. (b) Frequency of consumption per week. Dairy products (milk, yogurt, and cheese), cereals (bread, cereals, rice, pasta, and couscous), meat (red meat, white meat, and processed meat), Legumes (Split Peas, Dry Beans, Lentils, Chickpeas) and Vegetables (excludes potatoes) Variables are presented as mean (standard deviation). Statistic significant differences are defined as $P < 0.05$ for t-test for Equality of means or for the Chi2 test according to categories of adherence to Mediterranean diet.

with the recommendations of the Mediterranean dietary pyramid were initially evaluated. This analysis included the study of the dietary intake from food groups according to the categories of adherence to the Mediterranean diet and to the region of residence. In a second step, the relationship of adherence to MD with the populations' socio-demographic and the lifestyle factors in both regions was also analyzed.

Analysis of the data on food intake indicates that women in El Jadida, which belongs to the Casablanca-Settat region, have a higher weekly consumption of meat (red, white, processed), vegetables, cereals, potatoes and a daily consumption of herbs, spices, garlic, onions, water and infusions than the Tetouan region. This result can be due, mainly, to the geographical nature made of vast fertile fields and to a strong agricultural activity in El Jadida region compared to that of Tetouan which is located in the mountain range of the Rif. The red meat production in the Casablanca-Settat region represents indeed, 11% of national production, with a processing and processing capacity of 150,000 tons/year (Ministry of Agriculture, Fisheries, Rural Development, n.d.). The region that includes the Tetouan province, located in the north of the country close to the Mediterranean coast, is an important basin of olive groves and productions of legumes, olives, nuts and seeds, given the climatic conditions particularly rainfall, improved irrigation facilities and production methods. The yields of olive oil production vary from 1.6 to 3 tons/Ha and contribute up to 16% to the deficit in edible oils (50,000 tons) [30, 31].

Many rating systems expressed as indices or scores have been used to measure adherence to the Mediterranean diet. However, the different definition of the maximum and minimum possible values as well as the definition of compliance levels (low, medium and high), make inter-study comparison difficult [32, 33]. In this study, the score used is the Simplified Mediterranean Food Score [28] adapted from the Mediterranean Food Score (MDS) [8]. Although the latter is the first most used score developed for Mediterranean countries, the MDS cannot faithfully reflect the respect of traditional eating habits specific to the Moroccan study population, mainly those related to religion, such as abstinence from alcohol.

Our data showed that the simplified Mediterranean dietary score is below that reported in other Moroccan studies [28, 34] as only 2 out of 5 participating women had their eating habits compliant with at least 40% of the MD characteristics. The same proportion of adherence was found before, in the same region of El Jadida in 2015 but using another Mediterranean score [35]. Although the majority of the study population consumes most of the food groups concerned, this study shows that the present women population does not fully follow the recommendations of the Mediterranean diet pyramid. The most concrete examples are those of the consumption of olive oil and red meat which are 86.2% and 95.8% respectively in the whole population studied while only 2 % and 32.1% have respectively this consumption consistent with the Mediterranean diet pyramid norms. This low compliance with the recommendations of the Mediterranean diet pyramid has been registered in other studies.

These results are in agreement with other studies carried out in other provinces such as Casablanca with regard to the consumption of fish, potatoes and olive oil, but not for the consumption of red meat, dairy products and sweets [34]. The comparable fish consumption is probably linked to the position of these towns on the coast, with greater accessibility to seafood products. The consumption of white meat, processed meat, vegetables and eggs in Casablanca than in the study population, would be due to the fact that the former is the economic capital with largest agglomeration in the country [24], where most people eat out of home or on their workplace. On the other hand, in comparison with the population of Fez [28], the province of Tetouan has similarities in terms of geographical proximity. El Jadida is also comparable with the province of Fez for all food groups except legumes and olives.

The present data show that in general, the high adherence to the Mediterranean diet found among women in the two regions studied, is characterized by a high consumption of vegetables, fresh fruits, legumes, cereals, fish, oil olive oil and low meat consumption (non-significant decrease). However, the consumption of dairy products which is supposed to be lower does not show a significant difference. This is certainly attributed to the non-compliance on the part of the subjects studied of nearly 60% of the recommendations of the Mediterranean diet, which largely explains the low distribution of adherence to the DM which is 44.8%.

The food profile studied shows a marked deviation from the traditional Mediterranean diet, particularly in terms of consumption of animal products (meat and dairy products), sweets, sugar and olive oil. The most surprising low consumption of the latter is probably due to its limited affordability, which could constitute an obstacle to a healthy diet [36, 37, 38, 39] not only in the two regions of study, but also in most Mediterranean countries [40, 41]. These eating habits changes, through the adoption of cheaper and unhealthy foods, will switch to a cheap and westernized diet high in empty calories as part of a nutritional transition expressed in parallel to an increase in the production of food industry, increasing urbanization, economic development and the effect of globalization [11, 12,

20]. On the other hand, the correlations studied of the DM adherence are different from those observed in other Mediterranean countries. Generally, the analysis results do not indicate any detected significant impact of socio-demographic or lifestyle factors on the level of adherence to the Mediterranean diet.

While age was a predictor of adherence to the Mediterranean diet in most Mediterranean areas [32, 41, 42, 4, 44, 45, 46], no association was revealed in the present study, between age groups and adherence to MD. This result corroborates with those of a study previously carried out in the country [28]. In Mediterranean countries, especially those experiencing a nutritional transition, young people are more adopting the Western dietary pattern [45, 47] characterized by, modern, fast and off-home food, while older peoples stick to the traditional lifestyle and eating habits they grew up with and avoid modern dishes and fast foods. However, the present study data do not support this hypothesis, probably because Morocco is in an earlier stage of the nutritional transition in Morocco [20] and to cultural and traditional lifestyle differences between Moroccans and other populations of the Mediterranean Basin. Furthermore, the study sample does not include young aged people (children and adolescents) under the age of 18.

Similarly, no significant differences of the adherence to the Mediterranean diet were revealed according to average family income or education. These results are consistent with those found in other national population studies [28, 34], and could be explained by the characteristics of the study population that is mostly illiterate or poorly educated and of low income. Nevertheless, it is important to emphasize that a good family income regardless of the level of education does not automatically guarantee good nutritional status. Indeed, traditional food, which is rather less expensive and within reach of poor households, was traditionally more plant-based, diversified and healthy. Therefore, the deviation from the traditional MD is mainly linked to changes in eating habits associated with factors related to the overall transition underway in the country. It is rather a lack of nutritional education, associated with the economic and political transition following a set of mutations and changes that the country has experienced and which have given rise to great socio-economic disparities and social categories with contradictory characteristics. These are accompanied by an improvement in the economic level of households associated with the abundance and poor quality of food and living conditions inducing a direct impact on health and nutritional status [11, 48]. Other studies have, conversely, shown a significant association between a healthier diet and higher levels of education and income while, low income affects the type of food products consumers buy in terms of quality and variety [35, 49, 50]. On the other hand, a low level of education is generally associated with limited nutritional knowledge and low awareness of food-related issues.

Contrary to what is reported in the literature, in the present study being married was not associated with the highest adherence to the Mediterranean diet [50, 51]. This finding may be related to weakening potential family influences and the changing role of women in the family over the past decades. Indeed, traditional meal preparation by housewives has declined and eating outside the home has become more common. Regarding housing classes, no association was found between a healthier diet and living in luxurious housing. This is consistent with the result discussed above showing that adherence to the Mediterranean diet is not significantly associated with higher income or education levels in this study population, and consumers can meet their nutritional needs in terms of food quality and diversity. A contradictory result was reported in the province of Fez in Morocco by [28, 34], which showed that the inhabitants of the old and the new medina (town), still retain their traditional way of life while people living in luxurious dwellings may have lower adherence to the Mediterranean diet or the opposite.

Tobacco use was not widespread among the studied women, who were 98.3% non-smokers. As in such communities, it is difficult to determine the actual number of women smokers given the social and cultural considerations preventing them from answering this question. Smoking has already been reported to be positively associated with unhealthy eating habits [52] but also negatively associated with adherence to the traditional Mediterranean diet [53, 54].

The Mediterranean lifestyle also includes regular physical activity. In this study, no relationship was observed between physical activity at work and during leisure time with MD adherence, although the majority of the study population (86.8%) practiced physical activity. The beneficial health effect attributed to MD against coronary artery disease and other related diseases, is not the result of diet alone, but also includes in part a more active leisure lifestyle of those who adhere to the traditional Mediterranean diet. This association has also been confirmed by previous studies [47, 55]. Furthermore, the present study did not reveal any significant impact of medical history or cardiovascular risk factors on the level of adherence to MD. The only noticeable effect was on heart rate which was, on average, slightly lower in participants with high MD adherence scores compared to those with lower ones; this difference was however, not statistically significant. Nevertheless, the cross-sectional design of the present study does not make

it possible to establish the causality between these cardiovascular risk factors and adherence to DM. The high prevalence of these risk factors in previous Moroccan data [56, 57, 58] could partly be explained by the abandonment of the traditional MD, which constitutes a culinary heritage of Morocco and other Mediterranean countries.

CONCLUSIONS

The data from this study show that adherence to the Mediterranean diet is comparable in the two provinces studied regardless of their geographical proximity to the Mediterranean. These results do not reveal any significant association between socio-demographic factors, lifestyle or medical history with MD adherence, regardless of the location or distance of residence from the Mediterranean. The adherence rate to the MD obtained in this work, comparable result and in agreement with that previously reported in the same population and of El Jadida province studied using an adapted score [35] constructed on the first score developed by underlines the validity of the simplified score of the MD used in this study. Furthermore, the data from this work underline a comparable deviation from the traditional Mediterranean diet in the two provinces.

The shift away from MD model observed in the two populations suggests a change in adopted eating habits associated to a loss of culinary traditions and the introduction of fast and modern food. Hence, there is an urgent need for education about healthy properties and benefits of the MD diet through public awareness in order to re-establish the Mediterranean model of diet and lifestyle.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgments

The authors wish to thank the participants to this study, the local authorities of El Jadida Province and the Ministry of Interior for their help with the data collection and their cooperation. The survey was supported by the Moroccan Ministry of Higher Education and Research.

Conflicts of interest

The authors declare that they have no competing interest.

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Receive: 15.06.2023
Accepted: 12.02.2024

Rocz Panstw Zakl Hig 2024;75(1):59-65
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0295

ORIGINAL ARTICLE

ADAPTATION OF THE SCALE OF EFFECTS OF SOCIAL MEDIA ON EATING BEHAVIOR IN HUNGARIAN UNIVERSITY STUDENTS

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ABSTRACT

Background. People live in a technological world, where social media is used very commonly. Social media has effects on eating behaviors, as in other aspects. For this reason, it is important to measure social media effect.

Objective. This study aimed to adapt the Scale of Effects of Social Media on Eating Behaviour (SESMEB) that examines the effect of social media on eating behavior in Hungarian university students.

Material and methods. The SESMEB was translated into the target language by taking various stages. The online questionnaire including general information, social media use, and the eighteen-item SESMEB was used to collect data. The scale was administered to the study group consisting of 213 Hungarian university students, and data from 203 of them were analyzed. Confirmatory factor analyses were performed to test construct validity, and the Cronbach alpha coefficient was calculated for the reliability of the scale in Hungarian.

Results. Total correlation value was higher than 0.50 for all items of the scale. The fit indices were at an acceptable level or had a perfect fit. The t-values were significant at the level of 0.1 and ranged between 2.927 and 5.706. The Spearman–Brown coefficient was calculated at 0.894. The reliability coefficient of the scale was calculated to be 0.866. SESMEB scores were different according to spending time daily, sharing content, and using filters or Photoshop on social media ($p<0.05$).

Conclusions. Higher than 0.80 Cronbach's alpha coefficient and other results show that Hungarian SESMEB is a valid and reliable tool. Therefore, Hungarian SESMEB will be useful for further studies to determine the impact of social media on eating behaviors.

Key words: *eating behavior, Hungarian university students, social media effect, social media scale, reliability, validity*

INTRODUCTION

Current technological developments have a remarkable effect on numerous aspects of people's lives, including the way they spend their free time, read the news, and keep in touch with their friends and family [1]. For this reason, especially Internet usage which is one of the most important technological advancements is on the increase all over the world, social media and social networks are gaining importance day by day. As of April 2021, 4.72 billion people around the world use the Internet, which means 60% of all the people on Earth. Also, social media use continues to grow, with more than 55% of the total world population, and today each global user spends an average of 2 hours and 22 minutes on any social media every day [2]. This situation regarding the Internet and social media usage is also not different in Hungary, and 88% of Hungarian households had an Internet connection in 2020, this shows Internet usage in Hungary is expanding like the globe, and one of the aims of Internet usage is that used social media with percentage %74 [3].

Social media influences its users in various ways, and eating behavior is one of them. When considering the widespread use of social media, it is normal for people to be exactly exposed to norms in our wider social environment, and they influence our eating behavior, and potentially, body mass index [4]. Usage of social networking sites may be contributing to body image concerns, disordered eating, and body change behaviors [5, 6, 7]. As a result of a systematic review

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

that included 20 studies, social networking site usage is associated with body image and eating disorders without depending on the gender of participants [8]. Especially Instagram has become an unofficial health promotion education resource. Health and well-being posts on Instagram can lead people to think more related to highlighting the importance of losing and managing weight [5, 6]. Therefore, upon this effect of social media, it is inevitable for individuals to also change their eating behavior.

Regarding all this information, the fact that with the widespread use of social media, we begin to acquire a social personality in the virtual world and that social media dominates every aspect of life and affects people, is known. Therefore, it is necessary to measure the effect of social media on eating behaviors, especially in young people. Validity and reliability studies should be conducted in the target language to use scales. Therefore, this study aimed to adapt the scale of effects of social media on eating behavior (SESMEB), a scale developed in Turkish, into the Hungarian language.

MATERIAL AND METHODS

Participants

Considering that the developed scale consists of 18 items and one dimension, at least 5 and 10 individuals per item were needed to participate in the study. In this case, a minimum of 90 participants was required. Inclusion criteria of the study: (i) being a volunteer (ii) aged 19-30 (iii) being a Hungarian native speaker (iv) using social media (v) being educated at University of Debrecen (bachelor students, master students, doctoral students) (vi) not educated departments related to nutrition (vii) not having any chronic or mental illness or eating disorder.

The students were informed and invited on the Facebook site, and the online platforms, and 213 students paid attention to this study. Two of them did not agree to attend the study. Eight of them were aged below 19 or above 30. Thus, the data of 203 students were analyzed.

Measures

Content of online questionnaire

The questionnaire was created online way using Google Forms. The introduction part of the online questionnaire form consists of informative sentences related to the study for participants. Before completing the questionnaire, they were required to read this informed consent form, to approve that that they volunteered via a check box and that they met the inclusion criteria. The first part of the questionnaire was related to students' social media use habits. The second part included the SESMEB, and the third part was on the socio-demographic background. This online questionnaire took approximately 5-7 minutes to complete.

Steps of the data collection

Step 1: Translation of SESMEB from Turkish (the main language) to Hungarian (the target language). In the first step of this study, an expert, whose native language was Hungarian and who also knew Turkish translated this scale from Turkish into Hungarian independently.

Step 2: The experts controlled the scale that was translated into Hungarian in terms of terms, language, and understandability.

Step 3: Back translation. To avoid possible language mistakes, a different person from the first step translated the scale from Hungarian to Turkish again to avoid possible language mistakes.

Step 4: The Turkish authors examined the scale translated into Turkish in the third step to check for possible errors and compare it with the original scale.

Step 5: The pilot study. The scale's items were tested by online interviews with eight people who represented the whole student population to test the understandability Hungarian form of the scale.

Step 6: Internal-external validity phase. The final scale with other socio-demographic questions was sent to students.

Assessment of the SESMEB

The scale is a five-point Likert scale (never "1", rarely "2", sometimes "3", often "4" and always "5") which is used to express the level of participation of the items in the scale. The scoring applies to all items. There is no reverse-coded item in the SESMEB. The total score can be calculated. According to this, 18 points in minimum, and 90 points in maximum from the SESMEB scale can be taken as the total point. As a result, the increase in someone's scale score means that eating behaviors are affected by social media (9).

Data analysis

Descriptive statistics were represented as mean and standard deviation or median and interquartile range for continuous variables based on the normality assumption while for categorical variables frequency and percentages were given. The normality assumption for continuous variables was specified with the Kolmogorov-Smirnov test, histogram, and boxplot. To compare two independent groups and more than two independent groups, the Mann-Whitney U test and the Kruskal-Wallis variance analysis were conducted. For pairwise comparisons when the statistical significance was obtained after the Kruskal-Wallis variance analysis, the Dunn-Bonferroni test was applied. The construct validity was examined

with the confirmatory factor analysis (CFA). The factor loadings, item statistics, and model fit indices were evaluated. For reliability, the Cronbach's alpha coefficient, the Spearman-Brown formula for split-half reliability, and the correlation between two halves of the scale were investigated. $p<0.05$ was considered statistically significant. Data were analyzed using the statistical software package IBM SPSS statistics for Windows v.23.0 (10) and AMOS 23.0. (11).

RESULTS

Characteristics of the participants

203 university students from 11 different faculties (except for departments related to nutrition), and most of them were from the Faculty of Economics and Business, attended this study. 35.0%, 63.5%, and 1.5% of participants were male, female, and of other sex, respectively. The mean age of 203 university students was 21.11±2.21 years. The majority of them (92.1%) were single and bachelor's students (Table 1).

Validity of the Hungarian SESMEB

Figure 1 shows the path diagram of the one-factor scale when the standardized factor loadings and error variances were examined for the model.

