

ISSN 0035-7715
eISSN 2451-2311

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE



Quarterly
2023
Volume 74
Number 4 - DECEMBER

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

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

(ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE)

Published since 1950

Quarterly, 4 issues in 1 volume per year (No 1 - March, No 2 - June, No 3 - September, No 4 - December)

The journal is devoted to research studies on food and water safety, nutrition, 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

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The printed version of the journal is identical to the on-line version.

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ISSN 0035-7715
eISSN 2451-2311

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– NATIONAL RESEARCH INSTITUTE
Warsaw, Poland

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24 Chocimska Street, 00-791 Warsaw, Poland
<http://www.pzh.gov.pl>

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]

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NUTRITIONAL VALUE OF GLUTEN-FREE PRODUCTS USING THE FRONT-OF-PACK LABELING NUTRI-SCORE

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ABSTRACT

Background. Nutri-score is a useful and comprehensible system of extended nutrition labeling of food, which is intended to provide the consumer with simple guidance in choosing food products, taking into account the consumer's healthy diet. In several countries, in addition to the mandatory nutritional value of food indicated on the product packaging, the use of the so-called food traffic lights, which, based on a simple graphic display, make it easier for consumers to concentrate on choosing healthier food options.

Objective. The aim of the work was to evaluate the nutritional composition of gluten-free food products based on the nutritional data indicated on the packaging of these products in order to find out how useful the use of Front-of-Pack labeling (FOPL) Nutri-score will be in distinguishing the nutritional value of products.

Material and Methods. We analyzed 206 randomly selected gluten-free food products obtained from commercial retail chains (semi-finished products, other bakery products, biscuits, flour mixtures, porridges, pasta, muesli, snacks, confectionery, etc.) intended for celiacs. Based on the obtained data, we evaluated the composition of the products using a modified algorithm for calculating the Nutri-score.

Results. We found that gluten-free products are a very rich source of energy, especially fats, carbohydrates and sugars, while the proportion of fiber and protein is very low. More than one third of the products had a nutritional score of category A or B, which are healthier variants, but over 40% of the analyzed products already fell into categories D or E. We found the lowest average energy value in the case of products classified in category B, the lowest average fat content and saturated fatty acids were found in products labeled A, the highest sugar content was found in products labeled D and E, the highest average protein content in products labeled A. The highest average salt content was found in products labeled C, fiber in products labeled B and A.

Conclusions. Nutritional profiling can significantly contribute to several health-beneficial decisions, especially when choosing and buying healthier food options, including gluten-free foods.

Key words: nutrients, gluten-free foods, nutrition, healthy eating, Nutri-score

INTRODUCTION

Gluten-free foods are intended for special dietary use. They are formulated, processed or manufactured for special nutritional purposes in people with gluten intolerance. A medical prescription is not required to purchase gluten-free food for special dietary use. They are commercially available, not only for gluten-intolerant subjects, but for anyone who voluntarily chooses to follow a gluten-free diet or consume these products [30]. Foods for special medical/nutritional purposes should contain nutrients at the level of nutrients found in the foods to be replaced. Nowadays, in developed countries, thanks to marketing strategies and trends related to healthy foods, as well as self-

diagnosis of disorders related to the consumption of gluten or wheat, the representation of the population eating gluten-free is increasing. This diet is currently one of the three most popular diets in the world, along with low-carbohydrate and fat-free diets [3]. These trends have boosted the global market for gluten-free and reduced-gluten products. Several factors affect the quality of gluten-free products. The quality of the input raw materials is important, as well as the production technology [13]. After overcoming earlier technological obstacles, the attention of producers and developers focused on the texture, taste and above all the nutritional quality of gluten-free products. Their quality is getting closer and closer to products containing gluten. One of the possibilities of increasing

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their nutritional value is fortification (with vitamins, minerals) or adding nutritionally rich raw materials to recipes [20, 27]. The nutritional quality of gluten-free food has been a persistent problem for consumers. Studies have shown that the total fat content of gluten-free bread is almost double the amount found in their gluten-containing counterparts. Conversely, many gluten-free pastas are significantly higher in carbohydrates and sodium. Gluten-free products are generally poorer sources of protein and fiber. The glycemic index of these products varies depending on the type and quality of the ingredients used, as well as on the processing of food and the technological process of production [28]. Since gluten-free products are not usually fortified, they are usually deficient in folate, iron, niacin, thiamin and riboflavin. According to Barone et al. [1] patients with celiac disease consume significantly higher amounts of fat, sugar and lower amounts of fiber compared to healthy individuals.

Recently, the issue of mandatory labeling of the nutritional value of foods on the front of the packaging, the so-called Front-of-Pack nutrition labeling (FOPL). Front-of-pack nutrition labeling is one measure that has recently been introduced in combination with educational campaigns to promote healthy eating and prevent diet-related chronic diseases [5, 44]. FOPL nutrition labels use nutritional profiling models to assess the nutritional quality of food products, which is then transformed into a simple visual form. The purpose of FOPL is to simply and succinctly clarify mostly back-of-package nutritional information to help consumers make healthier food choices and to encourage the food industry to improve the nutritional composition of their products [26]. Discussions are currently underway in the EU and in several countries, including Slovakia, about the introduction of the FOPL label as a measure in the field of public health, which will direct consumers to healthier food choices. Nutri-score represents an alternative supported by several actors. The FOPL Nutri-score labeling scheme is a scientifically validated five-colour system developed by independent French researchers [24]. It was inspired by the British Food Standards Agency's nutritional profiling system, which was originally developed to regulate television advertising for children [34,35]. The algorithm assigns a given food/beverage a total score according to its nutritional composition. Based on this total score, Nutri-score categorizes food products into five color categories that reflect their nutritional quality [24]. Each color is also associated with a letter from A (dark green) to E (dark orange) to make labeling more accessible and understandable for consumers. With the exception of some specific commodities, FOPL also applies to gluten-free products.

The objective of our work was to evaluate the nutritional composition of selected gluten-free products based on the nutritional information provided on the packaging of these products in order to find out how useful the FOPL Nutri-score will be in distinguishing the nutritional value of products.

MATERIAL AND METHODS

Study design

Our study was methodically based on the evaluation of the nutritional values of randomly selected gluten-free products based on the nutritional information provided on the product packaging. We assessed the representation of basic nutrients such as proteins, fats, as well as the total energy value of the products and specific components such as saturated fatty acids, sugars, fiber and salt. We focused on gluten-free products of various types and from various manufacturers. In total, we analyzed 206 randomly selected gluten-free products obtained from commercial retail chains. These were mainly semi-finished products, other bakery products such as bars, soles, crackers, flour mixes, porridge, pasta, muesli, snacks, confectionery, etc. intended for celiacs. Based on the obtained data we evaluated the composition of the products using a modified Nutri-score algorithm [37]. The score calculation algorithm is based on the assessment of the presence and amount of unfavorable components of the food, such as energy (kJ per 100 g or 100 ml), sugars (g per 100 g or 100 ml), saturated fatty acids (g per 100 g or 100 ml) and salt (g per 100 g or 100 ml); and the favorable ones such as proteins (g per 100 g or 100 ml), fiber (g per 100 g or 100 ml) and the proportion of fruits, vegetables and legumes (%). The resulting score decides what class and color distinction the given product will receive. Final Nutri-score thresholds include five scaling classes A to E and five colors from dark green to dark orange, with the former being the healthiest variants and the latter the least healthy.

Statistical analysis

We used Microsoft Office Excel 2016 (Los Angeles, CA, USA) in combination with XLSTAT (version 2019.3.1) for statistical data processing. We performed descriptive analysis using mean \pm standard deviation. For monitored parameters, we present additional statistical characteristics: max (maximum), min (minimum), med (median). We performed statistical analysis using the computer software STATISTICA 13 (TIBCO Software, Inc., Palo Alto, CA, USA, version 13.5.0.17) and MedCalc® Statistical Software version 22.009 (MedCalc Software Ltd, Ostend, Belgium; 2023). The level of statistical significance was set as $P < 0.05$. Using one-way analysis of variance (ANOVA),

we tested differences between variables and compared using *Tukey's post hoc* test.

RESULTS AND DISCUSSION

We evaluated more than two hundred gluten-free products from the point of view of the nutritional information mandatory listed on the back of the product packaging. The values of the selected nutritional data of the examined gluten-free products are shown in Table 1.

The average energy value of the products was 1533 ± 390 kJ.100 g⁻¹ or 100 mL⁻¹. Furthermore, we found an average fat content of 10.19 ± 9.29 g.100 g⁻¹ or 100 mL⁻¹, of which saturated fatty acids 4.14 ± 5.58 g.100 g⁻¹ or 100 mL⁻¹, content sugars 13.82 ± 16.45 g.100 g⁻¹ or 100 mL⁻¹, protein content 5.43 ± 2.94 g.100 g⁻¹ or 100 mL⁻¹, salt content 0.73 ± 0.73 g.100 g⁻¹ or 100 mL⁻¹ and fiber content 3.05 ± 2.81 g.100 g⁻¹ or 100 mL⁻¹.

Based on the obtained values of nutritional data and calculations of the nutritional score, we found that 33 (16%) products were included in category A, i.e. the category with the best nutritional profile, category B 41 (19.9%), category C 47 (22.8%), category D 50 (24.3%) and category E 35 (17%) of gluten-free products. The average values of nutritional data of gluten-free products of individual categories A to E according to Nutri-score are shown in Table 2. Figure 1 shows the distribution of the energy value of gluten-free products adjusted according to the classification of products in individual Nutri-score categories. We found the lowest average energy value in the case of products classified in category B. As expected, the highest average energy value was found in category E. The energy value of gluten-free products in categories A, B and C differed significantly from the values in categories D and E ($P < 0.001$). The energy value of the products is also significantly influenced by the fat content (Figure

Table 1. Basic descriptive characteristics of the evaluated sample of gluten-free products

N=206	Mean	SD	Min	Max	Med
Energy (kJ.100 g ⁻¹ or 100 mL ⁻¹)	1533	390	142	2260	1515
Total fat (g.100 g ⁻¹ or 100 mL ⁻¹)	10.19	9.29	0.1	32.19	6.60
Saturated fatty acids (g.100 g ⁻¹ or 100 mL ⁻¹)	4.14	5.58	0	22.90	1.50
Sugars (g.100 g ⁻¹ or 100 mL ⁻¹)	13.82	16.45	0	65.70	3.85
Proteins (g.100 g ⁻¹ or 100 mL ⁻¹)	5.43	2.94	0.31	14.30	4.60
Salt (g.100 g ⁻¹ or 100 mL ⁻¹)	0.73	0.73	0	4.00	0.68
Fibres AOAC (g.100 g ⁻¹ or 100 mL ⁻¹)	3.05	2.81	0	13.20	2.85

Table 2. Statistical evaluation of the nutritional value of gluten-free products profiled using Nutri-score

	Nutri-score A	Nutri-score B	Nutri-score C	Nutri-score D	Nutri-score E
	n=33	n=41	n=47	n=50	n=35
Energy (kJ.100 g ⁻¹ or 100 mL ⁻¹)	1391 ^a	1264 ^a	1362 ^a	1660 ^b	2032 ^c
Total fat (g.100 g ⁻¹ or 100 mL ⁻¹)	2.76 ^a	3.45 ^a	7.89 ^b	12.24 ^c	25.25 ^d
Saturated fatty acids (g.100 g ⁻¹ or 100 mL ⁻¹)	0.67 ^a	0.68 ^a	1.66 ^a	4.43 ^b	14.40 ^c
Sugars (g.100 g ⁻¹ or 100 mL ⁻¹)	0.85 ^a	2.53 ^{ab}	6.89 ^b	25.46 ^c	31.94 ^d
Proteins (g.100 g ⁻¹ or 100 mL ⁻¹)	7.09 ^a	5.14 ^b	4.58 ^b	5.12 ^b	5.75 ^{ab}
Salt (g.100 g ⁻¹ or 100 mL ⁻¹)	0.28 ^a	0.67 ^b	0.98 ^c	0.90 ^{bc}	0.66 ^b
Fibres AOAC (g.100 g ⁻¹ or 100 mL ⁻¹)	3.58 ^a	3.98 ^a	3.24 ^a	2.95 ^a	1.34 ^b

^{abcd} Different symbols in a line mean significant differences between samples

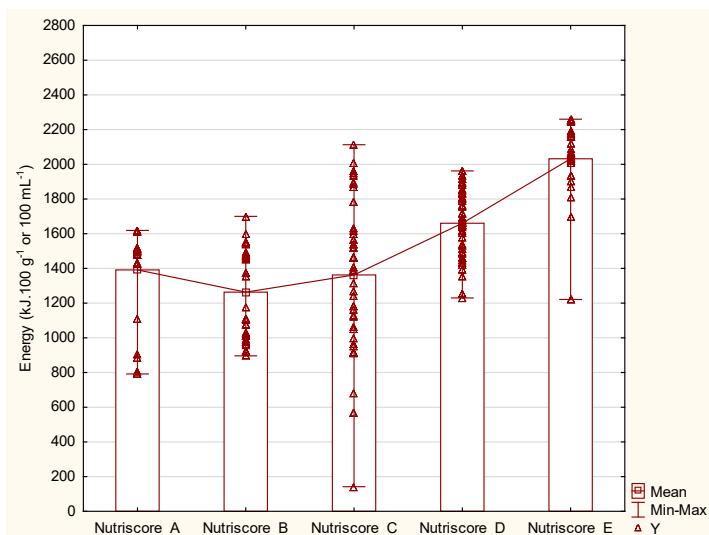


Figure 1. Variability of the energy value of gluten-free products adjusted according to Nutri-score

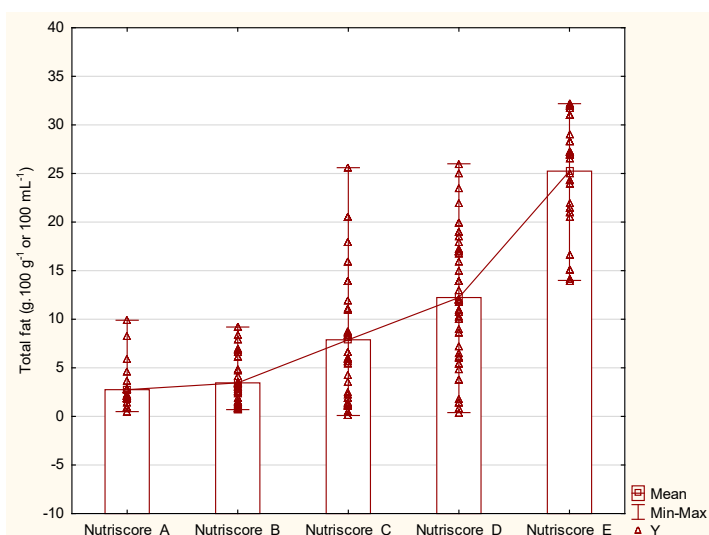


Figure 2. Variability of the total fat value of gluten-free products adjusted according to Nutri-score

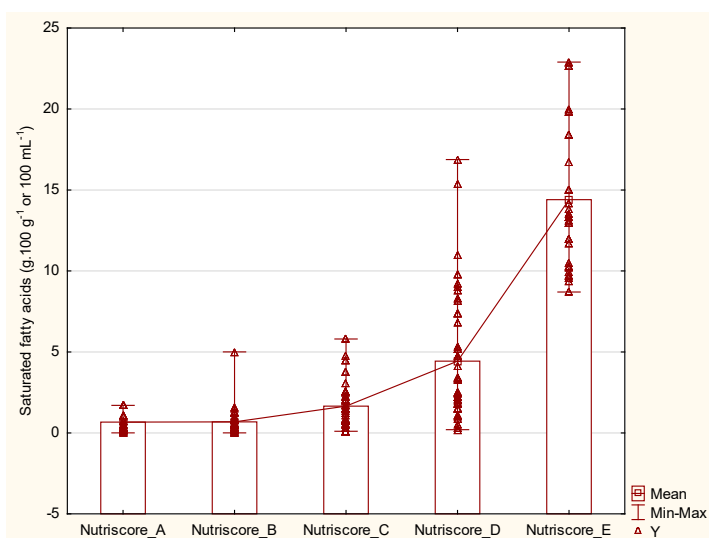


Figure 3. Variability of the saturated fatty acids value of gluten-free products adjusted according to Nutri-score

2). We found the lowest average fat content in the case of products labeled A and similar values in products labeled B. We did not find a significant difference between these categories ($P > 0.05$). In the case of the other categories (C, D, E), a significant difference in fat content was observed ($P < 0.001$). This indicator also confirmed the expected highest average fat content in products marked E. Similar results were also found in the case of saturated fatty acids, but we did not detect significant differences between products marked A, B and C in connection with this parameter ($P > 0.05$; Figure 3). Sugar content also contributes to the energy value of food (Figure 4). As expected, we found a higher content in products labeled D and E, whose values differed significantly from other products classified in categories A to C ($P < 0.001$). At the same time, in their case, we also found a relatively large variability of values. Proteins are a nutrient that is perceived very positively by consumers, especially in athletes and in baby food. The protein content of gluten-free products fluctuated considerably in all Nutri-score categories (Figure 5). We found the highest average protein content in the case of products marked A, which was significantly higher than in the case of the other categories ($P < 0.001$). Unexpectedly, we found the second highest average protein content in the case of products labeled E. Based on the above, we can conclude that products with a worse score and label can also be a good source of protein. Figure 6 expresses the salt content of the products. We found the highest average salt content in products labeled C, the lowest in products in category A ($P < 0.001$). Paradoxically, we again found the second lowest average salt content in the case of products marked E. The fiber content is shown in Figure 7. We found the highest average content of this health-protective component in products marked B and A. The fiber content differed significantly only in the case of products of category E, which was also the lowest ($P < 0.001$).

As we can see in Table 3 in the correlogram of mutual correlations between individual parameters, a high score (negative status) is clearly related to a higher content of total and saturated fats, as well as energy and sugars. In their case, we found significant positive correlations. A negative correlation was confirmed for protein and fiber in relation to the score.

Health in connection with food consumption is perceived and defined by

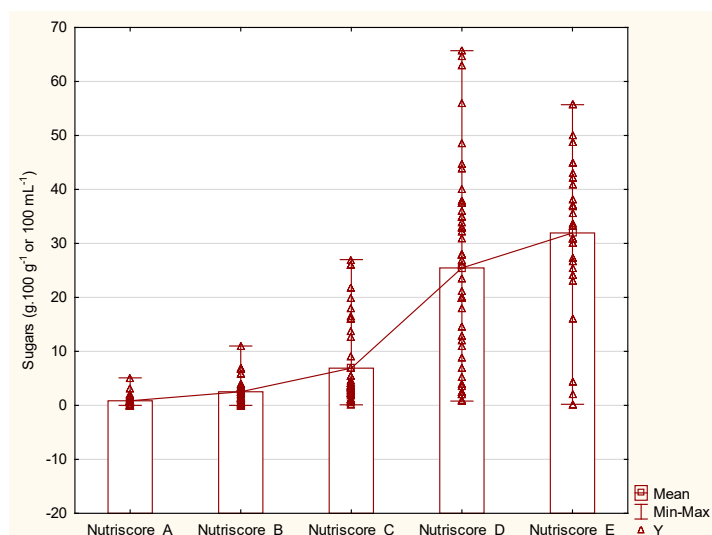


Figure 4. Variability of the sugars value of gluten-free products adjusted according to Nutri-score

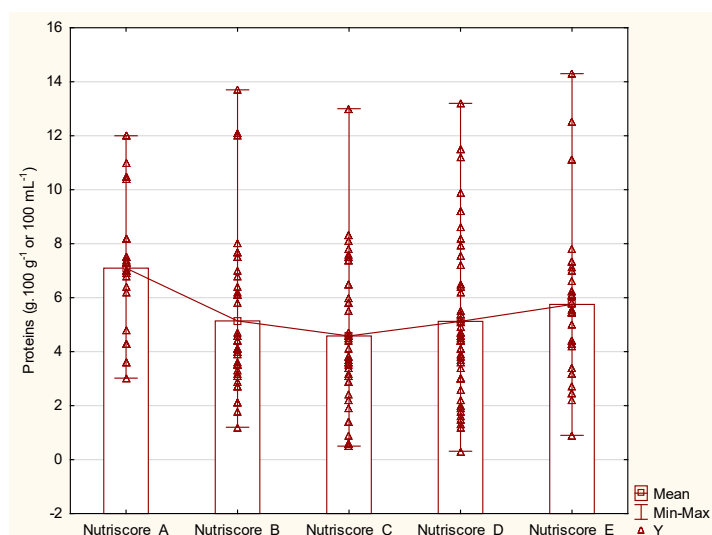


Figure 5. Variability of the proteins value of gluten-free products adjusted according to Nutri-score

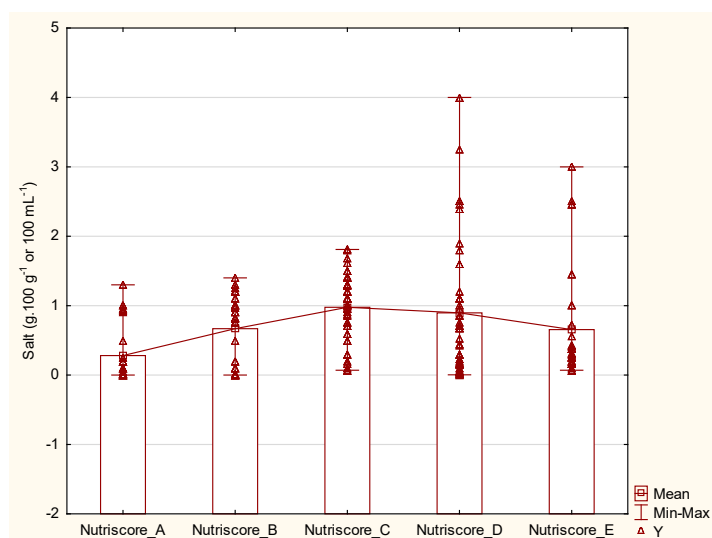


Figure 6. Variability of the salt value of gluten-free products adjusted according to Nutri-score

consumers precisely by the quality of food [18]. The perceived quality of food is derived from the likelihood that the product supports health and a healthy lifestyle. However, the perception of products from a nutritional point of view depends on nutritional labeling [31]. Consumers rely on available nutritional information to form opinions about food commodities because nutrients in a product are invisible and intangible [4]. Since 2011, all food manufacturers are obliged to provide information on the nutritional value of food by stating mandatory data on the product packaging [36]. The original intention was to make it easier for the consumer to navigate between products and choose healthier options. However, the consumer effect was not achieved, because it is not easy to find your way around the numbers and evaluate them correctly from the point of view of the degree of suitability and nutritional balance. This is also why the European Union plans to introduce a unified system of nutritional labeling of food with the aim of building better lifestyle habits and reducing the obesity epidemic. The objective of Nutri-score is not to divide food into healthy and unhealthy, but to make the consumer choose a healthier version of the product based on the label. In the case of a worse score, the consumer can evaluate which parameter of the nutritional value reduced the score of the product and also evaluate the risks that would be related to the eventual consumption of the product. It has been found that consumers generally perceive food to be of high quality if the calorie, sugar and fat content remains low [19]. Consumers evaluate the quality of products both at the point of purchase through nutritional labels and at the point of consumption, where the initial perception is either confirmed or refuted, which affects future food choices [43].

Several studies have shown that the basic Nutri-score algorithm is able to differentiate the nutritional quality of foods in a way that is mostly consistent with current nutritional recommendations [23, 40]. In addition, the Nutri-score format (i.e., an interpretive, color-coded summary nutritional rating system) was found to be well perceived and better understood by consumers than other FOPL nutrition labels [11, 15]. Nutri-score has also been shown to be relatively useful as a shopping aid tool, which is ultimately reflected in the nutritional quality of purchases [7, 9, 22]. In addition, in the long term, FOPL labels, and thus the Nutri-score, are thought to have the potential to help reduce

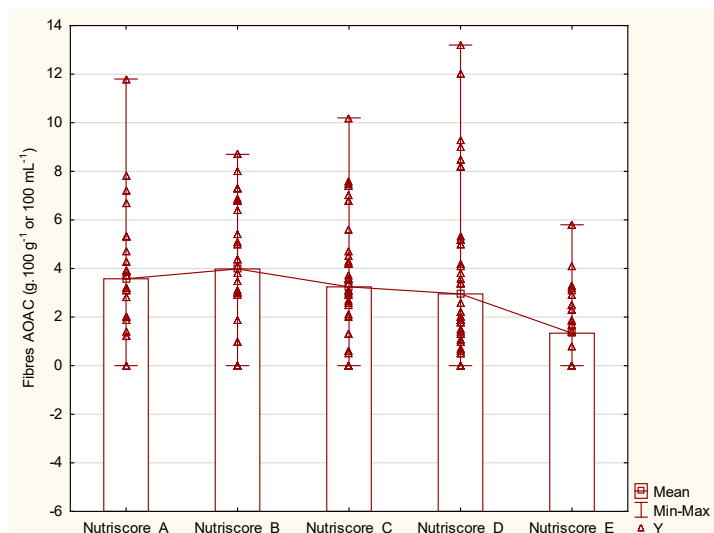


Figure 7. Variability of the fibres value of gluten-free products adjusted according to Nutri-score

mortality from civilization diseases by improving dietary habits [10].

The analysis of the gluten-free products showed that with the worsening of the score, all the parameters of the nutritional value did not have to deteriorate legally and proportionally. Exceeding the amount of even one component of the product is enough to worsen and reduce the score. The taste of gluten-free products is mostly associated with a higher caloric value of the products. Research has shown that the nutritional value of gluten-free products varies not only depending on the producer, but also on the country of origin, and they also found a higher content of fats, especially saturated, and carbohydrates, especially

simple sugars [29]. The raw materials from which gluten-free products are made usually have a higher energy value, thus increasing the energy value of the final product [30]. Studies analyzing gluten-free products have found that bread, pasta, various snacks and cakes have a significantly lower protein content than those that contain gluten. The gluten-free ones contained an average of 4.4 g per 100 g of protein compared to 10 g per 100 g in gluten-containing products [14]. It follows from many studies that gluten-free products are generally deficient in protein content. We reached a similar result in our analysis. Fats have an important sensory function and also affect the taste of gluten-free products. They are a very good source of energy for our body and its activity. In general, according to the principles of healthy nutrition, they should represent

a maximum of 30% of the total energy intake [39]. The basic components of fats are fatty acids and glycerol. It is the representation of fatty acids that determines the properties of fats. However, a high intake of saturated and trans fatty acids is not recommended. Their intake should be a maximum of 10% and 1% of the total daily energy intake, respectively [33]. The intake of simple sugars should not exceed 60 grams during the day. Their long-term and excessive consumption may lead to weight gain and other undesirable consequences of this consumption. The fiber content of gluten-free products is generally very low. Studies confirm that fiber intake in patients on a gluten-free diet is significantly lower compared to a normal diet. This

Table 3. Correlogram of mutual correlations between individual parameters

Parameters	Score	Total fat	Saturated fatty acids	Energy	Sugars	Salt	Proteins	Fibres
Score	1	0.822	0.802	0.663	0.745	0.137	-0.059	-0.276
		P<0.0001	P<0.0001	P<0.0001	P<0.0001	P=0.0492	P=0.3990	P=0.0001
Total fat	0.822	1	0.814	0.697	0.555	0.033	0.134	-0.179
	P<0.0001		P<0.0001	P<0.0001	P<0.0001	P=0.6334	P=0.0549	P=0.0101
Saturated fatty acids	0.802	0.814	1	0.621	0.625	-0.141	-0.008	-0.135
	P<0.0001	P<0.0001		P<0.0001	P<0.0001	P=0.0436	P=0.9136	P=0.0530
Energy	0.663	0.697	0.621	1	0.535	-0.2	0.171	-0.222
	P<0.0001	P<0.0001	P<0.0001		P<0.0001	P=0.0039	P=0.0139	P=0.0014
Sugars	0.745	0.555	0.625	0.535	1	-0.246	-0.016	0.041
	P<0.0001	P<0.0001	P<0.0001	P<0.0001		P=0.0004	P=0.8245	P=0.5592
Salt	0.137	0.033	-0.141	-0.2	-0.246	1	-0.155	0.083
	P=0.0492	P=0.6334	P=0.0436	P=0.0039	P=0.0004		P=0.0265	P=0.2343
Proteins	-0.059	0.134	-0.008	0.171	-0.016	-0.155	1	-0.044
	P=0.3990	P=0.0549	P=0.9136	P=0.0139	P=0.8245	P=0.0265		P=0.5291
Fibres	-0.276	-0.179	-0.135	-0.222	0.041	0.083	-0.044	1
	P=0.0001	P=0.0101	P=0.0530	P=0.0014	P=0.5592	P=0.2343	P=0.5291	

fact, in combination with other facts, increases the probability of developing diseases such as obesity, diabetes and various cardiovascular diseases [41]. It is important to pay attention to the increased fiber intake, because the average consumption is only about 15 g per day, while the recommended intake is around 30 g [17, 25]. Our body needs to receive even a small amount of salt for optimal functioning. However, most people consume significantly higher amounts than recommended. The main source of salt is processed food (including bakery products). We should not take more than 5-6 grams per day. However, today's salt consumption exceeds 10-12 g per day. High salt consumption with the participation of other nutritional deficiencies (e.g. potassium deficiency) increases blood pressure and thus promotes the development of not only hypertension, but also diseases of the heart and blood vessels and the development of a heart attack or stroke. Most people consume too much salt, due to excessive consumption of processed and ultra-processed foods and dishes that are either naturally rich in salt and sodium or have salt added to them for technological and sensory reasons (bread, pastries, meat products, instant semi-finished products and snacks, seasoning mixes, etc.). Food containing an excessive amount of salt is considered to have a salt content higher than 1.5 g per 100 g. Low salt foods have less than 0.3 g per 100 g. And it is precisely the amount of salt in the product that can affect the final Nutri-score score. The World Health Organization and its members (including Slovakia) have agreed to reduce salt intake by 30% by 2025. Manufacturers are gradually reducing the amount of salt in their products so that consumers get used to the new taste. With this trend, the manufacturer can also achieve an improvement in the score of its products within the Nutri-score. At the same time, the benefits of eating foods with a reduced salt content are being promoted through activities aimed at increasing consumer awareness. It is generally believed that the main source of salt in the diet is precisely the salt added to dishes during cooking. The fact is that most of our salt comes from processed foods [12, 21, 42, 45, 47]. Nutri-score can alert us to the presence of a higher amount of salt in the product, because the value of the score will be affected by the high salt content, of course negatively. In that case, the consumer has a choice whether to choose the green and healthier option, or the dark orange one, which is therefore the least healthy.