Factor loadings were above 0.50 for all items when the factors in Table 2. It was found that the t values were significant at the level of 0.1 and changed between 2.927 and 5.706. t values presented information on the

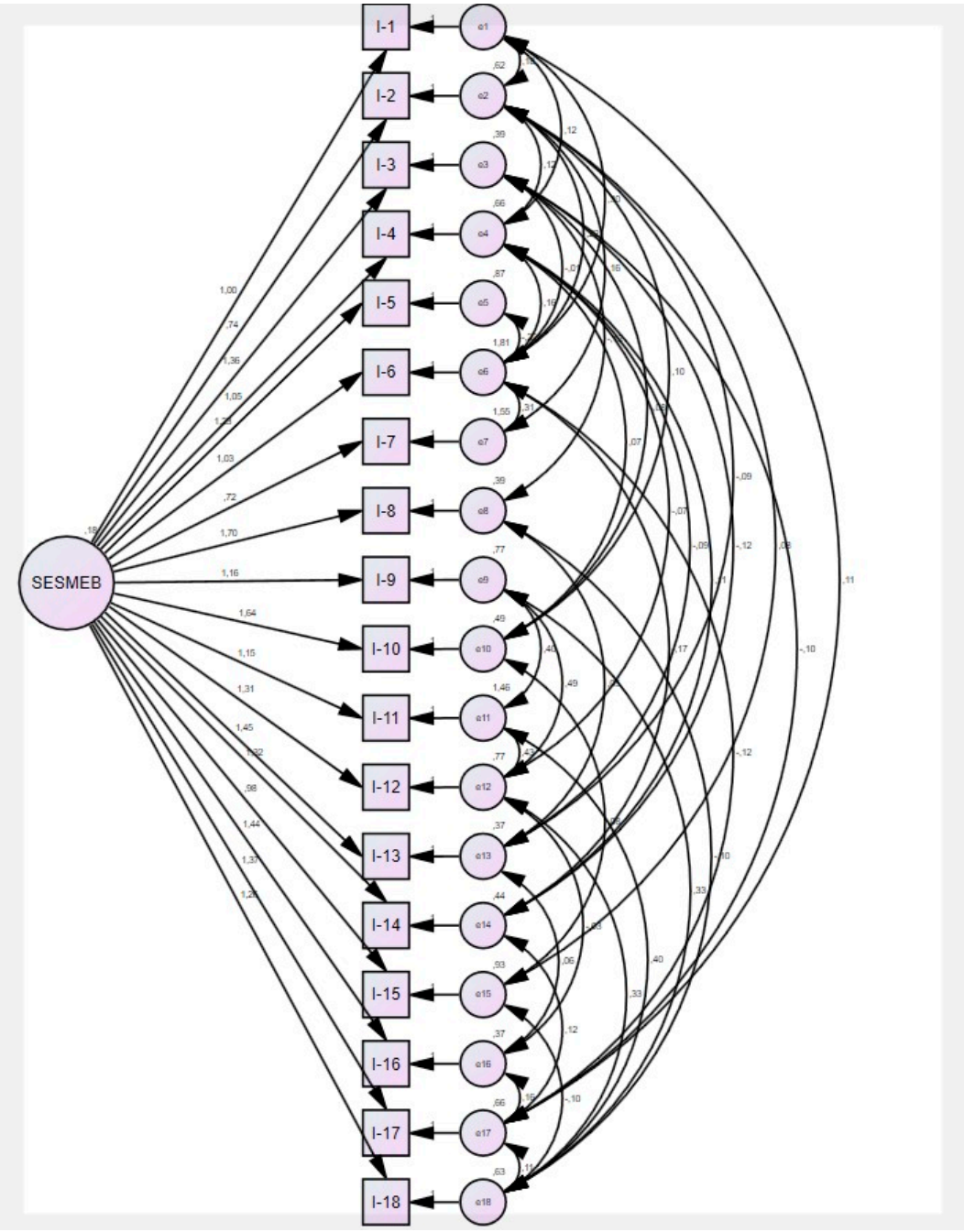

Figure 1. Standardized path coefficients of model

Table 1. Baseline characteristics of participants

Variable	Total Group (n=203)
Sex (M:F:O) n(%)	71(35.0) : 129(63.5) : 3(1.5)
Age (yrs) $\bar{X}$ ±SD	21.11±2.21
Marital status (M:S) n(%)	16(7.9) : 187(92.1)
Educational status (BS:MS:DS) n(%)	187(92.1) : 12(5.9) : 4(2.0)

M:male, F:female, O:other for gender; M:married, S:single for marital status; BS:bachelor student, MS:master student, DS:doctoral student for educational status

explanatory level of the theoretical structure (hidden variable) on the items (observed variables) [12, 13].

The fit indices χ2 /df, root mean square error of approximation (RMSEA), comparative fit index (CFI), adjusted goodness of fit index (AGFI), goodness of fit index (GFI), normed fit index (NFI), and Tucker-Lewis Index/non-normed fit index (TLI/NNFI) were used as a result of CFA. In examining the SESMEB values according to the criteria in Table 3, while χ^2/df (1.184), RMSEA (0.03), CFI (0.987), TLI (0.979) were at a good-fitting model level, AGFI (0.897), GFI (0.942), NFI (924) indicated the acceptable-fitting model level [14, 15].

Reliability of the Hungarian SESMEB

The Cronbach's alpha reliability coefficient was calculated and the coefficient is 0.866. The Cronbach's alpha coefficient was greater than 0.80 states that the

Table 2. Factor loadings, t-values and variances explained for the model

Items	$\bar{X}$ (SD)	λ	t	p
Item-1	2.56 (1.005)	0.42		
Item-2	2.86 (0.862)	0.371	4.65	<0.001*
Item-3	1.65 (0.845)	0.677	5.437	<0.001*
Item-4	2.11 (0.932)	0.476	5.115	<0.001*
Item-5	2 (1.069)	0.485	4.803	<0.001*
Item-6	3.22 (1.432)	0.308	3.945	<0.001*
Item-7	2.61 (1.283)	0.238	2.927	0.003
Item-8	1.76 (0.951)	0.755	5.706	<0.001*
Item-9	1.72 (1.007)	0.488	4.815	<0.001*
Item-10	1.96 (0.987)	0.701	5.594	<0.001*
Item-11	2.07 (1.303)	0.373	4.096	<0.001*
Item-12	1.81 (1.042)	0.535	5.042	<0.001*
Item-13	1.52 (0.864)	0.709	5.636	<0.001*
Item-14	1.71 (0.867)	0.645	5.42	<0.001*
Item-15	3.13 (1.052)	0.395	4.234	<0.001*
Item-16	1.48 (0.864)	0.707	5.666	<0.001*
Item-17	1.82 (1.01)	0.579	5.55	<0.001*
Item-18	1.74 (0.977)	0.553	5.096	<0.001*

**p<0.001*

Table 3. Compliance statistics of the SESMEB scale

Compliance indices	Criteria	SESMEB
χ^2/df	≤2	1.184
RMSEA	≤0.06	0.03
CFI	≥0.95	0.987
AGFI	0.85≤AGFI≤0.90	0.897
GFI	0.90≤GFI≤0.95	0.942
NFI	0.90≤NFI≤0.95	0.924
TLI (NNFI)	≥0.95	0.979

Hungarian SESMEB was reliable [12]. The correlation between two split parts of items and Spearman–Brown coefficient were calculated as 0.808 and 0.894, respectively. These results indicated a high correlation between two split parts of items and high reliability.

SESMEB scores of students

Instagram, Facebook, and TikTok were more commonly used social media platforms by students. In the examining of the SESMEB score, daily time spent on social media, and BMI of students, the mean SESMEB score was 37.72±10.27 (19-72), the mean time spent was 239.63±112.47 (60-720) minutes, and the mean BMI was 22.89±4.09 (15.67-41.62) kg/m^2. While the median scores were different according to spending time daily, sharing content, and using filters or Photoshop on social media ($p<0.05$), there was no significant difference between the median scores of those who had different main purposes while social media using ($p>0.05$) (Table 4).

DISCUSSION

In examining the literature, before the development of the scale of SESMEB, no tool measured the effect of social media on eating behaviors. Although this scale presents an assessment of eating behavior, the main language of it is Turkish. Due to changes that cannot be avoided during translation from the main language to the target language, there is a need to conduct a study to have a valid and reliable scale in the target language. For this reason, the eighteen-item form prepared in the target language by experts was administered to 203 Hungarian university students. As a result of the statistical analysis, Cronbach's alpha reliability coefficient of CFA was 0.866. Therefore, in this current study, the scale of effects of social media on eating behavior (SESMEB), which was a tool developed in Turkish, was proved that it is a valid and reliable scale in Hungarian. The Hungarian version of SESMEB can be used in scientific studies.

In this study, Instagram, Facebook, and TikTok were more commonly used social media platforms by participants. In 2021, the most common social platform is Facebook with 95.9% of Hungarian Internet users [16]. However, younger generations seem to prefer Instagram more than Facebook, while the biggest share of Instagrammers was between 18 and 24 years of age [1] similar to the results of this study. In addition, the effects of social media on the eating behaviors of Hungarian university students were measured via SESMEB. The eating behaviors of those who spend more time, sharing content, and using filters/Photoshop on social media, are more affected

Table 4. SESMEB scores of participants according to social media using and BMI

Variable	SESMEB Score Median (IQR)	p
Main purpose of social media using		
Friends group(s), or other group(s) (n=63)	37.00 (12.00)	0.875
Connecting with other people (n=18)	35.50 (24.75)	
To get information (n=37)	37.00 (10.50)	
Having fun, spending time (n=85)	36.00 (14.00)	
Daily time spent on social media (min)		
≤120 (n=41)	34.00 (11.00)	0.035*
>120 (n=162)	37.00 (12.25)	
Sharing frequency on social media		
Yes (n=65)	40.00 (17.00)	<0.001**
Rarely-never (n=138)	35.00 (10.25)	
Using filters or photoshop for your social media posts		
Yes (n=48)	38.00 (13.75) [a]	<0.001**
Sometimes (n=89)	39.00 (9.50) [a]	
No (n=66)	31.50 (11.25) [b]	
BMI		
Underweight (n=19)	35.00 (17.00)	0.844
Normal (n=136)	36.0 (12.00)	
Overweight (n=39)	35.00 (12.00)	
Obese (n=9)	38.00 (13.00)	

**p<0.05 **p<0.001*

by social media in the aspect of eating concerns. In the results of another current study, there was a strong and consistent association between social media use and eating concerns in a nationally representative sample of young adults aged 19 to 32 years [17].

On the other hand, although the SESMEB score exhibits the level of social media effect on eating behavior, it is not known whether this effect is positive or negative. While some of the items in SESMEB indicate the negative like item fifth *'After I started using social media, my fast-food/cook-chill food consumption increased'*, some of them can be bi-directional like *'the foods/dishes that I see on social media arouse my desire to eat'.* This situation makes us think relative to the issue of which direction the effect is. There is evidence on social media accounts that have a high number of followers promoting the healthy food consumption. Because these accounts can lead to social nudges from the people who take a look at them [18]. Therefore, social media can be a useful tool for the healthy eating behavior development and consequently a healthier lifestyle. In the study in which undergraduate students participated, participants were watched with either a positive social media feed (health) or not (taste and visual) content on Instagram, and their food choices were observed. Apple preference increased and chocolate preference decreased in participants who did not care about health and nutrition, after a positive social media feed (health) [19]. In the study where dietary diaries were used in a smartphone application to motivate university students to create personal awareness related to eating habits, it was emphasized that they were encouraged to develop their eating habits through interactions on social media [20]. However, this effect sometimes could be a dangerous issue that anyone can compose content on social media, mostly without the proficiency of education, qualification, or experience. In addition, influencers can reach lots of people and share plenty of posts including health claims, often without any formal training [21]. According to the results of a review conducted on the impact of routine social media use or exposure to image-related content on body image and food choices in young adults (19-30 years), these situations may negatively impact body image and food choices in some of them [22]. Also, experimental and observational studies show that exposure to social networking sites was associated with negative body image and disordered eating behaviors in all stages of life (children, pre-adolescent, adolescent, and young adult populations) in community, school, and college settings [8]. Considering all aspects, while examining the effect of social media on eating behavior, after being supported by other data such as food consumption records, nutritional habits, physical activity habits, questioning the nutritional patterns and lifestyles of individuals, a wide assessment should be made related to which direction this effect is.

CONCLUSIONS

There is a need to conduct studies to use SESMEB in other languages because it needs to be measured the impact of social media use on eating behaviors. According to this study's results, the Hungarian version of SESMEB is a valid and reliable tool that can be used to determine the effects of social media on eating behaviors.

Acknowledgements

The authors thank Hungarian university students who participated in this study and spent their valuable time. This study was conducted with the cooperation of Republic of Türkiye Ministry of National Education, General Directorate of European Union and Foreign Relations, and with the support of Stipendium Hungaricum program, and the authors thank those who contributed.

Conflict of interest

The authors declare no conflict of interest.

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Recived: 18.01.2024
Accepted: 12.03.2024

Rocz Panstw Zakl Hig 2024;75(1):67-73
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0287

ORIGINAL ARTICLE

EVALUATION OF LIPOPROTEINS AND HIGH SENSITIVITY CRP IN CONSUMERS OF BAKERY PRODUCTS

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ABSTRACT

Background. In recent years, a wider range of bakery products with a lower glycaemic response can be observed in the food industry. This contributes to the provision of a wider range of cereal bakery products. The gradual increase in the consumption of brown bread is significant, but despite this, white bread remains a part of the typical Western diet. Studies showed high intake of carbohydrates increase TG levels by enhancing hepatic synthesis of very low-density lipoprotein (VLDL) and decrease activity of lipoprotein lipase. White bread consumption has been therefore associated with an unhealthy lifestyle.

Objective. The aim of this study was to assess the influence of the consumption of gluten bakery products on lipids and inflammatory parameters of the probands.

Material and Methods. The monitored group consisted of 30 probands from the general population. The average age of the monitored group was 29.7 years. The intervention dose consisted of a different combination of several types of bakery products containing gluten (bread, pastries, soft pastries) within the individual weeks of consumption, while the intervention lasted 6 weeks. An intervention dose of 150 to 200 g per day was set for women and 200 to 250 g per day for men. Biochemical blood parameters were determined using a fully automatic Biolis 24i Premium blood serum biochemical analyzer, by end-point photometry method. We tested the differences between the biochemic parameters by one-factor analysis of variance (ANOVA) and compared them by Tuckey's Post Hoc Test.

Results. The measurement of the lipid profile showed that the average levels of total cholesterol (TC) were above the reference value (<5.00 mmol. l-1) in each of the three performed measurements ($P<0.01$). In the case of LDL, we found a similar trend in the development of lipoprotein values, while we positively evaluate a slight reduction of LDL in the measurement immediately after the intervention ($P<0.001$). Certain changes during the study were also noted in HDL parameters with high statistical significance ($P<0.001$). During the TG analysis, we found that probands have normal values (0.45-2.70 mmol. l-1). A reduction in average TG values was achieved in individual measurements, but without statistical significance ($P>0.05$). In high sensitivity CRP (hs-CRP) parameters was achieved a bell curve of the development of average values, with a maximum measured immediately after the intervention. Changes in hs-CRP during the study were without statistical significance ($P>0.05$)

Conclusions. The measurement of the lipid profile showed that the average levels of TC, LDL and HDL, there were above the reference value in each of the three measurements performed. Through the analysis of TG, we found normal values and during the study there was a slight decrease. Furthermore, we found that intervention with bakery products containing gluten was associated with an increase in hs-CRP levels in our probands.

Key words: *gluten bakery products, lipoproteins, CRP, health, inflammatory reaction*

INTRODUCTION

Foods made from cereals are necessary to fulfil the need for nutrients. Therefore, assessing and managing grain quality is critical to our health and well-being. Grains have always been essential in the diet, as evidenced by the fact that growing grain was a reason for people to settle in one place, because that was the only way they could sow the seed and harvest the harvest. The consequence of this act was the emergence of civilization [30].

The chemical composition of cereal grain varies according to the species, variety, soil and climatic conditions, agricultural techniques (fertilization, protection, etc.) and weather conditions in a particular year [18]. Cereals are mainly a source of carbohydrates (55-78%), mainly starch. The protein content is 7-19%. Cereal proteins are classified as incomplete,

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

the limiting amino acid is lysine [19]. They have a high content of proline and glutamine [9]. The fat content is in the range of 1-5%. Lipids should have a simple composition. Cereals are a source of vitamins of group B and vitamins of group E (tocopherols, tocotrienols), fiber, minerals, especially drugs, iron, magnesium, copper, manganese, zinc and phosphorus, antioxidants, and phytochemicals. Carotenoids (lutein), polyphenolic components (phenolic acids, alkylresorcinols, lignans), phytosterols and other biologically active substances (choline, betaine, etc.) play a significant role in nutrition and health [2, 6]. The content of individual nutrients in flour depends on the degree of milling [19].

The bread we eat today has changed little from the bread our ancestors ate a few millennia ago. There are hypotheses, partially supported by archaeological excavations, that mankind has known bread for about 6000 years. At the turn of the last century and the current one, there is also information that mankind consumed bread 15-20 thousand years ago. These opinions resulted from archaeological findings, allegedly also in the territory of Bohemia and Russia, where stones were found on which plant seeds were crushed and porridge was apparently made from this grain grinding [27].

Bread is an important stable food [8]. In recent years, a wider range of bakery products with a lower glycemic response can be observed in the food industry. This contributes to the provision of a wider range of cereal bakery products. The gradual increase in the consumption of brown bread is significant, but despite this, white bread remains a part of the typical Western diet [7].