Consumers usually do not read the information about the product on the back of the package during the purchase, whether it is nutritional information or the composition of the product. This is mainly due to lack of time or due to nutritional illiteracy and the inability to understand the meaning of nutritional information [2, 16, 38]. In this regard, FOPL graphic labels on the

front of the package provide simpler and more visible information about the important nutritional elements of food products, thereby facilitating and speeding up consumer decision-making [46]. As reported by *Poquet et al.* [32], Nutri-score appears to be more effective in stimulating healthy food choices and improving the nutritional quality of shopping baskets than other FOPL labels. Moreover, the Nutri-score was the only one that led consumers to add products with significantly lower amounts of (saturated) fat and salt to their shopping carts [7]. The results of the studies indicate the potential of Nutri-score to stimulate the purchase of healthy products (A and B) and to inhibit the purchase of less healthy foods (D and E). The presence of Nutri-score helped the respondents in the study by *de Temmerman et al.* [6] to more easily assess the nutritional value of products. Respondents rated products as healthier when the Nutri-score logo was present on their packaging. These findings resonate with previous research showing that respondents tend to choose more products with a green Nutri-score and less products with a dark orange Nutri-score [8].

CONCLUSIONS

Healthy and nutritionally balanced foods are an integral part of the diet not only of healthy consumers, but also of people with specific nutritional needs or restrictions, including celiacs. The introduction of FOPL labels is becoming common practice and also applies to gluten-free products. Their quality has increased considerably in recent years, both in terms of sensory and nutritional aspects. Nutritional profiling can significantly contribute to several health-beneficial decisions, especially when choosing and buying healthier food options, including gluten-free ones, if the consumer is dependent on their consumption, which will also favorably affect the consumer basket and ensure healthier eating practices.

Acknowledgment

The study was supported by the Educational Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (KEGA 003SPU-4/2022) and Grant Agency of Faculty of Agrobiology and Food Resources SUA in Nitra (GA FAPZ 1/2022).

Conflict of interest

The Authors declare no conflict of interest.

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Received: 16.08.2023

Accepted: 08.10.2023

Published online first: 23.10.2023

GENDER AS A FACTOR INFLUENCING THE FREQUENCY OF MEAT AND FISH CONSUMPTION IN YOUNG ADULTS

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ABSTRACT

Background. Meat and fish contain easily digestible whole protein, B vitamins and numerous minerals, such as zinc, phosphorus and iron, thanks to which these products have a high nutritional value.

Objective. The aim of the study was to assess the frequency of consumption of meat and fish in young adults depending on gender.

Material and Methods. Data was collected from 200 respondents aged 19-30 using online survey questionnaire. The questionnaire was divided into three parts. The first part contained questions about sociodemographic and anthropometric data, the second part contained a question regarding the self-assessment of the diet. Whereas, the third part of the questionnaire concerned the frequency of consumption of meat and fish. Statistical analysis of the results was performed using Statistica 13.3 software and statistical significance was assumed at the $p \leq 0.05$ level.

Results. Meat consumption was declared by 86.5% of the respondents (83% of women and 90% of men), usually 5-6 times a week (20%). Gender statistically significantly differentiated the frequency of meat consumption. Men significantly more often consumed total meat ($p=0.002$), red meat ($p=0.001$) and poultry ($p=0.004$) compared to women. Fish was eaten by 85% of the respondents, and 39% only 1-3 times a month. Respondents preferred oily fish. There were no statistically significant differences in the consumption of fish by men and women.

Conclusions. Considering the complexity of the relationship between men and women's meat and fish consumption and health, research is needed to clarify the amounts of meat and fish consumed, the degrees and how they are processed, and the reasons for eating or not eating them. This can be helpful in directions for nutritional education.

Key words: young adults, meat, fish, frequency of meat consumption, frequency of fish consumption, men, women

INTRODUCTION

In Poland in 2019, 41% of deaths were caused by cardiovascular diseases. The second leading cause of death in Poland was malignant neoplasms, which accounted for one-fifth of all deaths [40]. Diet and lifestyle have a big impact on human health and life expectancy. Both in the case of cardiovascular diseases and malignant neoplasms, it is possible to apply preventive measures to reduce the risk of their occurrence. Such activities include the consumption of food products low in cholesterol and saturated fats, and rich in health-promoting polyunsaturated fatty acids and antioxidants. Products such as meat and fish, like any animal product, contain cholesterol, but their nutritional value varies greatly. Existing differences concern not only the content of fat in consumed products, but also the profile of fatty acids [26].

The impact of meat consumption on health is particularly important for Poles, due to how important this product is in the Polish food ration, where the average diet is dominated by meat products, especially red meat and poultry [17]. On the one hand, these products can be a source of complete protein, easier to digest than vegetable protein. By providing important macro- and microelements, such as bioavailable heme iron, zinc, B vitamins and vitamin A, meat is considered a valuable and nutritious product. On the other hand, however, the relationship between the consumption of meat (especially processed and red meat) and many civilization diseases is emphasized. The negative impact of meat consumption on human health includes diseases such as type 2 diabetes, certain types of cancer (e.g. esophagus, stomach), cardiovascular diseases and increased mortality risk [28].

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

The subject of the impact of meat consumption on health is focused mainly on red meat. However, there are also studies showing a different effect of white meat, i.e. poultry, and red meat on the risk of certain diseases. In a study of nearly 500,000 adults, a higher consumption of both red and white meat was associated with a higher risk of diverticular disease and type 2 diabetes, but also a lower risk of iron deficiency anemia. However, high consumption of red meat alone was associated with a higher risk of coronary heart disease, pneumonia and colon polyps. Conversely, a higher risk of gastroesophageal reflux disease, gastroduodenitis, and gallbladder disease was observed with high consumption of only poultry meat [24]. It has been shown that excessive consumption of red meat may contribute to an increased risk of certain malignancies, adversely affect the functioning of the circulatory system, or disturb the lipid profile in the blood. Consumption of red meat in the amount of even 100 g per day increases the risk of stroke, malignant tumors of the breast, colon and prostate, and increases the risk of death from cardiovascular causes by as much as 15% [41]. High red meat consumption was also associated with non-alcoholic fatty liver disease and insulin resistance [43]. Another study also showed a significant link between higher consumption of red meat and cancer, especially colon, lung, esophageal and stomach cancers [18]. *Ibsen et al.* (2019) noted that replacing red meat intake with fish or poultry meat was associated with a lower risk of type 2 diabetes [10].

As part of the research, it has also been shown that the high nutritional value of meat can positively affect mental health. Conducted by *Dobersek et al.* (2021), a systematic review found that in eleven of the eighteen studies reviewed, not eating meat was associated with poorer mental health. Some studies have shown that the risk of depression, anxiety and/or self-injury was significantly higher in those who avoided meat [6]. Meat products can also be an important element in the nutrition of athletes due to the high content of: taurine, L-carnitine, coenzyme Q10, choline, alpha-lipoic acid, conjugated linoleic acid, glutathione, creatine, minerals such as iron and zinc and bioactive peptides. These ingredients may have the effect of protecting cell membranes from oxidative stress and controlling inflammation. Thus, meat consumption may help reduce signs of muscle damage and accelerate recovery from exercise [5].

Fish, on the other hand, are characterized by high nutritional value in terms of beneficial amounts of highly assimilable protein with a balanced amino acid composition, valuable lipids and essential biocomponents, such as vitamins and minerals [26]. Therefore, the consumption of fish, which has a lower caloric density and a higher content of omega-3

polyunsaturated fatty acids compared to land animals, is strongly associated with numerous positive health effects [37]. Fish is a food rich in components such as omega-3 acids: EPA and DHA, which are known for their health-promoting properties. They have a blood pressure lowering effect and also support the development of the nervous system in children [2]. *Kim et al.* [12] showed a positive effect on human health when consuming two to four servings of fish per week. This frequency of consumption resulted in a 12% reduction in cardiovascular disease mortality and a 21% reduction in coronary heart disease mortality (CHD). The frequency of fish consumption 2-4 times a week not only reduced the risk of death due to these diseases, but even their occurrence. This study also showed a beneficial effect of fish consumption in the prevention of cancer, metabolic syndrome, dementia, the onset of Alzheimer's disease, musculoskeletal and gastrointestinal problems. In turn, *Wang et al.* (2022) confirmed the positive impact of the consumption of marine fish and omega-3 fatty acids contained in them on higher survival among people with cancer [38]. Among the studies, there are also those indicate that with increased consumption of fish, the risk of metabolic diseases was reduced [12, 38] and bone mineral density was increased [19, 29].

In addition to the many positive effects of fish consumption on human health, there are also potential risks associated with the consumption of these products. These threats may result from human errors committed e.g. during thermal processing or due to environmental pollution, in particular water. Raw or undercooked fish can be carriers of tapeworms or their larvae, which, after getting into the human body, deplete it of vitamin B₁₂, and as a result can lead to anemia. Other potential risks associated with fish consumption include tetrodotoxin poisoning (a powerful neurotoxin found in fish), marine poisoning, bacterial poisoning from eating spoiled meat, and heavy metal poisoning such as mercury [22]. It is the heavy metals present in certain types of fish are often the reason why consumers choose not to buy fish for fear that it is contaminated [9]. Although heavy metals are indeed present in fish, in particular in predatory, large and older fish, there is a lot of evidence the values of these pollutants in most cases do not exceed the established standards [3, 16, 25].

MATERIAL AND METHODS

Sample and data collection

The study was conducted in the period from June 2021 to October 2022 using the online interview technique (CAWI) with the use of a questionnaire. The questionnaire was made available to the respondents using an Internet tool – Google Form. The study group

was selected using non-probability sampling methods. They were: purposeful selection (selected groups for young adults in social media), convenience selection (respondents among family and friends) and snowball selection (respondents shared the questionnaire with others). 206 people expressed their willingness to participate in the study, of which 200 respondents (100 women and 100 men) constituted the final research sample. Three people were excluded from the study because they did not meet the age criterion of 19-30 years, while the answers of the remaining 3 people regarding anthropometric questions were unreliable and were not included in the study. Figure 1 shows the sample selection scheme, taking into account the inclusion criteria and the reasons for exclusion.

The questionnaire was divided into three parts. The first part contained questions about sociodemographic and anthropometric characteristics. This part includes open questions about: age (years), height (m), body weight (kg) and closed questions about gender (female, male), education (primary, vocational, secondary or higher), place of residence (village, city < 100,000 inhabitants or city over 100,000 inhabitants), number of household members (<2, 3-4 or >5), professional status (I do not work, I work, I study/study and at the same time I work, I study I am studying and not working). In the first part of the survey, respondents were also asked about smoking (yes or no), self-rated

physical activity (no/low, medium or high), self-rated health (very good, good, average, poor/very poor or I don't know) and self-rated economic situation (very good, good, average, poor/very poor or I don't know).

The second part of the questionnaire contained a question regarding the self-assessment of the diet (very good, good, average, poor/very poor or I don't know).

The third part of the questionnaire concerned the frequency of consumption of meat and fish. In the opening instructions of this section of the questionnaire, the respondents were informed how to indicate the answers. Respondents were asked to indicate the frequency of consumption of: red meat (beef, pork, veal, lamb and mutton), poultry meat (chicken, turkey, duck and goose meat), fatty fish (salmon, herring, mackerel, tuna, halibut, eel, sprat, sardines and sea trout) and lean fish (cod, pike, zander, hake and perch). Consumption of these products was to include all meals and snacks as well as meals eaten at home and away. It was possible to choose only one answer that best describes the diet of the examined person in the last 6 months. Consumption frequency questions were closed and the response cafeteria included choices such as: never, <1/month, 1-3/month, 1/week, 2/week, 3-4/week, 5-6/week, 1/day or 2 and more/day.

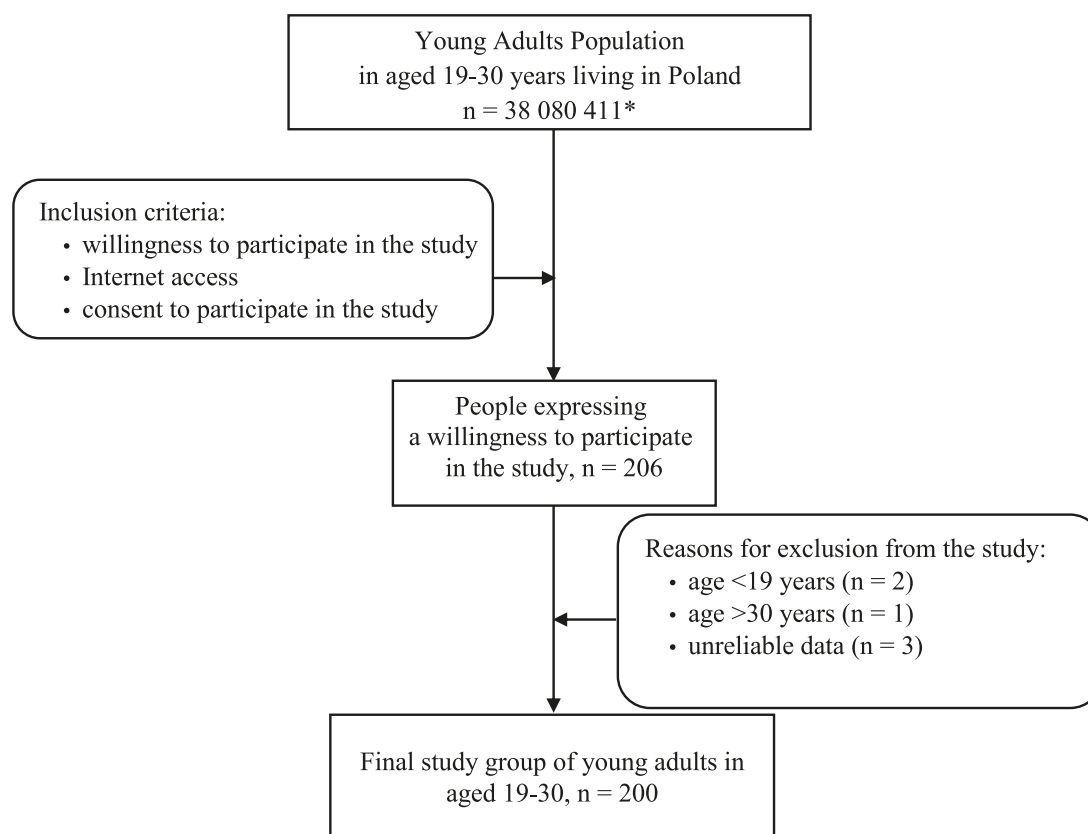


Figure 1. Scheme of selection of the research

*[32]

Statistical analyses

The obtained answers were transferred to Microsoft Excel in order to check them, assign numbers to the respondents and calculate the Body Mass Index (BMI). Obtaining information about the height and weight of the subjects made it possible to divide the research group. The respondents were divided into subgroups based on the classification of the World Health Organization (Tab. 1) [42] by calculating the body mass index (BMI) of each respondent according to the formula:

$$\text{BMI} = \frac{\text{body weight [kg]}}{\text{height}^2 \text{ [m}^2\text{]}}$$

Table 1. Categories of nutritional status based on body mass index [42]

Classification	BMI (kg/m ²)
Underweight	< 18.5
Normal body weight	18.5 – 24.9
Overweight	25.0 – 29.9
Obesity	≥ 30.0

The obtained results were statistically analyzed in the Statistica 13.3 program. The *Chi² Person* test was used to determine statistical relationships. It was used to determine the effect of gender on the frequency of consumption of various types of meat and fish by young adults. Differences calculated with the Pearson *Chi²* test were considered statistically significant at $p \leq 0.05$.

RESULTS

The surveyed group of young adults consisted of 200 respondents aged 19 to 30 (Tab. 2), with the average age of the respondents slightly above 23 years. The average BMI value in the study group was 23.01

(kg/m²) and meant normal body weight. Three-fourths of the subjects were of normal weight, i.e. BMI ranged from 18.5 to 24.9 kg/m². Among the respondents, about half were people with higher education and more than half were people who lived in large cities and every second respondent indicated 3-4 people living in his household. The largest percentage of respondents indicated they currently work, do not smoke, and mostly assessed their physical activity as moderate and health, diet and financial situation as good (Table 2). None of the respondents declared poor/very poor their status health.

In the own study, it was found meat in general (i.e. red and poultry) was eaten relatively often, every fifth respondent asked about the frequency of meat consumption declared meat consumption 5-6 times a week and this was the most frequently chosen answer (20% of respondents in total).

The largest percentage of men and women declared meat consumption 5-6 times, but with a predominance of men (Figure 2). Men, on the other hand, significantly outnumbered women when it comes to daily meat consumption. Every third surveyed man admitted that he eats meat at least once a day. Such frequency was declared by only 12% of the respondents. It can therefore be seen sex was a statistically significant differentiating factor in terms of the frequency of total meat consumption ($p=0.002$). Lack of consumption or a very low frequency of consumption of this product (1-3 times a month) was typical for women, while daily consumption concerned mainly men.

Red meat, including beef, pork, veal, lamb and mutton, was not eaten very often by respondents. Every fifth young adult did not eat it at all. A slightly smaller number of people eating this type of meat declared the frequency of consumption at the level of 1-3 times a month (19% of respondents in total). On the other hand, every third respondent (31%) ate red meat two to four times a week.

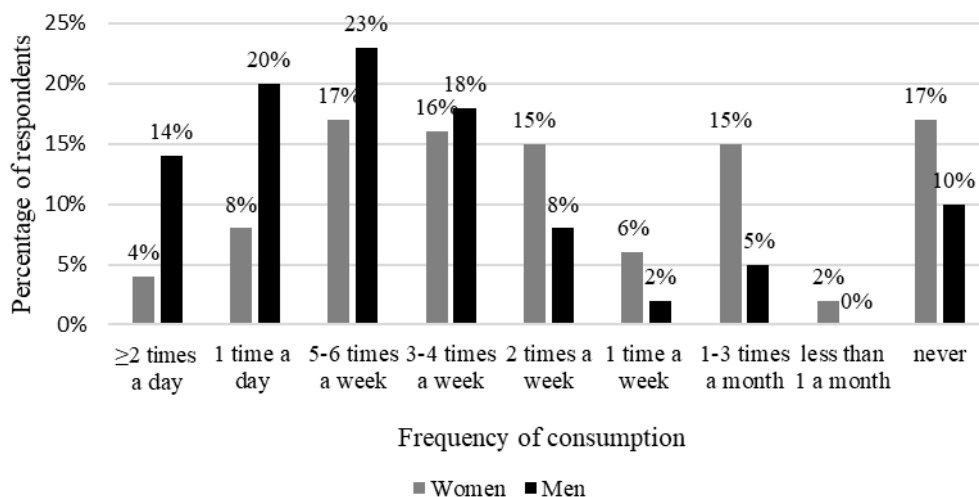


Figure 2. Frequency of total meat consumption in the study group of young adults

Table 2. Characteristics of the studied population

Variables	Group	Total (%)	Women (%)	Men (%)
Gender	Women	50	100	0
	Men	50	0	100
BMI	Underweight	5.5	9	2
	Normal body weight	70	75	65
	Overweight	20	11	29
	Obesity	4.5	5	4
Education	Primary	0	0	0
	Professional	4.5	3	6
	Secondary	50.5	50	51
	Higher	45	47	43
Place of residence	Village	19	24	14
	Town	20.5	14	27
	City	60.5	62	59
Number of people in the household	< 2	34.5	41	28
	3-4	51	44	58
	> 5	14.5	15	14
Professional status	I do not work	0	0	0
	I work	38.5	33	44
	I study/study and work	30	27	33
	I study	31.5	40	23
Smoking cigarettes	Yes	17	13	22
	No	83	87	78
Self-assessment of health	Very good	30.5	26	35
	Good	56.5	63	50
	Average	9	8	10
	I do not know	4	3	5
Self-assessment of physical activity	No/low	27	36	18
	Moderate	47	49	45
	High	26	15	37
Self- assessment of nutrition	Very good	8.5	13	4
	Good	44	50	38
	Average	36.5	34	39
	Poor/Very poor	10	3	17
	I do not know	1	0	2
Self-assessment of economic situation	Very good	17	24	10
	Good	49.5	51	48
	Average	32.5	23	42
	Poor/Very poor	1	2	0
	I do not know	0	0	0

There were statistically significant differences in the frequency of red meat consumption depending on the gender of the respondents ($p=0.001$). Every fourth woman did not eat red meat at all (Figure 3). In the case of men, one in four of them ate such meat twice a week. It was also observed that twice as many men than women declared consumption of red meat with a frequency of 3-4 times a week (Figure 3).

Poultry meat in the study included chicken meat, turkey meat, duck meat and goose meat and was more often consumed than red meat. Compared to a fifth of respondents not consuming red meat, 16% of respondents did not eat poultry. There were also relatively few people characterized by sporadic consumption of this meat (up to several times a month) – 13%, which is more than twice less than in the case

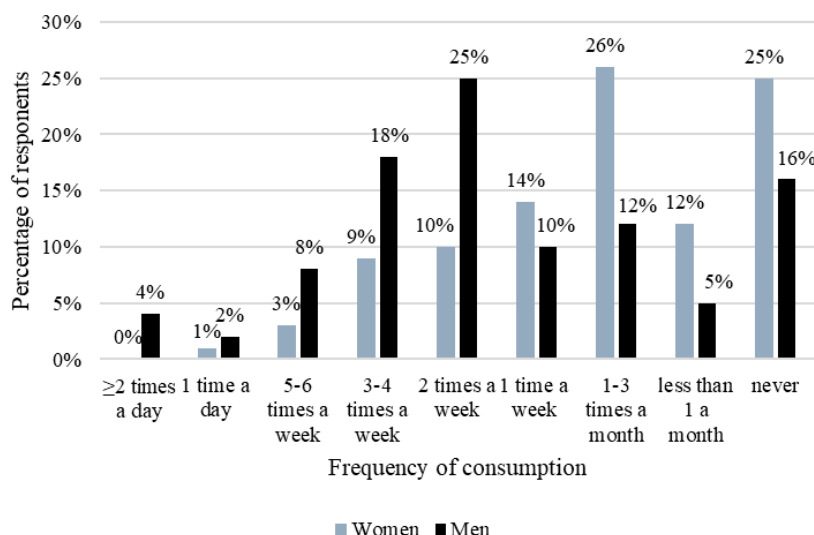


Figure 3. The frequency of red meat consumption in the study group of young adults

of red meat. This may be due to the fact of consciously reducing the consumption of red meat and replacing it with white meat. As many as one third of respondents declared poultry consumption at the level of 3-4 times a week. It can therefore be concluded that poultry meat was more common in the diet of the respondents.

The frequency of poultry meat consumption differed statistically significantly depending on the sex of the respondents ($p=0.004$). Men were more likely to consume this type of meat (Figure 4). Two-thirds of them ate poultry several times a week, of which the majority (40% of surveyed men) declared consumption 3-4 times a week. Such frequency of consumption was also most often declared by women, but it was only a quarter of the respondents who declared the consumption of this type of meat (Figure 4).

The group of fatty fish includes salmon, herring, mackerel, tuna, halibut, eel, sprat, sardines and sea trout. Cod, pike, zander, hake and perch were given as examples of lean fish species. The frequency of fish consumption (including fatty and lean fish) was very low. The largest percentage of respondents, about 40%

(all respondents) declared the consumption of fish 1-3 times a month.

Gender was not a statistically significant differentiating factor in the case of the overall frequency of fish consumption by the surveyed young adults (Figure 5). Both the majority of women and men indicated the consumption of fish from one to three times a month (36% and 42%, respectively). Among those who consume fish, the second most frequently declared frequency of consumption was once a week. A similar percentage of men and women declared fish consumption in accordance with the recommendations, i.e. twice a week (Figure 5).

Also, gender was not a statistically significant factor differentiating the frequency of consumption of fatty fish (Figure 6). The greatest difference in consumption between the sexes was observed with the consumption in accordance with the recommendations – twice a week. 12% of women and only 2% of men declared such a frequency. Men, however, were more likely than women to report higher intakes, three to six times a week. Assuming that the sufficient frequency of

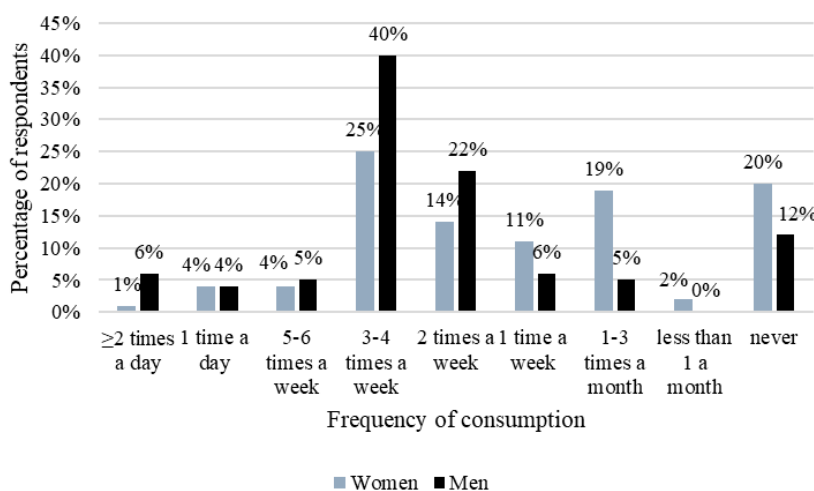


Figure 4. Frequency of poultry consumption in the study group of young adults

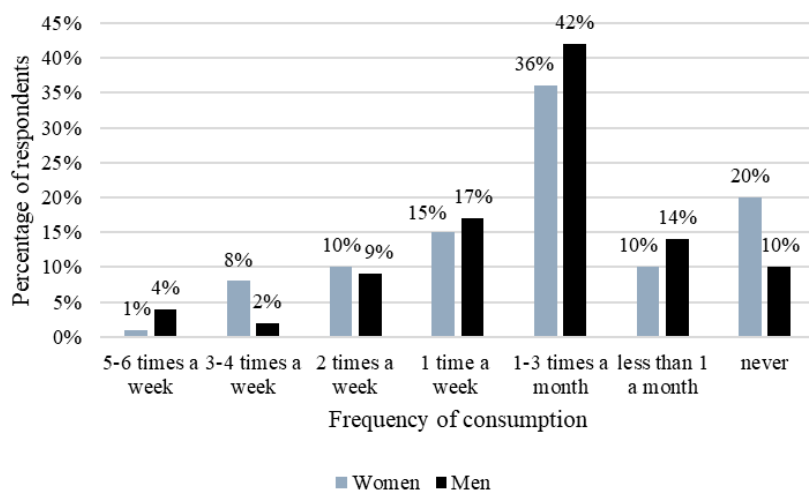


Figure 5. Frequency of total fish consumption in the study group of young adults

consumption of fatty fish is at least once a week, such consumption would be declared by a similar number of respondents of both sexes (28% of men and 30% of women) – Figure 6.

As in the case of total fish and oily fish, sex did not turn out to be a statistically significant factor

differentiating the frequency of consumption of lean fish (Figure 7). Consumption of this type of fish was declared by only slightly more than two-thirds of women and almost three-quarters of men. However, both sexes were characterized by a low frequency of consumption of these products – 63% of men and

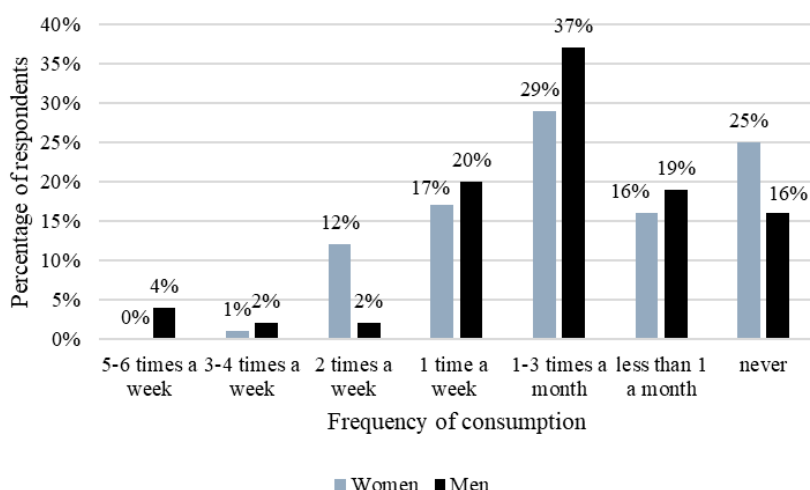


Figure 6. Frequency of fatty fish consumption in the study group of young adults

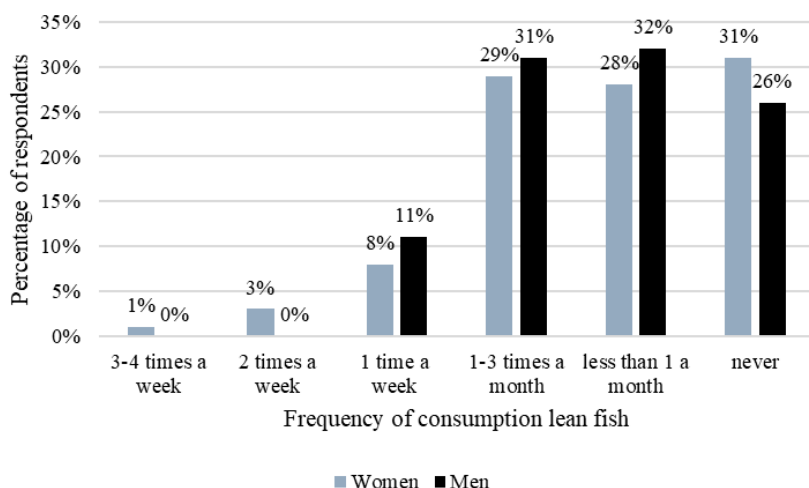


Figure 7. Frequency of lean fish consumption in the study group of young adults

57% of women declared the consumption of lean fish at a level not exceeding 3 times a month. Higher consumption, i.e. at least once a week, was very similar for both sexes, as 12% of women and 11% of men declared such frequency of consumption of lean fish (Figure 7).