In many European countries, the higher social classes more often prefer whole-grain and brown bread to white bread [5, 12, 17]. Total bread consumption is nevertheless often associated with low socio-economic status [12, 23, 25]. Grains without bran have higher glycaemic load and glycaemic index than whole grains and have adverse effect on health. [29]. Studies showed high intake of carbohydrates increase TG levels by enhancing hepatic synthesis of very low-density lipoprotein (VLDL) and decrease activity of lipoprotein lipase [15]. White bread consumption has been therefore associated with an unhealthy lifestyle [20].

The aim of this study was to monitor the influence of the consumption of gluten bakery products on selected biochemical blood parameters of the probands.

MATERIAL AND METHODS

The monitored group consisted of 30 probands from the general Slovak population who were obtained by random selection, regardless of gender and age. All participants took part in the study expressly on a voluntary basis, without any coercion or financial or other remuneration for participating in the study. The research was approved by the Ethics Committee at the Specialized Hospital of St. Svorada Zobor, n.o., Nitra, Kláštorská 131, 94901 Nitra under number 012911/2016. This study was part of a larger study, partly already published [14].

For inclusion, or exclusion of probands, the following inclusion and exclusion criteria were used. The main inclusion criteria were good health, the absence of serious acute or chronic diseases, the absence of food allergies and intolerances, as well as the absence of drug treatment, and pregnancy in the case of women, as these factors could affect the results of the clinical study. Other conditions were not to change your eating habits, diet, physical activity, or lifestyle during the entire duration of the study. The condition for participation in the study was oral and written submission of information about the nature, course, and conditions of the study, which all enrolled probands confirmed by signing the informed consent.

The group consisted of 13 men (43.3%) and 17 women (56.7%). The average age of the monitored group was 29.7 years, while the age range varied from 21 to 53 years. The average height in the group was 174.53 cm (height range 154-196 cm) and the average body weight was 71.24 kg (weight range 42.8-104.1 kg). The intervention dose consisted of a different combination of several types of bakery products containing gluten (bread, pastries, soft pastries) within the individual weeks of consumption. Their amount considered the recommendations for the intake of bakery products regarding the gender of the probands. An intervention dose of 150 to 200 g per day was set for women and 200 to 250 g per day for men.

The intervention lasted 6 weeks. All measurements were taken before, immediately after the intervention and at an interval of 8 weeks after the end of the intervention. For these reasons, the total duration of the study was predetermined at 14 weeks. Monitored parameters (TC; DL; HDL; TAG, hs-CRP) were determined using a fully automatic Biolis 24i Premium blood serum biochemical analyzer (Tokyo Boeki Medical System Ltd., 569-6, Nobe, Akiruno, Tokyo 197-0823, Japan). It is an automatic biochemical analyzer for determination of substrates, enzymes, electrolytes, and specific proteins. The analyzer is compact, has the functions of larger analyzers. Highly efficient, non-contact (by air pressure) mixing of the reaction mixture in the cuvette is unique and ensures protection against any mutual contamination of the stirrer. An automatic washing system is used to wash the cuvettes. Reagents are identified based on the ID position or barcode according to the setting. The method of determination in this device is end-point photometry (turbidimetry),

kinetics, homogeneous immunoanalysis. The device works at wavelengths: 40, 380, 405, 450, 505, 546, 570, 600, 660, 700, 750, 800 nm.

For statistical processing of the obtained data, we used the Microsoft Office Excel 2010 program (Los Angeles, CA, USA) in combination with XLSTAT (Version 2019.3.1). Statistical analyzes were also performed using the program Statistik Cz Version 10 (TIBCO Software Inc., Palo Alto, California, USA).

The resulting values of the measured parameters are presented as mean ± standard deviation (SD). Statistical significance levels were set at $P<0.05$ (*); $P<0.01$ (**); $P<0.001$ (***). We tested the differences between the anthropometric parameters by one-factor analysis of variance (ANOVA) and compared them by Tuckey's Post Hoc Test.

RESULTS AND DISCUSSION

The lipid profile represents a group of lipid parameters - total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and triacylglycerols (TG), based on which it is possible to determine the risk of primarily cardiovascular and, in some cases, metabolic diseases. The lipid profile is considered a good indicator of the risk of developing cardiovascular complications of atherosclerosis, i.e., myocardial infarction and sudden stroke.

The average values of the levels of individual lipoproteins, evaluated within the lipid profile of the probands during the study, are shown in Table 1. During a more detailed analysis of total cholesterol values, we found that before the intervention only 3 probands (1 woman and 2 men) had levels in the physiological range (<5.00 mmol. l^{-1}), the remaining 27 probands had elevated values above this limit. After the intervention, we recorded the physiological values of cholesterol in 6 probands (3 women and 3 men) and the number of probands with hypercholesterolemia decreased to 24. At the same time, there was also a decrease in the average value of cholesterol in the group of women and in the entire group of probands, a slight increase in the average value was noted in men total cholesterol. Subsequently, in measurements with an interval of 2 months, we observed the physiological TC values in 5 probands (2 women and 3 men), 25 probands had increased TC values. At the same time, there was an increase in the average values of TC both in the whole group and also when differentiating by gender. The mentioned differences between the 2nd and 3rd measurements were statistically significant ($P<0.01$).

LDL particles should be in the range of 1.20-3.00 mmol. l^{-1} in the blood of a healthy person. In this case too, we found a similar trend in the development of lipoprotein values. In the first measurement, only 8 probands (5 women and 3 men) reached the physiological limit of LDL and the rest had elevated LDL values, in the second measurement there was a relatively significant improvement in LDL values,

Table 1. Lipoprotein levels of probands

	Before intervention $\overline{X}$ ± SD	After 6-weeks intervention $\overline{X}$ ± SD	2 months after intervention $\overline{X}$ ± SD	P value (significance) */**/***
TC (mmol.l^{-1})				
All (n=30)	6.05±0.92	5.82±0.95	6.22±1.41	P<0.01(**)
Women (n=17)	6.33±0.92	5.84±0.97	6.30±1.37	P<0.01(**)
Men (n=13)	5.67±0.81	5.79±1.52	6.10±1.52	P>0.05
LDL (mmol.l^{-1})				
All (n=30)	3.56±0.67	3.11±0.85	3.74±1.10	P<0.001(***)
Women (n=17)	3.49±0.71	2.88±0.85	3.56±1.04	P<0.05(*)
Men (n=13)	3.64±0.64	3.41±0.78	3.99±1.18	P<0.001(***)
HDL (mmol.l^{-1})				
All (n=30)	1.75±0.85	1.75±0.52	1.93±0.54	P<0.001(***)
Women (n=17)	2.04±0.58	1.99±0.52	2.20±0.49	P<0.001(***)
Men (n=13)	1.36±0.29	1.42±0.32	1.56±0.35	P<0.05(*)
TG (mmol.l^{-1})				
All (n=30)	1.22±0.73	1.07±0.44	1.07±0.46	P>0.05
Women (n=17)	1.16±0.54	0.92±0.31	0.98±0.42	P<0.01(**)
Men (n=13)	1.30±0.95	1.26±0.53	1.20±0.50	P>0.05

TC – total cholesterol; LDL – low density lipoprotein; HDL – high density lipoproteins; TG – triacylglycerols; $\overline{X}$ – average; SD – standard deviation

as 15 probands (50%) had physiological values (12 women and 3 men) (P<0.001). We negatively evaluate the repeated decrease in the number of probands with physiological LDL values in the third measurement, only 8 probands (6 women and 2 men) reached them (P<0.001).

Certain changes during the study were also noted in the HDL parameter (P<0.001). The physiological range (women 1.20-2.70 mmol. l^{-1}; men 1.00-2.10 mmol. l^{-1}) was reached in the first measurement by 15 women (1 had reduced and 1 increased HDL values) and 12 men (1 man had reduced HDL values). In the second measurement, the situation in women did not change, while in men, 1 proband normalized HDL from a low value and the other proband reached values of 2.16 mmol. l^{-1}, which is already a value slightly above the norm. The situation in the third measurement in men did not change, in women compared to the second measurement we recorded an increase in HDL in one subject to a value of 2.73 mmol. l^{-1}, which is above the reference value.

By evaluating TG, we found that up to 29 probands had normal values (0.45-2.70 mmol. l^{-1}) in the 1st measurement, which is quite positive information from the point of view of evaluating their health and nutritional status. One proband (a man) had an initial TG value of 4.04 mmol. l^{-1}, which, among other things, may reflect not only errors in the diet, but also an incorrect lifestyle (irregular regime, little sleep, low level of physical activity, etc.). However, in the measurement after the intervention, his TG values decreased, so all probands reached optimal levels of these lipoproteins in the blood. In the third measurement, all probands again had normal TG values. Average TG values had a decreasing trend during the study (from 1.22±0.73 mmol. l^{-1} in the first measurement to 1.07±0.46 mmol. l^{-1} in the third measurement), but without statistical significance (P> 0.05).

In a study by Sawicki et al. [24], a higher intake of whole grains was associated with a greater increase in HDL cholesterol and a decrease in triglyceride concentrations, and conversely, a higher intake of refined cereal products was associated with a smaller decrease in triglyceride concentration (P<0.001). Evidence from observational studies found that greater consumption of whole grain bakery products is also associated with a lower risk of cardiovascular disease (CVD) [3], as well as obesity, type 2 diabetes, hypertension [1] and all-cause mortality [11, 31]. Nevertheless, intake of refined grains remains high. Some, but not all, studies suggest that a higher intake of refined grains is associated with a higher risk of CVD [13]. Higher intake of whole grains was associated with a smaller increase in waist circumference (about 1.4±0.2 cm compared to refined grains, where the increase in waist circumference was 3.0±0.1 cm (P<0.001). The changes were also in fasting glucose concentrations (an increase of 0.7±0.4 mg.dl^{-1} compared to 2.6±0.2 mg.dl^{-1} (P < 0.001)) and systolic blood pressure (by 0.2±0.5 mmHg compared to 1.4±0.3 mmHg (P<0.001). When stratified by sex, a stronger association of consumption of individual types of cereals with waist circumference was observed in women than in men [24]. Therefore, replacing refined grains with a higher intake of whole grains and their products is a potential dietary strategy to reduce CVD risk.

C-reactive protein (CRP) is considered the most sensitive reactant of the acute phase of inflammation, CRP concentrations rise very quickly during inflammatory processes to many times the norm. CRP activates the complement system, triggers opsonization and phagocytosis of damaged cells, but its main function is to bind and detoxify endogenous toxic substances arising as a product of tissue damage. About two decades ago, the importance of small, chronic elevations of CRP as an indicator of cardiovascular risk in healthy people was identified. A very sensitive method has been developed for measurement, therefore CRP measured by this method is called highly sensitive C-reactive protein (hs-CRP).

Several studies have concluded that the hs-CRP value can be used as an indicator of cardiovascular risk, i.e., a marker for predicting the risk of developing coronary heart disease in healthy people, as well as an indicator of the prognosis of cardiovascular events with its very close relationship to the "inflammatory" theory of the pathogenesis of atherosclerosis [26]. Both methods (CRP and hs-CRP) measure the same protein in the blood. "Classic" CRP is normal if the CRP concentration is less than 5 mg. l^{-1}. Accurate determination of CRP concentrations in the range of 0-5 mg. l^{-1} can only be realized using a special highly sensitive hs-CRP test. In healthy adults, the average value of CRP is 0.8 mg. l^{-1}, with a distribution of 90% of the population below 3 mg. l^{-1}. The concentration tends to be slightly higher in women compared to men. This difference is due to the effect of estrogen. CRP values increase with age, but only minimally. The basal CRP level can be influenced by several factors, mostly influenceable. CRP increases e.g., inappropriate lifestyle (smoking, sedentary lifestyle, higher BMI, overweight), metabolic disorders (obesity, low HDL level, high LDL level, high TG level, hypertension, metabolic syndrome, *diabetes mellitus*), chronic infections and inflammatory processes, use of estrogens and progesterone. Conversely, factors that reduce CRP levels are increased physical activity, weight loss, quitting smoking, moderate alcohol consumption, some medications - statins, etc. [4]. For these reasons, we decided to use hs-CRP to evaluate the inflammatory response and to estimate the risk of cardiovascular diseases. Before the intervention, hs-

CRP was present in the probands at an average value of 1.80±2.25 mg. l^{-1} (maximum 9.48 mg. l^{-1}; minimum 0.06 mg. l^{-1}, median 0.75 mg. l^{-1}). Immediately after the intervention, the average value increased to 3.25±5.66 mg. l^{-1} (maximum 26.59 mg. l^{-1}; minimum 0.12 mg. l^{-1}, median 0.56 mg. l^{-1}). 2 months after the end of the intervention, hs-CRP levels decreased to 1.75±2.76 mg. l^{-1} (maximum 12.39 mg. l^{-1}; minimum 0.07 mg. l^{-1}, median 0, 66 mg. l^{-1}). Changes in hs-CRP during the study were without statistical significance (P>0.05) (Table 2).

Table 2. Cardiovascular risk of probands by using the hs-CRP

hs-CRP (mg. l^{-1})				
	Before intervention $\overline{X}$ ± SD	After 6-weeks intervention $\overline{X}$ ± SD	2 months after intervention $\overline{X}$ ± SD	P value (significance) */**/***
All (n=30)	1.80±2.25	3.25±5.66	1.75±2.76	P>0.05
Women (n=17)	1.69±2.61	4.21±7.07	1.79±3.09	P>0.05
Men (n=13)	1.94±1.77	1.99±2.80	1.69±2.35	P>0.05
Cardiovascular risk of probands				
	Low risk (hs-CRP<1 mg.l^{-1})	Medium risk (hs-CRP 1-3 mg.l^{-1})	High risk (hs-CRP>3,0 mg.l^{-1})	
Before intervention	17 (12 women;5 men)	7 (2 women;5 men)	6 (3 women;3 men)	
After 6-weeks intervention	18 (9 women;9 men)	3 (2 women;1 men)	9 (6 women;3 men)	
2 months after intervention	18 (11women;7men)	8 (4 women;4 men)	4 (2 women;2 men)	

hs-CRP - high sensitivity C-reactive protein; $\overline{X}$ – average; SD - standard deviation.

As can be seen from the data in Table 2, based on the average levels of hs-CRP (> 3.0 mg. l^{-1}), were immediately after the intervention 9 probands (6 women and 3 men) at high cardiovascular risk. In the measurement with an interval of 2 months from intervention, we found that only 4 probands (2 women and 2 men) had a high cardiovascular risk. From which we could conclude that an inflammatory response occurred during the study, but we also need to think about other factors that affect the hs-CRP level. These results show that intervention with bakery products containing gluten was associated with an increase in hs-CRP levels. When assessing hs-CRP levels according to gender, we found a more massive increase in hs-CRP in women, which could have been contributed to by the presence of female sex hormones, as well as a higher proportion of adipose tissue in their body (part of CRP is also formed in adipose tissue). Similar results were reached by Taskinen et al. [28], who observed the effect of consumption of whole grain and refined (white) bakery products on hs-CRP levels. At the intervention dose of 92 g.day^{-1} in men and 75 g.day^{-1} in women, the average serum concentration of hs-CRP was 1.77 mg. l^{-1} in men and 1.96 mg. l^{-1} in women. They found that increasing the intervention dose by every 50 g.day-1 was associated with an increase in hs-CRP concentration by 0.23 mg. l^{-1}. They found no statistically significant interactions by gender or BMI.

Evidence from studies regarding the health consequences of consuming refined grains is inconsistent, with most studies reporting negative or neutral effects on the development of low-grade inflammation that accompanies an increase in hs-CRP [16, 22] and to the development of cardiovascular and metabolic diseases [1]. Data on the association between grain consumption and low-grade inflammation in intervention studies are somewhat conflicting. A recent meta-analysis of 14 randomized controlled trials found no significant effect of whole grain intake on serum concentrations of CRP, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), or plasminogen activator inhibitor type 1 (PAI-1) [21], while in another meta-analysis of 17 randomized controlled trials, whole grain intake resulted in significantly lower serum hs-CRP and IL-6, but not TNF-alpha, compared to consumption of refined (white types) bakery products [10].

CONCLUSION

Measurement of the lipid profile showed that mean TC and LDL levels were above reference values throughout the duration of the study. The slight reduction of LDL immediately after the 6-week intervention was positively evaluate. Certain changes during the study were also noted in HDL parameters. It was found that the average TG values had a decreasing

tendency, which is quite positive from the point of view of evaluating the health and nutritional status.

Furthermore, it was found that intervention with bakery products containing gluten was associated with an increase in hs-CRP levels. When evaluating hs-CRP levels by gender it was found a more massive increase in hs-CRP in women, which could have been contributed to by the presence of female sex hormones, as well as a higher proportion of adipose tissue in the body. Therefore, we consider it is necessary to improve the overall lifestyle of the general Slovak population as soon as possible. Further research is needed with a larger number of probands and the use of other methods of monitoring the nutritional and health status of the population.

Acknowledgments

This research was funded within the project of Grant Agency of Faculty of Agrobiology and Food Resources, SUA in Nitra „04/2023 – Blood biochemical parameters in the evaluation of the health and nutritional status of selected population groups".

Publication was also created thanks to the support of the KEGA 003SPU-4/2022 Integration of university education with practice through the implementation of practical training in the new subject Nutritional counselling.

Conflict of interest

The authors declare no conflict of interest.