DISCUSSION

Currently, it can be seen that vegetarian and vegan diets are becoming more and more popular in Poland, especially among young adults. People who give up meat in their diet are guided by various motives, including: religious, ethical or environmental reasons. The authors of studies on the frequency of meat consumption in most cases do not agree with each other. The results of studies conducted in Poland differ. These differences occur both in the percentage of respondents who do not consume meat and in individual categories of the frequency of its consumption. Researchers often also chose another differentiating factor, such as the age of the respondents, and not gender, as in the case of their own study.

In our own study, 13.5% of the respondents (17% of women and 10% of men) did not eat meat similarly to the *Olszak* study [23], in which 11.5% of young adults declared not eating meat. A similar number of people who did not eat meat (11%) was also found among students of Warsaw universities in the *Kucharska* et al. study [14]. A slightly lower percentage concerned all respondents who took part in the *Kowalcze* et al. study [13] it was 8% of the surveyed students of dietetics. In the *Malczyk* et al. study [21] among the surveyed adults, respondents who did not eat meat accounted for only 6.7% of all respondents. A similar percentage was obtained by *Adamski* et al. study [1], in which only 6% of respondents declared not eating meat.

In our own study, the respondents most often declared daily consumption of meat (23%) and with a frequency of 5-6 times a week (20% of the respondents) and 3-4 times a week (17% of the respondents). In the case of the *Malczyk* et al. study [21] it was respectively: 18.3%, 21.7% and 31.7% of the respondents. Meat consumption 3-4 times a week was declared by almost twice as many respondents as in the case of our own study. Perhaps this was due to the age of the respondents, who in this study were between 18 and 59 years old, i.e. they were older than those surveyed in our own study. A similar result to that in the own study was obtained by *Olszak* [23], in which students eating meat more than three times a week accounted for 33.5% of all respondents (in the own study it was 37%). Meat was eaten less than three times a week by 16.5% of the students, which is very similar to the results of our own study, in which 15.5% of the respondents ate meat with such frequency. Daily

consumption was declared by 33% of the respondents, which is 10% more than in the case of our own study.

Also in the case of examining the frequency of meat consumption, taking into account the division of respondents by gender, the researchers obtained different results. However, many studies have confirmed the relationship observed in our own study, i.e. the fact that women consume meat significantly less often than men. Such a conclusion was reached by researchers *Sidor* and *Rzymiski* [31], when 37.7% of the men surveyed consumed meat every day, while only 20.3% of the women surveyed. In our own study, it was 34% and 12%, respectively. Also *Szymandera-Buska* et al. [35] confirmed the influence of gender on the frequency of consumption of products that are a source of thiamine in the study group of students, especially meat. The surveyed women declared meat consumption in much smaller amounts and with less frequency than men.

Many researchers have decided to conduct research on the frequency of consumption of meat or its particular types, taking into account only one gender. Much more often these were studies in which the study group consisted of women only. It is possible that this is due to greater willingness of women to complete questionnaires than men, or the desire to learn more about women's eating habits, due to the fact that they are usually responsible for shopping and preparing meals in households. In our own study, 17% of the respondents did not eat meat. In the study *Gajda* [8], only 2.8% of women declared not eating meat, i.e. 6 times less than in the case of our own study. A very similar result to that obtained in our own study was obtained by *Szymańska* et al [36], who found women eating meat several times a month accounted for 14.8% of the respondents. These researchers also obtained almost the same percentage of women declaring meat consumption less than once a month – 1.9% (in their own study it was 2% of women).

In our study, the frequency of meat consumption concerned not only meat in general, but also its various types, i.e. red meat and poultry. In our own study, the most frequently consumed meat by the respondents turned out to be poultry meat. The preference for poultry meat has also been confirmed in many other studies. *Kowalcze* et al. [13] indicated the surveyed students were more likely to eat poultry (65% of respondents) than red meat (35% of respondents). *Adamski* et al. [1] obtained the similar result. Poultry meat was consumed by 96% of the respondents, the majority of whom (84%) indicated boiler chicken meat as preferred. *Kulesza* et al. [15] analyzing the consumption of meat in students, showed that most of them preferred white meat. In the *Szczepańska* et al. study [34] almost every tenth of the respondents consumed poultry meat every day, half of the

respondents several times a week, and once a week or less often 37% of the respondents .

Many authors of studies confirm a higher frequency of meat consumption or its particular types by men compared to women, which can be psychologically determined [30]. However, these differences were not always statistically significant. In many studies, it can also be seen that women much more often prefer chicken or turkey meat, while men are particularly eager to eat pork. This may be due to greater care for health among women, and thus, paying more attention to the nutritional value of meat. This is confirmed by the study *Ilow et al.* [11] conducted at the Medical University of Wrocław. Researchers, evaluating students' preferences regarding the fat content of various food products, showed that 41% of respondents always chose low-fat meat, while 15.9% of women did not pay attention to the fat content of meat. Among men, it was respectively 18.5% and 30.8% of respondents. In the *Gacek et al.* study [7], which was also conducted among students, men significantly more often than women ate red meat. However, the frequency of poultry consumption declared by male and female students was similar. Most respondents of both sexes ate white meat once a week. On the other hand, the study in which there were no statistically significant differences in the frequency of consumption of white meat, taking into account the gender of the respondents, was the study of *Malczyk et al.* [20].

The frequency of fish consumption (including fatty and lean fish) was very low. 15% of respondents completely eliminated fish from their diets. This is slightly more people than in the case of not eating meat (13.5% of respondents). Reasons for eliminating fish from diet include: their high price, poor availability of fresh fish, limited culinary skills or insufficient knowledge about the health-promoting properties of fish. Occasional consumption of fish, several times a year, was indicated by 12% of respondents. These are probably people who eat fish mainly on holidays. Most of the respondents, almost 40%, declared the consumption of fish from one to three times a month. According to the current recommendations of healthy eating, fish (especially fatty and marine species) should be eaten twice a week. However, such frequency of consumption was declared by less than one tenth of the respondents.

In our own study, as many as 15% of respondents declared not eating fish (both fatty and lean). The largest percentage of the respondents, constituting 39%, declared consumption of fish from one to three times a month, and consumption in accordance with the applicable recommendations, i.e. twice a week, was declared by only 9.5% of young adults. In the study *Cichocka and Krupa* [4] conducted among slightly younger respondents, a low frequency of

fish consumption was also obtained. However, the surveyed adolescents were characterized by a slightly higher frequency of fish consumption than young adults in our own study. Not eating fish was declared by three times less high school students, and more than half of the respondents indicated that they eat fish once a week. However, in the *Cichocka and Krupa* study [4], most of the young people surveyed admitted that they eat fish once a week, which may be due to the fact that high school students use the school canteen. Serving fish once a week, traditionally on Friday, is quite a common phenomenon in Polish schools. However, our study examined the frequency of fish consumption by young adults. Therefore, it seems more reasonable to compare their answers with those of students or slightly older adults, rather than children or teenagers of school age. *Kulesza et al.* [15], examining the eating habits of students, showed, as in her own study, that the most frequently declared answer was the consumption of fish several times a month. In this study, slightly fewer respondents (nearly one tenth) did not eat fish at all. As in our own study, there were no people who would eat fish every day among the surveyed students. Other results were obtained by *Kowalcze et al.* [13], when more than half of the students ate fish at least once a week, while in their own study it was one third of the respondents.

As the results of our own study show, gender was not a statistically significant factor differentiating the frequency of fish consumption. A different conclusion was obtained *Gacek et al.* [7], in which men consumed fish significantly more often than women . Students usually ate them once a week, and female students 1-3 times a month. *Malczyk et al.* [20] also found statistically significant differences in the frequency of fish consumption, taking into account the gender of the respondents. However, they obtained different results compared to the previously cited study. In this case, female students significantly more often than male students ate fish.

The authors of other studies rarely addressed the frequency of consumption of different types of fish in their studies. The own study included a division into fatty fish (such as salmon, herring, mackerel and tuna) and lean fish (such as pike and zander). In most cases, the former fish species inhabit the seas and oceans, while a significant proportion of lean fish species are found in fresh waters. Thus, analyzing the *Stoś et al.* study [33], a comparison of the frequency of consumption of marine fish with oily fish, and freshwater fish with lean fish, was adopted. In this study, the consumption of saltwater fish once a week was declared by every fourth respondent, and freshwater fish by every tenth. In our own study, however, it was on average every fifth and also every tenth respondent. Our own results indicate respondents of both genders preferred

fatty fish and consumed it more often than lean fish. Purkiewicz et al. [27] came to different conclusions. In the case of the frequency of consumption of fatty fish, the gender of the surveyed young adults was indeed not a statistically significant differentiating factor. The situation was different in the case of lean fish, the consumption of which by men was significantly more frequent. Lean fish was consumed by a much smaller percentage of respondents than in the study Purkiewicz et al. [27]. It was only 11% of both men and women. Such differences may be due to the fact that in the cited study as many as 67% of the respondents were at risk of developing eating disorders. This could therefore explain the high consumption of lean fish as an alternative to fatty fish in the case of, for example, orthorexia or excessive reduction of fat in the diet. The small study group, in which only 15% of the respondents were men, could also have contributed to the frequent consumption of lean fish, especially by men.

CONCLUSIONS

Men significantly more often than women consumed both total meat, as well as red and poultry meat. It seems important to explore this result in the context of excessive consumption of meat (especially red meat) and the risk of lifestyle diseases in men.

Gender was not a factor differentiating the consumption of total fish, fatty and lean fish. At the same time, the frequency of fish consumption by young adults was very low, regardless of gender. It is justified to conduct a study to explain the reasons for low consumption and to propose possible nutritional education in this regard.

Given the complexity of the relationship between men and women's meat and fish consumption and health, research is needed to clarify the amounts of meat and fish consumed, the degrees and how they are processed, and the reasons for eating or not eating them. This can be helpful in directions for nutritional education.

Conflict of interest

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

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Received: 24.08.2023

Accepted: 05.10.2023

Published online first: 23.10.2023

THE EFFECT OF 6-WEEK CONSUMPTION OF BAKERY PRODUCTS ON CHANGES IN SELECTED ANTHROPOMETRIC PARAMETERS IN WOMEN

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ABSTRACT

Background. The changes in consumers' eating habits are one of the most important factors affecting the consumption of selected foods. The most important factors for bakery products consumers are freshness, taste, price, durability, composition, and country of origin.

Objective. The aim of this study was to monitor changes in selected anthropometric characteristics in women from the general population in Slovakia, after 6 weeks of bakery products consumption.

Material and Methods. The study was conducted on healthy adult women from the general population (n=78), who were divided into four subgroups ("gluten" group, "gluten-free" group, "whole-grain" group and "control" group). The intervention dose consisted of a different combination of several types of bakery products (bread, pastries, soft pastries) within the individual weeks of consumption, while the intervention lasted 6 weeks, women consumed 150-200 grams per day. Anthropometric characteristics were measured by body composition analyser InBody 720 (Biospace Co. Ltd., Seoul, Republic of Korea), which works on the principle of bioelectrical impedance analysis. We also used the Lookin'Body 3.0 software to process the results. The collected data from these measurements were evaluated statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA). The levels of statistical significance were set at P<0.05 (*); P<0.01 (**); P<0.001 (***). Differences among subgroups of probands were tested with Pared Student t-test.

Results. The amount of fat mass (FM) in the participants changed along a similar curve as their body weight. After the 6-week intervention, there was a slight decrease in FM with statistical significance (P<0.01). In the third measurement, i.e., two months after the intervention, there was an increase in the amount of FM, but without statistical significance. Fat free mass (FFM) values in women increased very slightly after 6 weeks of consumption and remained approximately at the same level two months after the intervention (without statistical significance). We noted the most significant changes in the visceral fat area (VFA) parameter, which had a steadily increasing tendency. Changes in VFA values were statistically significant after the 6-week intervention (P<0.01) and also after two months of the intervention (P<0.05).

Conclusions. Changes in body composition in women were noted already after 6 weeks of bakery products consumption, especially in the FM, FFM, VFA and partially BMI parameters, but no changes were recorded in the WHR parameters during the entire duration of the study. One of the most significant results is the finding that there was an increase in VFA when consuming all types of bakery products (gluten, gluten-free and whole grain).

Key words: bakery products, anthropometric parameters, fat mass, FM, fat free mass, FFM, visceral fat area, VFA

INTRODUCTION

The challenge of provision of a much wider range of foods of relatively low glycaemic response than is currently available, especially in terms of cereal products, has been highlighted in recent years and this has relevance to bread consumption. Although there has been some transition to brown bread consumption, white bread remains a firm feature in the typical average western diet.

Food, which is the necessity of human life, is of critical importance in terms of community health. Bakery products and their derivatives have an important place in the food consumption all over the world [6, 9, 36]. Bread is, in fact, the only food known everywhere around the world, from the most developed to the most primitive cultures, and it is on the tables of everybody one way or another. Consumer perceptions and behaviour regarding this staple food are very important for the industrial bakery sector [29].

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

Bread with its high nutritional value, a neutral aroma, availability, and cheapness is the basic food source in bakery products [9]. The quality and nutritional value of dietary proteins are determined by the quantity, digestibility, and bioavailability of essential amino acids (EAA), which play a critical role in human growth, longevity, and metabolic health. Plant-source protein is often deficient in one or more EAAs (e.g., branched-chain amino acids, lysine, methionine and/or tryptophan) and, in its natural form, is less digestible than animal-source protein. Nevertheless, dietary intake of plant-source protein has been promoted because of its potential health benefits, lower cost of production and lower environmental impact compared to animal-source protein. Implementation of dietary strategies that improve both human and planetary health are of critical importance and subject to growing interest from researchers and consumers [6]. The grain-based products have been and still are an important part of people's regular diets. Their nutritional properties recommend them as significant energy sources for the human body [3, 33].

The health effects of cereal foods depend on the type of product. The intake of whole-grain cereal foods is consistently associated with health benefits at the population level, and increased consumption is therefore warranted and advocated in official dietary guidelines in many countries [22]. However, many observational studies have shown consistent inverse associations between high whole-grain intake and the risk of developing noncommunicable diseases such as type 2 diabetes, cardiovascular disease, colorectal cancer, and total and cause-specific mortality [19, 30]. In addition to observational studies, more than 200 dietary intervention studies have shown – depending on the cereal species – reduced body weight, decreased total cholesterol, improved systolic blood pressure [30] improved postprandial glucose and insulin homeostasis [21] decreased inflammatory markers, [10] and lowered total and low-density lipoprotein cholesterol [11, 13] when whole grains have been consumed, although it should be acknowledged that some results are conflicting. The evolution of wheat consumption has been of interest for a long time [1, 16]. However, concerns regarding the negative influence of its consumption through its derived products such as bread on health [5] or the environment [7] have increased. In view of the growing trend of obesity, some questions can be raised about the quality of nutritional information on food.

The changes in consumers' eating habits are one of the most important factors affecting the consumption of selected foods. The most important factors for bakery products consumers are freshness, taste, price, durability, composition, and country of origin. Rational aspects in the purchase of bakery products are factors

of composition and durability. The irrationality of consumers' purchasing behaviour can be accompanied by psychological factors, which include the perception of freshness and smell of bakery products, perception of the price, perception of the country of origin, as well as sensory aspects such as taste [18].

The most important factor influencing the purchase of bakery products is their price, but its importance decreased with the growth of consumer incomes. The quality of bakery products is more important to women, while special offers were more important to men. The frequency of buying bakery products is significantly influenced by age, with younger respondents buying more often than older categories of respondents. In addition, all age categories of consumers prefer supermarkets or hypermarkets to local stores [20].

Obesity is defined as an abnormal or excessive accumulation of fat that presents a risk to health [39]. Obesity, however, is also recognized as a heterogeneous disorder [14, 35]. Obese individuals are known to vary in their body fat distribution, and increasing evidence suggests that the regional distribution of adipose tissue might be more important than the total amount of body fat [15]. Body fat tissue has traditionally been thought to be distributed across two main compartments – subcutaneous fat, and visceral fat. These two different fat depots have been shown to have disparate functions, biochemical features, and metabolic characteristics [2].

The aim of the work was to evaluate the changes in the anthropometric characteristics of females from the general population after influence of 6 weeks of consumption of various bakery products.

MATERIAL AND METHODS

The study was conducted in healthy adult women from the general population ($n = 78$), who were divided into four subgroups ("gluten" group, "gluten-free" group, "whole-grain" group and "control" group). All participants signed written informed consent to participate in the study and were informed about the course and type of measurements, that will be used in the study. The research was approved by the Ethics Committee at the Specialized Hospital St. Zoerardus Zobor, n.o. (approval number 012911/2016).

Volunteers with the severe disease or with recommended special dietary regimen were excluded. Women of "gluten group" consumed gluten-containing bread and bakery products; women of "gluten-free group" consumed gluten-free bread and bakery products; women of "whole-grain" group consumed whole-grain containing bread and bakery products and women of "control group" not consumed bakery products. The amount of bread and bakery products consumed was determined according to

the recommended food consumption for the Slovak population, which means that women consumed 150-200 grams per day for 6 weeks. All participants were asked not to change their eating and physical activity habits.

Three measurements were taken during the study: (1) before the intervention; (2) after six-week intervention; (3) two months after intervention. Body height of probands was measured with device TANITA WB-380H, which measures body height and body weight at the same time. When measuring body height, the proband stands upright, his legs are close to him, his hands are next to his body. The head is in the orientation so-called Frankfurt location, i.e., his eyes are fixed on a point on the opposite side at the height of the person's eyes. The distance of the vertex from the plane on which the proband is standing is measured. The measured person stands without shoes, dressed only in underwear.

Anthropometric characteristics were measured by body composition analyser InBody 720 (Biospace Co. Ltd., Seoul, Republic of Korea), which works on the principle of bioelectrical impedance analysis. Proband was informed about measurements procedure, explained the possible risks of measuring in the case of pregnancy or having an artificial pacemaker at the heart. The measurement must be performed before a meal or 2 hours after a meal and after taking it toilets. It is not recommended to drink large amounts of fluids before the examination. There should be no major physical activity before the examination, as temporary changes in body composition may occur. When measuring during menstruation, there will be an increase in body water, so it is better to perform measurement only after its completion. It is also not advisable to perform the test after or after showering sauna, because sweating causes temporary changes in body composition. During testing it is suitable for a temperature of 20-25 °C. If a repeat test is performed, it is necessary him under the same conditions. We also used the Lookin' Body 3.0 software to process the results.

The collected data from these measurements were evaluated statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA). The levels of statistical significance were set at $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***). Differences among subgroups of probands were tested with Pared *Student t*-test.

RESULTS AND DISCUSSION

In the study, we monitored the influence of bakery product consumption on women's body composition. Anthropometric measurements were performed in 78 women with an average age of 31.64 ± 9.42 years (in "gluten" group it was 31.76 years; in "gluten-free"

group 29.11 years; in "whole-grain" group 44.08 years and in "control" group it was 27.86 years). To determine the body composition the bioelectrical impedance analysis was performed.

Single-frequency bioelectrical impedance analysis is recognized as an appropriate measurement of body composition [27, 34]. BIA is a method for estimating body composition, in particular body fat and muscle mass, where a weak electric current flows through the body and the voltage is measured in order to calculate impedance (resistance) of the body. Most body water is stored in muscle. Therefore, if a person is more muscular there is a high chance that the person will also have more body water, which leads to lower impedance. BIA determines the electrical impedance, or opposition to the flow of an electric current through body tissues which can then be used to estimate total body water (TBW), which can be used to estimate fat-free body mass and, by difference with body weight, body fat [31].

Table 1 shows the changes in anthropometric parameters of all study women ($n=78$). Average body weight ranged around 63.43 kg (maximum 101.53 kg; minimum 43.17 kg). By evaluating the changes in the body composition of the entire group of women during the study, we found that the body weight after the 6-week intervention decreased slightly with statistical significance ($P < 0.001$). However, with an interval of two months, an increase in body weight was recorded in the probands to a higher average value of body weight than before the intervention with a statistically significant difference ($P < 0.05$).

Body mass can be divided into two major components: body fat (energy stores) and lean mass (including muscle, organs, and bone), each of which has distinct biological significance and was likely subject to different selective pressures during human evolution. Humans have a high proportion of body fat compared to other primates, and to mammals more widely [28, 37, 41]. In contrast, skeletal muscle mass (a major constituent of lean mass) is low compared with our closest relatives [41], other primates [24].

When evaluating the changes due to the six-week consumption of various types of bakery products, we found that there were partial changes not only in body weight, but also in its individual components.

The amount of fat mass (FM) in the participants changed along a similar curve as their body weight. After the 6-week intervention, there was a slight decrease in FM with statistical significance ($P < 0.01$). In the third measurement, i.e., two months after the intervention, there was an increase in the amount of FM, but without statistical significance. The average values of FM during the study were 18.66 kg. The mean of the maximum during study was 51.33 kg; the mean of the minimum was 8.63 kg.

Table 1. Changes in anthropometric parameters of all study women (n=78)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	63.34±10.83	63.16±10.75	63.79±10.93
P value, Significance (*/*/*/**)	0.0873	0.0005***	0.0393*
FM (kg)			
mean±SD	18.64±7.83	18.43±7.74	18.91±7.91
P value, Significance (*/*/*/**)	0.0558	0.0011**	0.3006
FFM (kg)			
mean±SD	44.70±4.79	44.72±4.77	44.88±4.69
P value, Significance (*/*/*/**)	0.8180	0.4011	0.3177
VFA (cm²)			
mean±SD	75.38±27.96	75.02±28.29	76.81±28.20
P value, Significance (*/*/*/**)	0.4262	0.0027**	0.0426*
BMI (kg.m⁻²)			
mean±SD	22.81±3.68	22.74±3.63	22.95±3.75
P value, Significance (*/*/*/**)	0.0750	0.0004***	0.0330*
WHR			
mean±SD	0.87±0.06	0.88±0.05	0.88±0.06
P value, Significance (*/*/*/**)	0.2517	0.2418	0.0482*

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Fat free mass (FFM) values in women (average value 44.70 kg; maximum 57.60 kg; minimum 34.20 kg) increased very slightly after 6 weeks of consumption (average value 44.72 kg; maximum 57.70 kg; minimum 34.20 kg and remained approximately at the same level two months after the intervention (without statistical significance). The mean of the maximum FFM was 57.77 kg; the mean of the minimum FFM was 34.53 kg.

Visceral fat area is important factor used in the assessment of cardiometabolic risk and is correlated with the metabolic syndrome even at the normal body mass index indicating the absence of obesity [4, 17]. In this study, we noted the most significant changes in the visceral fat area (VFA) parameter, which had a steadily increasing tendency. Changes in VFA values were statistically significant after the 6-week intervention ($P < 0.01$) and also after two months of the intervention ($P < 0.05$). VFA values higher than 100 cm² indicate abdominal obesity. In the set of monitored women, we recorded VFA values above 100 cm² in 16 women, of which 11 women during all three measurements; in 3 women, there was an increase in the values above the 100 cm² limit until two months after the intervention; 1 woman had an increased VFA only before the intervention, and 1 woman had the VFA decreased up to two months after the intervention. 31 women had borderline VFA values (71-100 cm²), and

31 women had borderline VFA values (71-100 cm²). 30 women had normal values (41-70 cm²).

In this study, average values of VFA were achieved to 75.74 cm² (the mean of the maximum was 159.67 cm²; the mean of the minimum was 33.71 cm²). From the point of view of assessing cardio-metabolic risk, VFA values are very important, as the accumulation of fat in the abdominal cavity and waist area is a risk factor for the development of typical metabolic (insulin resistance, type 2 diabetes mellitus...) and many cardiovascular diseases. However, the fact is that even low VFA values (<40 cm²) are endocrinologically undesirable. We found them in 7 women.

WHO compiled uniform categories of BMI. Four categories were established: underweight, normal, overweight, and obese. An individual would be considered underweight if their BMI was between 15 and 19.9 kg.m⁻², normal weight if their BMI was 20 to 24.9 kg.m⁻², overweight if their BMI was 25 to 29.9 kg.m⁻², and for obesity, if it were 30 to 35 kg.m⁻² or bigger. Using linear regression, a BMI of 16.9 kg.m⁻² in men and 13.7 kg.m⁻² in women represents a complete absence of body fat stores [8, 25, 39].

Body mass index (BMI) is a metric currently used to define anthropometric height/weight characteristics in adults and to classify (categorize) them into groups. A common interpretation is that it represents an individual's fatness index. It is also widely used

as a risk factor for the development or prevalence of several health problems. BMI has been useful in population-based studies based on its wide acceptance in defining specific categories of body weight as a health problem. But it's becoming increasingly clear that BMI is a poor indicator of body fat percentage. Importantly, BMI also does not capture information about the amount of fat in different parts of the body [25], which also confirm our findings. Based on BMI, there were 15 women with underweight 44 women with normal weight, 13 women with overweight and 6 women with obesity. The average values of BMI during the study were 22.83 kg.m⁻². The mean of the maximum during study was 35.96 kg.m⁻²; the mean of the minimum was 17.37 kg.m⁻².

The average values of WHR of the entire group of women did not change during the study, BMI increased slightly after the 6-week intervention, then decreased only minimally two months after the intervention. We did not observe statistically significant differences in both indexed parameters (WHR and BMI). WHR has been used as an indicator or measure of health, and as a risk factor for developing serious health conditions. WHR is used as a measurement of obesity, which in turn is a possible indicator of other more serious health conditions. The WHO states that abdominal obesity is defined as a waist–hip ratio above 0.90 for males and above 0.85 for females, or a body mass index (BMI) above 30.0 [38]. If obesity is redefined using WHR

instead of BMI, the proportion of people categorized as at risk of heart attack worldwide increases threefold [40]. WHR may be less accurate in individuals with a BMI of 35 kg.m⁻² or higher, and more complex to interpret since an increased WHR may result from increased abdominal fat or decreased lean muscle mass around the hips [12]. The body fat percentage is considered to be an even more accurate measure of relative weight. Of these three measurements, only the waist–hip ratio takes account of the differences in body structure. Hence, it is possible for two people of the same sex to have different body mass indices but the same waist–hip ratio, or to have the same body mass index but different waist–hip ratios. WHR has been shown to be a better predictor of cardiovascular disease than simple waist circumference and body-mass index [23]. The average values of WHR during the study were 0.88. The mean of the maximum during study was 1.01; the mean of the minimum was 0.78. Women with a WHR value higher than 0.8 and men with a value higher than 1.0 have a higher risk of health complications due to inappropriate distribution of fat in the body. In our study, there were only 7 women with a WHR value below 0.8, and therefore up to 71 women had an increased risk of health complications related to fat distribution.

When dividing the probands in more detail based on the bakery products type consumed (i.e., with gluten content; gluten-free; whole grain and without

Table 2. Changes in anthropometric parameters of “gluten” group (n=17)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	61.39±11.64	61.54±11.27	61.45±10.67
P value, Significance (*/*/*/**)	0.6249	0.7917	0.8843
FM (kg)			
mean±SD	15.48±6.92	15.59±6.62	15.52±6.57
P value, Significance (*/*/*/**)	0.6156	0.7999	0.9221
FFM (kg)			
mean±SD	45.91±5.96	45.95±6.06	45.94±5.54
P value, Significance (*/*/*/**)	0.8680	0.9643	0.9369
VFA (cm²)			
mean±SD	63.26±28.51	63.86±26.64	64.26±26.92
P value, Significance (*/*/*/**)	0.5653	0.7324	0.5447
BMI (kg.m⁻²)			
mean±SD	21.58±3.19	21.63±2.98	21.61±2.79
P value, Significance (*/*/*/**)	0.6708	0.8956	0.8481
WHR			
mean±SD	0.86±0.06	0.86±0.05	0.86±0.06
P value, Significance (*/*/*/**)	0.4477	0.3109	0.1635

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Table 3. Changes in anthropometric parameters of “gluten-free” group (n=27)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	63.51±11.93	63.29±12.09	64.04±12.09
P value, Significance (*/*/*/**)	0.1550	0.0041**	0.093
FM (kg)			
mean±SD	18.60±9.44	18.66±9.27	19.36±9.44
P value, Significance (*/*/*/**)	0.8080	0.0038**	0.0171*
FFM (kg)			
mean±SD	44.91±4.08	44.63±4.05	44.68±4.19
P value, Significance (*/*/*/**)	0.2456	0.8033	0.5308
VFA (cm²)			
mean±SD	74.10±30.16	74.77±31.37	76.77±29.99
P value, Significance (*/*/*/**)	0.3732	0.0668	0.0231*
BMI (kg.m⁻²)			
mean±SD	22.75±4.12	22.67±4.13	22.94±4.19
P value, Significance (*/*/*/**)	0.1797	0.0043.94**	0.0900
WHR			
mean±SD	0.87±0.06	0.87±0.06	0.87±0.06
P value, Significance (*/*/*/**)	0.1726	0.7326	0.1109

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Table 4. Changes in anthropometric parameters of “whole grain” group (n=12)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	64.19±9.66	63.86±9.74	64.78±10.21
P value, Significance (*/*/*/**)	0.0487*	0.0061**	0.1152
FM (kg)			
mean±SD	20.19±6.46	19.68±6.72	20.28±6.91
P value, Significance (*/*/*/**)	0.0061**	0.0161*	0.9898
FFM (kg)			
mean±SD	44.00±4.68	44.19±4.61	44.51±4.69
P value, Significance (*/*/*/**)	0.2515	0.2298	0.0390*
VFA (cm²)			
mean±SD	82.45±24.19	80.81±25.47	83.31±25.88
P value, Significance (*/*/*/**)	0.0095**	0.0097**	0.6121
BMI (kg.m⁻²)			
mean±SD	23.48±3.47	23.36±3.46	23.65±3.71
P value, Significance (*/*/*/**)	0.0426*	0.0064**	0.1066
WHR			
mean±SD	0.89±0.05	0.89±0.05	0.89±0.05
P value, Significance (*/*/*/**)	0.8224	0.4661	0.5818

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Table 5. Changes in anthropometric parameters of “control” group (n=22)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	62.95±9.44	62.45±9.36	62.95±9.86
P value, Significance (*/**/***)	0.0358*	0.0997	1.0000
FM (kg)			
mean±SD	19.14±5.91	18.54±6.15	19.05±6.38
P value, Significance (*/**/***)	0.0165*	0.0713	0.7755
FFM (kg)			
mean±SD	43.81±4.64	43.91±4.58	43.90±4.55
P value, Significance (*/**/***)	0.6561	0.9392	0.7147
VFA (cm²)			
mean±SD	77.98±21.93	75.85±23.01	77.60±23.04
P value, Significance (*/**/***)	0.0069**	0.1054	0.7511
BMI (kg.m⁻²)			
mean±SD	22.80±3.52	22.61±3.44	22.80±3.68
P value, Significance (*/**/***)	0.0337*	0.0953	0.953
WHR			
mean±SD	0.88±0.04	0.87±0.04	0.87±0.04
P value, Significance (*/**/***)	0.3386	1.0000	0.5229

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

consumption of bakery products) we found certain differences. Tables 2, 3, 4 and 5 show the changes in anthropometric parameters of “gluten” group; “gluten-free” group; “whole-grain” group and “control” group.

After six weeks of intervention with different bakery products, we found that women in the “whole grain” group achieved a slight increase in fat-free mass (FFM) and this increase was maintained two months after the intervention ($P < 0.05$), similarly, the value of FFM increased at the same level in the other monitored groups. Only in the gluten-free group did the FFM values decrease (without statistical significance).

Furthermore, there was an increase visceral fat area (VFA) in the “gluten” and “gluten-free” groups, and a decrease VFA in the “control” and “whole grain” groups. The values of fat mass (FM) increased slightly in the “gluten” and “gluten-free” groups and decreased in the “whole-grain” and “control” groups.

The consumption of different types of bakery products thus caused changes in the body structure of women after only 6 weeks of intervention.

Some studies have suggested that promoting the Mediterranean diet as a healthy eating model may help prevent weight gain and the development of overweight/obesity. The consumption of bread, which was part of the traditional Mediterranean diet, has been declining because the general public believes that bread is fattening. A reduction in consumption of white bread, but not whole grain bread, within

a Mediterranean-style diet is associated with lower weight gain and abdominal fat. The difference in composition between whole grain bread and white bread appears to make a difference in its effect on body weight and abdominal fat [32].

Whole grains have received increased attention for their potential role in weight regulation. A high intake has been associated with smaller weight gain in prospective cohort studies, whereas the evidence from randomized controlled studies has been less consistent [26].

CONCLUSION

Changes in body composition were noted already after 6 weeks of consumption, especially in the FM, FFM, VFA and partially BMI parameters, but no changes were recorded in the WHR parameters during the entire duration of the study. One of the most significant results is the finding, that there was an increase in VFA when consuming all types of bakery products (gluten, gluten-free and whole grain), which represents a serious cardio-metabolic risk. Therefore, we consider it necessary to improve lifestyle factors in order to improve this unflattering situation of women from the general Slovak population. Further research is needed to monitor the effect of different types of bakery products on body composition, especially fat mass, including visceral fat, in a larger number of

volunteers with using daily records of physical activity during the entire study period.

Acknowledgments

This publication was created thanks to the support of the Grant Agency FAPZ SPU in Nitra 04/2023 'Blood biochemical parameters in the evaluation of the health and nutritional status of selected population groups' and 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: 16.08.2023

Accepted: 04.10.2023

Published online first: 23.10.2023

PREVALENCE AND PREDICTIVE DETERMINANTS OF OVERWEIGHT AND OBESITY IN CHILDREN AGED 0-24 MONTHS IN MOROCCO: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background. Preventing overweight and obesity in early childhood is a priority for healthcare systems worldwide due to the harmful effects on health and economy over the medium and long term.

Objective. The aim of this study is therefore the identification of the determinants predictive of overweight and obesity during the first 24 months of a child's life.

Material and Methods. From May 2021 to January 2022, 1012 mother-child pairs were included in this study and were interviewed at urban and rural health centers in the Skhirat-Temara in Morocco prefecture using a questionnaire. The anthropometric measurements of the children were also taken according to World Health Organization standards.

Results. The prevalence of overweight and obesity in children aged 0-24 months was 16.5%. This prevalence increased with age (12.5% for the 0-6 months group, and 15.5% and 21.5% respectively for the 7-12 months and 13-24 months groups). Cesarean delivery (aOR=1.78; 95%CI: 1.26-2.51; p=0.001), more than two living siblings in the household (aOR=1.48; 95%CI: 1.03-2.12; p=0.03), male gender (aOR=1.56; 95%CI: 1.10-2.20; p=0.01), and child age (aOR=0.94; 95%: 0.92-0.97; p<0.001) are significant predictors of overweight and obesity. Paternal smoking (aOR=2.16; 95%CI: 1.15-4.06; p=0.01), short sleep duration (aOR=4.05; 95%CI: 1.27-12.88; p=0.01) in children aged 7-12 months, and combined breastfeeding (aOR=5.88; 95%CI: 2.07-16.72; p<0.001) during the first six months in children aged 13-24 months are also predictive determinants for this problem.

Conclusion. The identification of early predictors of overweight and obesity can be used by public health decision-makers as a roadmap for action to prevent and improve health.

Key words: overweight, obesity, determinant, predictive, children, Morocco

INTRODUCTION

The evidence shows that the first 1,000 days of a child's life are a critical window of opportunity for the child's future development, growth and health. This period covers the period from conception to the end of the first 24 months of life [12]. Over this time, the foetus, the newborn and the infant are confronted with major challenges resulting from a combination of

biological, environmental, behavioral and individual factors that can affect their healthy development and expose them to the risk of overweight and obesity [26, 28].

In recent decades, overweight and obesity in early childhood has emerged as a serious public health problem due to the harmful repercussions that can result, and the major costs that can be incurred by the nations' healthcare systems [7, 63], particularly in the

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

knowledge that once established in early childhood, overweight and obesity can extend into adolescence and adulthood [42]. As a result, this problem has the potential to lead to a number of non-communicable diseases, such as type 2 diabetes, cardiovascular disease, high blood pressure, stroke, cancer, osteoarthritis and mental health disorders [40].

However, overweight and obesity could be prevented by identifying risk factors from an earlier stage, especially those most likely to overwhelm a child in the first 24 months of life [49]. Several studies have explored the determinants of overweight and obesity in early childhood, covering the period from conception to the second year of life. The main determinants are parental obesity, maternal weight gain during pregnancy, gestational diabetes, mode of delivery, birth weight of the newborn, maternal smoking, socio-economic status, sleep, breastfeeding, etc.[37, 27].

In fact, health policies throughout the world need resolve to tackle this growing epidemic, which has serious economic, social and health impacts, by planning, developing and strengthening programs to promote maternal and child health during this critical period in a child's life, covering diagnosis, monitoring, awareness raising and education.

The present study aims to identify and predict the determinants of overweight and obesity in children aged 0-24 months in the prefecture of Skhirat-Temara in Morocco, thus highlighting for public health decision-makers the possibilities for action in favor of modifiable risk factors for early and effective prevention of this problem.

MATERIAL AND METHODS

Study settings, design, period and population

This is a cross-sectional study with descriptive and analytical aims. It was conducted from May 2021 to January 2022 in urban and rural health centers in the prefecture of Skhirat-Temara, a province close to the capital Rabat, with a total population of 570887 and 134998 households, for which the estimated annual growth rate in population projections between 2014 and 2030 is 3%, the higher rate in the region [39]. The survey concerned consenting mother-child pairs, resident in the Skhirat-Temara prefecture, and whose children were aged 0-24 months, who consulted health centers for various preventive and curative primary health care services. Any couple whose child was born prematurely, had a history of infection or diarrhea within two weeks of the interview, or had a congenital malformation or metabolic disease affecting growth were excluded from the study.

Sample size determination

The estimated sample size was calculated on the following formula to ensure that the results of this study were representative [57]:

$$n = \frac{z^2 \times p \times (1 - p)}{m^2}$$

Where: n= sample size, z = 1.96 for a 95% confidence level, p is the estimated prevalence of childhood obesity, and m = 5% is the margin of tolerable sampling error.

The 2018 National Population and Family Health Survey [38] reported an obesity and overweight prevalence of 13.7% for children under 5 years of age in the Rabat-Salé-Kenitra region. Indeed, the sample size calculated for this study was 182 participants (with p=13.7%), although our study had included 1012 mother-child pairs.

Instruments and data collection

The study was carried out by a face-to-face questionnaire administered to the mothers, who were interviewed about their demographic, socio-economic and clinical background, as well as data on their partners and children. Based on evidence from previous studies [27, 41], these data represent the variables that have a potential risk for overweight and obesity:

Data concerning parents: the mother's age, the parents' level of education (illiterate, low, medium and high), the parents' occupation (working or not), the mode of delivery and the history of gestational diabetes. The mothers were also interviewed about the notion of passive smoking (smoking partner), as none of the mothers included in this study were smokers or ex-smokers.

Child data: the child's age, sex, rank in the siblings, the method of breastfeeding adopted during the first six months after birth: exclusive breastfeeding, combined breastfeeding (breast milk, artificial milk and other solid or pasty foods), or exclusive artificial feeding. Lastly, the hours slept by the child in accordance by age over a 24-hour period (compliant, non-compliant). According to the recommendations of the American Academy of Sleep Medicine [45]: for optimum health, the recommended hours of sleep for children aged 4-12 months are 12-16 hours in 24 hours, and 11-14 hours in 24 hours for children aged 12-24 months.

The household data: residence, number of living children and monthly household income, which was defined according to the Moroccan guaranteed minimum inter-professional income (SMIG) set at ≈ 2800 Moroccan dirhams (MAD) ≈ \$282. The dependent variable for this study was overweight and obesity in children aged 0-24 months. Then, anthropometric measurements were taken by the investigator for each child included in this study, according to WHO standards. The variables measured were:

The weight: We measured the child's weight using a digital baby scale with a maximum capacity of 20 kg. The scale was calibrated before each weighing session.

The height was determined using a toise. The same instrument was used throughout the survey. The body mass index (BMI) calculated using the formula: Weight (kg) / Height² (m²)

The WHO BMI-for-age (BMI/A) reference curves for girls and boys (0-24 months) were used to determine the BMI/A z-scores, and to classify the child's weight as normal, overweight, obese, wasted or severely wasted. For this study, overweight was defined as a weight-for-height Z score > 2 standard deviation (SD), obesity as a weight-for-height Z score >3SD, wasting as a weight-for-height Z score <-2SD and severe wasting as a weight-for-height Z score <-3SD in accordance with WHO recommendations [43].

Statistical analysis

In our study, the data collected were analysed according to three age groups: the first group-included children aged 0-6 months, while the second and third groups covered children aged 7-12 months and 13-24 months in succession.

The determinants: mode of breastfeeding and sleep duration, were only introduced into the analysis for the second and third classes in order to study the impact of the nature of breastfeeding during the first 6 months of the child's age on the development of possible overweight or obesity. Thus, the majority of studies looking at the evaluation of sleep duration, quality and behaviors have set 6 months as the youngest age at which to assess this determinant [64].

All data analyses for this study were performed using Jamovi statistical software, version 2.3.16. Statistical analyses were performed to generate: (i) the predictive factors of overweight and obesity in all the children participating in our study (children from 0 to 24 months; N=1012), and then to generate (ii) the predictive factors of overweight and obesity in the children for 3 age categories (children from 0 to 6 months, children from 7 to 12 months and children aged 13 to 24 months) after adjusting for confounding factors. The normality of quantitative variables was verified using the *Kolmogorov Smirnov* test. Quantitative variables with asymmetrical distributions are expressed as median and quartile, so the study of the association between the "overweight and obesity" outcome and the other quantitative variables investigated in this study was carried out using the *Mann-Whitney U* test. Categorical variables were summarized as frequencies and percentages. The *Chi-square* test and *Fisher's* exact test were conducted to compare differences in categorical variables among groups regarding the outcome variable (overweight and obesity).

Multiple logistic regression were used to generate adjusted odds ratios (aOR) for a 95% confidence interval. Variables with a p-value < 25% in the univariate analysis were entered into the multiple logistic regression models. The test was considered significant for a p-value < 0.05.

Ethical considerations

This study was approved by the Ethics Committee for Biomedical Research of the Faculty of Medicine and Pharmacy, Mohamed V University, Rabat, Morocco (ethical approval no. C68/20 issued on 18 February 2021). Prior to data collection, participants were informed of the purpose of the study and its benefits, as well as the respect of anonymity and confidentiality during processing and publication of results. Oral and written consent was then obtained from all participants.

RESULTS

Characteristics of the study participants

A total of 1012 mother-child pairs were included in this study. The prevalence of overweight and obesity among the children was 16.5% (167 overweight and obese children). The age median of the participating mothers was 29 years old, more than two thirds were illiterate or had a low level of education (64.9%), 6.4% had gestational diabetes. The study also revealed that the majority of participating households lived in urban areas (88.3%), 70.6% had 2 children at most, with less than \$282 monthly for 42.1% of participating households. The median age of the children in the study was 8 months, more than half were boys (50.9%), and 32.4% had been delivered by caesarean section. The *chi-square* test and *Fisher's* exact test showed that mode of delivery, number of live births, child age and sex were significantly associated with childhood overweight and obesity in the current study (Table 1).

Determinants predictive of overweight and obesity in participating children

For children from 0 to 24 months, multiple regression models showed that: Cesarean delivery (aOR=1.78; 95%CI: 1.26-2.51; *p*=0.001), more than two living siblings in the household (aOR=1.48; 95%CI: 1.03-2.12; *p*=0.03), male gender (aOR=1.56; 95%CI: 1.10-2.20; *p*=0.01), and child age (aOR=0.94; 95%: 0.92-0.97; *p*<0.001) are significant predictors of overweight and obesity (Table 1).

Table 2 shows the results of the predictive factors of overweight and obesity in children aged 0 to 6 months, where three factors are significantly associated with the outcome variable. Children with the second or higher sibling rank are more likely to be overweight or obese compared to first-born children (aOR=1.87;

Table 1. Univariate and multiple logistic regression analysis of risk factors for overweight and obesity in children aged 0-24 months (N=1012)

Variables	Total N = 1012	Overweight / Obesity		<i>p</i>	aOR (95%CI)	<i>p</i>
		Yes n (%) 167 (16.5)	No n (%) 845 (83.5)			
Maternal age (year)						
Median (IQR)	29 (25-34)	29 (25-35)	29 (25-34)	0.37 ^a		
Residence						
Urban	894 (88.3)	141 (84.4)	753 (89.1)	0.08		
Rural	118 (11.7)	26 (15.6)	92 (10.9)			
Mother's education level						
Illiterate	167 (16.5)	25 (15)	142 (16.8)	0.59		
Low	490 (48.4)	76 (45.5)	415 (49)			
Medium	174 (17.2)	31 (18.6)	143 (16.9)			
High	181 (17.9)	35 (21)	146 (17.3)			
Father's education level						
Illiterate	118 (11.7)	16 (9.6)	102 (12.1)	0.79		
Low	506 (50)	84 (50.3)	422 (49.9)			
Medium	222 (21.9)	37 (22.1)	185 (21.9)			
High	166 (16.4)	30 (18)	136 (16.1)			
Working mother						
Yes	168 (16.6)	31 (18.6)	137 (16.2)	0.45		
No	844 (83.4)	136 (81.4)	708 (83.8)			
Working father						
Yes	998 (98.6)	166 (99.4)	832 (95.5)	0.48 ^β		
No	14 (1.4)	1 (0.6)	13 (1.5)			
Monthly household income						
<\$282	426 (42.1)	67 (40.1)	359 (42.5)	0.44		
\$282– \$504	369 (36.5)	58 (34.7)	311 (36.8)			
>\$504	217 (21.4)	42 (25.2)	175 (20.7)			
Gestational diabetes						
Yes	65 (6.4)	9 (5.4)	56 (6.6)	0.55		
No	947 (93.6)	158 (94.6)	789 (93.4)			
Smoking husband						
Yes	241 (23.8)	49 (29.3)	192 (22.7)	0.06		
No	771 (76.2)	118 (70.7)	653 (77.3)			
Mode of delivery						
Cesarean	328 (32.4)	72 (43.1)	256 (30.3)	0.001	1.78 (1.26-2.51)	0.001
Vaginal	684 (67.6)	95 (56.9)	589 (69.7)		1	
Number of living sibling						
≤ 2	714 (70.6)	107 (64.1)	607 (71.8)	0.04	1	
> 2	298 (29.4)	60 (35.9)	238 (28.2)		1.48 (1.03-2.12)	0.03
Age of child (Month)						
Median (IQR)	8 (3-12)	9 (6-18)	7 (3-12)	< 0.001 ^α	0.94 (0.92-0.97)	<0.001
Sex of child						
Male	515 (50.9)	101 (60.5)	414 (49)	0.007	1.56 (1.10-2.20)	0.01
Female	497 (49.1)	66 (39.5)	431 (51)			
Rank in the sibling						
1 st	393 (38.8)	60 (35.9)	333 (39.4)	0.39		
2 nd and more	619 (61.2)	107 (64.1)	512 (60.6)			

Note: Most data values (except the median) are presented as frequencies and percentages; a *p*-value < 0.05 was statistically significant; α: Mann-Witney U test; β: fisher test

Table 2. Univariate and multiple logistic regression analysis of risk factors for overweight and obesity in children up to the age of 6 months (N=464)

Variables	Total N = 464	Overweight/Obesity		<i>p</i>	aOR (95%CI)	<i>p</i>
		Yes n (%) 58 (12.5)	No n (%) 406 (87.5)			
Maternal age (years)						
Median (IQR)	28 (24-33)	28 (25-33)	28 (24-33)	0.67 ^a		
Residence						
Urban	435 (93.8)	52 (89.7)	383 (94.3)	0.23 ^β		
Rural	29 (6.2)	6 (10.3)	23 (5.7)			
Mother's education level						
Illiterate	72 (15.5)	11 (19)	61 (15)	0.76		
Low	226 (48.7)	25 (43.1)	201 (49.5)			
Medium	70 (15.1)	10 (17.2)	60 (14.8)			
High	96 (20.7)	12 (20.7)	84 (20.7)			
Father's education level						
Illiterate	47 (10.1)	5 (8.6)	42 (10.3)	0.91		
Low	220 (47.4)	27 (46.6)	193 (47.3)			
Medium	108 (23.3)	13 (22.4)	95 (23.4)			
High	89 (19.2)	13 (22.4)	76 (18.7)			
Working mother						
Yes	393 (84.7)	7 (12.1)	64 (15.8)	0.46		
No	71 (15.3)	51 (87.9)	342 (84.2)			
Working father						
Yes	458 (98.7)	85 (100)	400 (98.5)	1 ^β		
No	6 (1.3)	0	6 (1.5)			
Monthly household income						
<\$282	188 (40.5)	20 (34.5)	168 (41.4)	0.38		
\$282-\$504	171 (36.9)	21 (36.2)	150 (36.9)			
>\$504	105 (22.6)	17 (29.3)	88 (21.7)			
Gestational diabetes						
Yes	31 (6.7)	3 (5.2)	28 (6.9)	0.78 ^β		
No	433 (93.3)	55 (94.8)	378 (93.1)			
Smoking husband						
Yes	101 (21.8)	15 (25.9)	86 (21.2)	0.41		
No	363 (78.2)	43 (74.1)	320 (78.8)			
Mode of delivery						
Vaginal	314 (67.7)	35 (60.3)	279 (68.7)	0.20	1	
Cesarean	150 (32.3)	23 (39.7)	127 (31.3)		1.83 (1-3.34)	0.04
Number of living sibling						
≤ 2	331 (71.3)	35 (60.3)	296 (72.9)	0.04		
> 2	133 (28.7)	23 (39.7)	110 (27.1)			
Median (IQR)	3 (2-4)	4 (3-6)	3 (2-4)	<0.001 ^α	0.63 (0.53-0.76)	<0.001
Sex of child						
Male	30 (49.6)	33 (56.9)	197 (48.5)	0.23		
Female	234 (50.4)	25 (43.1)	209 (51.5)			
Rank in the sibling						
1 st	193 (41.6)	17 (29.3)	176 (43.3)	0.04	1	
2 nd and more	271 (58.4)	41 (70.7)	230 (56.7)		1.87 (1-3.48)	0.04

Note: Most data values (except the median) are presented as frequencies and percentages; a *p*-value < 0.05 was statistically significant; α: Mann-Witney U test; β: fisher test

Table 3. Univariate analysis and multiple logistic regression of risk factors for overweight and obesity in children aged 7-12 months (N=302)

Variables	Total N = 302	Overweight/Obesity		<i>p</i>	aOR (95%CI)	<i>p</i>
		Yes n (%) 56 (18.5)	No n (%) 246 (81.5)			
Maternal age (years)						
Median (IQR)	29 (25-34)	29.5 (26-35)	29 (25-34)	0.43		
Residence						
Urban	236 (78.1)	42 (75)	194 (78.9)	0.52		
Rural	66 (21.9)	14 (25)	52 (21.1)			
Mother's education level						
Illiterate	48 (15.9)	8 (14.3)	40 (16.3)	0.93		
Low	141 (46.7)	26 (46.4)	115 (46.7)			
Medium	62 (20.5)	11 (19.6)	51 (20.7)			
High	51 (16.9)	11 (19.6)	40 (16.3)			
Father's education level						
Illiterate	36 (11.9)	5 (8.9)	31 (12.6)	0.47		
Low	157 (52)	29 (51.8)	128 (52)			
Medium	58 (19.2)	9 (16.1)	49 (19.9)			
High	51 (16.9)	13 (23.2)	38 (15.5)			
Working mother						
Yes	57 (18.9)	11 (19.6)	46 (18.7)	0.87		
No	245 (81.1)	45 (80.4)	200 (81.3)			
Working father						
Yes	298 (98.7)	55 (98.2)	243 (98.8)	0.56 ^α		
No	4 (1.3)	1 (1.8)	3 (1.2)			
Monthly household income						
<\$282	131 (43.4)	23 (41.1)	108 (43.9)	0.6		
\$282-\$504	102 (33.8)	22 (39.3)	80 (32.5)			
>\$504	69 (22.8)	11 (19.6)	58 (23.6)			
Gestational diabetes						
Yes	20 (6.6)	3 (5.4)	17 (6.9)	1 ^α		
No	282 (93.4)	53 (94.6)	229 (93.1)			
Smoking husband						
Yes	74 (24.5)	21 (37.5)	53 (21.5)	0.01	2.16 (1.15-4.06)	0.01
No	228 (75.5)	35 (62.5)	193 (78.5)		1	
Mode of delivery						
Vaginal	204 (67.5)	33 (58.9)	171 (69.5)	0.12		
Cesarean	98 (32.5)	23 (41.1)	75 (30.5)			
Number of living sibling						
≤ 2	212 (70.2)	37 (66.1)	175 (71.1)	0.45		
> 2	90 (29.8)	19 (33.9)	71 (28.9)			
Age of child (month)						
Median (IQR)	9 (9-12)	9 (9-12)	9 (9-12)	0.91	1 (0.84-1.2)	0.92
Sex of child						
Male	157 (52)	33 (58.9)	124 (50.4)	0.24		
Female	145 (48)	23 (41.1)	122 (49.6)			
Rank in the sibling						
1 st	112 (37.1)	22 (39.3)	90 (36.6)	0.7		
2 nd and more	190 (62.9)	34 (60.7)	156 (63.4)			
BF method						
Exclusive BF	144 (47.7)	22 (39.3)	122 (49.6)	0.32		
Combined BF	120 (39.7)	27 (48.2)	93 (37.8)			
Exclusive AF	38 (12.6)	7 (12.5)	31 (12.6)			
Sleep duration						
Non-compliant	13 (4.3)	6 (10.7)	7 (2.8)	0.01 ^α	4.05 (1.27-12.88)	0.01
Compliant	289 (95.7)	50 (89.3)	239 (97.2)		1	

Note: Most data values (except the median) are presented as frequencies and percentages; a *p*-value < 0.05 was statistically significant. BF= breastfeeding. AF= artificial feeding; α: Mann-Witney U test; β: fisher test

Table 4. Univariate and multiple logistic regression analysis of risk factors for overweight and obesity in children aged 13-24 months (N=246)

Variables	Total N = 246	Overweight/Obesity		<i>p</i>	aOR (95%CI)	<i>p</i>
		Yes n (%) 53 (21.5)	No n (%) 193 (78.5)			
Maternal age (years)						
Median (IQR)	30 (25-36)	30 (24.5-36)	30 (25-35.5)	0.78 ^α		
Residence						
Urban	223 (90.7)	47 (88.7)	176 (91.2)	0.59 ^β		
Rural	23 (9.3)	6 (11.3)	17 (8.8)			
Mother's education level						
Illiterate	47 (19.1)	6 (11.3)	41 (21.2)	0.1		
Low	123 (50)	25 (47.2)	98 (50.8)			
Medium	42 (17.1)	10 (18.9)	32 (16.6)			
High	34 (13.8)	12 (22.6)	22 (11.4)			
Father's education level						
Illiterate	35 (14.2)	6 (11.3)	29 (15)	0.59		
Low	129 (52.4)	28 (52.8)	101 (52.3)			
Medium	56 (22.8)	15 (28.3)	41 (21.3)			
High	26 (10.6)	4 (7.6)	22 (11.4)			
Working mother						
Yes	40 (16.3)	13 (24.5)	27 (14)	0.06		
No	206 (83.7)	40 (75.5)	166 (86)			
Working father						
Yes	242 (98.4)	53 (100)	189 (97.9)	0.58 ^β		
No	4 (1.6)	0	4 (2.1)			
Monthly household income						
<\$282	107 (43.5)	24 (45.3)	83 (43)	0.07		
\$282-504	96 (39)	15 (28.3)	81 (42)			
>\$504	43 (17.5)	14 (26.4)	29 (15)			
Gestational diabetes						
Yes	14 (5.7)	3 (5.7)	11 (5.7)	1 ^β		
No	232 (94.3)	50 (94.3)	182 (94.3)			
Smoking husband						
Yes	66 (26.8)	13 (24.5)	53 (27.5)	0.66		
No	180 (73.2)	40 (75.5)	140 (72.5)			
Mode of delivery						
Vaginal	166 (67.5)	27 (50.9)	139 (72)	0.004	1	
Cesarean	80 (32.5)	26 (49.1)	54 (28)		1.96 (1-3.84)	0.04
Number of living Sibling						
≤ 2	171 (69.5)	35 (66)	136 (70.5)	0.53		
> 2	75 (30.5)	18 (34)	57 (29.5)			
Age of child (Month)						
Median (IQR)	18 (18-18)	18 (18-19)	18 (17.5-18)	0.007 ^α	0.9 (0.78-1.03)	0.15
Sex of child						
Male	128 (52)	35 (66)	93 (48.2)	0.02	1.97 (1-3.87)	0.04
Female	118 (48)	18 (34)	100 (51.8)		1	
Rank in the sibling						
1 st	88 (35.8)	21 (39.6)	67 (34.7)	0.5		
2 nd and more	158 (64.2)	32 (60.4)	126 (65.3)			
BF method						
Exclusive BF	128 (52)	13 (24.5)	115 (59.6)	< 0.001	1	
Combined BF	96 (39)	30 (56.6)	66 (34.2)		5.88 (2.07-16.72)	< 0.001
Bottle feeding	22 (9)	10 (18.9)	12 (6.2)		1.79 (0.67-4.74)	0.23
Sleep duration						
Non-compliant	40 (16.3)	9 (17)	31 (16.1)	0.87		
Compliant	206 (83.7)	44 (83)	162 (83.9)			

Note: Most data values (except the median) are presented as frequencies and percentages; a *p*-value < 0.05 was statistically significant. BF= breastfeeding. AF= artificial feeding;

95%CI: 1-3.48; $p=0.04$). Children delivered by cesarean section have a 1.83 greater risk of being overweight or obese than those delivered vaginally. Likewise, the risk of overweight and obesity increases by 0.63 fold when the child's age is advanced by one month (aOR=0.63; 95%CI: 0.53-0.76; $p<0.001$).

Tables 3 and 4 present the results of univariate and multivariate logistic regression analysis for the age range of 7 to 12 months, and over 12 months successively. Paternal smoking (aOR=2.16; 95%CI: 1.15-4.06; $p=0.01$) and inadequate sleep duration (aOR=4.05; 95%CI: 1.27-12.88; $p=0.01$) were associated with an increased risk of overweight and obesity in children aged 7 to 12 months. Beyond this age, delivery by caesarean section (aOR=1.96; 95%CI: 1-3.84; $p=0.04$), combined breastfeeding (aOR=5.88; 95%CI: 2.07-16.72; $p<0.001$) during the first six months of the child's life, and masculinity (aOR=1.97; 95%CI: 1-3.87; $p=0.04$) were found to be predictive of overweight and obesity in children in this age group.

DISCUSSION

In this study, the prevalence of overweight and obesity among all children in our population was 16.5%. However, this was higher than in Vietnam and Colombia (10.7% and 5.8% respectively) [19, 2], but slightly lower than in South Africa (17.3%) [56]. In addition, the distribution of children in the 3 age groups revealed an increase in the prevalence of overweight and obesity with age (12.5% for the 0-6 months group, and 15.5% and 21.5% respectively for the 7-12 months and 13-24 months groups). This could be explained by the dietary habits at this stage of life, the low physical activity levels, and screen time (television, tablet, mobile phone), especially as the period of this study was during the Covid-19 pandemic, which was characterised by confinement and a restriction on children playing outside [5, 62]. Furthermore, our study presents a series of predictors of overweight and obesity in relation to the socio-economic context, the peri- and post-natal context and other child-related determinants.