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Received: 07.09.2023
Revised: 27.01.2024
Accepted: 30.01.2024

Rocz Panstw Zakl Hig 2024;75(1):75-81
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0292

ORIGINAL ARTICLE

ASSESSMENT OF PHENOLIC AND FLAVONOID CONTENTS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF MOROCCAN PROPOLIS

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ABSTRACT

Background. Despite the extensive literature focused on propolis extract, few data exists on the bioactive compounds and biological activities in the Moroccan propolis and its economic value is low.

Objective. In this research, the aim was to evaluate the total content of phenols and flavonoids as well as the antioxidant, antibacterial and antifungal activities of Moroccan propolis.

Material and Methods. The polyphenol and flavonoid content of the Moroccan propolis from three geographic regions, was quantified in the ethanolic extract by colorimetric methods using folin-ciocalteu and aluminum chloride. The antioxidant activity was evaluated by the DPPH test and expressed as IC50. Disk diffusion and broth microdilution methods were used to examine in vitro antimicrobial activity against known human microorganism pathogens.

Results. The obtained data revealed that Moroccan propolis samples presented significant variations in total polyphenols and flavonoids. All samples showed significant antioxidant activity with IC50 values ranging from 4.23±0.5 to 154±0.21 µg/mL. A strong correlation between total phenolic activity, flavonoids and antioxidant activity was found. The in vitro study of antibacterial activity showed that the propolis samples exhibited a range of growth inhibitory actions against all bacterial strains tested with the highest activity against gram-positive bacteria. Only propolis from the Sidi Bennour region demonstrated an antifungal activity.

Conclusion. The study data show that Moroccan propolis extracts have a promising content of antioxidant and antimicrobial compounds that could be exploited to prevent certain diseases linked to oxidative stress and pathogenic infections.

Key words: *propolis, polyphenols, antibacterial activity, antifungal activity, Morocco*

INTRODUCTION

Propolis is a sticky, resinous, darker-green material produced by *Apis mellifera* honey bees using buds and exudates of various plants of the native vegetation near their hive [1]. Honeybees use propolis as an insulating material and as a sealant for cracks and openings in the hive, also as a barrier against external reats and climatic conditions [2]. Since the antiquity, propolis has been used by Romans, Egyptians, Greeks and Persians civilizations as a raw material for numerous pharmaceutical preparations [3]. The composition of this beekeeping product is quite complex and varied throughout the world. Generally, raw propolis consisting of resin and vegetal balsams (~50%), wax (~30%), essential and aromatic oils (~10%), pollen (~5%) and other substances including, minerals, polysaccharides, proteins, vitamins, aliphatic and aromatic acids and esters, flavonoids and other plant phenolics and terpenoids [4]. The chemical composition is considerably affected by botanical source, collection region, harvest season, climate environmental conditions [5]. Different biological and pharmacological effects have been linked to propolis constituents. These include, but not limited to antioxidant, antimicrobial, antiviral, antitumor, antidiabetic, anti-inflammatory, antiallergic activities. It would also have immunomodulatory, cardioprotective, hepatoprotective, neuroprotective, and hypoglycemic properties [1, 2, 6]. Propolis

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

has become the subject of extensive biological and chemical studies in recent decades, mainly for its applications in medicine, the food industry, cosmetics, veterinary medicine and animal husbandry. However, Moroccan propolis is little studied and few studies have recently addressed its chemical composition and biological properties [7-12]. With a view to contributing to the knowledge of Moroccan propolis, the aim of this present work was to determine the chemical composition, antimicrobial and antioxidant activities of three samples taken from regions of different geographical origins in Morocco.

MATERIAL AND METHODS

Propolis samples

Propolis samples were collected by professional beekeepers from *Apis mellifera* hives located in three regions of Morocco. Table 1 provides detailed information on the floral origin, area of origin, geographic location and date of collection of the propolis samples.

Equipment

A Spectrophotometer-UV/Visible (Jenway 6300, USA), biomerieux densitometer (ATB 1550, Italy), Büchi rotavapor (Type R110, Switzerland) were used.

Chemical reagents

Sodium carbonate, gallic acid, folin-ciocalteu reagent, sodium nitrite, aluminum chloride, sodium hydroxide, quercetin, 2,2-diphenyl-2 picrylhydrazyl and ethanol were obtained from Sigma – Aldrich (Germany).

Propolis extraction

After their collection, the propolis samples were cut into small portions and ground in a mortar to obtain homogeneous powders and then extracted by maceration method using ethanol 70% (70:30 ethanol: water) as a solvent, they underwent extraction by maceration method using 70% ethanol as a solvent (ethanol 70:30 water). The 70% ethanol is one of the most used solvents for propolis extraction, particularly for the extraction of phenolic and flavonoid compounds [13]. The extract obtained is then filtered through Whatman filter paper (No. 4) and the solvent is removed by evaporation at 40°C under reduced pressure using a rotavapor.

Determination of total phenolic content

The quantification of the total phenolic compounds (TPC) in the propolis extracts, was carried out by the colorimetric method of folin-ciocalteu according to Loizzo et al. [14]. Gallic acid was used to calculate the standard curve and the resulting values were expressed in milligrams per gram of gallic acid equivalent (mg GAE/g).

Determination of total flavonoids content

The content of the total flavonoid compounds (TFC) was quantified in the propolis extracts by the colorimetric method of aluminum chloride as previously used according to Aboukhalaf et al. [15]. The mean of three readings was used and total flavonoids was expressed as milligrams per gram of catechin equivalent (mg CE/g).

In vitro antioxidant evaluation

The free radical scavenging potentials of propolis ethanolic extracts were assessed using the 2,2-diphenyl-2 picrylhydrazyl (DPPH●) method described by Aboukhalaf et al [15]. The test was performed in triplicate and expressed as an IC50 value (concentration in µg/ml needed to scavenge the 50% of initial DPPH). Ascorbic acid was used as standard antioxidant.

Antimicrobial activity

Microorganism strains

Four strains of bacteria (two strains of gram-positive bacteria and two strains of gram-negative bacteria) and two strains of fungi were obtained from the American Type Culture Collection (ATCC) and the Institute Pasteur Paris Collection (CIP), and were used in this study: *Escherichia coli* (CIP54127), Pseudomonas sp, *Enterococcus faecalis* (ATCC19433), *Staphylococcus aureus* (ATCC25923), *Cryptococcus neoformans* (CIP 960) and *Candida albicans* (48.72). All the strains were maintained at freeze temperature until use.

Disk diffusion assay

Screenings of extract for antimicrobial activity was done using the disc diffusion method [16]. In order to do this, bacterial and fungal sterile physiological saline suspension was prepared to 0.5 of the McFarland standards (1.5 × 10 8 CFU/mL) from bacterial colonies grown on nutrient agar overnight

Table 1. Floral origin and geographical location of the propolis samples

Sample	Area	Geo-localisation	Vegetation origin	Collection date
1	Sidi Bennour	32°39’ 7» N 8° 26’ 35» W	*Eucalyptus- Rosmarinus Thymus- Citrus- Olea*	May 2021
2	Benslimane	33° 36’ 44»N 7° 07’ 16»W	*Eucalyptus* and *Quercus*	May 2021
3	Sidi Ifni	29° 23′ 0″ N, 10° 10′ 0″ W	*Argania* and *Euphorbia*	June 2021

at 37 °C and yeast grown on Sabouraud at 28 °C for 48 h. Then the bacterial suspensions were spread on Mueller Hinton Agar and the yeast suspensions were spread on Sabouraud Dextrose Agar. A paper discs (6 mm diameter) that were impregnated with 60 µl of extract at concentration of 100 mg/ml, were placed on the inoculated agar surface. Petri dishes were left for 2h at 4 °C to allow the diffusion of the extract before incubation at 37 ± 2 °C for 18–24 h for bacteria and at 28 ± 2 °C for 48 h for the yeast activity. After incubation, the diameters of the inhibition zones were measured in mm. Fluconazole and Ampicillin were used respectively as positive controls and methanol as negative control.

Determination of the minimum inhibitory concentration (MIC)

The MIC was defined as the least concentration of extracts that totally inhibited microbial growth. MIC was determined by the quantitative method of microdilution. Volume of 8.7 ml of Mueller-Hinton broth or Sabouraud broth were placed in test tubes and 0.2 ml of each extract, were adjusted to (0.5-100 mg/mL), and added to the test tubes. To each tube containing the mixture, 0.1 ml of the microbial suspension (1×10^7 CFU/ml) was added, prepared in Mueller-Hinton Broth for bacteria and prepared in Sabouraud broth for yeast. The tubes were incubated for 24–48 h at 37 ± 2 °C and for 48–96 h at 27 ± 2 °C respectively for bacteria and yeasts. Microbial growth in each tube is indicated by turbidity at the bottom of the tube.

Statistical analysis

SPSS software version 26 was used to statistical analysis. All studied data are expressed as mean ± standard error means (SEM). ANOVA one way with a confidence level of 95% was used to look for differences among the study results. A p value<0.05 was considered significant.

RESULTS AND DISCUSSION

Total phenolic content

The TPC determined in the Moroccan propolis samples analyzed in this study, are summarized in Figure 1. The data showed that TPC content in the sample extracts ranged from 12.26 ± 1.46 to 100.13 ± 1.23 mg GAE/g. The propolis of Sidi Bennour region demonstrated the highest value of phenols (100.13 ± 1.12 mg GAE/g). While, the propolis of Sidi Ifni region demonstrated the lowest value of phenols. These results are similar to those reported by El Menyiy el al. [9] and by Laaroussi et al. [8] in propolis samples collected from different regions of Morocco in which the TPC ranged from 6.74 ± 1.17 mg FAE/g to 149.13 ± 2.12 mg FAE/g and from 5.99 ± 0.86 mg GAE/g to 117.81 ± 5.43 mg GAE/g respectively. These values are however, higher than those reported for the neighbor countries as Tunisia (17.34 to 33.4 mg GAE/g) [17] and Algeria (0.81±0.16 to 8.97±0.25 mg GAE/g) [18].

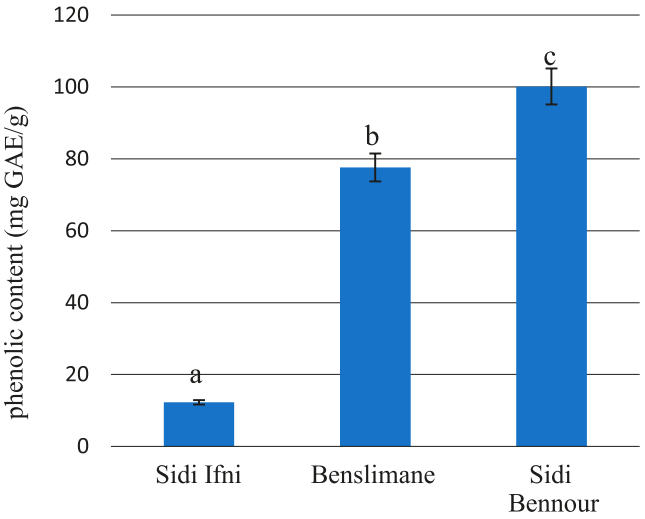

Figure 1. Total phenolic content of ethanolic extract of propolis samples collected from different Moroccan regions Means with different letters are significantly different at $P < 0.05$.

Total flavonoids content

Flavonoids are one of the most important polyphenolic classes found in many plants and plant-derived products. Numerous studies have shown that flavonoids exhibit a broad range of biological properties, including, antifungal, antibacterial, antiviral, antioxidant anti-inflammatory, anti-allergic, hepatoprotective activity, anticancer and cardiovascular protection effects and so on [19, 20, 21]. In studied propolis samples the TFC was 73.46±2.12 mgQE/g for the propolis of Sidi Bennour region, 34.7±1.12 mgQE/g for the propolis of Benslimane region and 3.56 mgQE/g for the propolis of Sidi Ifni region. These values are similar to those obtained for different other Moroccan propolis samples reported to have TFC values ranging between 0.16-80 mgQE/g [22]. On the other hand, the present results are higher than those obtained for the propolis obtained from Indonesia 0.76- 3.39 mgQE/g [23] and South Korea, Australia, Brazil, and China (33–53 mg QE/g) [24]. The obtained results were however, lower than those found in the propolis from Mexico (13-379 mg mgQE/g) [25]. The variations in TPC and TFC are dependent on type of vegetation predominant in the region of the sample collection, the environmental conditions, the seasonal variation as well as the extraction method [10], Table 2.

Antioxidant activity

The results found concerning the antioxidant activity of the selected propolis samples as determined

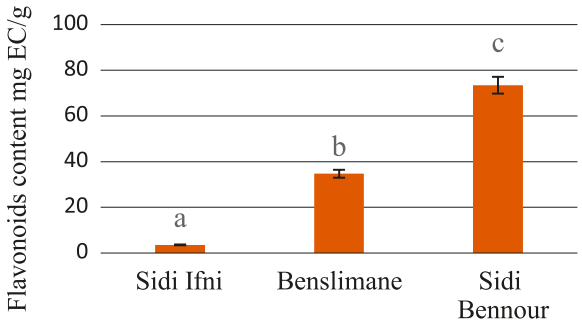

Figure 2. Total flavonoids content of ethanolic extract of propolis samples collected from different Moroccan regions Means with different letters are significantly different at $P < 0.05$.

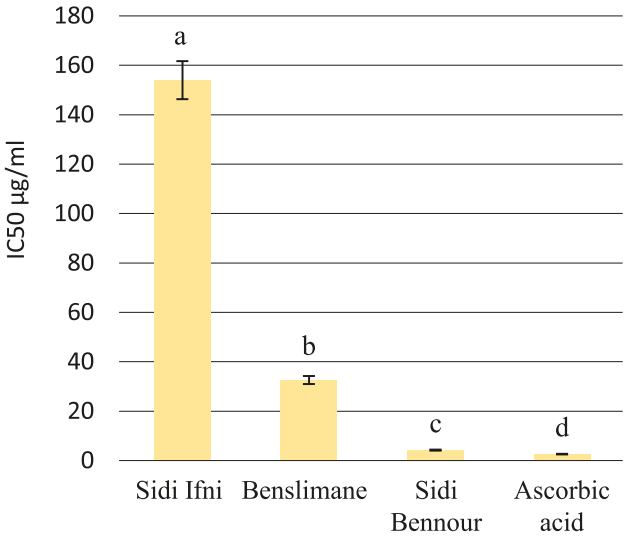

Figure 3. IC50 values of ethanolic extract of propolis samples collected from different Moroccan regions Means with different letters are significantly different at $P< 0.05$.

using the free radical scavenging assay of the 2,2-diphenyl-2 picrylhydrazyl (DPPH●) method are expressed as IC50 values and shown in Figure 3. All the propolis samples examined have a considerable antioxidant activity with IC50 between 4.23 ± 0.5 and 154 ± 0.21 µg/ml. The sample from the Sidi Bennour region expressed the highest antioxidant potential (IC50 = 4.23 ± 0.5 µg/ml) that was close to that of ascorbic acid used as a standard antioxidant. The sample from Sidi Ifni that was with the smallest contents of phenolic and flavonoids, demonstrated the lowest free radical scavenging capacity (IC50 = 154 ± 0.21 µg/ml).

Using the Pearson's correlation analysis showed a significant negative correlation between IC 50 values and total phenols (r = −0.946), and total flavonoid content (r = −0.817), (Table 2). The high correlations prove the role of phenolic and flavonoids compounds as the major contributor to the antioxidant properties of Moroccan propolis extracts. High correlation between the total phenolic and flavonoids content and the antioxidant capacity has also been revealed in other propolis samples from Morocco [9, 10, 22], Algeria [18], South Korea [24], Mexico [25] and Poland [26]. These data allow us to confirm the beneficial use of propolis as valuable tool in food and pharmaceutical industry.

Table 2. Spearman's correlation coefficients between total polyphenols, total flavonoids and antioxidant activity

	Flavonoids	Polyphenols	IC50
Flavonoids	1.000	0.879**	-0.817**
Polyphenols	0.879**	1.000	-0.946**
IC50	-0.817**	-0.946**	1.000

** Correlation is significant at the 0.01 level.

Antimicrobial activity

The present study examined also the antimicrobial effects of the ethanolic extracts of propolis against known human microorganisms' pathogens, by the disk diffusion method. The results of the tested propolis samples using this method are shown in Table 3. All propolis samples exhibited varying degrees of antibacterial activity against all strains tested, markedly against gram-positive bacterial strains, with inhibition zones ranged between 12±1.03mm and 22±0.5 for *S. aureus*, followed by *Pseudomonas* sp with a diameter ranged from 10±0.08mm to 20±0.02mm and *E. faecalis* with a diameter varied

Table 3. Disk inhibitory zone of propolis extracts against microorganisms

	Disk inhibitory zone (mm)					
Microorganisms	Gram negative bacteria		Gram positive bacteria		Yeasts	
Sample	*Pseudomonas sp.*	*E. coli*	*S. aureus*	*E. faecalis*	*C. albicans*	*C. neoformans*
Sidi Bennour	20±0.02	17±1.04	22±0.5	19±0.6	9±0.6	10±0.2
Benslimane	10±0.08	13±2.52	12±1.03	13.5±0.04	NI	NI
Sidi Ifni	11±0.58	12±0.35	NI	11±0.4	NI	NI
Ampicilline	24±0.77	22±1.4	22±0.83	23±0.9	ND	ND
Fluconazole	ND	ND	ND	ND	20±0.1	21±0.1

ND: not determined; NI: no inhibition

from 11±0.4mm to 19±0.6mm. The *E. coli* has the lowest value of the inhibition diameter (12±0.35mm to 17±1.04mm). The propolis of Sidi Bennour region which has high phenolic and flavonoids contents and higher antioxidant potential was the most effective against all the tested bacteria.