Socio-economic determinants

In contrast to previous studies [8, 54, 70] our study found that, children with more than 2 siblings, and particularly those who are not the eldest, are more likely to be overweight or obese. Possible explanations for this are: the low level of education, the poor standard of living in our population, or the high stress levels of mothers with many children, which inhibit them from adopting appropriate and healthy dietary behaviors [1, 6, 11, 50] in accordance with the child's age, knowing that the first four months of a child's life is a key period when the risk of developing obesity

later in childhood is high [30]. It is therefore essential to have a good control of these determinants, which are closely linked and interact with each other, especially during the first 24 months of a child's life, in order to determine a child's nutritional status [26, 53].

Peri- and postnatal determinants

In this study, birth by caesarean section and paternal smoking were shown to be predictive risk factors for overweight and obesity in the child's first 24 months of life, however, contrary to previous research [3, 16, 28, 59], our study revealed no association of gestational diabetes with the risk of overweight and obesity in our population. In the first six months of a child's life and after the first year, birth by caesarean section successively represents a risk of overweight and obesity of 1.83 and 1.96 compared with vaginal birth. This finding is consistent with the results of previous studies that have demonstrated an increased risk of overweight and obesity during the first six months and at 24 months of age for Caesarean delivery [23, 31, 47, 65, 69]. In addition, scheduled caesarean section has been found, although not confirmed by other study [39], to increase the risk of overweight and obesity at the age of 12 months [10, 36, 69]. A series of studies have suggested an association between caesarean delivery and a predisposition to obesity, indicating that delivery by caesarean section alters the colonisation of the neonatal microbiome by vaginal microbes, leading to a lower abundance and diversity of intestinal microbiota [36, 55]. Nevertheless, exposure of the newborn to vaginal flora during vaginal birth is important and beneficial for improving metabolism and immune system function [18]. Therefore, the choice of caesarean section as the mode of delivery should be based on a medical indication to prevent the risk of overweight and obesity in newborns.

The involvement of the mother and her responsibility for the child's well-being or risk has always been emphasised, even before conception, but the father's contribution has often been discreet or underestimated, especially as regards the risk of overweight and obesity in the child [35]. Several studies have shown that direct exposure to tobacco in utero has a considerable impact on the risk of overweight and obesity in early childhood compared with exposure to paternal or family smoking [6, 22, 28]. In our study, children whose fathers smoked had a two times greater risk of developing overweight or obesity than children whose fathers were non-smokers.

In agreement with our finding Lindholm et al [29] revealed that paternal smoking was well associated with rapid weight gain between 6 and 12 months. Several other studies have also shown that passive smoking and paternal smoking, even well before and after conception, may be associated with the risk

of overweight in the child [9, 48, 66, 68]. Although the mechanism between exposure to smoking and overweight is not well defined, it is supposed that exposure to tobacco may cause metabolic and hormonal dysfunctions [25].

Child-related determinants

This study showed that the risk of overweight and obesity changes with age. Overall, the risk of overweight and obesity increases 0.94 fold when the child's age advances by one month between 0 and 24 months. It is lower during the first 6 months of life (aOR=0.63). These results are inconsistent with those reported in the study by *Gebremichael* et al, which revealed that the risk of overweight and obesity was higher in children aged less than 6 months (aOR = 5.19), followed by children aged 6 to 24 months (aOR = 1.97) [17]. The variation in these findings may suggest that these age groups represent periods when various factors contribute significantly to the development of overweight and obesity in children, highlighting the potential importance of infant diet in comprehending these outcomes [62].

Similar to our study, numerous studies revealed that the risk of overweight and obesity is greater in boys than in girls [1, 8, 15, 17, 54, 61]. The fact that boys are more overweight and obese than girls could be explained by genetic, metabolic and environmental differences [61]. In that context, studies have shown a positive association between obesity and the Y chromosome suggesting that paternal behaviors and the environment faced by fathers represent a critical challenge to the future health of the offspring through potential damage and modification of male germ cells. However, the evidence is currently limited and there is only hypothesis [13, 20, 35, 60, 68].

Various studies revealed an association between the risk of overweight and obesity in early childhood and later life with the feeding mode and duration, rather than the age of diversification and introduction of solid foods [44, 46, 50, 52, 70]. In our study, combined breastfeeding during the first 6 months of a child's life was a predictive risk factor for overweight and obesity from the age of 13 months. Our results are consistent with those of other studies which have shown that the introduction of complementary foods before the age of 6 months has a negative effect on the development and optimal growth of children. The benefits of exclusive breastfeeding have been demonstrated as a protective factor against overweight and obesity, as well as other virtues such as protection against short- and moderate-term illness [4, 50, 51, 67]. However, the beneficial effect of breastfeeding may be reduced in the long term due to a combination of genetic, environmental and behavioral factors [51]. The duration and choice of exclusive breastfeeding may depend on other factors,

such as caesarean delivery, which appears to have a negative influence on the initiation of exclusive breastfeeding and to reduce the period to 3 months [50]. Moreover, a high level of maternal education makes mothers more vigilant, aware and favorably selective in terms of dietary habits and the lifestyle to be established in their households [11, 50].

Our study showed that children with inadequate sleep duration were five times more likely to be overweight or obese, but only in the 7-12 month age group. However, epidemiological studies have suggested that sleep duration may be associated with the risk of overweight and obesity throughout an individual's life [21]. In a systematic review of prospective studies, the authors revealed that insufficient sleep represents both a high risk of overweight or obesity in infants, children and adolescents, and also a modifiable risk factor expressed in the inverse relationship between the number of hours of sleep and the change in BMI z-scores and BMI [14, 34]. Similar results have been reported in Chinese studies reporting an inverse relationship between sleep duration and BMI z-scores in children aged 1-24 months [58, 71]. However, the mechanism by which sleep deprivation affects weight gain is not entirely clear, and studies have suggested that in early childhood sleep duration and quality may be at the origin of overweight and obesity through hormonal changes, especially in leptin and ghrelin secretion, in addition to specific dietary behaviors involving diet quality and meal timing [24, 32, 33]. Based on the studies available, parental awareness of the need to promote healthy and optimal sleep quality and quantity from early childhood is necessary to prevent the risk of overweight and obesity.

CONCLUSION

In this study we found an increase in the prevalence of overweight and obesity with age during the first 24 months of a child's life. Moreover, the risk of being overweight or obese depends on a combination of multiple genetic, environmental, behavioral and individual determinants, and the main ones being: gender, sibling rank, number of living siblings, caesarean delivery, paternal smoking, combined breastfeeding during the first 6 months of life, and inadequate sleep duration. However, this problem can be prevented by developing public health programs aimed at identifying and intervening early in modifiable risk factors, as well as consolidating monitoring and management programs for diagnosed cases. In addition, raising parents' awareness and educating them are colossal measures for preventing unhealthy dietary patterns and promoting appropriate behaviors, such as preparing children properly for sleep in order to improve its quantity and quality.

Strength and limitations of the study

To the best of our knowledge, our study is the first to investigate the predictive factors of overweight and obesity during the first 24 months of a child's life in the prefecture of Skhirat-Temara; however, several limitations can be identified; firstly, the type of cross-sectional study does not allow us to establish a causal relationship. Then, all the mothers classified as having a history of gestational diabetes had no laboratory evidence, even for the variable "number of hours of sleep of the child", which was assessed on the basis of the mothers' reports and not on the basis of objective measurements such as actigraphy.

Acknowledgements

The authors would like to thank all the people who collaborated in carrying out this research, in particular the mother-child pairs participating in the study and the health staff from the health centers in the Skhirat-Temara prefecture for their cooperation in organizing interviews with the participants, and taking anthropometric measurements.

Funding sources

This research received no external funding.

Conflict of interest statement

The authors have no conflict of interests to declare.

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Received: 14.10.2023

Accepted: 25.11.2023

Published online first: 01.12.2023

STUDY OF THE DIETARY AND ANTHROPOMETRIC PROFILE AND IDENTIFICATION OF DETERMINING FACTORS IN ADOLESCENTS AT SCHOOL IN THE KHEMISSSET REGION OF MOROCCO

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ABSTRACT

Background. The nutritional status and dietary diversity of adolescents play a crucial role in their growth, development, and overall health.

Objective. The aim of this study is to assess the nutritional status and determine the dietary diversity score in urban and rural school settings among Moroccan adolescents.

Material and Methods. We conducted a descriptive cross-sectional study in public schools in Khemisset in the municipality of Sidi Allal El Bahraoui, Morocco. We used a questionnaire to collect sociodemographic data and a 24-hour dietary recall to calculate the dietary diversity score.

Results. This study included 215 students. The mean BMI was 21.06 ± 0.23 kg/m², with 60.8% of students having a normal BMI, 24.5% being underweight, 11.8% overweight, and 2.8% obese. The mean dietary diversity score was 5.672 ± 0.956 , with 21% having a low dietary diversity score, 12% having a high score, and 67% having a moderate score. Factors associated with nutritional status were place of residence, type of delivery, and age, while the dietary diversity score was associated with school level and BMI of adolescents.

Conclusion. The data from the study show that the diet of adolescents in the Khemisset region of Morocco is moderately diversified. In addition, the prevalence of underweight and overweight was worrying, underlining the need for multiple strategic interventions to improve the health status of adolescents.

Key words: adolescents, dietary survey, risk factors, nutritional status, dietary diversity, Morocco

INTRODUCTION

Chronic non-communicable diseases (NCDs), mainly cardiovascular diseases, cancer, diabetes and chronic respiratory diseases are the main causes of mortality, with 70% deaths worldwide. They share common risk factors such as smoking and alcohol overdose, lack of exercise, unbalanced diet. They cause public health problems in all populations [1].

In fact, a balanced diet combined with sufficient physical activity is considered essential for maintaining a healthy state of health and reducing the risk of chronic disease [2].

Food is a multidimensional concept that combines several dimensions. During a meal, many foods containing different nutrients are eaten at the same time. The rhythm and context of meals, food choices and food combinations are influenced by many factors, including genetic, cultural, social, environmental,

economic, health and lifestyle factors [3]. Public health recommendations are increasingly stressing the need for people to pay attention to the food they eat [4].

Socio-demographics factors such as gender, age and family composition are correlated with behaviors related to nutrition. As a result, the major food families do not occupy the same place in the consumption patterns of men and women [5]. Analysing the social aspects of diet in terms of health recommendations, and more generally that of social and cultural practices, provides valuable insights into the processes by which certain chronic diseases, such as diabetes, hypertension or hypercholesterolemia (and obesity) develop. But let's not forget that deficiencies exist in certain situations and are also a public health concern [6].

Analysis of the nutritional situation of the Moroccan population involves analysis of its socio-demographic evolution, its epidemiological profile

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

and the evolution of its food consumption, which is at the root of a dietary change that is itself at the root of a nutritional transition [7]. The dietary transition is caused by the transition from a traditional diet, based on cereals and vegetables, to a diet that includes more products of animal origin, and tends to become rich in energy needs. Adolescents are the first to adopt these dietary changes [8].

In Morocco, there is a lack of studies on eating habits, eating behaviors, nutritional status and associated factors among adolescents. With this in mind, we conducted this study to describe the nutritional status, dietary profile, lifestyle and eating habits of a sample of school-going adolescents in the Khemisset region, as well as to explore the impact of socio-demographic factors on their diet.

MATERIAL AND METHODS

A survey was carried out from December to February 2020 in the Khemisset region on a sample of 215 children and adolescents aged 13 to 19. The study protocol was approved by the Provincial Directorate of National Education and Sport in Khemisset, which granted permission to carry out the study. Before their participation, the objectives and methodology of the study were explained to the students and their consent was obtained. The students participated in the study were randomly selected. The classes assessed third year college (3TC) and second year college (2SC)) were also chosen at random.

The survey was based on a standardized questionnaire following a specific protocol to ensure the same procedure in all classes. Participants were asked to answer all the questions and encouraged to report any questions about parameters they did not know or understand. The questionnaire was completed in private so as to guarantee the confidentiality of the information and to allow them to express themselves freely. The first part of the questionnaire included socio-demographic information such as age, sex, living environment, weight, height, level of education, daily physical activities, etc. To study the food intake patterns of adolescents, we used a 24-hour recall in which the subjects surveyed declare the different food groups or drinks consumed, or during the last 24 hours (the food survey takes place in two days between them a month).

RESULTS

Socio-demographic characteristics

This study involved 215 pupils, 61.9% of whom were in third Year College (3TC) and 38.1% in second Year College (2SC). The gender breakdown of our sample shows that 61.9% (n=133) are female and 38.1%

(n=82) are male. The sex ratio was not balanced (F/M= 1.62; $p<0.001$). Age distribution showed that the average age of the students was 14.75 ± 1.38 years, with a minimum of 13 years, a maximum of 19 years and a median of 15 years. The sample was composed of 82 boys (mean age = 15.10 ± 1.28 years; range: 13-18 years) and 133 girls (mean age = 14.54 ± 1.39 years; range: 13-19 years).

The breakdown by place of residence shows that 73% (n=157) were from urban areas (girls=95 and boys=62) compared with 27% (n=58) from rural areas (girls=38 and boys=20).

As regards the parents' occupation, 70.7% (n=152) of fathers were in employment (with an occupation) and 88.8% (n=191) of mothers had no occupation. The breakdown of pupils by type of birth shows that 71.6% (n=154) were born naturally through the vagina (vaginal birth) and 28.4% (n=61) were extracted from the mother's uterus through an incision in the Abdomen (caesarean section). 13.02% (n=28) of these students reported having had a previous birth, half of whom were born by caesarean section (n=14).

Anthropometric characteristics

Body mass index (BMI) is calculated by dividing weight in kilograms by the square of height in meters ($BMI = \text{weight (kg)} / \text{height (m)}^2$). The distribution of pupils surveyed according to BMI shows that the average is $21.06\pm0.23 \text{ kg/m}^2$, with a minimum BMI of 14.28 kg/m^2 and a maximum BMI of 31.63 kg/m^2 and a median of 20.51. The distributions of BMI categories are 60.8% of pupils have a normal BMI, 24.5% are underweight, 11.8% are overweight and 2.8% are obese. The results of the χ^2 test between BMI and certain socio- demographic variables are shown in Table 1.

In terms of place of residence, among pupils from rural areas, 12.28% were underweight and 14.3% were overweight or obese. Among urban pupils, 29.03% were underweight and 14.84% were overweight or obese.

There was a significant relationship between type of delivery and BMI ($p<0.05$). In fact, among the students extracted from a caesarean delivery, 21.67% were underweight and 4 students were overweight or obese. Among students who had undergone a vaginal delivery, 20.39% were underweight and 17.76% were overweight or obese.

Age is a factor significantly linked to BMI ($p<0.006$). In addition, 30.70% of pupils under 15 were underweight compared with 8.47% of pupils over 15, while 8.47% of pupils over 15 were underweight and 20.34% were overweight or obese.

Table 1. χ^2 test between BMI and certain socio-demographic variables

Variable	Modality	BMI				Total	χ^2 (p value)
		under weight	normal	overweight	obese		
Sex	Male	21	51	6	3	81	2.69 (p<0.44)
	Female	31	78	19	3	131	
Total		52	129	25	6	212	
School level	2TC	26	52	3	0	81	14.29 (p<0.003) **
	3SC	26	77	22	6	131	
Total		52	129	25	6	212	
Living environment	Rural	7	42	5	3	57	9.11 (p<0.028) *
	Urban	45	87	20	3	155	
Total		52	129	25	6	212	
Childbirth	Normal	31	94	22	5	152	7.5 (p<0.05) *
	Caesarean section	21	35	3	1	60	
Total		52	129	25	6	212	
Age	<15 years	47	87	16	3	153	12.2 (p<0.006) **
	> 15 years	5	42	9	3	59	
Total		52	129	25	6	212	

BMI: body mass index. *: significant difference; **: highly significant difference; 2SC: second year college; 3TC: third year college

Study of dietary diversity in the survey population

Dietary diversity is a qualitative measure of food consumption that reflects the variety of foods to which individuals or households have access. Before calculating dietary diversity score (DDS), the food groups to be included are defined in a way that is compatible with the eating habits and practices of the population under study. The most commonly used classification is that of the Food and Agriculture

Organization (FAO). The DDS is then calculated by a simple count of the food groups that a household or individual has consumed in the period preceding the interview. The number of food groups consumed reflects the degree of diversity in the diet.

Figure 1 gives the comparative results for consumption by the different food groups over the four meals and between the two sampling periods. The graph shows the following information:

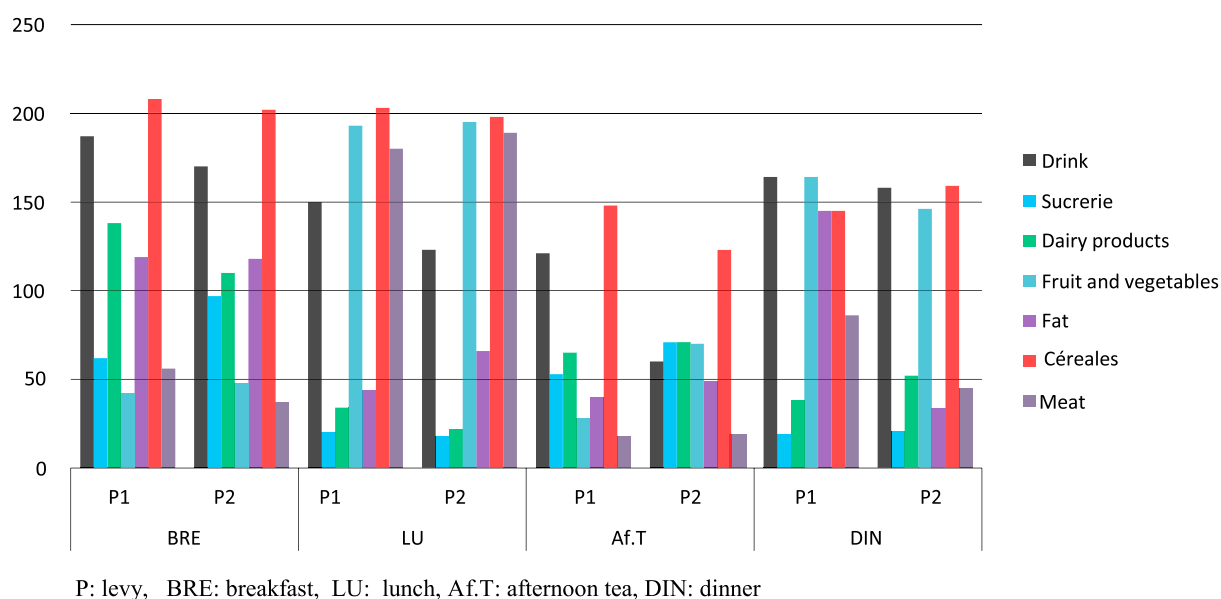


Figure 1. Distribution of participants according to food group consumption frequency

1. The most consumed products in all meals are, in ascending order, cereals (96.7% of respondents), followed by drinks (tea, water, etc.) (87% answered "yes"), then dairy products and fats (64.2% and 55.3% respectively).
2. The same trend was observed during the second survey period, but with a decrease in frequency.
3. The respondents' answers confirm that the food group consumed most frequently is cereals, with a frequency of 68.8%.

The mean dietary diversity score was 5.672 ± 0.956 , with a minimum of 3, a maximum of 8 and a median of 6. In order to identify the categories, given the lack of a threshold defining the different classes of dietary balance, we proposed translating the scores into reduced centered variables (Z score). While adopting the following classification:

Class 1 - Z less than -1: low dietary diversity

Class 2 - Z between -1 and + 1: average dietary diversity

Class 3 - Z greater than + 1: high dietary diversity

The distribution of children surveyed according to DDS class and Z score is shown that 21% have a low DDS, 12% have a high DDS and 67% have an average DDS.

Table 2 presents the results of the χ^2 test of independence between the DDS category and certain socio-demographic variables. The table shows that only school level showed a significant association with DDS ($\chi^2=7.17$; $p<0.028$). Among pupils in the 2nd year of secondary school, 12.19% showed low DDS and 8.54% showed high DDS. In the third year of

secondary school, 12.03% had a low DDS and 22.56% a high DDS.

Figure 2 shows the results of the distribution of respondents according to DDS and BMI. The χ^2 test of independence shows a strong relationship between these two factors (DDS and BMI) ($\chi^2=42.42$; $p<0.000$). Indeed, of the pupils with a normal BMI, 14.04% had a low DDS compared with 12.36% with a high DDS. Furthermore, of the overweight or obese pupils, 2 % of respondent had a low DDS and 40% had a high DDS.

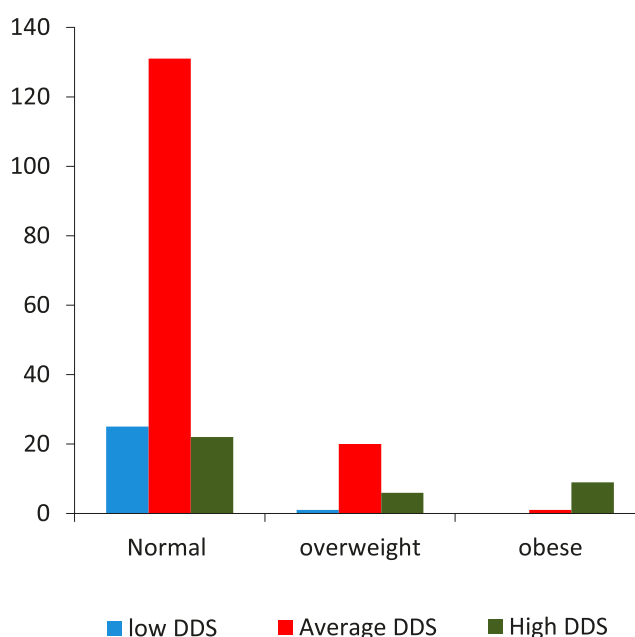


Figure 2. Distribution of respondents according to DDS and BMI

Table 2. χ^2 test of independence between DDS and certain socio-demographic variables

Variable	Modality	DDS			Total	χ^2 (p value)
		low	average	high		
Gender	Male	12	58	12	82	1.22 (p<0.54)
	Female	14	94	25	133	
Total		26	152	37	215	
Level of education	2SC	10	65	7	82	7.17* (p<0.028)
	3TC	16	87	30	133	
Total		26	152	37	215	
Living environment	Rural	8	42	8	58	0.76 (p<0.68)
	Urban	18	110	29	157	
Total		26	152	37	215	
Childbirth	Normal	19	108	27	154	0.76 (p<0.96)
	caesarian	7	44	10	61	
Total		26	152	37	215	
Age	<15 years	19	112	25	156	0.56 (p<0.75)
	>15 years	7	40	12	59	
Total		26	152	37	215	

*: Significant difference, 2SC: second year college, 3TC: third year college ; DDS : dietary diversity score

DISCUSSION

The purpose of our study is to assess the nutritional status of adolescents attending schools in urban and rural areas of the Sidi Allal El Bhraoui Khemisset region in Morocco.

The chosen parameter is body mass index (BMI). However, the average BMI is $21.06 \pm 0.23 \text{ kg/m}^2$, with 60.8% of students having a normal body weight, 24.5% being underweight, 11.8% being overweight, and 2.8% being obese. This result is consistent with surveys conducted in other Moroccan cities. A cross-sectional survey conducted in 2014 on a sample of 2271 students in the eastern region of Morocco found a prevalence of overweight and obesity of 12.2% and 3.0% respectively (14.2% in girls versus 10.4% in boys) [9]. Another study in the commune of Bitit Ait Oualal in the El Hajeb province of Morocco, conducted by *Nour El Yakine* et al. [10], showed that stunting, overweight, and obesity affected 25.9%, 7.7%, and 2.6% of the overall sample, respectively, with no significant difference between girls and boys.

Our mean BMI of $21.06 \pm 0.23 \text{ kg/m}^2$ is comparable to that reported by *Fedala* et al. [11] in Algeria in a school setting (BMI 19.03 ± 3.77). However, in the Taza region of Morocco, the mean BMI among students was higher ($28.26 \pm 3.04 \text{ kg/m}^2$) [12]. Additionally, our results are similar to those reported in a study in Congo, which found a prevalence of overweight and obesity among adolescents of 8% and 1% respectively, with a mean BMI of $18.82 \pm 3.15 \text{ kg/m}^2$ [13]. *Khader* et al. [14] reported that in Jordan, the overall prevalence rates of overweight and obesity among adolescents were 13.7% and 10.0% respectively.

The prevalence of overweight and obesity in our study is lower than in some developed countries such as the United States [15], Canada [16], and Italy [17], where the prevalence of overweight ranges from 22% to 36% and obesity from 4.15% to 9.45%. In France, a survey of 1,507 adolescents aged 11 to 17 years showed that 17.6% of students were overweight (including obesity) [18]. *Prado* et al. [19] found that in Spain, overweight was present in 29% of adolescents aged 13 to 16 years. There have been numerous studies conducted to assess the nutritional status of adolescents, aiming to understand nutritional intake and factors influencing their nutritional status. According to our survey, some selected variables were found to be associated with BMI, except for gender. Contradictory results regarding the distribution of overweight and obesity by gender have been observed in different countries, including Canada [16] and Italy [17], where the prevalence was higher in boys than in girls. In a population of Algerian adolescents aged 10 to 19 years in a school setting, boys were found to have higher rates of overweight and obesity (6.6%

and 2.42%) compared to girls (2.16% and 0.54%) [11]. However, among students with overweight, 74.19% are from urban areas, while 25.80% of overweight or obese students are from rural areas. This shows that urban residence is a risk factor associated with overweight and obesity, which is consistent with several studies in the literature [20]. This can be explained by changes in dietary habits and lifestyle due to urbanization, which contributes to weight gain. The same relationship was found in a cross-sectional survey conducted by *Nouayti* et al. [9] among a sample of 2,271 students in the eastern region of Morocco. Additionally, a higher income of the father (including obesity) was associated with overweight.

In our study we note a significant relationship between the type of delivery and BMI. Indeed, among students born by caesarean section, 21.67% are underweight. Whereas among students born by vaginal delivery, 20.39% are underweight and 17.76% are overweight or obese. Age is also significantly associated with BMI. Furthermore, among students under 15 years old, 30.70% are underweight compared to 8.47% among students over 15 years old. Among students over 15 years old, 8.47% are underweight.

In a study by *El Kabbaoui* et al [10], found that overweight in adolescents is influenced by various social factors, including family income. The prevalence of overweight and obesity increases with family income. In developing countries, excess weight is primarily found in children and adolescents from higher socioeconomic families. Consequently, their children have access to highly energy-dense foods. Thus, motorized transportation to school, using the computer for more than 4 hours a day, and frequent consumption of sodas and carbonated beverages are reported as risk factors that contribute to overweight and obesity.

This study also aimed to evaluate dietary habits in order to determine the Dietary Diversity Score (DDS) and its relationship with certain sociodemographic factors. The calculation of the DDS, as well as the list of food groups used, was established according to FAO recommendations. The most consumed food groups in our sample for the four meals were cereals, beverages, fruits, and vegetables. The average DDS was 5.672 ± 0.956 out of a total of 9. It skewed towards higher values, with a minimum of 3 and a maximum of 8. However, 21% had a low DDS, 12% had a high DDS, and 67% had a moderate DDS. This class needs to be closely monitored as children in this class can easily shift to a low or high DDS class. Educational level showed a significant association with DDS, with higher DDS observed among 3TC students. Another association was found between DDS and BMI. Among students with normal BMI, 14.04% had a low DDS, compared to 12.36% with a high DDS. Furthermore,

among overweight or obese students, the majority had a high DDS.

Our results are consistent with a study conducted on a sample of 463 children aged 9 to 17 years from the city of El Jadida, Morocco. This study found that 2.4% of children had a low DDS, 55.7% had a moderate DDS, and 41.9% had a high DDS. The mean DDS was 5.67 ± 1.03 among children. Additionally, data analyses showed that children of parents with low education had higher mean DDS. Furthermore, children of parents with high economic status had lower mean DDS and Dietary Variety Score compared to those with low economic status. Moreover, high dietary diversity was less suitable among underweight and overweight children compared to those with normal weight [21].

In parallel, a national nutrition survey conducted in 2019 by the Moroccan Ministry of Health among children aged 6 to 12 years revealed that 39.1% of them had a very low dietary diversity, consuming less than 4 food groups per individual. Additionally, 49.9% of children had moderate dietary diversity, while 11% had a highly diverse diet (≥ 6 groups). The statistical analysis showed a significant difference between urban and rural areas, with more diversified consumption among children in urban areas [22]. In Algerian adolescents, the situation is not better. The results of the study conducted by Karoune et al. [23] among 327 adolescents aged 11 to 19 years in Eastern Algeria showed that the Dietary Diversity Score (DDS) had an average of 3.19 ± 1.10 . It ranged from 0 to 7. Adolescents with low DDS accounted for 19.6% of the total, while only 9.2% had a high DDS. Illiteracy was more common among parents of adolescents with low DDS. According to this study, parents' level of education was a more determinant factor of DDS than occupation. It had a negative relationship with dietary diversity among adolescents. Among Iranian adolescents aged 10 to 18 years, Mirmiran et al. [24] recorded a DDS of 6.25 ± 1.08 out of a maximum of 10. This score reflects a diversified diet. Yi Zhou et al. [25] in a cross-sectional survey in central China between 2015 and 2020, found that 78.3% had insufficient dietary diversity, while nearly 1% reported sufficient dietary diversity. Yi Zhou also confirmed that high fat/sugar/salt dietary practices can lead to low dietary diversity, while high dietary diversity can have effects on BMI. This study highlighted the importance of increasing dietary diversity in a balanced and healthy manner.

The results of our study should be interpreted in light of certain limitations. Firstly, it was a cross-sectional study, and the results did not imply a cause-and-effect relationship but rather valid interpretations that could be used for further research. Additionally, the data were self-reported, which may introduce data bias.