The efficacy tests of the propolis extracts on the bacterial strains used, was assessed by determining the minimum inhibitory concentration (MIC) (Table 3). The MIC of tested bacteria was between 5 to 40 mg/ml. Among the bacterial strains tested, the gram-positive *E. faecalis* was the most susceptible as compared to other bacteria, while the gram-negative *E. coli* was the most resistant. These results have also been confirmed by numerous studies demonstrating that gram-positive bacteria are more sensitive to propolis extracts than gram-negative bacteria [9, 10]. The propolis sample from Sidi Bennour demonstrated the best antibacterial activity with the lowest value of MIC (MIC=1.25, MIC=1.25, MIC=2.25, MIC=5 mg/ml) for *S. aureus*, *E. faecalis*, *E. coli* and *Pseudomonas* sp respectively, while the propolis sample from Sidi Ifni has the low antibacterial effect, with MIC values of 40, 10, 5 and 5 mg/ml for *E. coli*, *Pseudomonas* sp, *S. aureus* and *E faecalis*, respectively. Only the propolis of Sidi Bennour region showed an antifungal activity against both *C. albicans* and *C. neoformans* with inhibition zones of 9±0.6 and 10±0.2 mm respectively, and MIC value of 100 mg/ml (Table 4). Several authors have reported antimicrobial activity of propolis from different regions of the world [27, 28, 29]. The antimicrobial effects of propolis extracts has been attributed to its phenolic and flavonoid content. As example, a study conducted by Hegazi et al. [30], has found that pinocembrin, benzyl caffeate and *p*-coumaric acid isolated from French propolis were partially responsible for its antimicrobial activity against *S. aureus; E. coli,* and *C. albicans.* Moreover, methyl ferulate, vanillin and 4-coumaric acid, are among the molecules reported to exhibit antibiofilm activity against several microorganisms, including *C. albicans* [31]. It is well known that phenolic compounds and flavonoids of propolis affects microbial viability through disruption membrane permeability, inhibition of ATP and DNA and RNA synthesis, decreasing microbial mobility as well as other activities [32, 33, 34].

CONCLUSION

The present study report high contents of bioactive compounds in Moroccan propolis from three geographic regions. The data revealed also high antioxidant, antibacterial and antifungal activities in these samples. Propolis from Sidi Bennour region was found to have the highest amount of phenolic and flavonoids compounds, and a stronger antioxidant and antimicrobial activities, making of it promising natural antioxidant and antimicrobial agents useful to combat diseases related to oxidative stress and pathogenic infections. Moreover, it could be also used as a suitable natural product in food industry to ensure safety and improve the food products quality. More detailed studies are required to identify the qualitative chemical composition of this Moroccan propolis.

Conflict of interest

The authors declare no conflict of interest.

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Table 4. Minimum inhibitory concentration of propolis extracts

	Minimum inhibitory concentration (mg/ml)					
Microorganisms	Gram negative bacteria		Gram positive bacteria		Yeasts	
Extracts	*Pseudomonas sp.*	*E. coli*	*S. aureus*	*E. faecalis*	*C. albicans*	*C. neoformans*
Sidi Bennour	5±0.0	2.25±0.0	1.25±0.0	1.25±0.0	100±0.0	100±0.0
Benslimane	7±0.0	5±0.0	6.5±0.0	4±0.0	ND	ND
Sidi Ifni	10±0.0	40±0.0	5±0.0	5±0.0	ND	ND

ND: not determined

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Received: 18.12.2023
Revised: 08.02.2024
Accepted: 18.02.2024

Rocz Panstw Zakl Hig 2024;75(1):83-91
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0289

ORIGINAL ARTICLE

CHANGES IN DIET AND PHYSICAL ACTIVITY IN STUDENTS DURING LOCKDOWN BY EXAMPLE OF COVID-19 PANDEMIC

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ABSTRACT

Background. Due to the spread of COVID-19 infections around the world, in early 2020, the World Health Organization (WHO) announced a global pandemic, i.e. an epidemic of particularly large dimensions affecting countries and entire continents. Long-term stay at home and self-isolation may have significantly impacted lifestyle, diet, food choices and access to food, as well as physical activity in the entire population, including students.

Objective. The aim of the study was to examine the impact of social isolation caused by the coronavirus pandemic on changes in diet, lifestyle and body mass index in a group of students, so that we would be better prepared for future new viral infections with characteristics similar to Covid-19.

Material and Methods. The study was conducted in 2021 using a cross-sectional online survey (using the CAWI technique). The survey was addressed to students of universities in Poland who were over 18 years of age. After excluding forms completed incorrectly or with incorrect data, the final analysis of the results included the responses of 196 respondents. Statistical analyzes were performed in STATISTICA 13.3. Statistical significance was assumed at the level of $p \leq 0.05$.

Results. The study involved 136 women and 60 men with an average age of 23. The majority of respondents were residents of cities with over 500,000 inhabitants (50%), were students of 1st degree (45%) in medical/natural sciences (36%). The largest percentage of respondents (above 70%), before the pandemic and during isolation, had normal body weight, according to the BMI. There were significant statistical differences between gender and changes during COVID-19 pandemic in sleeping (p=0.013), physical activity (p=0.028), as well as the consumption of tea (p=0.047), milk and dairy products (p=0.041), alcohol (p=0.001) and red meat (p=0.003), vegetables (p=0.049), sweets (p=0.029) and fast food (p=0.004).

Conclusions. Due to the fact that the impact of the coronavirus pandemic on the diet and lifestyle has been demonstrated, it is very important that the recommendations of public health organizations spread the message about rational nutrition and physical activity in the event of new viral infections among young people, including students.

Key words: *COVID-19 pandemic, social isolation, changes in diet, beverages, food products, lifestyle, students*

INTRODUCTION

As the result of the spread of a new disease called coronavirus (COVID-19), at the beginning of 2020, the World Health Organization declared a global pandemic. Due to the declaration of a global pandemic, on March 20, 2020, the Polish government officially announced the state of epidemic in the country, and then on March 25 it ordered the introduction of the obligation to social distance, stay at home for self-isolation, remote work and further closure of kindergartens, schools and universities [7, 13, 16]. Public health recommendations and government restrictions have led to a radical change in everyday life, including social distancing and isolation at home. Prolonged stay at home and self-isolation can significantly impact lifestyle, diet, food choices and access to food, as well as physical activity in the entire population, including students [13]. It was the first situation of this kind in the world, the effects of which can be used as models in scientific research, also in the field of human nutrition. Scientific publications define the pandemic as a sudden phenomenon characterized by the multidimensionality of medical and socio-economic experience, the effects of which are currently visible and the repercussions will be felt in the future [6].

According to the definition of the World Health Organization, coronavirus (COVID-19) is a disease caused by infection with the SARS-CoV-2 virus (severe acute respiratory syndrome coronavirus 2). Most people infected with this virus experience mild or moderate respiratory symptoms and become infected

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

without specific treatment. However, in some cases, the infection causes serious symptoms and requires special treatment and hospitalization. Older people, as well as people suffering from diseases such as circulatory system diseases, diabetes, chronic respiratory diseases or cancer, are more vulnerable to a serious course of the disease. Anyone can get sick with COVID-19 and experience serious symptoms or die, regardless of age [26].

The virus spreads through droplets from an infected person's mouth or nose, in small particles of liquid when they cough, sneeze, talk, sing or breathe. Transmission takes place mainly through larger droplets and respiratory aerosols [19]. How the virus infection progresses varies greatly depending on the patient's individual characteristics and the dose of the virus to which the patient was exposed [4]. Symptoms of COVID-19 infection appear on average 5-6 days after exposure to the virus, and the infection usually lasts up to 14 days [25].

Physiological states of the body and comorbidities potentially related to poor health, such as old age, obesity, diabetes or hypertension, have been identified as risk factors for severe and fatal courses of the disease. It has been found that people with diseases associated with organ damage, mainly the heart, liver and kidneys, are even more at risk of fatal virus infection [23]. In Spanish study carried out a retrospective cohort in patients between 18 and 70 years of age, included students diagnosed with COVID-19 and hospitalized. As a main result, it was observed that patients with a sedentary lifestyle had an increase in mortality from COVID-19 regardless of other previous risk factors. The authors concluded that a sedentary lifestyle increases the mortality of patients hospitalized with COVID-19 [18].

Safety measures and restrictions on movement, combined with the need for isolation and social distancing, are important in reducing the number of infections. However, restrictions may negatively impact people's mental health and lifestyle, including their diet, behavior and physical activity [1]. Students are among the group of people who are at risk of adverse reactions as a result of the restrictions introduced. Research indicates that students show high levels of stress and depression associated with isolation [21, 27]. In addition, fear of contracting COVID-19 and social isolation and reduced physical activity may lead to the consumption of excessive amounts of energy through food, which results in weight gain and eating disorders [2, 20, 22].

The impact of the COVID-19 pandemic on social functioning is very broad and complex. No aspect of normal social functioning was spared. Quarantine and social distancing are necessary measures to prevent the spread of the virus, but they can lead to repercussions related to both physical and mental health [15]. They can radically change individuals' daily habits, including related behaviors with lifestyle. Long-term stay and work at home may affect diet, food choice and access to food, and at the same time significantly limit physical activity [13]. Therefore, the aim of the study was to examine the impact of social isolation caused by the coronavirus pandemic on changes in diet and physical activity in a group of students, which could be a reflection and basis for information for future pandemics based on the lessons learned from COVID-19.

MATERIAL AND METHODS

Sample

The survey was conducted from November to December 2021, after several periods of COVID-19 quarantine in Poland and self-isolation, learning and remote work, as well as difficult access to internal and external exercise areas. The survey was addressed to students of universities in Poland who were over 18 years of age. The questionnaire was completed by 206 people. After excluding incorrectly completed forms or with incorrect data, the responses of 196 respondents were included in the final analysis of the results.

Data collection

The original questionnaire was used, which was administered as a Google form distributed in social media and included questions on socio-demographic and anthropometric data, questions regarding changes in diet and physical activity during social isolation caused by the pandemic. Questions about socio-demographic data concerned, among others, information related to gender (female or male), age, place of residence (village; town up to 500,000 inhabitants or city over 500,000 inhabitants), as well as the degree of study (1st degree - engineering/bachelor's degree; 2nd degree - master's degree; long-cycle degree - master's degree or 3rd degree - doctoral school), mode (full-time; part-time or evening) and field of study (artistic; economic/administrative; humanities/philological; medical/natural sciences; socio-pedagogical; technical or tourist/sports). The anthropometric data collected was height and body weight declared by respondents (before the pandemic and during the study). The collected anthropometric data, body mass (kg) and height (m) of the respondents, were used to calculate the Body Mass Index (BMI). The formula BMI = body weight/(height)^2 (kg/m^2) was used for the calculations four ranges of values were adopted: <18.5 (kg/m^2) - underweight, 18.5-24.9 (kg/m^2) - normal body weight, 25.0-29.9 (kg/m^2) - overweight and >30.0 (kg/m^2) - obesity [24]. Questions about changing diet (no consumption; less consumption; no changes or more consumption) concerned the amount of meals prepared at home before the pandemic and during

isolation, problems with access to food (yes or no) and the consumption of selected groups of food, such as: vegetables, fruits, legumes, whole grain products, eggs, white meat (e.g. poultry), red meat (e.g. beef), processed meat products, fish and seafood, fast food, sweets and sweet snacks, homemade sweets, salty snacks, as well as selected beverages eg.: milk and milk products, fermented milk products, water, fruit juices, sweetened drinks, coffee, tea, energy drinks and alcohol. The next stage of the survey included questions about the level of physical activity before the pandemic and its change during isolation, as well as changes in time spent in front of screens of electronic devices (for purposes other than studying or work) and changes in time spent on sleep (decreased; no changes or increased) [5, 13, 17].

Statistical analyses

The percentages of respondents were calculated, and the Pearson Chi^2 test was used for the statistical analysis. Statistical analysis of the results was performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, California, USA). Statistical significance was assumed at $p \leq 0.05$.

RESULTS

In the study took part 135 female and 60 male. The average age of the surveyed people was 23 years. Half of the respondents taking part in the study were people living in large cities with over 500,000 inhabitants. The other half of the surveyed group consisted of people living in towns up to 500,000 inhabitants (31%) or village (19%). In the study, the largest group, consisting of 45% of respondents were students of 1st degree, then students of 2nd degree (34%). The smallest percentage of respondents were students of 3rd degree (4%). Most respondents were full-time students (70%). Part-time students accounted for 29%, and evening students - 1%. The largest group of respondents were students of medical/natural sciences (36%). Students of economic and administrative faculties constituted 20% of the respondents, socio-pedagogical students 16%, technical studies 12%, and humanities/philosophical studies 10%. The smallest group were respondents declaring an artistic field (3%) or tourism/sports field (3%).

Table 1 shows changes in BMI and selected lifestyle factors during the COVID-19 pandemic. The majority

Table 1. Changes in BMI and lifestyle during COVID-19 pandemic in students (n=196)

Variables	Female 69.4% (n=136)	Male 30.6% (n=60)	p-value
BMI before pandemic:			
underweight	11.8 (16)	5.0 (3)	
normal weight	73.5 (100)	80.0 (48)	0.347
overweight	12.5 (17)	10.0 (6)	
obesity	2.2 (3)	5.0 (3)	
BMI during pandemic:			
underweight	10.3 (14)	(0)	
normal weight	75.0 (102)	81.7 (49)	0.274
overweight	14.0 (19)	15.0 (9)	
obesity	0.7 (1)	3.3 (2)	
Sleep time changes:			
decresased	16.9 (41)	15.0 (9)	0.013
no changes	30.2 (23)	51.7 (31)	
increased	52.9 (72)	33.3 (20)	
Screen time changes:			
decresased	2.9 (4)	1.7 (1)	0.608
no changes	19.9 (27)	15.0 (9)	
increased	77.2 (105)	83.3 (50)	
Physical activity changes:			
decresased	47.1 (64)	58.3 (35)	0.028
no changes	25.7 (35)	31.7 (19)	
increased	27.2 (37)	10.0 (6)	
Difficulties with food availability during pandemic:			
no	90.4 (123)	90.0 (54)	0.543
yes	9.6 (13)	10.0 (6)	
Changes in total food consumption during pandemic:			
less consumption	9.5 (13)	13.4 (8)	0.180
no changes	30.9 (42)	33.3 (20)	
more consumption	59.6 (81)	53.3 (32)	

of people participating in the study, both before and during the pandemic, had a normal BMI. During the pandemic, a decrease in the percentage of respondents who are underweight and obese was observed compared to before the pandemic. During the pandemic, the percentage of students whose BMI indicated overweight, increased. No statistically significant differences were found between the BMI value and the gender of the examined persons. The largest percentage of the examined students declared increased sleep time, increased screen time and increased total food intake during the pandemic, compared to time before. The largest percentage of respondents declared reduced physical activity and no difficulties with food availability during the pandemic. In the study group, a statistically significant difference was found between

Table 2. Changes in beverages consumption during COVID-19 pandemic in students (n=196)

Variables	Female 69.4% (n=136)	Male 30.6% (n=60)	p-value
Tea intake:			
non consumption	2.2 (3)	1.7 (1)	
less consumption	19.1 (26)	21.7 (13)	0.047
no changes	39.0 (53)	56.7 (34)	
more consumption	39.7 (54)	19.9 (12)	
Coffe intake:			
non consumption	11.8 (16)	21.7 (13)	
less consumption	27.2 (37)	35.0 (21)	0.105
no changes	38.2 (52)	25.0 (15)	
more consumption	22.8 (31)	18.3 (11)	
Water intake:			
non consumption	0.7 (1)	(0)	
less consumption	18.4 (25)	6.7 (4)	0.159
no changes	39.7 (54)	43.3 (26)	
more consumption	41.2 (56)	50.0 (30)	
Milk and milk prodacts intake:			
non consumption	6.0 (8)	3.4 (2)	
less consumption	15.4 (21)	15.0 (9)	0.041
no changes	52.9 (72)	69.9 (42)	
more consumption	25.7 (35)	11.7 (7)	
Fermented milk drinks intake:			
non consumption	14.7 (20)	15.0 (9)	
less consumption	11.8 (16)	3.4 (2)	0.124
no changes	53.8 (73)	68.3 (41)	
more consumption	19.7 (27)	13.3 (8)	
Sugar-sweetened beverages intake:			
non consumption	24.3 (33)	11.7 (7)	
less consumption	22.8 (31)	23.3 (14)	0.229
no changes	35.3 (48)	43.3 (26)	
more consumption	17.6 (24)	21.7 (13)	
Fruit juices intake:			
non consumption	8.9 (12)	6.7 (4)	
less consumption	19.1 (26)	28.3 (17)	0.359
no changes	52.9 (7)	53.3 (32)	
more consumption	19.1 (26)	11.7 (7)	
Energy drink intake:			
non consumption	44.1 (60)	33.3 (20)	
less consumption	19.7 (27)	28.3 (17)	0.286
no changes	26.5 (36)	23.3 (14)	
more consumption	9.7 (13)	15.1 (9)	
Alcohol consumption:			
non consumption	16.2 (22)	11.7 (7)	
less consumption	28.8 (39)	58.3 (35)	0.001
no changes	35.3 (48)	18.3 (11)	
more consumption	19.7 (27)	1.7 (7)	

the gender in the change in the sleep time and physical activity during the pandemic. Women slept longer (p=0.013) and showed less physical activity (p=0.028) compared to men.

The study showed changes in the consumption of selected beverages that occurred during the COVID-19 pandemic (Table 2). It was found that at that time the largest percentage of people declared higher water consumption and lower alcohol consumption, while the consumption of other drinks remained unchanged. Statistically significant differences were found between gender and the change in consumption of tea (p=0.047), milk and dairy products (p=0.041) and alcohol (p=0.001). It was found that during the pandemic, a larger percentage of men compared to women consumed less alcohol and more of them kept their consumption of tea, milk and dairy products unchanged.

Table 3 shows changes in the consumption of selected food products that occurred during the COVID-19 pandemic. It was found that the largest percentage of people at that time declared a higher consumption of homemade sweets and a lower consumption of fast food, while the consumption of other products remained unchanged. Statistically significant differences were found between gender and the change in consumption of red meat (p=0.003), vegetables (p=0.049), sweets and sweet snacks (p=0.029) and fast food (p=0.004). It was found that during the pandemic, a greater percentage of men compared to women consumed more vegetables, less fast food, and more of them kept their consumption of red meat, sweets and sweet snacks unchanged.

Table 3. Changes in food consumption during COVID-19 pandemic in students (n=196)

Variables	Female 69.4% (n=136)	Male 30.6%(n=60)	p-value
Whole grain products intake:			
non consumption	4.4 (6)	5.1 (3)	
less consumption	14.7 (20)	11.7 (7)	0.951
no changes	61.0 (83)	63.3 (38)	
more consumption	19.9 (27)	19.9 (12)	
Eggs intake:			
non consumption	4.4 (6)	5.1 (3)	
less consumption	14.0 (19)	18.3 (11)	0.539
no changes	55.9 (76)	60.0 (36)	
more consumption	25.7 (35)	16.6 (10)	
Fishes ans seafood intake:			
non consumption	16.1 (22)	13.4 (8)	
less consumption	18.4 (25)	18.3 (11)	0.777
no changes	51.5 (70)	58.3 (35)	
more consumption	14.0 (19)	10.0 (6)	
Processed meat intake:			
non consumption	21.3 (29)	8.3 (5)	
less consumption	16.9 (23)	20.0 (12)	0.168
no changes	50.0 (68)	60.0 (36)	
more consumption	11.8 (16)	11.7 (7)	
Red meat intake:			
non consumption	29.4 (40)	10.0 (6)	
less consumption	21.3 (29)	13.4 (8)	0.003
no changes	41.9 (57)	61.5 (37)	
more consumption	7.4 (10)	15.1 (9)	
Wheat meat intake:			
non consumption	16.1 (22)	6.7 (4)	
less consumption	14.9 (20)	11.7 (7)	0.138
no changes	52.9 (72)	55.0 (33)	
more consumption	16.1 (22)	26.6 (16)	
Vegetables intake:			
non consumption	0.7 (1)	(0)	
less consumption	19.9 (27)	15.1 (9)	0.048
no changes	50.0 (68)	36.7 (22)	
more consumption	29.4 (40)	48.2 (29)	

Fruits intake:			
non consumption	0.7 (1)	(0)	
less consumption	16.1 (22)	10.0 (6)	0.509
no changes	52.3 (71)	61.5 (37)	
more consumption	30.9 (42)	28.5 (17)	
Legumes intake:			
non consumption	5.9 (8)	11.7 (7)	
less consumption	21.3 (29)	6.7 (4)	0.059
no changes	61.8 (84)	66.5 (40)	
more consumption	11.0 (15)	9 (15.1)	
Homemade sweets intake:			
non consumption	8.0 (11)	5.0 (3)	
less consumption	19.1 (26)	18.3 (11)	0.828
no changes	30.2 (41)	35.0 (21)	
more consumption	42.7 (58)	41.7 (25)	
Salty snacks:			
non consumption	5.2 (7)	1.6 (1)	
less consumption	23.5 (32)	21.7 (13)	0.132
no changes	35.3 (48)	51.7 (31)	
more consumption	36.0 (49)	25.0 (15)	
Sweets and sweet snacks:			
non consumption	2.9 (4)	(0)	
less consumption	25.0 (34)	28.3 (17)	0.029
no changes	29.4 (40)	46.7 (28)	
more consumption	42.7 (58)	25.0 (15)	
Fast food intake:			
non consumption	14.7 (20)	3.4 (2)	
less consumption	33.1 (45)	58.3 (35)	0.004
no changes	28.7 (39)	25.0 (15)	
more consumption	23.5 (32)	13.3 (8)	

DISSCUSION

In this study, it was shown that the largest percentage of respondents, before the pandemic and during isolation, had normal body weight, according to the BMI index. We observed the largest percentage of the examined students declared increased sleep time, increased screen time and increased total food intake during the pandemic compared to time before. It was found that significantly more women admitted an increase in sleep and a decrease in physical activity, compared to men. We observed the largest percentage of participants declared more consumption: water and homemade sweets and less consumption: alcohol and fast food during pandemic. The significant statistical differences between gender and changes during COVID-19 pandemic in the consumption of tea, milk and dairy products, alcohol and red meat, vegetables, sweets and sweet snacks and fast food were observed.

In our study, we found that the largest percentage of respondents before the pandemic and during isolation had normal body weight and decreased physical activity. Research shows similar results regarding the decline in physical activity, but different results for body weight [8, 9, 12]. In study Giustino et al. [12] analyzed the change in the level of physical activity based on energy expenditure (min/week) among the physically active population of Sicily before and during the last seven days of quarantine. The relationship between this parameter and specific demographic and anthropometric variables was also analyzed. It was found that quarantine had a negative impact on the level of physical activity, with the greatest effect among men and overweight people. With respect to age groups, adolescents, young adults, and adults were more affected than older adults and seniors. In the study Dobrowolski and Włodarek [8] was described the impact of social isolation in the first phase of the pandemic on body weight, physical activity and eating behavior of Poles. Almost 50% of the surveyed people observed an increase in body weight due to a decrease in physical activity and an increase in food consumption. For people whose overall weight did not decrease or change, the increase was approximately 2 kg during the short period of pandemic restrictions. There was a general decrease in the level of physical activity among the respondents, as well as changes in the amount of food consumed and individual groups of food products, including alcohol. Another study conducted in a group of Polish women focusing on the impact of the pandemic and social isolation on

body weight changes showed that the isolation period caused bidirectional changes in body weight in the above-mentioned group. More than half of the study participants experienced a change in body weight, 34% of women gained weight, while 18% lost weight. In the weight gain group, women increased their body weight by an average of 2.8 kg, and about 65% of them increased their total food intake. As a negative effect of the pandemic, the percentage of women who were underweight before the pandemic whose body weight decreased and women who were obese before the pandemic who gained weight during social isolation should be assessed [9].

We observed the largest percentage of the examined students declared increased sleep time and increased screen time. Similar results were obtained by Wlazło et al. [22]. The surveyed students from Poland and Turkey also declared an increase in sleep time and time spent in front of TV or computer. However, in the study by Górnicka et al. [13], a similar result was obtained regarding the highest percentage of people who declared an increase in the time spent in front of a computer or TV during the pandemic, while sleep duration remained unchanged in a larger percentage of people at this time.

We found the largest percentage of the examined students declared increased total food intake during the pandemic compared to time before. Similar results were obtained in studies by other authors. The sanitary and epidemiological situation caused by the global spread of SARS-CoV-2 had directly influenced consumer behavior in areas such as gastronomy, grocery shopping and the precautionary measures taken. Panic related to the possibility of COVID-19 infection played a significant role and largely forced customers to change the typical way of shopping for groceries and using the services of catering establishments [14]. For comparison, the main findings in the study Gallo et al. [11] in the early phase of the pandemic, during isolation and a break in attendance for full-time academic classes, there was an increase in total energy consumption, frequency of eating and energy density of consumed food in the group of women, as well as a decrease in the level of physical activity both among women and men. Also in study Ammar et al. [2] reported that students' consumption patterns changed towards more frequent binge eating and uncontrolled eating during lockdown. During the pandemic among a significant number of respondents, there was an increase in the frequency of eating meals prepared at home and eaten among household members. The respondents derived greater joy from cooking and experimenting with recipes. They also reported reducing the frequency of using ready-made meals [10]. However, there were also those who declared that one of the reasons for ordering food from restaurants is the lack of time to prepare it at home and the general lack of desire to prepare dishes [14]. Interesting results were obtained in Rodriguez-Perez et al. [17] study, which was also conducted in the early phase of the pandemic in 2020 among the adult population. This report was the first to indicate the direction of changes in the diet of Spaniards for the better during social isolation caused by restrictions and social distancing. Respondents showed increased interest in the dietary patterns of the Mediterranean diet. This diet is considered a model of proper and healthy nutrition, it is dominated by vegetables, fruits and olive oil, and is also recommended as one of the ways to strengthen and maintain the proper functioning of the immune system.

In our study we found that largest percentage of participants declared more consumption water and homemade sweets and less consumption alcohol and fast food during pandemic. The significant statistical differences between gender and changes during COVID-19 pandemic in the consumption some of food products were observed. Other studies have observed various changes in the amount of food consumed [3, 10, 13, 22]. In September 2020, *EIT Food* (part of the European Innovation Institute and Technology) [10] conducted a survey among 5,000 consumers in ten European countries, including Poland. It was found there has been a significant increase in the frequency of online and wholesale purchases, as well as an increase in the consumption of almost every food group, especially fruit, vegetables and flour. On the other hand the Canadian study on the impact of the COVID-19 pandemic on college students' food intake, physical activity and lifestyle found that their diet during lockdown was worse than their usual pre-pandemic diet. Reduced frequency of consumption of cereals, fruits, vegetables, dairy products, nuts, meat and its plant alternatives was observed. Reducing the consumption of these products has contributed to an insufficient supply of both macro- and micronutrients and an increase in the occurrence of nutritional deficiencies [3]. Changes in the frequency of consumption of vegetables, fruit, legumes, milk and fish before and during the pandemic were also found among Polish and Turkish students [22]. Whereas, in the study Górnicka et al. [13], the largest percentage of respondents did not declare any changes in the consumption of main groups of selected food products during the pandemic.

It seems that in the event of the above threat among students, it would be helpful to create the position of a coordinator for counteracting viral infections at universities, as well as an appropriate website. The above would provide information on the correct way to deal with an emergency situation by including dietary recommendations that are aimed at supporting the immune system, and would also encourage physical activity.

Strengths and limitations

The present study had some limitations. First, our sample size was not large and we cannot generalized our results. Second, our data was based on self-assessment selected anthropometric measurements and change in food consumption. There is a lack of quantitative data on food consumption. Third, our study only examined the short-term impacts of the pandemic on food consumption, lifestyle and body weight.

The strengths of the study was used an online survey because epidemic situation makes it impossible to conduct research in stationary conditions. Moreover, our study allowed us collected data about lifestyle, selected anthropometric measurements and food consumption before and during COVID-19 pandemic.

CONCLUSION

1. It was shown that the largest percentage of respondents, before the pandemic and during isolation, had normal body weight, according to the BMI index.
2. The largest percentage of the examined students declared increased sleep time, screen time and total food intake during the pandemic compared to the time before. It was found that significantly more women admitted an increase in sleep and a decrease in physical activity, compared to men.
3. We found the largest percentage of participants declared more consumption: water and homemade sweets and less consumption: alcohol and fast food during pandemic. The significant statistical differences between gender and changes during COVID-19 pandemic in the consumption of tea, milk and dairy products, alcohol and red meat, vegetables, sweets and sweet snacks and fast food were observed.
4. In the event of the appearance of new viral infections and the need to introduce isolation in social life, it is very important to educate the public by public health organizations by providing dietary recommendations and maintaining physical activity among young adults.

Conflict of interest

The authors declare that there are no conflict of interest regarding the publication of this paper.

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Received: 13.01.2024
Revised: 01.02.2024
Accepted: 06.02.2024

Rocz Panstw Zakl Hig 2024;75(1):93-99
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0291

ORIGINAL ARTICLE

PREPAREDNESS, RESPONSE, AND PREVENTIVE BEHAVIORS FOR A PANDEMIC DISASTER CAUSED BY COVID-19 AMONG PRIMARY SCHOOL STUDENTS IN KRABI PROVINCE, THAILAND

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ABSTRACT

Background. The global impact of the Coronavirus Disease 2019 (COVID-19) pandemic has been enormous. The primary school's role and responsibilities include providing students with education and training, cooperating and supporting educational management, and monitoring, following up, and evaluating the school's operations.

Objective. The goals of this study were to evaluate the conditions of preparation for the COVID-19 epidemic's prevention and response, as well as to investigate the relationship between predisposing, enabling, and reinforcing factors in preventing the spread of COVID-19 among primary school students in the educational area.

Material and Methods. A cross-sectional analytical study was used as the research design. The informants are kids in grades 4-6 at Anuban Krabi School in Krabi province. Data is gathered in a computational quantitative manner. Purposive sampling was used to choose the informants. The researchers employed a questionnaire with a content validity index (CVI) of 0.89 and a reliability coefficient (Cronbach's Alpha Coefficient) of 0.89. For quantitative data analysis, the Pearson Correlation Coefficient was used.

Results. In preparation for the COVID-19 outbreak, there is a high degree of knowledge, behaviors, and self-conduct ($\bar{x}$=2.78, 2.55, 2.66, and 2.78). The perception and awareness of the COVID-19 epidemic were statistically significant at $P < 0.01$ in terms of the relationship between perception, awareness, behavior, and self-conducting in stopping the spread of COVID-19. Conclusion: At $P < 0.01$, regarding the relationship between COVID-19 epidemic perceptions, behaviors, and self-conducting. The correlation between awareness, behaviors, and self-conducting in relation to the COVID-19 outbreak was statistically significant at $P<0.01$.

Conclusions. To prepare and response for COVID-19 enhance and concern perceived policy and COVID-19 information, participation with stakeholders or communities emphasize COVID-19 prevention and all of organization and family.

Key words: *preparedness, response, preventive behaviors, disaster, COVID-19, Thailand*

INTRODUCTION

COVID-19 is an emerging disease that began to spread worldwide in December 2019. The World Health Organization (WHO) officially announced the disease on February 11, 2020 as Coronavirus Disease 2019 [12]. WHO declared COVID-19 an epidemic on March 11, 2020 with 731,453 cases and 34,660 deaths globally [13]. The outbreak was first reported from Wuhan, Hubei Province, China [10, 11]. The disease is primarily transmitted by inhalation of droplet transmission from a patient who coughs, sneezes or exhales and is invisible to the eyes about one to two meters distance is the distance that can be infected when approaching the patient [17]. WHO is issuing the COVID-19 Strategic Preparedness and Response Plan (SPRP) for 2021, aimed at guiding the coordinated action to overcome the ongoing challenges in the response to COVID-19, address inequities, and plot a course out of the pandemic [14]. The incubation period for the disease is mostly about 4.5 to 5.8 days (average 5.2 days) and the basic reproductive number R(0) was 2.2 at the onset of the outbreak [9]. Coronavirus infection (COVID-19) can be asymptomatic or cause mild to severe clinical illness [8]. As of June 29th, 2021, the current situation of the COVID-19 epidemic has been reported worldwide with 182,277,428 confirmed cases, 3,947,643 deaths, Case Fatality Rate (CFR) was at 2.17% [15]. Globally, the mortality rate is estimated about 3.4% of reported COVID-19 cases have died,

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

possibly as high as 12 percent of patients that higher effected by underlying disease and older age [4, 11]. The outbreak effects with over 3.5 million recorded deaths from COVID-19, an estimated loss to the global economy of 22 trillion US dollars and new variants compounding explosive outbreaks [16]. The outbreak situation in Thailand as of June 29th, 2021, reported 254,515 confirmed cases, 1,970 deaths and Case Fatality Rate (CFR) was at 0.77% [4]. Particularly, in upper southern region consists of 7 provinces, which are Nakhon Si Thammarat, Surat Thani, Chumphon, Ranong, Krabi, Phang Nga, and Phuket, with 6,537 confirmed cases, 1,523 being hospitalized, and 38 deaths. According to Krabi Provincial Office report on June 29th, 2021, the situation of COVID-19 in Krabi province, there are 404 confirmed cases, Therefore all countries should increase their level of preparedness, alert and response to identify, manage and care for new cases of COVID-19 [18]. In the context of schools and learning situations, which address techniques that can assist reduce sickness exposure and lower the likelihood of transmission among students, instructors, and non-teaching staff. Schools and early care and education (ECE) programs, as well as social, physical, behavioral, and mental health services, are provided by schools are critical components of a community's infrastructure [4]. Educational institutions are therefore very important to promote health and prevent disease, or it can be compared to "school" as a "shelter" for students, which must be considered and given priority to such matters. Under the epidemic situation of coronavirus disease 2019 or COVID-19, which currently has no vaccine to prevent COVID-19 and there is no suitable medicine for children, it is imperative that educational institutions are prepared to deal with the epidemic situation that will greatly affect the teaching and learning system and the health of students and staff in schools. Henceforth, the most important thing is that administrators, teachers, students, parents and personnel of educational institutions must adapt to a new way of living, known as "New Normal", emphasizing practice under measures to prevent the spread of COVID-19 strictly to make educational institutions a safe place from COVID-19, enabling students to learn to their full potential and be safe from disease [7]. The preparation before reopening schools after closing is of great necessity according to rigorous evidence relevant to the COVID-19 emergency to formulate recommendations for policymakers on five critical dimensions of school reopening and recovery including: (1) Engaging communities in reopening plans, (2) Targeting resources to where they are most needed, (3) Getting children back to school, (4) Making school environments safe, and [5]. Recovering learning loss and building back better [1]. According to Guidelines for educational institutions to prevent the spread of COVID-19, Thailand, Department of Health Ministry of Health at 20 May 2020, consists of 6 points: (1) screening must be screened for body temperature, (2) Wearing mask throughout their time at school, (3) Hand-washing with soap and water for at least 20 seconds or use an alcohol gel, (4) Maintaining social distance a distance of at least 1-2 meters between people, (5) Open doors and windows to allow ventilation if air conditioning is needed; set time limits for turning on and off air conditioners; open doors and windows for ventilation every 1 hour; and cleaning classrooms and surrounding areas and providing rubbish bins with lids and collecting garbage from the classroom for disposal every day, (6) Reduce congestion (reduce the time of work, reduce the duration of the activities as much as possible and avoid group activities [5]. The COVID-19 epidemic situation has a huge impact on all sectors. When the situation improves, the reopening of onsite schools after the closure is necessary to be prepared. Strict behavior among students and personnel in schools or educational institutions will reduce the chance of infection and prevent the spread of COVID-19 and ensure safety for everyone. Therefore, there should be an assessment of the preparedness and responded for the term to begin and establish a connection with the preventive measures of the Center for COVID-19 Epidemic Situation Management to prevent the spread of COVID-19. Krabi, in Southern Thailand, is a tourist city with people traveling in and has a large number of schools and students, which are directly affected by the COVID-19 epidemic, and there is no research work on the preparation of primary schools in the area. This study aimed to assess the following community factors for COVID-19 prevention using the PRECEDE model as a conceptual framework [6]. The PRECEDE model was applied for social and ecological assessment and is comprised of predisposing, enabling, and reinforcing factors related to behaviors in Predisposing factors such as perceived risk and the advantage of risk prevention, enabling factors such as: school sanitation, and reinforcing factors such as perceived policy and COVID-19 information, participation with stakeholders or communities emphasize COVID-19 prevention. The conceptual of the study showed as Figure 1.