CONCLUSION

In our study, we found that the diets of the adolescents surveyed were moderately diversified. However, we also found a worrying prevalence of malnutrition. These indicators enable us to identify population groups at risk of poor nutrition. It is therefore crucial to take into account certain socio-economic, cultural and environmental factors, such as place of residence, type of childbirth, age, level of education and BMI, when devising strategies aimed at improving the dietary diversity and nutritional status of adolescents.

Acknowledgments

The authors would like to thank the provincial Directorate of National Education and Sport of Khemisset, as well as all the educational teams of the schools which participate in our work and to all the children and adolescents for their participation. This work was supported by the Moroccan Ministry of Higher Education and Research of Morocco.

Funding

No funding was received for this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Received: 06.09.2023

Accepted: 18.10.2023

Published online first: 20.11.2023

BIO-DEMOGRAPHIC CHARACTERISTICS OF HOUSEHOLDS AND RISK FACTORS FOR DOWN SYNDROME IN MOROCCO

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ABSTRACT

Background. The most studied risk factors for Down Syndrome (DS) were: region of residence, exposure to chemicals, parents' education level, cigarette and alcohol use by father or mother or both, and oral contraceptive (OC) use.

Objective. The aim of this study was to compare certain variables considered as risk factors on DS such as parental age at birth, OC use, child's sex, and rank of birth between children with DS and their siblings without DS as well as to determine the socio-bio-demographic characteristics of the families studied compared with the general Moroccan population.

Material and Methods. We conducted a cross-sectional analysis of 277 families with 925 siblings and at least one child with DS (279 with DS) between 2014 and 2017. The data are collected using a standardized questionnaire in Marrakech-Safi region. Data were entered and analyzed using the statistical program SPSS statistics software for Windows (version 20.0). Chi-square (χ^2) and Student t tests were used for testing statistical significance. Differences were considered significant when the p-value <0.05.

Results. The binary logistic regression analysis between DS and non-DS children in their bio-demographic characteristics studied (sex, maternal age at birth, paternal age at birth, oral contraceptive (OC) use, length of oral contraceptive use before pregnancy and rank of birth) showed that only maternal age and paternal age at birth and OC use were associated with DS birth (OR= 1.16; 95% CL: 1.11-1.21, OR= 1.05; 95%CL: 1.01-1.09 and OR= 0.01; 95%CL: 0.00-0.003, respectively). In the other hand, the comparison between socio and bio-demographic characteristics of households studied with data from National Population Survey and Family health (2018) showed a higher level of education in women and men in our sample. Similar results were shown in rate of men and women in paid employment, the rate of smoking and alcohol consumption among men and the rate of OC use before pregnancy among women.

Conclusion. These results will help to sensitize the Moroccan population about risk factors for DS.

Key words: Down syndrome, socio-bio-demographic factors, oral contraceptive, maternal age, paternal age, children, Morocco

INTRODUCTION

Down syndrome (DS) was discovered by *John Langdon Down* in 1866 [1], who described individuals with a common phenotype that called by his name.

Subsequently, the development of cytogenetic karyotyping has shown that DS is caused by the presence of extra chromosome 21 [2, 3], because of a meiotic nondisjunction error in the segregation of chromosome 21 during ovogenesis or spermatogenesis.

This extra chromosome 21 most often originates from the mother in approximately 95% of the cases of free trisomy 21 [4]. The most common type is associated with advanced maternal age, especially from approximately 35 years of age and above [5].

In addition to these genetic associations, several epidemiologic studies have shown a significant association between DS birth and environmental risk factors [6, 7, 8]. The most studied risk factors were region of residence, exposure to chemicals, parents'

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

education level, cigarette and alcohol use by father or mother or both, and oral contraceptive use. Within this list of risk factors, oral contraceptive (OC) use was of particular interest, as it is a more common habit than the others. The association of DS birth with this factor has been confirmed in several studies [7, 9] but contradicted in others [10, 11, 12].

The objectives of this study is to compare certain variables considered as risk factors on DS such as parental age at birth, OC use, child's sex, and rank of birth between children with DS and their siblings without DS as well as to determine the socio-bio-demographic characteristics of the families studied compared with the general Moroccan population.

MATERIAL AND METHODS

Subjects

Between May 2014 and November 2017, 277 families with DS child were enrolled in 11 associations and health centers that provided care and support for individuals with DS in; Marrakech, Safi, Chichaoua, El Kelâa of Sraghna, and Al Haouz in the Wilaya of Marrakech. Regarding the centers chosen, we placed a request attached to the questionnaire to allow our interview with parents and explain the objectives of the study to the responsible of all centers and associations providing help to people with DS in Marrakech Safi region. The eleven centers in which the study was conducted came to those who accepted our request. Families with at least one child clinically and/or cytogenetically confirmed DS were included.

For this study, pregnancies resulting in live births without perinatal mortality, including deaths in the first week of life and fetal deaths (stillbirths) [13], were included. Our cases were children with DS (N=279), all the cases in this study were sporadic, except for two couples who had two children with DS. Siblings without DS were used as controls (N=646).

Procedures

Data were collected using a descriptive, retrospective, and analytical cross-sectional survey. The material support of our survey consists of a standardized questionnaire providing information about the identity, socio-economic, bio-demographic, and cultural conditions of parents. The socio-bio-demographic variables used in this study were mean size of households, woman's level of education, man's level of education, mean paternal age at first marriage, mean age difference between spouses, kinship, synthetic fertility index SFI, mean number of live births, contraceptive use before pregnancy, cigarette smoking and alcohol consumption.

The independent variables used in this study were parents' age at birth, length of OC use before pregnancy,

sex of birth and rank of birth. The dependent variable was adjusted for children with DS.

Our study was designed in accordance with the Declaration of Helsinki. It was conducted in full respect of local ethical considerations, namely obtaining prior authorization from the competent authorities of the University and the responsible 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 volunteering for participation as well as the confidentiality and anonymity of the questionnaire were respected. A written parental consent form was received before the study.

Statistical analysis

Statistical analysis was done using the statistical program SPSS software for Windows (version 20.0). A descriptive analysis was performed using means, standard deviations (SD), and proportions as appropriate. To estimate the significance of the differences observed between the means, the student t test for normally distributed data was used. *Chi-square* (χ^2) test was used for categorical variables and differences were considered significant when the p-value was <0.05 . The binary logistic regression analysis which allows the elimination of confounding factors and entering the weight of the associated variables with DS birth in the bivariate analysis ($P<0.2$), was used to identify factors independently associated with DS birth. Associations were measured in odds ratio (OR) with 95% confidence intervals (95% CL).

RESULTS

Comparison of bio-demographic characteristics between DS and non-DS children studied

Table 1 gives the results of bivariate comparison of sex, maternal age at birth, paternal age at birth, oral contraceptive use, length of oral contraceptive use before pregnancy, and rank of the birth between DS and non-DS children studied. Except for the sex of the children, who is not statistically significant between groups (children with DS and their siblings without), all others variables studied, are strongly associated with DS. The mean maternal and paternal age at birth with DS was higher than that of non-DS birth, with a very significant statistical difference (35.95 ± 6.45 years vs. 28.49 ± 6.47 years and 42.20 ± 7.94 years vs. 35.56 ± 7.13 years, $p<0.0001$, respectively). The rate of OC use before DS pregnancy was higher than that of non-DS one (60.9% vs. 45.2%, $p<0.0001$). The mean length of OC use before DS pregnancy was longer than that of non-DS pregnancy (3.39 ± 2.48 vs. 2.15 ± 1.74 years, $p<0.0001$). Regarding the rank of birth, we

Table 1. Distribution of the nature of birth according to oral contraceptive (OC) use and bio-demographic characteristics of the families surveyed

Variables	Modalities	DS birth	Non-DS birth	Test
Child sex	Male	(160 /279) 57.3%	(363/646) 56.2%	$\chi^2=0.10$ ns
	Female	(119/279) 42.7%	(283/646) 43.8%	
OC use before pregnancy	Yes	(167/274) 60.9%	(284/629) 45.2%	$\chi^2=19.05$ ***
	No	(107/274) 39.1%	(345/629) 54.8%	
Length of OC use (years), (Mean \pm SD)		3.39 \pm 2.48	2.15 \pm 1.74	t =5.64***
Maternal age at birth (years) (Mean \pm SD)		35.95 \pm 6.45	28.49 \pm 6.47	t =16.10***
Paternal age at birth (years) (Mean \pm SD)		42.20 \pm 7.94	35.56 \pm 7.13	t =11.79***
Rank of birth (Mean \pm SD)		3.07 \pm 1.88	2.51 \pm 1.74	t =4.38***

SD - standard deviation; χ^2 - *Chi*² test ; t - *Student* test; ns - not significant; ***: P< 0.0001

Table 2: Result of Binary Logistic Regression Model. Analysis between Down and non-Down syndrome variable and bio-demographic variables studied

Variables	Adjusted OR	(CL 95%)	p
Sex	1.04	(0.66-1.66)	0.84
Oral contraceptive use	0.001	(0.00-0.003)	<0.0001
Length of oral contraceptive use before pregnancy (years)	1.11	(0.99-1.25)	0.06
Maternal age at birth	1.16	(1.11-1.22)	<0.0001
Paternal age at birth	1.05	(1.00-1.09)	0.01
Rank of birth	0.86	(0.73-1.00)	0.06

OR = odds ratio; p= significance; CL= confidence limits, underlined= significant at 5%

found that children with DS are born in advanced ranks compared to non-DS children (3.07 \pm 1.88 vs. 2.51 \pm 1.74, p<0.0001).

To eliminate the confounding factors we applied the logistic regression method (Table 2). Through this analysis, maternal age at birth, paternal age at birth and OC use before pregnancy were the only variables that determine independently the recourse to birth with DS (OR= 1.16; 95%CL: 1.11-1.21, OR= 1.04; 95%CL: 1.00-1.09 and OR= 0.00; 95%CL: 0.00-0.003, respectively).

Socio-biodemographic characteristics of households

Table 3 gives the number and the percentage of modalities of qualitative variables and mean \pm standard deviation for quantitative variables of DS households studied and their comparison with data from national Moroccan population surveys [14, 15].

The results showed that the rate of women and men who have a higher level of education is higher than what was recorded at the ENPSF in 2018 [14] at national level and in Marrakech-Safi region (16.3%, 20.9%; 8.8%, 10.1% and 7.0%, 7.9%, respectively). The rate of men and women with paid employment in our sample is higher than the rate recorded nationally and regionally (82.7%, 21.4%; 62.0%, 13.1% and 63.0%, 9.3%, respectively). The rate of smoking among

men in our study is higher than the rate recorded nationally and regionally (50.8%, 21.9% and 22.4%, respectively). With regard to alcohol consumption, the consumption rate among our sample was higher than that recorded among young Moroccans according to the 2011 National Youth Survey [15] (29.0% against 15.0%). The couples who are kinship in our study represent 11.6%, this rate is lower than that recorded at the national and the regional level (23.4% and 20.6%, respectively). The investigation on the use of OC use before pregnancy showed that 60.9% of the women in our study used the pill before child birth with DS, while this rate is around 48.7% nationally and 59.1% regionally. The other variables studied did not show any difference between the two groups.

DISCUSSION

Maternal age at birth in our study influences DS birth. The median maternal age at DS birth was 35.95 years versus 28.49 years of non-DS (Table 1). This result was similar to that found in other studies [5, 16, 17, 18]. Likewise, the paternal age showed a statistically significant difference with DS birth in our study. This finding was agree with what does found by several studies especially when paternal age was adjusting for

Table 3. Number and percentage for modalities of qualitative variables and mean \pm standard deviation for quantitative variables of DS households studied and their comparison with data from National Moroccan Population Surveys (ENPSF, 2018, ENJ, 2011)

Variables	Modalities	Present study (%)	National data (%)	Marrakech-Safi data (%)	Survey
Woman's level of education	None	35.1	39.5	44.2	ENPSF 2018
	Primary	22.1	24.2	26.3	
	Secondary	26.5	27.5	22.4	
	Superior	16.3	8.8	7.0	
Man's level of education	None	22.7	22.7	27.4	
	Primary	22.7	29.8	31.6	
	Secondary	33.7	37.4	33.0	
	Superior	20.9	10.1	7.9	
Paid employment	Man	82.7	62.0	63.0	ENPSF 2018
	Woman	21.4	13.1	9.3	
Smokers	Man	50.8	21.9	22.4	
	Woman	0.0	1.0	1.1	
Alcohol consumption	Man	29.0	15.0	-	ENJ 2011
	Woman	0.0	0.4		
Mean age at first marriage (years)	Man	30.63 \pm 6.28	31.9	30.4	ENPSF 2018
	Woman	24.59 \pm 6.88	25.5	23.8	
Kinship		11.6	23.4	20.6	
Oral contraceptif use before pregnancy		*60.9	48.7	59.1	
Mean size of Moroccan households (persons)		5.42 \pm 2.02	4.5	4.9	
Mean age difference between spouses (years)		6.36 \pm 6.7	7.9	8.1	
Synthetic Fertility Index SFI (years)		1.92	2.38	-	
Mean number of live births		2.96	2.6	2.8	

SD - standard deviation , * - Down syndrome pregnancy

maternal age [19, 20, 21] and contradictory with other studies [22, 23].

The results of our study suggest a role of OC use in DS birth. These results agree with those found by Ghosh et al, who confirmed the effect of OC use in DS birth, based on observation of an increasing frequency of OC use with advancing maternal age [7]. However, our results concerning the length of OC use before pregnancy were contradictory to those reported by Nagy et al. [10]. That study suggested that for women giving birth at advanced maternal age, the history of longer OC usage before pregnancy might lower the risk of common trisomy of the fetus. Even longer oral contraception use before pregnancy in women of advanced reproductive age can reduce the risk of common fetal trisomy [12].

The rank of birth did not show significant differences for DS in our sample even though it was found that children with DS are born in the third rank on average (3.07 \pm 1.88). The study of Jaouad et al showed that 40% of DS patients were born after at least four healthy births [24].

Regarding parents sociodemographic level evaluated by the level of education and paid employment we noticed a clear difference concerning the higher level of education and high rate of people who exercise paid employment in favor of our sample. This difference may be explained by the region of residence location of the majority of the population studied in urban area. This result was contradicts with what has been shown by Hunter et al [25] that a low socio-economic level of parents increases the risk of having a child with DS.

Parental habits such as smoking cigarettes and consuming alcohol are environmental risk factors among other widely studied whose impact on DS birth was confirmed by several studies [8, 26]. Concerning our study, there was a marked increase in the consumption of cigarettes and alcohol among men compared to what is recorded at the Moroccan national level [14,15].

The rate of kinship in families studied (11.6%) was lower than that recorded at the national and the regional level (23.4% and 20.6%, respectively). This result is in agreement with several studies which have

not shown an association with DS, especially when consanguinity is evaluated alone as a risk factor [26, 27, 28], but confirmed in other studies either alone [29] or in combination with other bio-socio-demographic risk factors [6, 8].

Limitations

Limitations of our study are related to the population of studied families, without taking into account families with non-malformed children and to the small sample size. This is justified by the constraints of time and limited resources that have not allowed us to expand our research.

CONCLUSION

Our results showed that parents included in this study had a higher educational level, were more involved in paid employment and had more smokers and alcohol consumption in men. Among the risk factors studied only the maternal age at birth, the paternal age at birth and the use of oral contraception before pregnancy that have shown an association with the birth with Down syndrome.

Disclosure of interest

The authors declare that there is no conflict of interests.

Acknowledgment

We gratefully acknowledge all the parents whose participation has made this study possible. In addition, we want to thank all personnel at each visited association and health center that provide care and support for individuals with Down syndrome in Marrakech-Safi region, for helping and supporting us to complete this study. We would like to thank Mr. Harich Nourdin for reviewing this article

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Received: 23.08.2023

Accepted: 07.11.2023

Published online first: 24.11.2023

WORLD HEALTH ORGANIZATION MPOWER FOR TOBACCO CONTROL IN INDIA: A 6-YEAR RETROSPECTIVE ANALYSIS

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ABSTRACT

Background. Prevalence and practices of tobacco usage in India are diverse and incongruent and Government of India has enacted various laws to overcome this burden. To make tobacco control measures effective and powerful, WHO introduced MPOWER in 2004 and India was one of the first countries that implemented the MPOWER.

Objective. This study is aimed to quantify the implementation of MPOWER tobacco control policies in India.

Material and Methods. In this retrospective analysis, data was gathered from the WHO MPOWER of India from 2015 to 2021. This analysis was based on the checklist which was designed previously by Iranian and international tobacco control specialists in their study on tobacco control.

Results. In the present comparative analysis, India was categorized by scores and these were acquired from each indicator for each activity and 2021 year got the highest scores as compared to the previous year scores i.e. 27 in 2015. In context to individual indicators, noticeable increase in scores has been seen in both health warning on cigarette packages and adult daily smoking prevalence, whereas no progress was observed in smoking related policies.

Conclusion. Although MPOWER programmes are widely accepted by the Indian government, but still substantial improvement in fewer sections is required.

Key words: MPOWER policies, tobacco, WHO, smoking, policies

INTRODUCTION

Tobacco sector employment is an important public health concern which is a common cause of addiction, preventable illness and various categories of fatal and disabling diseases including cancerous conditions, cardiovascular and chronic respiratory diseases [2, 3]. Tobacco practice imposes a huge and mounting public health burden in India with 275 million adults consuming different tobacco products and it is growing at a rate of 2-3% per annum [6]. In India, tobacco promptly recognized itself by Portuguese in the 17th century in Goa and the country steadily became the second leading purchaser of tobacco worldwide [6]. Tobacco is consumed in many different forms such as smoking, chewing, applying, sucking, gargling, etc and a few of these products are industrially manufactured either on a huge or diminutive scale. Few are prepared by a trader and some may be primed

by the user depending upon the need [2]. Although, consumption of tobacco is socially disapproved due to diverse ill-effects but still its cultivation is unrelenting because of domestic and international demand.

Tobacco usage in any form is treacherous and is the solitary preventable cause of death. The Government of India enacted diverse legislations and comprehensive tobacco control measures during mid-1970s to cut down consumption of tobacco. In 1975, Cigarettes Act (Regulation of Production, Supply and Distribution), which was India's first national level anti-tobacco legislation was passed followed by other regulations. India was also among the first few countries to endorse World Health Organization, Framework Convention on Tobacco Control (WHO FCTC) in 2004 [8].

Basically WHO FCTC is providing and supporting the foundation for countries to execute and deal with tobacco control and to help formulate this into pragmatism by introducing the MPOWER in New

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

York City on February 7, 2008. MPOWER with its six evidence-based components: **M**onitor tobacco use and prevention policies, **P**rotect people from tobacco smoke, **O**ffer help to quit tobacco use, **W**arn about the dangers of tobacco, **E**nforce bans on tobacco advertising, promotion and sponsorship, **R**aise taxes on tobacco is projected to support the various countries for execution of effectual interventions to diminish the stipulate for tobacco consumption contained in the WHO FCTC. MPOWER is the only document of a somewhat tactical nature that is a resource of information on the extent of tobacco epidemic, as well as for suggestions pertaining to precise actions for supporting the wrestle against this epidemic [4, 8].

These tobacco control programmes have been followed by different countries with diverse levels of triumph and in order to find out about their constructive nature, timely assessment is required over the time. The foremost such assessment was conducted with the help of MPOWER in 2006 in European countries by Joossens et al [5]. In India, a study was conducted by Malhi et al in 2015 (assessment from 2009 to 2013) [4]. After 2015, no such study has been reported till date to enumerate the upgrading in tobacco control measures [8]. Therefore, a contemporary analysis was conducted with the help of six measures of MPOWER and WHO reports for examining the accomplishment of tobacco control programs from 2015 to 2021 in India.

MATERIAL AND METHODS

Information and scoring criteria

In this paper, secondary investigation information was composed from the WHO report which is prepared once in two years on the Global Tobacco Epidemic Program, India for the year 2015, 2017, 2019 and 2021 and MPOWER was used to appraise the progress/accomplishment in Tobacco Control Initiative. This investigation was based on the checklist which was premeditated formerly by Iranian and International tobacco control specialists in their study on tobacco control and its cut-offs were set according to the scoring of key sections of the 2011 MPOWER report [4]. There were seven questions with five options ranging from least 0 to utmost 4 scores, and three questions ranging from least 0 to utmost 3 scores as per measures reported in the report and 0 score was given to point for which data was not available (NA). So, the overall score was 37 ($7 \times 4 + 3 \times 3$) as shown in Table 1.

Training and data collection

A day of training session was carried out for standardization and calibration of the readings. The internal reliability for the examiners was assured using Cronbach's alpha coefficient (0.85). After recording,

Table 1. WHO MPOWER score on tobacco control based on WHO report [4]

Indicator	Point scoring
Adult daily smoking prevalence	(4)
Estimates not available	0
30% or more	1
20%–29%	2
15%–19%	3
< 15%	4
Monitoring: prevalence data	(3)
No known data or no recent data or data that is neither recent nor representative	0
Recent and representative data for either adults or youth	1
Recent and representative data for both adults and youth	2
Recent and representative data for either adults or youth	3
Smoke-free policies	(4)
Data not reported	0
Up to 2 public places compl. smoke-free	1
3–5 public places completely smoke-free	2
6–7 public places completely smoke-free	3
All public places completely smoke-free	4
Cessation programmes	(4)
Data not reported	0
None	1
NRT and/or some cessation services (neither cost-covered)	2
NRT and/or some cessation services (at least 1 cost-covered)	3
National quit line, and both NRT and some cessation services cost-covered	4
Health warning on cigarette packages	(4)
Data not reported	0
No warnings or small warnings	1
Medium-sized warnings missing some appropriate characteristics	2
Medium-sized warnings with all appropriate characteristics	3
Large warnings with all appropriate characteristics	4
Anti-tobacco mass media campaigns	(4)
Data not reported	0
No campaign conducted between January 2009 and August 2010	1
Campaign conducted with 1–4 appropriate characteristics	2
Campaign conducted with 5–6 appropriate characteristics	3
Campaign conducted with all appropriate characteristics	4

cont. Table 1.

Indicator	Point scoring
Advertising ban	(4)
Data not reported	0
Complete absence of ban in print media	1
Ban on national television, radio and print media only	2
Ban on national and some international television, radio and print media	3
Ban on all forms of direct and indirect advertising	4
Taxation	(4)
Data not reported	0
25% of retail price is tax	1
26%–50% of retail price is tax	2
51%–75% of retail price is tax	3
75% of retail price is tax	4
Compliance with bans on advertising	(3)
Complete compliance (8/10 to 10/10)	3
Moderate compliance (3/10 to 7/10)	2
Minimal compliance (0/10 to 2/10)	1
Not reported	0
Compliance with smoke-free policy	(3)
Complete compliance (8/10 to 10/10)	3
Moderate compliance (3/10 to 7/10)	2
Minimal compliance (0/10 to 2/10)	1
Not reported	0

Table 2. Ranking of India according to WHO score on tobacco control [10,11,12,13]

Indicator	Points			
	2015	2017	2019	2021
Adult daily smoking prevalence	4	4	4	4
Monitoring: prevalence data	2	0	1	3
Smoke-free policies	3	3	3	3
Cessation programmes	3	4	4	4
Health warning on cigarette packages	1	4	4	4
Anti-tobacco mass media campaigns	4	3	3	3
Advertising bans	3	3	3	3
Taxation	3	2	3	3
Compliance with bans on advertising	2	3	2	3
Compliance with smoke-free policy	2	2	2	2
Affordability	----	YES	YES	YES
Total number	27	28	29	32

all the scores were recorded by one person who acted as an investigator, and established by two proficient persons who acted as chief supervisors. Entry of data was done autonomously by the investigator itself followed by inspection which was done by the supervisors with the checklist.

Data analysis

The scores were summed and the rankings were computed. The checklist with its scoring and scale is shown in Table 1. It was found that proper explanation of the items was essential for understanding of the concept which prevents misinterpretation. The chief supervisors made sure that proper explanation of each item was done.

RESULTS

The current secondary analysis was done with the help of WHO report (2015, 2017, 2019 and 2021) on tobacco control using MPOWER and we found the changes in the scores over six years (2015-2021) after comparing the scores for the each year. In the present study, India was ranked by scores and these scores were obtained from every indicator for each activity. Table 2 shows the changes in the scores and after analysing the six main MPOWER measures, year 2021 getting the highest scores in India [10, 11, 12, 13].

It has been observed through this secondary analysis that data remained unbothered i.e. 04 for all the respective six years for adult daily smoking prevalence whereas for monitoring the prevalence data initially in 2015, score was 02 later on declined in 2017. In 2019 was 01 but again raised to 03 in 2021. As far as appraisal is concerned for smoking related policies and advertisements, we can see that scores remained same throughout all the six years i.e. 03 (2015-2021). In the case of health warnings over cigarette packaging, the score was 01 (least) in 2015 but rapidly increased to 04 in 2017 and remained same throughout the remaining years (2019 and 2021). Compliance with bans on advertisement / taxation policies remained unchanged throughout all the years with a score of 02 (2015-2021). In context to taxation, scores were 03 initially in the year 2015, then suddenly declined to 02 but increased to 03 again for remaining years whereas for anti-tobacco mass media campaigns, scores declined from 04 in year 2015 to 03 for the rest of the years (2017-2021).

DISCUSSION

Tobacco imposes a gigantic encumber of ailments leading to calamitous health. Prevalence and practices of tobacco usage in India are diverse and incongruent. Various anti-tobacco programmes are

conceded out among different countries in order to curtail the tobacco practices. The past decade has seen a considerable swing in policies regarding tobacco which further lead to a momentous diminution of tobacco usage in numerous countries [1, 8]. The present secondary analysis was done for the six years (2015-2021) year with the help of WHO report (2015, 2017, 2019 and 2021) on tobacco control using MPOWER. The changes in the scores have been noted for these six years after comparing the scores for each year and year 2021 got the highest scores in India.

In this study, adult daily smoking prevalence scores remain constant i.e. 04 for all the respective six years which meant smoking prevalence was less than 15%. This indicates that India is rolling towards tobacco control as in the previous studies it was reported that the prevalence of tobacco use among adults (15 years and above) was 35%. The prevalence of both smoking and chewing tobacco varied considerably among different states in India. In context to monitoring the prevalence data, initially in 2015, score was 02 which later on declined in 2019 (01) but again got escalated to 03 in 2021 as recent, representative and periodic data was made available in the 2021. However, this data was not available previously in 2017 as score was zero for this year which meant that no known data was available in the Tobacco Control survey globally. This might be due to the incomplete monitoring of tobacco use within the populations. It can be stated that monitoring should be done more in affordable way with thoughtful integration of health systems.

Although WHO approximated the prevalence of tobacco consumption of all forms on the basis of studies conducted at smaller level but focus was more on smoking form due to limited availability of literature with regard to other forms of consumption.

As far as assessment is concerned for smoking related policies and advertisements, we can see that scores remained same throughout all the six years i.e. 03 (2015-2021). This depicts that 6-7 public places are mostly smoke free in various regions of India and ban on advertising at national level is more focused and fruitful. Even to some extent, ban on international advertisements were also contributing towards smoking prohibition. It has also been stated through WHO Global report on tobacco control that in India that cessation services have also been started to control this tobacco usage and 2.1 million users have registered themselves. Some authors also reported in their study that there is apparent evolution in tobacco control in India due to enforcement and incessant monitoring but assessment of these policies is still a challenging task [14]. Fewer studies have reported that Indian courts have issued quite a few instructions regarding ban on advertisements, endorsement and sponsorships by the tobacco manufacturers in many states. The best

examples in this case are removal of advertisements of tobacco displayed on the public transport vehicles in Gujarat State and The Honourable Karnataka High Court engaged the Indian Government to extract the funding support by the tobacco industry or board [8].

The Cigarettes Act, 1975 was regulated by the Government of India which made mandatory to exhibit a constitutional health warning on all packages with the rationale of informing the citizens about the adverse effects of smoking so that the demand for cigarettes would be reduced [4, 8]. Even through a memorandum was issued by the Cabinet Secretariat in 1990, it has been said that there is prohibition over tobacco smoking in all of the health care sectors, like educational institutions, domestic flights, air-conditioned coaches in trains, suburban trains and air-conditioned buses, etc. Moreover after an extensive legal battle and interventions by the civil society, revised smoke-free rules came into effect from 2nd October, 2008 where ban over smoking in work places also got included. This can be witnessed with remarkable change in scores from 01 to 04 points from year 2015 to 2021 as no data was reported in 2015 but rapidly scores got increased which depicts the large coverage of health warnings over the packages. It has been said that pictorial warnings are easiest and successful way of illustrating the detrimental effects of tobacco usage. Some authors are in agreement with this as they reported affirmative response in their study on general population for execution of health warnings on tobacco products thus motivating the local population to change their behaviour and discouraging them for not adopting the smoking habits (Figure 1) [9]. Although taxation was less i.e. just 50% in 2017 but it got enhanced to 75% in the year 2021. In 2013, it was also reported that smoke free legalisation and tobacco taxation is one of the effective strategies for tobacco control at larger scale in India [7]. Even the cigarettes got less affordable since 2017 onwards which remained consistent throughout the remaining years which are one of the better approaches towards tobacco control. This might be due to the reason of increased taxation over the tobacco related products that lead to higher cigarette prices even after adjusting for purchasing power parity. Affordability is a strong measurement for all countries especially like India and similar other countries; increasing the excise taxes can make the cigarettes less affordable. Even fewer methods to curb tobacco usage have proven to be efficient such as taxation and media awareness of health risks associated with tobacco. It has been observed that worldwide effective tobacco control can be achieved through various approaches with much more emphasis on reduction on utilization of tobacco, raising taxes, bans on advertising and promotion. These affirmative results in tobacco control can further



Figure 1. Various health benefits associated with quitting tobacco (WHO) [9]

facilitate and encourage the governmental agencies to fortify the tobacco control efforts at more of superior level. Henceforth newer innovative methods in tobacco control programmes should be implemented by assembling various fiscal and human resources.

CONCLUSION

In the last 15 years since WHO's MPOWER tobacco control measures were introduced globally, smoking rates have fallen. Smoke-free public spaces is just one policy in the set of effective tobacco control measures, MPOWER, to help countries implement the WHO Framework Convention on Tobacco Control and curb the tobacco epidemic. Eight countries (Ethiopia, Iran, Ireland, Jordan, Madagascar, Mexico, New Zealand, and Spain) are just one MPOWER policy away from joining the leaders in tobacco control. There is still much work to be done as still 44 countries remain unprotected by any of WHO's MPOWER measures. Although Indian Government have endorsed various laws, regulations such as taxation/health warnings

but it could only be improved by focusing more over the policies or training of health care workers/ school teachers etc. By implementing more of evaluation or monitoring of these policies, attainment of a smoke-free society can be done so that we can shield health of the upcoming generations.

Conflict of interest

The Authors declare no conflict of interest.

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Received: 11.10.2023

Accepted: 15.11.2023

Published online first: 22.11.2023

FACTORS RELATED TO ALCOHOL DRINKING BEHAVIORS ON SECONDARY SCHOOL STUDENTS GRADE 1-3 IN THE PROVINCE OF NAKHON SI THAMMARAT THAILAND

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ABSTRACT

Background. Globally, alcohol consumption is the major cause of mortality and disease. Future health and life quality may also be affected by early initiation of alcohol consumption. It is a cross-sectional design for a research survey.

Objective. This study aimed to investigate alcohol drinking behavior, the effects of alcohol consumption, and factors linked to alcohol drinking behavior among 410 secondary school students in grades 1-3 in the province of Nakhon Si Thammarat in southern Thailand.

Material and Methods. A simple sample was selected from a stratified random sample of 210 individuals. The data collection instrument was a questionnaire comprised of five sections and 78 items in total. The index of item objective congruence (IOC) of 0.90 and the reliability of 0.74 was used to evaluate the tool's quality. To analyze the data, descriptive statistics and *chi-square* statistics were employed.

Results. The results of the study showed that a total of 40.0% of the participants had previously consumed alcohol, that men were more likely than women to drink (54.8%), that beer was the most popular alcoholic beverage (57.7%), that most people drank alcohol at night while at home (47.6%) and with friends (83.6%), the majority of participants (13.9%) drank alcohol one to three times per week, and their attitudes toward alcohol were moderate (85.7%). Religion, knowledge about alcohol use, and the amount of money received each month from habitat for humanity were not found to be linked to drinking alcohol.

Conclusion. The conclusion is that the use of alcohol by students is influenced by a wide variety of circumstances. Health professionals, educational institutions, and relevant agencies should use these factors to promote alcohol use prevention behaviors and formulate guidelines and measures to prevent this by designing an effective prevention program to reduce the alcohol use behaviors of children and young people in the appropriate manner.

Key words: *alcohol drinking behaviors, secondary school students, Nakhon Si Thammarat, Thailand*

INTRODUCTION

According to the World Health Organization's (WHO) Global Action Plan for the Prevention of Non-Communicable Diseases (NCDs), alcohol consumption is one of four major risk factors for non-communicable diseases (NCDs). Alcohol consumption should be protected in all countries around the world. By 2020, it is intended that the dangers associated with drinking will be reduced by 10% [1]. Furthermore, according to the Sustainable Development Goals (SDGs) for 2030 that was declared by the United Nations (UN), alcohol is a major impediment, as it is involved in 13

of the 17 primary goals [2]. The situation of alcohol use is always evolving, particularly among young people, who have a tendency to drink more and begin drinking at an earlier age. This is especially true in the United States. They are referred to as "new drinkers" due to the fact that people who begin drinking at a younger age have been discovered to have certain characteristics. This not only raises the risk of health repercussions and psychological illnesses connected with alcohol use, such as dangerous drinking behavior [2, 3], but it also increases the possibility that the individual would abuse other substances. [4, 5], has an effect on one's capacity for learning, and has an impact

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

on both social difficulties and dangerous behaviors [6, 7]. As a result, there should be risk surveillance of alcohol consumption behavior in a variety of settings as a guide for the development of policies to regulate and avoid issues brought on by alcohol consumption.

Drinking alcohol is a problem for the person, the family, society, the economy, and the country as a whole. Because it is connected with five times as many causes of mortality and disability as narcotics, it requires a budget in order to prevent and treat the different issues that it creates. Accidents involving drunk drivers, homicides, and other violent crimes account for thirty percent of all fatalities, whereas one in every three deaths is the result of a mental health condition. According to the World Health Organization (WHO) alcohol consumption statistics in 2018, alcohol is the cause of approximately 1 in 20 deaths worldwide every year [8]. Additionally, according to the WHO-Alcohol Consumption Database, it is found that Thais are the 5th most likely to consume alcohol in the world and the first in Asia [9], with a percentage increase in new drinkers of 4.96 per year, and found that they were regular drinkers among all 42.20 percent of the total population. [10]. Worryingly, there are approximately 1.06 million male adolescents between the ages of 11 and 19 who drink alcohol, which accounts for 21.2% of the population in this age group. Additionally, during the past seven years (1996-2003), there has been a group of female teenagers between the ages of 15 and 19 who have engaged in alcohol consumption. The survey indicated that students who drank alcohol in high school and at the vocational certificate level started drinking at a younger age, with almost half of those students starting drinking at a younger age. This trend was seen in both male and female students 15 years, and children who begin consuming alcohol before the age of 13 years have a greater risk of developing an addiction to alcohol until they reach adulthood [11].

According to the information shown above, the problem of underage drinking among young people has been shown to diminish at younger ages, which implies that in today's culture, children and young people have a changing mindset and behavior in regard to consuming alcohol [12] which Thailand has given significance to managing the use of alcoholic beverages among children and young people with the Alcohol Control Act B.E. [12] which Thailand has given importance to controlling the use of alcoholic beverages among children and young people. Under the National Education Act, Article 27 forbids the selling of alcoholic drinks at educational institutions, and Article 29 prohibits the sale of alcoholic beverages to anyone under the age of 20 [13].

In 2017, 32.3% of the population aged 15 and over consumed alcoholic drinks, according to the

National Statistical Office. In 2017, 55.9 million 15- to 24-year-olds were identified as alcohol drinkers: around 15.9 million individuals (or 28.4%) are drunk, 6.98 million people (or 12.5%) always drink, and 8.91 million people (or 15.0%) seldom drink. 16.7 years of age is the average age at which people begin drinking. Young people consume alcohol for the following reasons: 1) socializing or partying (41.9%); 2) following friends or friends who invite them to drink (27.3%); and 3) desiring to try drinking (24.4%) [14]. According to the report cited above, adolescents are being encouraged to use more alcohol, indicating that prior alcohol control measures have not been effective in reducing the number of new drinkers as they should have if the government and society do not take appropriate action. When the future of Thai children is filled with alcohol drinkers who cause problems after drinking alcohol, such as road traffic accidents, which are severe accidents that cause many injuries and deaths, particularly during the holiday season with many consecutive holidays like New Year's Eve, Songkran festival, etc., where alcohol is the leading cause of death from traffic accidents 90% of the year, killing 26,000 people, the majority of whom are young adults [10].

The province with the biggest population and second-largest land area is Nakhon Si Thammarat. The assessment of the quality of life of the people of Nakhon Si Thammarat in 2017 [15] indicated that more than fifty percent of the working-age population in the southern region had the highest alcohol consumption rate in Thailand. This is consistent with the 2011 Nakhon Si Thammarat Province Alcohol Consumption Situation Report. The prevalence of adult drinkers is highest in the 20–59 age range, the highest of any population in the province, as evidenced by festival accident statistics. New Year's and Songkran Festival 2018-2019 in Nakhon Si Thammarat province has the highest number of drunk driving accidents and injuries in the country [16].

Therefore, the researcher wished to investigate the factors associated with alcohol drinking behavior and the impacts of alcohol use among secondary school students in grades 1-3 in the province of Nakhon Si Thammarat, categorizing the factors as follows: 1) predisposing factors were gender, age, religion, level of education, monthly income, knowledge, and attitude toward alcohol; 2) reinforcing factors included residential characteristics, family relationships, and access to alcoholic beverages; and 3) enabling factors included support from family, friends, educational institutions, and local government organizations. This research can be utilized as a foundation for monitoring and increasing knowledge, preventing factors that attract students to drink alcohol, and collectively resolving major problems from all parties

concerned, including family institutions, educational institutions, etc.

Objectives

To investigate the drinking behavior and effects of alcohol among secondary school students grade 1-3 in Nakhon Si Thammarat Province, as well as the relationship between drinking behavior and related factors.

MATERIAL AND METHODS

This study is a cross-sectional survey. The population consists of students in grades 1-3 during semester 2 of the Academic Year 2018 in Nakhon Si Thammarat Province schools. A sample of 210 participants was calculated from a population of 410. The population sample size was determined using stratified random sampling and *Yamane's* algorithm [17] with a tolerance of 0.05. All questionnaire responses were collected using sampling and simple random sampling. The period of data collection was one month following the validation of the questionnaires. Encoding and analysis of data using descriptive statistics such as mean, frequency, percentage, and analytical statistics such as *Chi-squares*.

Research instruments

This study's data-gathering instrument was a questionnaire comprised of five sections. The 78 items are as follows:

Part 1 is a general information questionnaire. It is a questionnaire with 12 questions regarding personal aspects, such as gender, age, religion, level of education, grades, family status, housing, and monthly income.

Part 2 is a 20-question survey about predisposing factors, which is divided as follows:

1. Inquire about your knowledge of alcohol consumption. There is a rating scale (estimation measure) with three options: yes = 1 score, no = 0 score, and unsure = 0 score. Regarding the negative questions, the scores were inverted, with a mean score of 0.00–59.99 indicating a low level of knowledge, 60.00–79.99 indicating a moderate level of knowledge, and 80.00–100.00 indicating a high level of knowledge.
2. It used rating scales to evaluate people's views regarding alcohol drinking, with an average score of 1.00–2.33 indicating a negative attitude, 2.34–3.66 indicating a moderate attitude, and 3.67–5.00 indicating a good attitude.

Part 3. The section consisted of a questionnaire of reinforcing factors containing questions regarding home characteristics, family relationships, and access to alcohol trading platforms. The average score for

the 12 rating scale questions was between 1.00 and 2.33. This suggests that living conditions, familial relationships, and alcohol distribution are insufficient for the availability of alcoholic beverages. A mean score between 2.34 and 3.66 indicated a moderate level of housing characteristics, family relationships, and access to sites for trading alcoholic beverages, while a mean score between 3.67 and 5.00 indicated a high level of housing characteristics, family relationships, and access to sites for trading alcoholic beverages.

Part 4: The section is a questionnaire regarding enabling factors, which includes inquiries about family, friends, school, and local government assistance. There are a total of 14 questions and a grading scale with three possible responses. Frequent reception (once per week) equals 2. Points are occasionally awarded (at least once each month); 1 point is not awarded for every rejection question. A typical score between 1.00–1.66 indicates inadequate social support. The average score ranges between 1.67–2.33, representing moderate social support. A mean score between 2.34–3.00 suggests some level of social support.

Part 5: This section is a questionnaire about the behavior and effects of alcohol consumption, including how much you drank when you initially started drinking, why you drink, the sort of alcoholic beverage you consume, the frequency and duration of your drinking, and drinking parties. How much to drink, where to drink, etc. There are twenty questions in total, which form a checklist.

To evaluate the study instrument, the researcher did the following: Following a validity check by three experts, the questionnaire was tried on a sample representative of the actual sample, and the internal consistency reliability of the questionnaire (rating scale) in the index of item objective congruence (IOC) of 0.90 and the reliability of 0.74 was used to evaluate the tool's quality.

Protecting research participants' rights

In this study, the researcher reported the research project to the Human Research Ethics Committee at Walailak University, which issued certification number WUEC-18-071-01 for the project. In this study, the researcher has paperwork stating to the sample of participants answering the questionnaire that they are protecting the researcher's rights and gaining the sample's consent to engage in the study.

RESULTS

Part 1. General aspects

The general information research of the respondents revealed that the majority were female (55.2%), 44.8% were male students, 14 years old (34.8%), 15 years old (32.9%), and 13 years old (28.6%), and that 97.1% were

Buddhists. 49.0% with a cumulative GPA between 2.00 and 3.00, followed by 28.6% with a cumulative GPA of 3.00 or above and 22.4% with a cumulative GPA below 2.00. The majority of them received money from their parents, with 72.4% receiving an average of 2,001–3,000 baht per month or higher; 70.0% lived with their parents, while 21.4% stayed with relatives. 56.9% were divorced or legally separated, compared to 18.7% and 16.6%, respectively. Monthly family earnings were from 5,001 to 10,000 baht (43.8%), 10,001 to 20,000 baht (35.2%), and 20,000 baht or more (16.2%), respectively.

Part 2. General characteristics of predisposing, reinforcing, and enabling factors

The predisposing factors demonstrated that the majority of students had moderate and low knowledge of alcohol (48.1% and 33.8%, respectively). 74.8% of the population had moderate attitudes about alcohol drinking, while 23.8% had favorable sentiments. The attitude against alcohol use in the questionnaire was at its greatest level of positivity, namely that consuming alcohol is not a solution to one hundred life issues (59.0%), and that alcohol increased the danger of accidents and other diseases among students (41.9%). The questionnaire generated the most negative attitudes toward alcohol consumption. Alcohol consumption was a common practice among teenagers (23.8%), and alcohol consumption can alleviate stress (20.5%).

The reinforcing factors were shown that 45.2% of the dwelling features of college students were favorable to easy access to alcoholic drinks. Sex, age, level of education, attitude toward alcohol, family relationship, availability to alcohol trade, peer support, families, educational institutions, and local administrative organizations were statistically significant characteristics related to alcohol consumption. And 51.8 and 48.1% of them were at a good level and 60.5% of them found that their family relationships were also at a moderate and good level, respectively which to access have access to a source of alcohol trading with the majority indicating that family members understood one another and provided support when problems arose with students.

The enabling factors found that family support was at a medium level, for example, parents had warned and taught about alcohol drinking 78.6%. For friend support: 31.6% of low levels of alcohol use and peers often helped students avoid alcohol. As for the support from educational institutions, it was found that the students perceived that the educational institutions had clearly announced a policy prohibiting alcohol consumption at 69.5%. Campaign to reduce the consumption of alcoholic beverages in the community from local administrative organizations 64.8%

Part 3. Alcohol drinking behaviors of secondary school students

For the drinking behaviors of 84 students, it was found that a percentage of them used to drink. 40.0% of the sample group started drinking at the ages of 11–13 years (26.2%), 14–16 years (32.6%), and under 10 years (17.9%) of the year. Most of the reasons that they drank for the first time were wanting to try it (52.4%), followed by friends asking for a drink (28.6%), and stress relief (17.1%). The type of alcohol that was drunk the most was beer (45.2%) and wine (36.9%), and the frequency of drinking was 1–11 times a year, 58.2%. followed by 1–3 once/month, or 26.2%, most of the time they drank late at night (21.00 p.m.–04.59 a.m.), (47.6%), 42.9% in the afternoon (16.00 p.m.–21.00 p.m.). 55.2% of alcohol purchases were made at convenience stores, 43.2% at grocery stores, 45.2% at parties, 28.6% on New Year's Eve, and 17.9% on birthdays. 70.2% of friends, 17.9% of relatives, 47.6% of drinking places where their own homes, 34.5% of friends' houses, and 8.3% of restaurants or entertainment venues, respectively. The cost of each drink was 69.0% less than 200 baht and 12.2% from 200–500 baht. And while drinking, other drugs were also involved, including smoking (10.7%) and cough syrup (2.4%).

Part 4. Effects of alcohol consumption among junior high school students

The results revealed that the majority of samples were influenced by alcohol use as follows: blurring, forgetfulness, absenteeism (21.4%), worse grades (3.6%), accidents (4.2%), and sex (2.4%). Conflicts with family and friends as a result of alcohol consumption. Alcohol-related health issues such as headaches, palpitations, and exhaustion account for 21.4%, 4.2%, and 2.4%, respectively.

Part 5. The relationship between predisposing factors, reinforcing factors, and enabling factors for the alcohol-drinking behavior of junior high school students

Predisposing factors: sex, age, level of education, and attitude toward alcohol were associated with alcohol-drinking behavior among secondary school students ($p < 0.001$), according to the research of the primary variables connected to alcohol use among that age group. According to Table 1, religious characteristics, monthly student income, and alcohol knowledge had no statistically significant relationship with alcohol drinking behavior.

Reinforcing factors: family relationship variables and availability of alcoholic beverage sources were shown to be associated with drinking behavior among secondary school students, according to the findings of housing characteristics into the determinants of alcohol

Table 1. The relationship between predisposing, enabling, and reinforcing factors and the drinking behavior of junior high students (n=210)

Factors	Alcohol consumption behavior		χ^2	p-value
	Drinking (n=84)	No drinking (n=126)		
Predisposing factors				
Gender				
Male	46	48	5.662	0.017*
Female	38	78		
Age				
12-13 years	15	51	17.341a	0.001*
14-15 years	69	75		
Education				
Secondary school grade 1	18	48	6.715	0.035*
Secondary school grade 2	33	36		
Secondary school grade 3	33	42		
Income (Baht per month)				
1,000-2,000 baht	16	31	2.986a	0.372
2,001-3,000 baht or more	68	94		
Knowledge of alcoholic beverages				
Excellent	13	25	1.180	0.554
Moderate	44	57		
Low	27	44		
Attitudes toward alcoholic beverages				
Excellent	11	39	9.412	0.004*
Moderate	73	87		
Enabling factors				
Housing				
Excellent	15	32	3.317	0.177
Moderate	36	59		
Low	33	35		
Family relationship				
Excellent	39	88	12.502	0.001*
Moderate	45	38		
Access to consumption of alcoholic beverages				
Excellent	25	76	19.350a	0.001*
Moderate	59	50		
Reinforcing factors				
Family support				
Moderate	57	93	4.099a	0.143
Low	30	30		
Friend support				
Moderate	9	46	18.350	0.001*
Low	55	64		
Institute support				
Moderate	16	47	8.071	0.015*
Low	53	63		
Local administrative support				
Moderate	24	66	12.004	0.003*
Low	52	54		

a = Fisher's Exact test, *p < 0.05

use ($p < 0.001$), however, there was no significant link between housing and alcohol use (Table 1).

Enabling factors: peer support, educational institutions, and local administrative organizations were shown to be connected with alcohol-drinking behavior among junior high school students ($p < 0.05$), according to research examining variables related to alcohol use. According to Table 1, there was no statistically significant link between family support and alcohol consumption.

Part 6. The relationship between alcohol drinking behavior and the effects of alcohol consumption among junior high school students

Research examining the association between alcohol drinking behavior and the consequences of alcohol consumption found that the cause of the initial alcohol intake and the frequency of drinking were connected to the effects of alcohol usage ($P < 0.05$). There was no correlation between the length of drinking and drug usage during drinking and the consequences of alcohol consumption.

Frequency of drinking was connected with post-alcohol accidents ($p < 0.05$), according to the findings of a study examining the association between alcohol use and subsequent accidents. The reasons of initial alcohol intake, length of drinking, and drug usage during drinking were not substantially associated with alcohol-related accidents as Table 2.

The results of the study examining the association between alcohol-drinking behavior and alcohol-related health issues revealed that the use of other substances while drinking was connected with alcohol-related health problems ($p < 0.05$). According to Table 2, initial alcohol consumption, drinking frequency, and drinking interval were not substantially associated with alcohol-related health concerns.

The findings of the study examining the association between alcohol-drinking behavior and brawling after alcohol consumption revealed that the reason for first-time alcohol consumption was connected to brawling after alcohol consumption ($p < 0.05$). According to Table 2, drinking frequency, drinking interval, and drug usage during drinking were unrelated to fights following alcohol intake as Table 2.

DISCUSSION

The following is a summary of the findings from the research on drinking behavior and its impacts on secondary school students in grades 1-3 in Nakhon Si Thammarat Province.

40% of junior high school students drank alcohol, 54.8% of whom were male and 54.8% of whom were female, according to their alcohol consumption patterns. 45.2 is a comparable percentage. Those

who drank for the first time because they wanted to try it with their friends were 11–2 years old when they began, and 54.8% were female students. 45.2 is a comparable percentage. First-time drinking was motivated by a desire to sample alcohol with friends. I began drinking between the ages of 11 and 2 (56.0%) Beer and spy wine coolers are the most common alcoholic drinks. They do not become intoxicated and consume less alcohol. Places to drink include your house, the homes of your friends, and entertainment venues. The average cost of each drink is between 100 and 200 baht. The frequency of alcohol use is one to three times each month. The drinking hours are from 9 p.m. to 4:59 a.m. Participants drank with family and friends. During celebrations such as birthdays and New Year's, the chance of consuming alcoholic drinks was greater, and 10.7% of drinkers also smoked cigarettes and used cough syrup. This is consistent with the findings of Chomsri et al [18], who found that more alcoholic beverages are used to celebrate various occasions because they are easy to obtain illegally and come in a wide variety of styles and flavors, which affects young drinkers who are passionate, curious, and eager to be socially accepted. There is some drinking, but the samples are unable to earn money, so their purchasing power is low, and the majority of the family lives, indicating that other causes are at play. However, poverty is a common reason why students consume alcohol. The effects of alcohol usage on junior high school kids include absenteeism, worse marks, and so on. Health difficulties such as headaches, palpitations, exhaustion, accidents, early sex, etc., and social issues such as disputes with friends were reported as having moderate to poor views of the impacts of alcohol consumption. Younger individuals used less alcohol, with easy access to trading sources and peer support having a role (see Table 1). This is consistent with data from the Office of the Alcohol Control Board [19] and the study by Treemek [20], which indicate that alcoholic beverages may be a predisposition to violence such as quarrels, assaults, killing others, etc., as well as social problems including drunk driving accidents, rape, and other forms of substance abuse.

The link between predisposing variables, reinforcing factors, and enabling factors for the alcohol-drinking behavior of junior high school students was examined in the research.

The drinking habit was substantially connected with predisposing factors variables such as gender, age, educational level, and attitude. Males were substantially more associated to drinking behavior than females, according to the findings ($p < 0.05$) because male teenagers are inherently inquisitive and desire peer acceptance, the opposing sex is more likely to be welcomed by society. While women were taught to abstain from alcohol use, this was consistent with

Table 2. The relationship between drinking behavior and alcohol's repercussions among junior high school students. (n=84)

Alcohol consumption behavior	Effects of alcohol consumption			Accident after drinking alcohol			Alcohol abuse causes health concerns			Brawl after drinking alcohol		
	Ever (23)	Never (61)	χ^2	p-value	Ever (3)	Never (81)	χ^2	p-value	Ever (3)	Never (81)	χ^2	p-value
Initial alcoholic beverage consumption causes												
1) Friend encouraged to drink	5	19	11.02 ^a	0.07*	0	24	11.35 ^a	0.23	0	24	18.41 ^a	0.07*
2) Broken heart	1	4			0	5			0	5		
3) Imitate advertisements	1	1			1	1			1	2		
4) Hoping to attempt	11	33			2	42			0	44		
5) Family problems	1	0			0	1			0	1		
6) Relax	4	2			0	6			0	6		
Frequency of alcohol consumption												
1) Drinking 3-6 days a week.	4	4	11.49 ^a	0.01*	2	6	6.88 ^a	0.08*	1	7	7.09 ^a	0.24
2) Drinking 1-3 days per month.	11	16			0	27			0	27		
3) Drinking 1-11 days per year.	8	41			1	48			0	49		
Period of drinking alcoholic beverages												
1) 16.00 p.m.– 20.59 p.m.	11	35	2.08 ^a	0.58	2	44	3.05	0.72	1	45	6.23 ^a	0.52
2) 21.00 p.m.– 04.59 a.m.	13	27			1	39			0	4		
Co-use of additional drugs												
1) Use	1	3		1.00	1	3		0.14	1	3		0.05*
2) No use	22	58			2	78			0	80		

a = Fisher's Exact test, *p < 0.05

the findings of *Maneeapat Saimek's* [21] study, which indicated that gender was a factor affecting alcohol consumption. This is consistent with the research of *Ponpaipan et al.* [22] which concluded that gender is a factor related to the alcohol-drinking behavior of students, and with the research of *Ucharattana et al.* [23] which concluded that gender was associated with alcohol-drinking behavior, which explains why social norms accepted more male alcohol-drinking behavior than female students, but this study found that female students had higher alcohol-drinking rates than male students. Age was connected with alcohol use ($p = 0.001$). The average age at which people began drinking for the first time was revealed to be between 10 and 11 years old. Currently, the majority of drinking pupils are between the ages of 13 and 15, which is the younger age range. The age of legal access to alcoholic drinks suggested that the samples were from new drinkers who had begun drinking during the last one to two years. There is a correlation between education level and drinking habits. Drinking alcohol ($p < 0.05$), with moderate alcohol-drinking behavior, found that the majority were in the second and third grades, can be explained by the fact that the years of study may not represent a significant difference in age, so there may be no difference in the concepts and behaviors displayed. This contradicts the findings of *Rattanamanee* [24], who discovered that age had no correlation with alcohol drinking attitude and that academic year had no correlation with alcohol consumption behavior among Bangkok undergraduates.

The correlation between the student's attitude toward alcohol consumption and alcohol consumption behavior ($p < 0.05$) explains why the sample group of students with a favorable attitude toward alcohol use drank alcohol. The group with a negative attitude regarding alcohol use exhibited little alcohol usage. According to the data in Table 2, the participants showed a positive attitude toward alcohol intake. Because the students considered that consuming alcohol was a normal teenage habit and easily socialized with their classmates, the level was good and moderate. This behavioral belief is a perception of the possibility of engaging in alcohol-drinking behavior, as seen in Table 2, which reveals that the sample group of students exhibited a high degree of alcohol-drinking activity. The sample with a medium drinking attitude implies that these students are more inclined to use alcohol. This is consistent with *Whiteside et al.* [25] research on the characteristics that influence students' intentions to smoke and drink in southern Africa. Attitude toward alcohol consumption was 7.70% predictive of intention to consume. Religion, parental monthly income, and alcohol knowledge were shown to be negligible, statistically significant, and linked with the sample group's alcohol consumption. It can

be explained because the sample consists of teenagers who have not yet attained moral maturity and their alcohol-drinking behavior tends to be shared with companions who share the average costs. Since alcoholic beverages are affordable, they do not impact the monthly income received from parents. In addition, the majority of the sample is aware of the effects of alcohol on health and pregnancy. Accidents, brawls, and legislation regulating alcohol use are moderate and low. This is comparable to the study by *Hashim et al.* [26] which explored the factors impacting high school students' alcohol use. Conscious of the knowledge, comprehension, significance, and repercussions of alcohol consumption. To limit alcohol use among children and adolescents, we must educate them about the hazards and bad consequences of alcohol drinking throughout the time short- and long-term.

Significantly correlated with the alcohol-drinking behavior of the sample group were family relationships and access to alcoholic beverage trade sites, which enabled factors to the alcohol-drinking behavior of junior high school students. It was shown that 60.5% of family ties were positive, indicating that students whose family members had positive relationships were encouraged, discussed, and displayed less alcohol-related behavior. Those with fewer family links are more likely to use alcohol than those with strong family ties [27, 28, 29]. Therefore, having strong family relationships is the first step in keeping students from abusing alcohol. It was determined that access to trading was moderate at 51.0%, leading to increased alcohol-drinking behavior and frequency. There was a strong association between easy access to alcoholic drinks and alcohol-drinking behavior. It was connected with increased alcohol use compared to students who had difficulty gaining access to alcohol, since alcohol was readily available from convenience stores, extensively distributed, affordable, and accessible to students. Even if the policy of the law is applied, such as regulating the age or time for purchasing alcohol, students have easy access to establishments that do not adhere to the law and are persuaded by advertising. Alcohol-drinking behavior is connected with public relations regarding alcoholic drinks [30, 32]. And this study found that there was no statistically significant correlation between living arrangements and alcohol consumption, which may explain why this sample group consisted of junior high school students, the majority of whom lived with their parents, and that living arrangements can prevent students from engaging in alcohol consumption.

High levels of peer support were identified as an additional factor associated with the alcohol use of junior high school pupils. *Chanamanee et al.* [33] revealed that the person with whom teenagers drink alcohol the most is the average of their friends.

($P=0.001$) showed that the majority of adolescents' alcohol intake resulted from peer soliciting by drinking with friends the most. In line with *Amanda's* research, *Chaikan* et al. [34] concluded that the majority of students' alcohol consumption behavior was connected to alcohol intake with friends and peer influence. It might explain why the sample group with alcohol-drinking close friends consumed more alcohol than the sample group without alcohol-drinking close friends. There was little support from educational institutions. Community support by local government organizations is low since educational institutions have established a clear alcohol prohibition policy and a campaign to prevent and closely monitor students' alcohol-drinking behaviors. This study found that family support was not substantially associated with students' alcohol intake. It can be explained by the fact that the majority of the target population lived with parents who closely monitored their children and disapproved of alcohol consumption as a factor that could prohibit their drinking behavior. On the other side, the fragmentation of families in modern nations has contributed to an increase in alcohol dependence among children and adolescents.

CONCLUSION

The respondents are female (55.2%), 14 years old, enrolled in grade 3 (35.7%), with a cumulative GPA between 2.00 and 3.00 (4.9%), receiving 2,001 to 3,000 baht or more per month from their parents (72.4%), reside with their parents (70.0%) and with relatives (21.45). It was discovered that knowledge and attitudes concerning alcohol were at a moderate level, particularly favorable views that drinking alcohol was not a solution to life issues and the perception that alcohol increased the chance of injury. The level of hostility against alcohol drinking was at its maximum. It was a common occurrence among teens (23.8%) and can help alleviate stress (20.5%). There were three factors: 1) student housing characteristics that facilitated access to alcoholic beverages by students living with friends and in student housing near alcohol stores; 2) access to trading facilities for alcoholic beverages by being able to drink alcohol easily; and 3) family relationships in which family members understand each other and encourage the students to deal with their problems. The reinforcing factors revealed that family support was moderate, as parents had warned and educated their children about alcohol consumption, while school support was low, as students perceived that the school had clearly announced a prohibition policy on drinking alcohol, and support from local governments was low, as students had experienced campaigns to reduce alcohol consumption in the community. It was observed

that the drinking habits of the pupils had remained unchanged. The most often consumed alcoholic beverage was beer (45.2%), the frequency of drinking was 1-11 times a year (58.2%), and most drinking occurred between 9 p.m. and 5 a.m. Other substances were used while drinking (47.6%), alcohol was obtained at a convenience shop (55.2%), and alcohol was purchased from a store (55.2%). In addition, smoking (10.7%) and cough medication (2.4%) were included. According to the findings of a study of the leading factors related to alcohol consumption, sex, age, education level, and attitude toward alcohol were statistically significantly related to alcohol drinking behavior ($p<0.001$). Among the reinforcing factors to alcohol consumption, family relationships and access to alcoholic beverages were found to be significantly associated with alcohol-drinking behavior ($p<0.001$). Peers, educational institutions, and local government organizations were discovered to have a statistically significant relationship with alcohol-drinking behavior ($p<0.05$).