MATERIALS AND METHOD

A cross-sectional analysis study to assess the situation Preparedness, Response and Preventive Behaviors for a Pandemic Disaster Caused by COVID-19 among Primary School Students in Krabi Province, Thailand. The study was approved by the Ethics Committee in Human Research Walailak

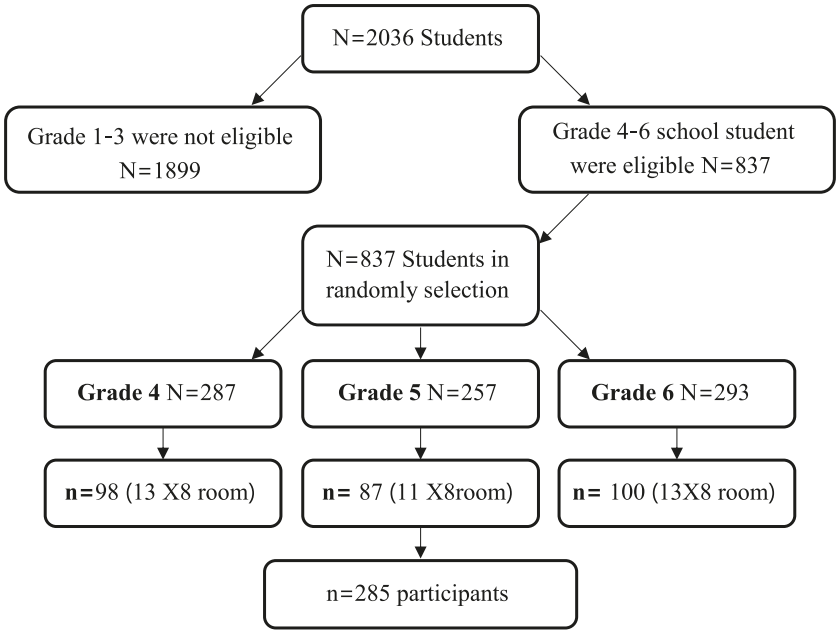

Figure 1. Flowchart for sample selection

University Project No. WUEC-21-244-01 on September 2rd, 2021.

Population and samples

There are 211 schools under the Krabi Primary Educational Service Area Office and 2 schools under the Special Education Bureau. Krabi province has 1 primary education area in Krabi province, covering the entire province of Krabi. From the target population of 837 people, 271 people were calculated using Krejcie and Morgan formula from the target population and added a 5% error in data collection to cover the grades 4-6, totaling 285 students in grades 4-6 were participated in this research as flowchart in Figure 1.

Methods

The research instrument was a questionnaire studied personal factors, predisposing factors (perceived of risk, perceived severity), enabling factors (well-being environment, access disease prevention equipment and activities to promote disease prevention), reinforcing factors (Policy and standard of prevention COVID spreading in primary school) and social support. Content validity and the reliability of the study instruments were checked by 3 experts (CVI = 0.89, Cronbach's Alpha Coefficient = 0.89). The questionnaire consisted of 5 parts, the first part consisted of 6 items of personal data, and the second part was 11 risk perception and benefit on COVID-19 prevention, the third part was a 21 questions of the enabling factors and social support, the forth part were 12 questions on preventive behavior in outbreak, and the fifth part about the student preparation of the COVID-19 epidemic, totaling 16 items, and the third part was a questionnaire on preparation pattern of students for COVID-19 prevention, 22 items.

Data collecting. Due to data collecting during COVID-19 situation, the researchers collected the data using questionnaire through the Google Forms.

Statistical analysis

The study was used a SPSS version 17.5 program and descriptive statistics were used to analyze personal data such as gender, age, religion, educational level of students parents' and occupation parent's income and analyzed the situation assessment and preparation pattern for preventing the spread of COVID-19 among schools in the Krabi educational area by frequency distribution, percentage, and analyzed the relationship of predisposing factors, enabling factors, and reinforcing factors in preventing the spread of COVID-19 among students in Krabi education area using Pearson's Correlation coefficient.

RESULTS

The results showed that 285 students participated in the research were mostly female (53.7%) because Anuban Krabi School had a total of 2,035 students, comprising 1,060 female students (52.1%) and 975 male students (47.9%) (Krabi Secondary Education Service Area, 2021). The research subjects were aged between 9-13 years old. Majority of participants were 11 years (38.2%) and were Buddhist (73.7%). Students' parents were merchant/personal business (47.8%) and their parents' income was 0 – 15,000 US a month (35.4%) (Table 1).

Table 1. Frequency distribution of students by sex, age, region, level of education, parents' occupations and parents' incomes (n = 285)

Variables	Group	Number	Percentage
Sex	Male	132	46.30
	Female	153	53.70
Age	9 years	47	16.50
	10 years	75	26.30
	11 years	109	38.20
	12 years	50	17.50
	13 years	4	1.40
Religion	Muslims	74	26.00
	Christians	1	0.40
	Buddhism	210	73.70
Grade of education	Grade 4	98	34.40
	Grade 5	87	30.50
	Grade 6	100	35.10
	Unemployed	15	5.30
	General contractor	20	7.00
	Government officer	106	37.20
	Merchant/Personal	136	47.80
	Agricultural	8	2.80
Incomes (US/month)	0 – 500	101	35.40
	501 – 1,000	94	33.00
	1,001 – 3,000	31	10.90
	> 3,000	59	20.70
Mean(SD) = 1,194.05 (1592.48)Min:Max = 1,000:15,000			

Level of student assessment and schools' self-assessment

In the assessment of students, the researcher studied all 5 factors as follows: Students' perception (SP) with 12 items, Students' Behaviors (SB) with 5 items, School Environment management (SE) with 10 items, Readiness (RE) with 12 items and Parents' Support (PS) with 11 items. For Schools' self-assessment 44 items. The cut-off point of all factors' level was considered using the Ministry of Education's preparatory criteria for school openings in Thailand; Low level (not allowed to open) = less than 50% of score, Moderate level (openable with conditions) = 51%-99% of score and High level (Fully on-site open) = 100% of score.

The results showed that most of Students' Perception (SP) was Moderate level (99.60%) and Low level (0.40%), the all average of SP was Moderate level with (mean, SD: 29.91, 2.38). The Students' Behaviors (SB) showed High level (21.10%) and Moderate level (78.90%), the average of SB was Moderate level with (mean, SD: 13.28, 1.44). School Environment (SE), showed High level (21.40%) and Moderate level (78.60%), the average of SE was Moderate level with (mean, SD: 28.15, 1.76). In terms of Readiness (RE), the result showed High level (26.70%), Moderate level (72.30%) and Low level (1.10%), the average of RE was Moderate level with (mean, SD: 33.03, 3.83). For the Parents' support (PS), the average majority were Moderate level (64.90%), High level (32.60%) and Low level (2.50%) with (mean, SD: 30.13, 3.97).

In the other hand, The Schools' self-assessment showed the high level (100%) in all aspects that was contrast from the Student Assessment Levels that was Moderate level (Table 2).

The analysis of variation in the scores from Grade 4 to Grade 6 students in the Students' perception (SP) was done by one-way ANOVA test, which shown significant increase with the increasing education level of study to Grade 6. However, Students' behaviors (SB), School Environment (SE), Readiness (RE) and Parents' support (PS) had no difference in each education level. (Table 3)

Predicting factors by multiple linear regression (Enter)

To explore the predictor variables of Readiness for preventing the spread of COVID-19 among students, the main independent variables such as Students' perception (SP), Students' behaviors (SB), School Environment (SE) and Parents' support (PS) model constructs entered the regression model analysis. The model accounted for 61% of the variance of Readiness. According to the results of Table 4, increasing the score of Students' perception (Beta 0.314, P<.001) and School Environment (Beta 0.587, P<.001) were positively with Readiness. The positive relationship

Table 2. Number, percentage, mean, standard deviation of variables in preventing the spread of COVID-19 among students and Schools' self-assessment (n = 285)

Variables	Student assessment levels					Schools' self-assessment
	Low n (%)	Moderate n (%)	High n (%)	Mean (SD)	Level	
Students' perception	1(0.40)	284 (99.60)	-	29.91 (2.38)	Moderate	High
Students' behaviors	-	225 (78.90)	60 (21.10)	13.28 (1.44)	Moderate	High
School environment	-	224 (78.60)	61 (21.40)	28.15 (1.76)	Moderate	High
Readiness	3 (1.10)	206 (72.30)	76 (26.70)	33.03 (3.83)	Moderate	High
Parents' support	7 (2.50)	185 (64.90)	93 (32.60)	30.13 (3.97)	Moderate	High

Table 3. One-way ANOVA test for the comparison of mean scores of predisposing factors, enabling factors, and reinforcing factors based on level of education (n = 285)

Variables	Mean±SD			F value	P value
	Grade 4	Grade 5	Grade 6		
Students' perception (SP)	29.67±2.37	29.58±2.83	30.45±1.84	3.920	.021*
Students' behaviors (SB)	13.23±1.41	13.10±1.55	13.48±1.36	1.662	.192
School Environment (SE)	28.20±1.55	27.85±2.07	28.38±1.64	2.164	.117
Readiness (RE)	33.14±3.55	32.45±4.68	33.44±3.22	1.579	.208
Parents' support (PS)	29.98±3.80	30.66±3.79	29.82±4.26	1.160	.315

* P<0.05 is significant

Table 4. Predictors in Multiple Linear Regression (The beta Coefficients)

Variables	B	Std. Error	95%CI	Beta	t	p
(Constant)	-15.588	2.697	[-20.896, -10.280]		-5.781	0.000
SP	0.505	0.090	[0.328, .681]	0.314	5.625	<0.001*
SE	1.276	0.102	[1.075, 1.477]	0.587	12.482	<0.001*
SB	-0.132	0.139	[-0.405, .142]	-0.050	-0.948	0.344
PS	-0.022	0.036	[-0.093, .049]	-0.023	-0.605	0.546

Note. R^2_{Adj} = 0.61 (n=285, p=0.000), CI = Confident interval for B, * P<0.01 is significant

indicated that student who had higher Students' perception (SP) and School Environment (SE) scored higher on Readiness for preventing the spread of COVID-19. (Table 4).

DISCUSSION

Closures of schools during coronavirus disease pandemic may not be well grounded for SARS-CoV-2 pandemic. Children are less affected by this virus and clinical attack rates in the 0–19 age group are low. Experts opine that school closures might have negative effects on scholastic abilities of children. Based on the results of the study of school preparation for preventing the spread of COVID-19 in all 5 aspects, the overall average preparation is at a high level, consistent with research on Readiness for School Opening in Outbreak of Coronavirus Disease 2019 For Wang Saphung Municipal School 1, Wang Saphung Municipality, Loei Province (Jaruwan Boonsorn, 2021). The research found that Wang Saphung Municipal School 1, Wang Saphung Municipality, Loei Province, is ready to open onsite classes in the situation of the 2019 coronavirus epidemic. Overall, it is at the highest level.

Perception and awareness about the spread of COVID-19epidemic were statistically significant low positively correlated at p-value <0.01 (r =0.248, P Value 0.000). It has been shown that perception raises awareness and can be applied to prevent COVID-19. Therefore, perception is associated with awareness and has a protective effect against COVID-19, which is consistent with the research on Information Exposure Behaviors and Awareness of Udon Thani Students During the COVID-19 Epidemic (Seksun Saiseesod, 2021). Regarding awareness of the COVID-19 epidemic, it was found that students had the highest level of awareness. Perception and behavior and self-conducting in the COVID-19 epidemic had a statistically significant moderate positive correlation at p–value <0.05 (r =0.455, P Value 0.000), showing that awareness can lead to behaviors that can prevent COVID-19. Consequently, perception is correlated with behavior and has a protective effect against COVID-19, consistent with research on Perceptions and Preventive Behaviors of the COVID-19 among High School Students in Bangkok, Thailand [19], which is found that the perception of Coronavirus disease 2019 was moderately positively correlated (r = 0.436) with Coronavirus 2019 prevention behaviors statistically significant at the .01 level. Awareness and behavior and self-conducting in the COVID-19 epidemic had a statistically significant moderate positive correlation at p<0.05 (r = 0.455, P Value 0.000) means awareness contributes to behaviors in the prevention of COVID-19. Consequently, awareness is correlated with behavior and affects the prevention of COVID-19, consistent with research Hand Hygiene, Mask Wearing Behaviors and Its Associated Factors during the COVID-19 Epidemic: A Cross-Sectional Study among Primary School Students in Wuhan, China [3]. It was found that primary school students in Wuhan, China, had good hand washing and masking behaviors.

CONCLUSIONS

To prepare and response for COVID-19 enhance and concern perceived policy and COVID-19 information, participation with stakeholders or communities emphasize COVID-19 prevention and all of organization and family.

Acknowledgments

The authors would like to thank all participants for providing all essential information and pass the gratitude to the Bachelor of Public Health Program, School of Public Health, Walailak University, that provides funding for research expenses and also be thankful to EC for DACH (Excellence Center for Dengue and Community Public Health) the sample group. In addition, author thanks school's director and all teachers of Anuban Krabi School. for helping gathering research data successfully.

Ethical consideration

This research was Compliance with Ethical Standards have been approved by an appropriate ethics committee, Research Institute Walailak University (Project No. WUEC-21-244-01 on September 2rd, 2021) and all participants were informed and consent given.

Conflict of interest

The Authors declare no conflict of interest.

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Received: 09.12.2023
Accepted: 08.02.2024

Rocz Panstw Zakl Hig 2024;75(1):101-106
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2024.0286

ORIGINAL ARTICLE

PARENTS' PERCEPTIONS OF MORBIDITIES AND SOME FUNCTIONAL ABILITIES IN PEOPLE WITH DOWN SYNDROME IN MOROCCO

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ABSTRACT

Objective. This study aimed to assess parental perceptions of morbidity and certain functional abilities in people with Down syndrome (DS) and their variability according to age and sex in Morocco.

Material and Methods. A retrospective and analytical survey was conducted between May 2014 and November 2017, and addressed to the parents of 279 individuals with DS, including 161 boys (57.7%) aged 1-40 years. The sample was subdivised to tree age groups, children under 10 years old, adolescents aged 10-18 years and adults aged ≥ 18 years. Information about the identity of parents, age and sex of people with DS, their morbidity during the two years preceding the survey, and some functional abilities was collected. Data were entered and analyzed using the statistical program SPSS statistics software for Windows (version 20.0). Chi-square ($\chi 2$) test was used for testing statistical significance. Differences were considered significant when the p-value <0.05. The multivariate analysis were used to identify the causes of morbidies independently associated with age and sex of child. Associations were measured in Odds ratio (OR) with 95% confidence intervals (95% Cl).

Results. The most common factors of morbidity registered in the study sample with DS, included respiratory infections, visual disturbances, oral pathologies, and cardiac problems (75.4%, 72.1%, 59.3%, and 44.9%, respectively). The hearing deficit, cardiac problems, respiratory infections, and oral pathologies showed statistically significant differences among the three age groups. According to the participants parents' perceptions, half of them (50%) were able to walk at 30 months, talk at 72 months, sit at 16 months, crawl at 16 months and eat alone at 48 months old.

Conclusion. People with DS at different ages present a set of potentially treatable diseases that require multidisciplinary medical monitoring. They also need early paramedical care to improve their functional abilities.

Key words: *Down syndrome, morbidity, functional abilities, Morocco*

INTRODUCTION

The life expectancy of people with Down syndrome (DS) has increased considerably over the past few decades. Since the 1980s, the improvement in the survival of children with DS has led to a spectacular improvement in the life expectancy of this population. The median age at death for American adults with DS passed from 25 years in 1983 to 49 years in 1997 [1], reaching the seventh decade of life in developed countries for many older adults with DS [2]. This increase in life expectancy has been linked to significant medical developments in recent decades, such as improvements in cardiac surgery, prevention of childhood infections, wider access to standard care, and better global psychosocial support for the population with DS [2].

Children with DS have an increased risk of birth defects and a wide range of treatable medical problems with 40-60% of DS babies having congenital heart disease. This make of importance to establish a cardiac malformation assessment for all babies with DS before the age of 6 weeks [3]. There is also a significant risk of hearing loss (75%), acute otitis

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

media (50%-70%) [4], visual disturbances (60-70%) [3, 4], and thyroid disease (4% –18%) [5, 6, 7, 8]. In children under 19 years of age with DS, respiratory infections are the second leading cause of death (after heart diseases) and the respiratory problems are the leading cause of hospitalization and extended stays [3]. On the other hand, people with DS are at an increased risk of developing infectious oral diseases such as periodontitis and caries lesions with more serious consequences than those observed in the general population [9]. As dental caries can appear as soon as teeth appear, initiating rigorous daily dental hygiene after tooth eruption, as well as dental visits after 18 to 24 months are reported to be important in this category [10]. In addition, there is generally a delay of psychomotor development in children with DS compared to their counterparts in the general population. Indeed it is reported that children with DS can roll around 6 months (51%), sit around 12 months (78%), crawl around 18 months (34%), walk around 24 months (40%), run, walk up the stairs, and jump around 5 years (45% to 52%) [11]. Also, about 75% of people with SD can eat alone with their fingers at the age of 20 to 22 months and eat alone with a fork around 5.5 to 7.5 years [12].

To our knowledge, no data highlighting the characteristics of the clinical profile and functional abilities of people with DS are available in Morocco. Therefore, the present study aimed to describe the main causes of morbidities in people with DS and to analyze their differences according to age group, sex and psychomotor development.

MATERIAL AND METHODS

Participants

The data were collected using a retrospective survey, conducted between May 2014 and November 2017 on a sample of 277 parents of children, adolescents and adults with DS. The sample was recruited from 11 sites that are associations and health centers providing care and support for individuals with DS in 5 provinces, Marrakech, Safi, Chichaoua, El Kelâa of Sraghna**,** and Al Haouz belonguing to Marrakech-Safi region. The study was conducted based on an information note accompanying the questionnaire placed at the survey centers inviting for interview with the parents. The study objectives were explained to the representives responsible of all centers and associations. The eleven centers in which the study was conducted were those who accepted our request. Families with at least one child clinically and/or cytogenetically confirmed DS were included. Except for two couples who had two children with DS, all participants in this study were sporadic.

Procedures

The survey tool used is a standardized questionnaire to collect information on the identity of the parents, age and sex of people with DS, functional abilities and state of psychomotor development. Information on morbidity and certain medical conditions as well as and the age at which the child was able to sit, crawl, walk, talk and eat alone are also collected. The sample contains 279 people with DS, however the number of responses obtained varies depending on the questions. This number varies between 277 for ear problems and 115 for thyroid dysfunction.

Our study was designed in accordance with the Declaration of Helsinki. It was conducted in full respect of local ethical considerations, namely obtaining the prior authorization of the competent authorities of the university and the responsibles of the visited centers. We contacted the parents/guardians of people with DS to whom we presented the objectives of the investigation and enlightened them on their rights. The principle of voluntary participation and the confidentiality and anonymity of the questionnaire were respected. Written parental consent was obtained before participation in the study.

Statistical analysis

Statistical analysis was performed using the SPSS software for Windows (version 20.0). Nominal and ordinal qualitative variables were presented as percentages of the different modalities. For descriptive analysis of functional abilities and because of the small sample size, the median and interquartile ranges (IQR) (25th percentile and 75th percentile) were used. The differences between groups for these variables for age groups and sex were tested using the Pearson's *Chi*-square ($\chi 2$) test. Differences were considered significant when the *p*-value <0.05.

The multinomial logistic regression model and the binary logistic regression model which allows the elimination of confounding factors of the associated variables with age and sex of child in the bivariate analysis ($P<0.2$), were used to identify the causes of morbidies independently associated with age and sex of child. Associations were measured in Odds ratio (OR) with 95% confidence intervals (95% Cl).

RESULTS

Morbidity of people with Down's syndrome

Table 1 summarizes the main causes of morbidity in people with DS. Overall, 51.1% of the participants had ≥3 pathologies. Even though the age of the participants did not show a statistically significant difference in the total number of pathologies, it is clear from Table 1 that the incidence of ≥3 pathologies increases with age (45.9% in children under 10 years old, 55.3% in

Table 1. Differences between modalities of causes of morbidity of people with DS and age groups

Diseases	Modalities	Total n (%)	Age < 10 years n (%)	Age 10-18 years n (%)	Age ≥ 18 years n (%)	χ2 ; significance
Number of pathologies	<3	131 (48.9)	85 (54.1)	34 (44.7)	12 (34.3)	5.25 ; n.s
	≥3	137 (51.1)	72 (45.9)	42 (55.3)	23 (65.7)	
Visual disturbances	Yes	150 (72.1)	81 (73.0)	46 (68.7)	23 (76.7)	0.74 ; n.s
	No	58 (27.9)	30 (27.0)	21 (25.6)	7 (23.3)	
Hearing deficit	Yes	14 (9.9)	6 (8.3)	2 (3.9)	6 (31.6)	12.29 ; **
	No	128 (90.1)	66 (91.7)	49 (96.1)	13 (68.4)	
Ear problems	Yes	47 (17.0)	28 (17.4)	11 (13.8)	8 (22.2)	1.31 ; n.s
	No	230 (83.0)	133 (82.6)	69 (86.2)	28 (77.8)	
Cardiac problems	Yes	101 (44.9)	74 (54.8)	21 (31.8)	6 (25.0)	13.77 ; **
	No	124 (55.1)	61 (45.2)	45 (68.2)	18 (75.0)	
Respiratory infections	Yes	205 (75.4)	131 (81.9)	54 (69.2)	20 (58.8)	10.24 ; **
	No	67 (24.6)	29 (18.1)	24 (30.8)	14 (41.2)	
Thyroid dysfunction	Yes	23 (20.0)	16 (18.8)	6 (27.3)	1 (12.5)	1.08 ; ns
	No	92 (80.0)	69 (42.9)	16 (19.5)	7 (87.5)	
Oral pathology†	Yes	143 (59.3)	52 (40.9)	59 (74.7)	32 (91.4)	40.45 ; ***
	No	98 (40.7)	75 (59.1)	20 (25.3)	3 (8.6)	

† For the analysis of the prevalence of dental caries, we excluded children under 3 years of age.
χ2 – *Chi*-square test; n.s - not significant; ** p<0.01; *** p <0.0001.

Table 2. Result of Multinomial Logistic Regression Model. Analysis between age groups and morbidities studed

Age groups	Morbidities	Modalities	Adjusted OR	(Cl 95%)	p
< 10 years	Number of pathologies	<3	0.32	(0.04-2.50)	0.283
	Hearing deficit	Yes	0.08	(0.008-0.79)	0.031
	Cardiac problems	Yes	3.44	(0.50-23.77)	0.209
	Respiratory infections	Yes	9.57	(1.55-58.90)	0.015
	Oral pathologies	Yes	0.07	(0.01-0.51)	0.009
10-18 years	Number of pathologies	<3	0.67	(0.11-4.12)	0.669
	Hearing deficit	Yes	0.06	(0.007-0.65)	0.020
	Cardiac problems	Yes	2.52	(0.37-17.08)	0.344
	Respiratory infections	Yes	2.23	(0.47-10.45)	0.309
	Oral pathologies	Yes	0.72	(0.10-4.85)	0.744
The reference modality is age ≥ 18 years					

OR = odds ratio; p= significance; Cl= confidence limits

preadolescents and adolescents aged 10-18 years and 65.7% in adults aged ≥ 18 years). The most common groups of diseases in the whole sample were respiratory infections (75.4%), visual disturbances (72.1%), oral pathologies (59.3%), cardiac problems (44.9%), thyroid dysfunction (20.0%) and ear problems (17.0%). Visual disturbances were particularly prevalent in all age groups, ranging from 73.0% in children under 10 years, 68.7% in preadolescents and adolescents aged 10-18 years old to 76.7% in adults aged ≥ 18 years (χ2 = 0.74, p not significant).

The distribution of ear problems and thyroid dysfunction by age group in our sample also did not show significant differences among the three groups. The hearing deficit, cardiac problems, respiratory infections and oral pathologies showed statistically significant differences among the three groups. Oral pathologies was very common in adults (91.4%; p<0.0001). Respiratory infections and cardiac problems were significantly common in children under 10 years of age with rates of 81.9% and 54.8%, respectively.

Multinomial logistic regression model analysis between morbidities and age groups with age ≥ 18 years as reference modality showed statistically significant differences between age < 10 years and age ≥ 18 years in hearing deficit, respiratory infections and oral pathologies (OR= 0.08; 95%Cl: 0.008-0.79, OR= 9.57; 95%Cl: 1.55-58.90 and OR= 0.07; 95%Cl: 0.01-0.51, respectively). While the age group 10-18 years showed only one statically significant difference compared to the age ≥ 18 years in favor of hearing deficit (OR= 0.06; 95%Cl: 0.007-0.65).

The distribution of diseases studied by sex (Table 3) did not show any statistically significant difference in the morbidities studied eccept for thyroid dysfunction ($\chi 2$ =3.92 ; p = 0.048). To take into account and simultaneously all the variables selected in this study, we applied the binary logistic regression method (Table 4). The results obtained showed that none of the morbidities studied allowed the observation of statistically significant differences by sex.

Psychomotor development

Data analysis concerning the perceptions of parents surveyed, regarding the functional abilities of their children with DS is presented in percentiles in Table 5. In the sample of people with DS studied, the median age where they were able to walk was 2.5 years IQR (2.0-4.0), to speak was 6.0 years IQR (4.0-7.0), to sit was 16.0 months IQR (10.75-24.0), to crawl was 16.0 months IQR (12.0-24.0), and to eat alone was 4.0 years IQR (3.0-6.0).

DISCUSSION

The present study reports data on the types of certain morbidities and their incidence within a sample of people with DS in Morocco as well as the median age at which they were able to aquire certain functional abilities according to their parents' perception. The results show that more than half of the participants (51.1%) had ≥3 types of morbidities. This rate is lower than that recorded previously by de Asua et al in a group of Spanish adults who presented in average 5 ± 2 clinical problems [13]. The lower rate observed in the present study population could be related to the age difference between the two cohorts.

Contrary to the Pikora et al [14] study reporting age differences in rates of diseases in school-age children (5–17 years) and young adults (16–30 years), the distribution of the number of illnesses did not show any statistically significant difference by age group in the present study.

The most common diseases in the whole sample were respiratory infections in 75.4%, visual disturbances in

Table 3. Differences between modalities of causes of morbidities in people with DS and sex of child

Diseases	Modalities	Male n (%)	Female n (%)	$\chi 2$; significance
Number of pathologies	<3	69 (45.1)	62 (53.9)	2.04 ; n.s
	≥3	84 (54.9)	53 (46.1)	
Visual disturbances	Yes	82 (69.5)	68 (75.6)	0.93 ; n.s
	No	36 (30.5)	22 (24.4)	
Hearing deficit	Yes	7 (9.0)	7 (10.9)	0.15 ; n.s
	No	71 (91.0)	57 (89.1)	
Ear problems	Yes	33 (20.8)	14 (11.9)	3.80 ; n.s
	No	126 (79.2)	104 (88.1)	
Cardiac problems	Yes	52 (40.3)	49 (51.0)	2.56 ; n.s
	No	77 (59.7)	47 (49.0)	
Respiratory infections	Yes	120 (77.4)	85 (72.6)	0.81 ; n.s
	No	35 (22.6)	32 (27.4)	
Thyroid dysfunction	Yes	9 (13.6)	14 (28.6)	3.92 ; *
	No	57 (86.4)	35 (71.4)	
Oral pathologies†	Yes	90 (63.8)	53 (53.0)	2.84 ; n.s
	No	51 (36.2)	47 (47.0)	

† For the analysis of the prevalence of dental caries, we excluded children under 3 years of age; $\chi 2$ – *Chi*-square test; n.s - not significant; * p<0.05

Table 4. Result of Binary Logistic Regression Model. Analysis between sex of child and morbidities studed

Variables	Adjusted OR	(CL 95%)	p
Number of pathologies	0.29	(0.07-1.11)	0.07
Ear problems	2.18	(0.39-12.02)	0.37
Cardiac problems	0.88	(0.28-2.71)	0.82
Thyroid dysfunction	0.30	(0.08-1.16)	0.08
Oral pathologies	0.60	(0.19-1.89)	0.38

OR = odds ratio; p= significance; Cl= confidence limits

Table 5. Medians and interquartile ranges (IQR) of age (months) when functional abilities are well achieved

	Q25	Q50	Q75
Walking (n = 240)	24.00	30.0	48.00
Talk (n = 76)	48.00	72.00	84.00
Sit (n = 230)	10.75	16.00	24.00
Crawling (n = 157)	12.00	16.00	24.00
Eating alone (n = 206)	36.00	48.00	72.00

72.1%, oral pathologies prevalent in 59.3%, cardiac problems in 44.9%, thyroid dysfunction in 20.0% and ear problems in 17.0% of the study people. In comparison with the rates found by Pikora et al [14], the visual disturbances are the most frequent (73%) followed by the hearing problems (45%), respiratory problems (25%), and the cardiac problems (36%). Another study conducted in a group of school-aged children with DS by Leonard et al [15] showed that heart and bowel problems are prevalent in less than half of the children. More than half had ear problems and more than three-quarters had eye problems. Also, ear, nose and throat (ENT) professionals were the specialists the most frequently consulted.

In the present study, the visual disturbances were particularly prevalent in all age groups, ranging from 73.0% in children under 10 years of age, 68.7% in adolescents to 76.7% in adults aged ≥18 years ($\chi 2 = 0.74$, not significant). These rates were lower than those reported by de Asua et al [13].

The hearing deficit, respiratory infections and oral pathologies showed statistically significant differences between children under 10 years of age and adults aged ≥18 years. Whereas concerning pre-adolescents and adolescents aged 10-18 years old, only the hearing deficit which showed statistically significant differences compared to adults aged ≥18 years. As reported by Picciotti et al [16], hearing loss was common in adults with DS and increases gradually with age. The Stensson et al [17] study data on how the parents perceived the general and oral health of their children with DS, showed that 70% of parents climed that general health and 74% the oral health of their child's as good or very good.

In addition, the respiratory infections and cardiac problems were significantly common in children under 10 years of age with rates of 81.9% and 54.8%, respectively. Findings from Zachariah et al [18] study showed that the risk of being hospitalized with lower respiratory tract infection is significantly higher in children of less than 2 years old with DS than in those without DS. The literature has revealed also that congenital heart defects and respiratory problems are the main causes of death in people with DS [19]. Congenital heart disease is one of the main causes of morbidity and mortality in this population [20, 21, 22].

On the other hand, the distribution of ear problems and thyroid dysfunction by age group did not show significant differences among the three groups in this study population. The effect of age in thyroid dysfunction has been shown by some studies [23, 24] and has been banished by others [25]. Another factor examined is the effect of sex on the distribution of the diseases studied. This effect was not observed in the present study corroborating the results of several previous studies [13, 23].

Analysis of the present survey data regarding the parents' perceptions on their children functional abilities, showed that the median age of people with DS when they were able to walk was 2.5 years IQR (2.0-4.0), to talk was 6.0 years IQR (4.0-7.0), to sit was 16.0 months IQR (10, 75-24.0), to crawl was 16.0 months IQR (12.0-24.0) and to eat alone was 4.0 years IQR (3.0-6.0). Comparing these results to those found by *de Graaf* et al [26] in people with DS from the United States and the Netherlands, showed that people with DS were able to walk at 5 months earlier and speak reasonably well late at about 6 years, while they were able to maintain their hygiene by the age of 13 and to work independently by the age of 20 yrs old.

Limitations

This study has limits related to the sample size, which is insufficient for the particularity of the population studied and justified by the constraints of the limited number of centers that provide care and support to people with DS. The data on morbidity and functional abilities were those declared by the parents. Although these data are subjective and reflect only the perception of the parents, they remain important in so far as they allow the analysis and understanding of certain related aspects.

There may also have been a level of missing data due to the failure to perform the clinical examinations and biological analysis necessary to confirm the involvement of certain pathologies. This aspect may have contributed to missing data, which limited the interpretation of some results.

CONCLUSION

The results of the present study showed that the main causes of morbidities in Down Syndrome people surveyed are respiratory infections, oral pathologies, visual disturbances, cardiac problems, ear problems, and thyroid diseases. The data revealed an effect of age on morbidity with impact on the hearing deficit, cardiac problems, respiratory infections, and oral pathologies. This fact was clearly showen between age < 10 years and age ≥ 18 years.

However, the effect of sex factor on morbidity did not show any statistically significant difference. In addition, the data on functional abilities, showed that the majority of people with DS were able to achieve acceptable levels of mastery of certain functional abilities, according to their parents, at the usual age. The study draw attention on the necessary early medical and paramedical care, to avoid any complications related to congenital malformations and to help the child with DS to have a significant level of autonomy by acquiring certain functional abilities early.

Acknowledgements

The authors would like to thank all the families who participated in this study. Special thanks go to the associations and health centers that provide care and support for individuals with DS in the region of Marrakech-Safi.

Disclosure conflict of interest

The authors declare that there is no conflict of interests.

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Received: 08.06.2023
Revised: 28.11.2023
Accepted: 15.12.2023

Rocz Panstw Zakl Hig 2024;75(1):107-110
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ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
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Volume 75 **2024** **Number 1**