Recommendations

1. It was discovered that motivating, contributing, and enabling variables impacted the drinking behavior of students, particularly gender, age, and drinking attitudes. Family, availability to alcohol trafficking and social support were connected with heavy alcohol consumption. Therefore, preventative strategies should begin at the family, school, and local community levels and be pushed at the national level, especially in limiting underage access to alcohol, which is important.
2. This study was a quantitative examination of the behaviors and variables associated with a junior high school student's alcohol use in Nakhon Si Thammarat Province. It was impossible to accurately reflect the whole teenage population. To acquire a better understanding of the problem, further qualitative studies were undertaken on various demographic groups, focusing on the personal, behavioral, and attitudinal aspects that influence students' alcohol use.

Authors' contributions:

Conceptualization, P.T.; methodology, R.S. P.T.; formal analysis, S.M. P.B.; investigation, S.S.; writing original draft preparation, R.S., S.S.; writing, review and editing, S.S., and S.M.; supervision, P.T., P.B.

Funding

This project was also partially funded by the Excellence Center for DACH of the School of Public Health Walailak University, and the School of Public Health Walailak University Nakhon Si Thammarat Province, Thailand.

Data Availability Statement

The data used to support the findings of this study are included in this article.

Acknowledgment

We are very grateful to the students who participated acknowledged supported in part by the Excellence Center for DACH and the School of Public Health Look University Nakhon Si Thammarat Province.

Conflicts of interest

This publication is confirmed as having no known conflicts of interest. This endeavor has not been supported by any significant financial donator/grantor, which could have affected its outcome.

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Received: 31.01.2023

Accepted: 24.11.2023

Published online first: 28.11.2023

EFFECTS OF GENISTEIN SUPPLEMENTED BEFORE OR AFTER IRRADIATION ON DNA INJURY IN HUMAN LYMPHOCYTES *IN VITRO*

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ABSTRACT

Background. Ionizing radiation (IR) carry adequate energy to ionize or remove electrons from an atom. Particles interact with water to produce reactive oxygen species (ROS). Genistein (GEN) is a naturally occurring phytoestrogen and the basic isoflavonoid in soybeans and soybean-enriched products and is believed to have the strongest antioxidant activity.

Objective. The study aimed at the investigation if application of GEN at different time prior or past irradiation may ameliorate or reduce injury of DNA in human lymphocytes.

Material and Methods. The isolated lymphocytes were exposed to X-irradiation (0.5; 1 Gy). GEN (1 µM/ml; 10 µM/ml) was appended to attempts at various times prior or past irradiation (1 h prior, immediately prior, immediately past, 1 h past). We joined each X-rays dose with each GEN dose. After 1h of incubation DNA damages were examined using Comet assay.

Results. Combination of 1 µM/ml of GEN given 1 h before irradiation with low or high dose markedly decreased induced by irradiation DNA injury. Higher dose of GEN applied immediately before or after irradiation markedly extended the frequency of DNA injury generated by irradiation. The result of application 1 µM/ml GEN 1 h after irradiation was not significantly different compared to control. The effect of 1 Gy + 10 µM/ml GEN was not significantly lower compared to each agent alone.

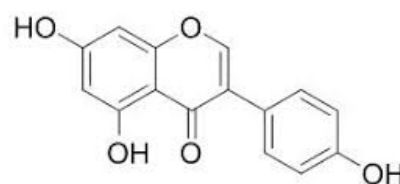
Conclusions. Only a very low concentration of GEN applied before irradiation, may be considered as a potential radiomitigator/radioprotector. High doses of GEN work as a radiosensitizer and may potent the effects of radiotherapy.

Key words: radioprotection, radiomitigation, DNA damage, genistein, irradiation

INTRODUCTION

Genistein (4',5,7-trihydroxyisoflavone, GEN) is a naturally occurring phytoestrogen and the basic isoflavonoid in soybeans and soybean-enriched products [26]. As the most rich in genistein is indicated psoralea (*Psoralea corylifolia*). Miscellaneous legumes like soybean (*Glycine max* L.), green bean (*Phaseolus vulgaris* L.), alfalfa sprout (*Medicago sativa* L.), mung bean sprout (*Vigna radiata* L.), cowpea (*Vigna unguiculata* L.), kudzu root (*Pueraria lobata* L.), red clover blossom and red clover sprout (*Trifolium pratense* L.) are also the source of genistein. They have been investigated for their estrogenic activity [6]. Since GEN is structurally similar to 17β-estradiol, it can compete with this estrogen and bind to estrogen receptors. GEN shows a much higher relationship toward the estrogen β (ER-beta) than toward estrogen α (ER-alfa) receptor [22]. At physiological

concentrations, GEN activates both nuclear estrogen α and β receptors ER and influences TGF-beta signaling pathways [8, 18, 21, 22].



The chemical structure of genistein

Isoflavones, which belong to the family of naturally occurring isoflavonoids, may protect oxidative damage by direct influence on free radicals or antioxidant scavenger enzymes. Among them GEN is believed to have the strongest antioxidant activity [19]. GEN is a component of the human diet, especially Asian soy-based foods, infant formulas, and dietary supplements. It is believed to be an anticancer, antiproliferative,

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

cardioprotective, and/or chemopreventive agent due to its ability to act as an inhibitor of tyrosine kinase, histidine kinase, and topoisomerase [1, 4]. Moreover, GEN showed beneficial effects on hypercholesterolemia and osteoporosis [46].

Ionizing radiation (IR) is described as electromagnetic waves and particles that carry adequate energy to ionize or remove electrons from an atom. Two principal mechanisms of IR interactions with tissues are known: direct and indirect. In the direct action, radiation simply disrupts the molecular structure. Indirect action occurs when noncharged particles interact with water to produce reactive oxygen species (ROS) [3, 34]. Ionization generated by radiation induces several chemical reactions, which are the reason of aberrations in atoms and molecules. Above changes may lead to cell damage, induction of mutation, carcinogenesis, or lethality.

Two kinds of electromagnetic waves can ionize atoms, X-rays and γ -rays. Among the damages induced by them, 60% is caused by indirect action. The total effect of IR depends on the physical attributes of radiation type, dose, and whether the exposure is acute, fractionated, or chronic. Biological responses to irradiation are addicted to the age, the kind of tissue, genetic background, and physiological status of the exposed individual [3, 10, 47].

IR induced cellular injuries are caused mainly by free radicals, so molecules with direct free radical scavenging features are likely to act as so-called radiation modifiers/protectors, factors which apply prior to or shortly after irradiation modify the response of tissues to radiation. Similarly, agents which may be used to reduce toxicity even applied after radiation are usually called mitigators [9]. During the last years, numerous compounds, including these coming from plants, have been discovered as radioprotective agents. Due to their antioxidant properties, they may serve as radioprotective agents to protect from irradiation damage [23]. Agents acting as radioprotectors/radiomitigators should be nontoxic, cheap, and easy to use. Such factors might be very helpful in health prevention, mainly when used after irradiation when damages already exist.

The aim of the study was the investigation if application of genistein at different times before or after irradiation may prevent or reduce radiation-induced injury of DNA in human white blood cells.

MATERIAL AND METHODS

Isolation of lymphocytes

The lymphocytes were isolated from samples of human peripheral blood. For this aim, blood was aseptically collected in heparinized sterile tubes from a nonsmoking, healthy individual (female, 35

years) according to the procedure of *Anderson et al.* [2]. Whole blood was then mixed 1:1 with phosphate buffered saline (PBS). From this mixture was taken 5 ml and cautiously placed on top of 2.5 ml of lympho separation medium (MP Biomedicals) and centrifuged at $918 \times g$ for 20 min at room temperature. After isolating the lymphocyte layer, they were mixed with 10 ml PBS and centrifuged at $450 \times g$ rpm for 10 min. The supernatant was then removed. The precipitated cell pellet was transferred to Eppendorf tubes (50 μ l of cell suspension for each tube).

Preparation of genistein

Genistein (GEN) was prepared as follows: 2.7 mg of GEN (purity $\geq 98\%$, $M = 270$, 24 g/mol; ROTH GmbH, Germany; item number: 0716.1) was dissolved in 1 ml of ethanol (EtOH). From this solution, two different concentrations (1 and 10 μ M/ml) of GEN were taken and added to the cells in Eppendorf tubes. The choice of doses for this study was based on a previous study [2]. Then the RPMI 1640 medium was added to the lymphocytes in such an amount that each Eppendorf tube contained 1 ml of solution. As a control, 0.1% concentration of EtOH in the test tubes was used, which corresponds to the maximum concentration of alcohol in the test samples. In a similar way were made up the controls for each dose of GEN and for the indication of vitality analysis of lymphocytes with trypan blue.

Treatment of the cells

The secluded lymphocytes (past indication of their vitality) were X-irradiated at doses of 0.5 and 1 Gy. Control cells were not irradiated. A therapeutic Roentgen unit, Medicor type THX-250, served as the X-rays origin. It was served with the succeeding factors: 155 kV, 18 mA, additional filtration, 0.25 mm Cu, and HVL 2 mm Al. Lymphocytes were irradiated at the dose rate of 0.2 Gy/min. GEN dissolved in EtOH at diverse doses, was inserted to test attempts at various times prior or past irradiation (1 h prior, just prior, just past, and 1 h past). The time points were selected based on scientific literature and our own earlier investigation. We have introduced a scheme to join every X-ray dose (0.5 and 1 Gy) with every GEN dose (1 μ M/ml and 10 μ M/ml). Afterwards, the cells were kept for 1h in a water bath at 37°C. Simultaneously, control cells (negative control), cells subjected single to GEN and single to X-rays. Three irrespective ($n = 3$) experiences were executed. The blood from the donor was donated at 3 various days in one month. The frequency of DNA injury was assessed by alkaline comet assay.

Comet assay

To assess the effect of GEN on X-ray induced DNA damage in lymphocytes, single cell gel electrophoresis (comet assay) was used according to the procedures of Singh et al. [38] and Anderson et al. [2].

At first, every lymphocyte specimen was swirled at 1778 x g for 3 min. The supernatant was then eliminated and 75 µl of 0.5% low melting point agarose (LMPA) at 37 ° C was inserted into the pellet remaining in the Eppendorf tube. The resulting solutions were mixed and mounted on microscope slides that had earlier been coated with 1% normal melting point agarose (NMPA). The slides were then coated with cover slips and stored in fridge (4 °C) to concentrate the agarose. Coverslips were removed after concentration and the next layer of LMPA was inserted and the slides were again coated with coverslips and permitted to solidify again at 4 ° C. Post removing the coverslips, the slides were submerged in a lysis solution (2.5 M sodium chloride – NaCl, 100 mM ethylenediaminetetraacetic acid – EDTA, 10 mM Tris, 1% sodium lauryl sarcosinate, pH 10, plus 1% Triton-X and 10% dimethyl sulfoxide - DMSO) until morning at 4 °C. The slides were then kept in the electrophoresis solution (10 N NaOH, 200 mM EDTA – pH 10 in distilled water at 4 °C) for 20 min to permit DNA to develop. Alkaline electrophoresis was carried out for 20 min at 4 °C, 0.6 V/cm, and 300 mA. The level of the electrophoresis buffer was around 0.25 cm above the slides. Post alkaline electrophoresis, the slides were neutralized and dyed with ethidium bromide (EtBr).

The DNA damages in lymphocytes were examined using a fluorescence microscope. To this aim, images of 100 randomly selected lymphocytes from each sample were recorded and analyzed with the CASP

image analysis software [20]. As the parameters for analysis Tail Moment and Percentage of DNA in Comet Tail (Tail DNA %) were chosen.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine any significant differences between the results from different groups. The Fisher's test was used as a *post hoc* test. The values $p < 0.05$ were considered statistically significant.

RESULTS

DNA injury in human lymphocytes treated with genistein 1 h before irradiation is shown in Figure 1. The cell viability varied between 62 in the group of 1 Gy + 10 µM/ml GEN and 92 % in controls. The Tail Moment and % Tail DNA for low dose of GEN and both doses of irradiation as well as for solvent control were not markedly varied like to negative control, however, the impacts after irradiation to 1 Gy were several times higher than control values. Combination of 1 µM/ml of GEN with low as well as high doses of irradiation markedly but not significantly decreased induced by irradiation DNA injury. The dose of 10 µM/ml GEN significantly enhanced the DNA injury in human lymphocytes compared to controls. Similarly, application of 10 µM/ml GEN 1 h before irradiation enhanced the DNA damages compared to those noted after irradiation with 0.5 or 1 Gy.

DNA injury in human lymphocytes treated with genistein immediately before irradiation is presented in Figure 2. The cell viability ranged from 62 in 0.5 Gy + 10 µM/ml GEN group to 92 % in the control. Results of solvent control and low dose of GEN were

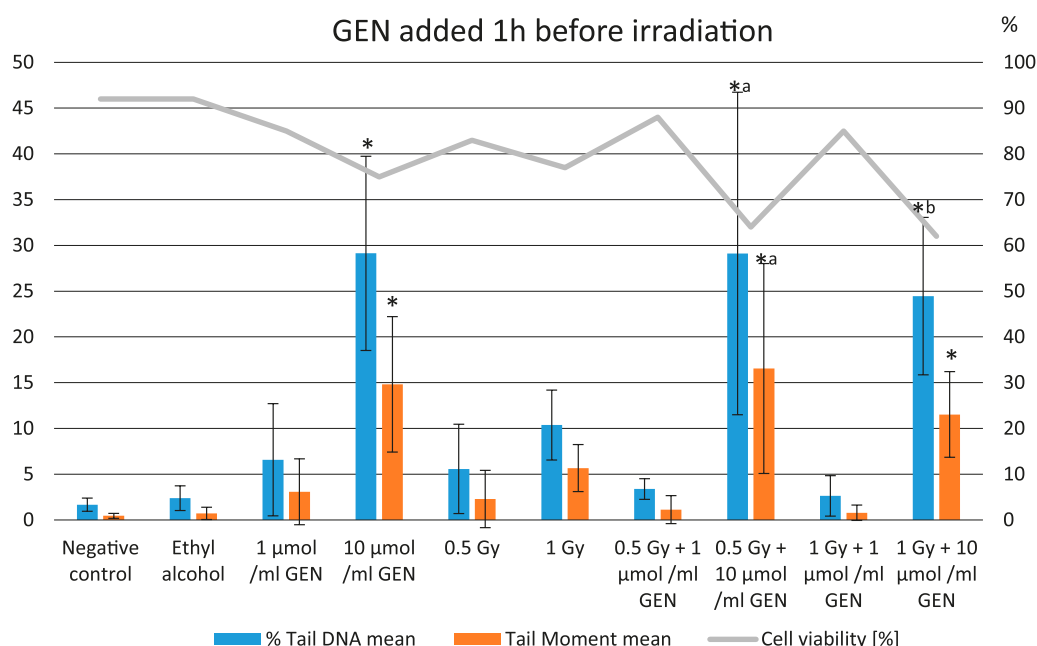


Figure 1. The effect of genistein supplementation 1 h before irradiation on the DNA damage of human lymphocytes

not significantly different compared to the negative control. Results of irradiation alone, especially at high dose were markedly higher than the negative controls but not markedly varied. Higher dose of genistein significantly enhanced the frequency of DNA injury acting alone as well as in combination with irradiation. The results after exposure to 0.5 Gy + 10 $\mu\text{M/ml}$ GEN were significantly higher compared to 0.5 Gy or 1 Gy alone, whereas 1 Gy + 10 $\mu\text{M/ml}$ GEN in comparison to 1 Gy alone only. Treatment with low dose of GEN and each dose of irradiation induced a similar levels of DNA injury that a single exposure to irradiation or GEN.

DNA damage in human lymphocytes treated with genistein immediately after irradiation is shown in Figure 3. The cell viability ranged from 70 in the group 1 Gy + 10 $\mu\text{M/ml}$ GEN to 92 % in the control group. Results of solvent control and low dose of GEN were not significantly differed from the negative control. The DNA damage (% Tail DNA and Tail Moment) after irradiation alone, especially at high dose were markedly higher than in negative control but not markedly varied. Genistein at the dose of 10 $\mu\text{M/ml}$ acting alone as well as in a combination with irradiation significantly enhanced the level of DNA injury. The results after exposure to 0.5 Gy + 1 $\mu\text{M/ml}$ GEN and to 1 Gy + 1 $\mu\text{M/ml}$ were slightly higher but not markedly varied as compared to negative control, 0.5 Gy or 1 Gy alone.

DNA injury in human lymphocytes treated with genistein 1h after irradiation is presented in Figure 4.

The cell viability varied between 68 in 0.5 Gy + 10 $\mu\text{M/ml}$ GEN group and 92 % in the control group. Results of solvent control and low dose of GEN were not significantly different like to the negative control. DNA damage after a higher dose of irradiation was almost twice higher compared to that after the lower dose, but both were not markedly varied compared to control. Higher dose of genistein significantly enhanced the frequency of DNA injury. The results of application of GEN alone 1 h after irradiation 0.5 Gy + 1 $\mu\text{M/ml}$ GEN and 1 Gy + 1 $\mu\text{M/ml}$ GEN were similar and not markedly varied compared to control. Treatment with high dose of GEN and low dose of irradiation enhanced the level of DNA injury. The effect of 1 Gy + 10 $\mu\text{M/ml}$ GEN was not significantly lower compared to each agent acting alone.

DISCUSSION

People may be exposed to ionizing radiation constantly due to their occupation or accidentally due to the breakdown of nuclear power plants or terroristic attacks, which may cause a temporally enhanced levels of radiation leading to harmful health effects. A number of jobs may be connected with an enhanced levels of exposure to man-made sources of radiation by employees. There are, for example, medical personnel, uranium miners, nuclear plant workers, and other employees using radiation for industrial and scientific purposes [45]. Moreover, patients ongoing radiotherapy may be exposed to an overdose of radiation.

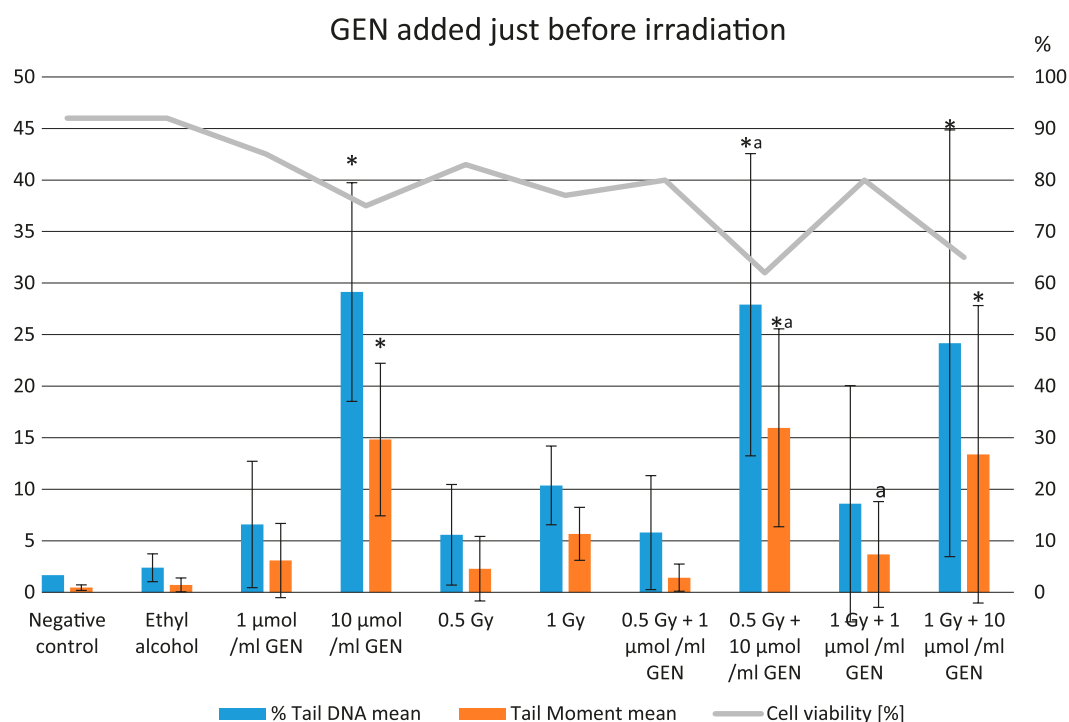


Figure 2. The effect of genistein supplementation immediately before irradiation on the DNA damage of human lymphocytes

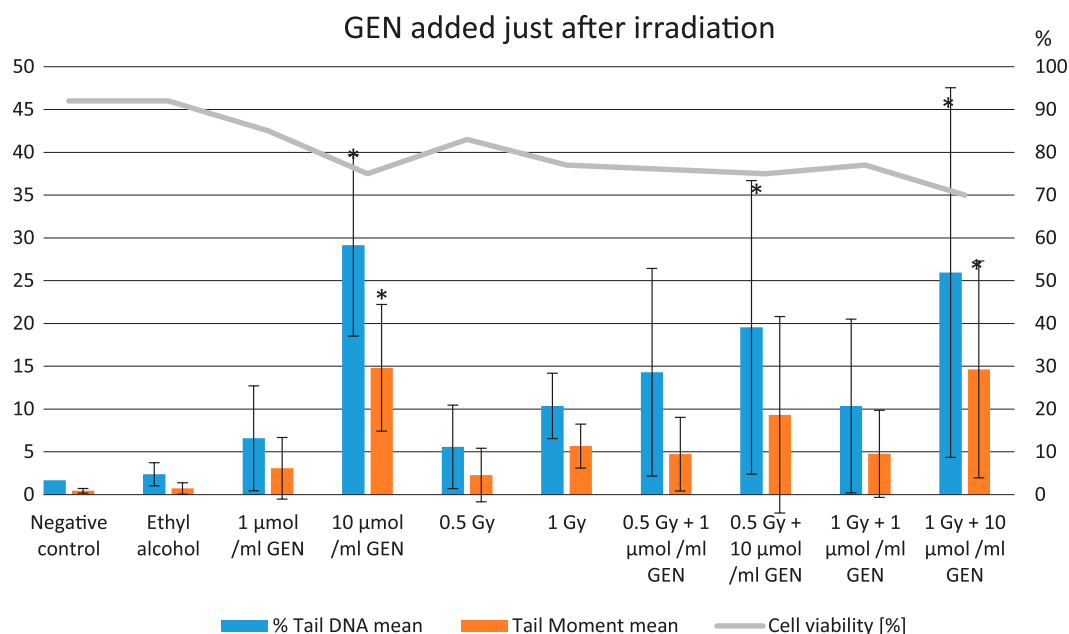


Figure 3. The effect of genistein supplementation immediately after irradiation on the DNA damage of human lymphocytes

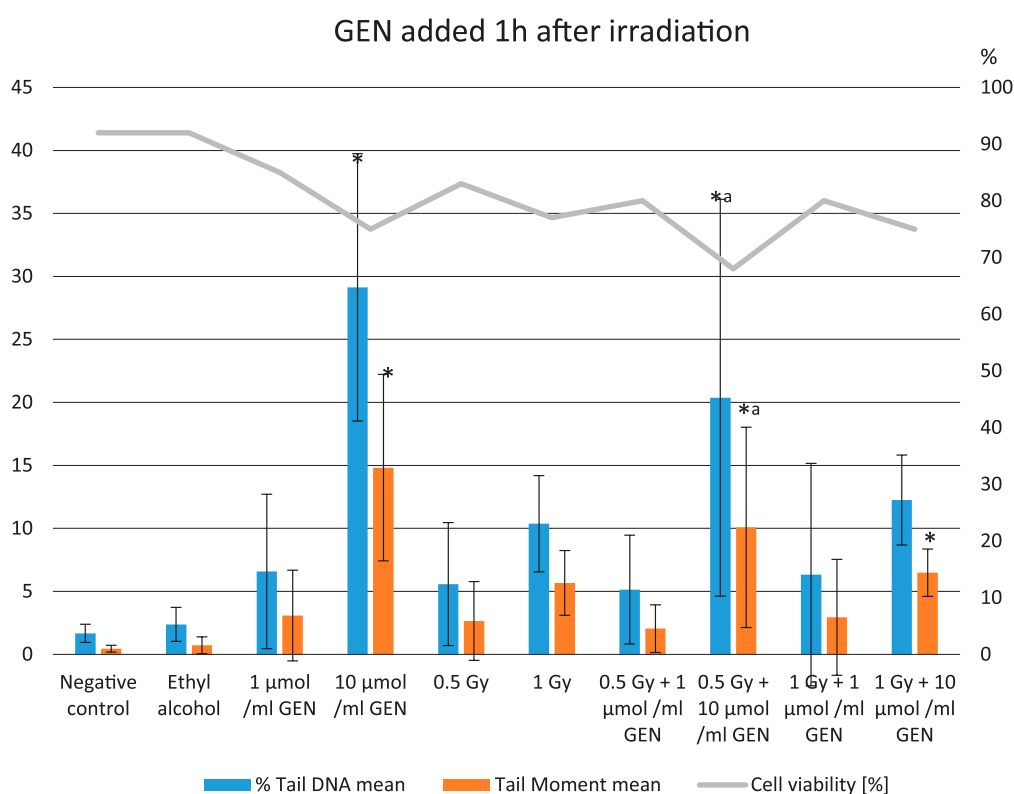


Figure 4. The effect of genistein supplementation 1 h after irradiation on the DNA damage of human lymphocytes

Ionizing radiation may lead to damage of living tissue, and later to induction of mutations, cancer, or cell death. Potentially dangerous cells are eliminated from the organism by lethal mutations. The impact of miss-repaired damage may be chromosomal damage or mutations. The above modifications may be transmitted to further generations of cells and may eventually lead to cancer [13, 40, 41].

Increased production of reactive oxygen (ROS) and nitrogen (RNS) species may be a reason of oxidative stress. ROS might oxidize cellular biomolecules, like carbohydrates, proteins, lipids, and DNA. Approximately 60 % of the damage induced by ionizing radiation is caused by reactive oxygen species (ROS). The widespread ROS are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl

radicals (OH⁻) [25]. ROS may also have the beneficial effect on the use of normal circular metabolism at low or moderate concentration when regulating the physiological function of cells [14, 44]. At higher concentrations, radicals become toxic and disrupt the antioxidant defense system leading to oxidative stress [32]. Major ROS mediated reactions include lipid peroxidation, removal of thiol groups from cellular and membrane proteins, strand break and base alterations leading to DNA damage [37]. Oxidative modification of important functional groups in essential membrane proteins (ion channels) leads to changes in purine and pyrimidine bases, single- and double-strand breaks, removal of bases, and cross-linking of DNA with DNA or adjacent proteins [42, 48].

Irradiated cells that avoid death may undergo mutations, which induce defects in the DNA scheme leading to altered gene expression and protein modifications, like peptide bond cleavage and cross-linking. It may influence protein localization, interactions and change enzyme activity [23]. Double-strand breaks (DSBs) are more difficult to repair than single-strand breaks and cause frequently mutagenesis or cell death [29]. Radiation also generates point mutations and deletions [28]. ROS-mediated DNA injury can finally evolve into cancer, particularly if the regulation of tumor suppressor genes is impaired [49].

In the present study, the effect of lower dose, i.e. 1 μ M/ml GEN was not significantly different compared to negative and solvent control. The higher dose 10 μ M/ml GEN induced significant damage to DNA of human lymphocytes. In the previous study, both doses of GEN did not induce DNA injury in human lymphocytes, however, the higher dose induced damage to DNA of human sperm [2]. In a hamster lung cell line (V79), GEN was found to induce micronuclei at concentrations ranging from 5 to 25 μ M, which declined at higher doses, whereas the comet assay showed the induction of DNA damage only at high concentrations [12].

The connection between genetic modifications by isoflavones and oxidative damage was described by *Cantanhêde et al.* [7] The study showed that soy isoflavones are nontoxic and efficient in reducing genomic lesions and the frequency of micronuclei induced by meglumine antimoniate. *Sarkar and Li* [36] showed a protective role of GEN against the genotoxic effects of cancer-induced agents. According to other papers, GEN has strong antioxidant activity against radiation-induced oxidative stress through upregulating endogenous glutathione levels and glutathione peroxidase activity [15]. Due to the recognized antioxidant capacity, GEN is a deliberated as an important agent in cancer prevention [27, 31, 33, 35].

GEN works by enhancing the expression of genes that engages in the detoxification of ROS, such as

superoxide dismutase, glutathione peroxidase, and catalase [11]. Similarly, there was noted that GEN was able to induce a significant increase of expression of the gene *GPx-1* in human prostate cancer cell lines (LNCaP and PC-3), which help in antioxidant defense and inhibition of proliferation of cancer cells [43].

GEN administration prior to irradiation protects mice against liver injury [46]. An alternative likely mechanism to clarify the antimutagenic impact of genistein is connected with its ability to stimulate the expression of genes involved in DNA repair, such as *BCRA1* and *ATM* and *p53* [5, 50]. *Song et al.* [39] stated that GEN was capable to increase expression of genes involved in DNA repair, such as *hHR23A*, *HUS1*, *RAD1*, and *RAD9* to prevent and repair radiation-induced DNA damage in HL-7702 cells. The study of *Hillman* [16] showed that soy isoflavones radiosensitized cancer cells. The mechanisms of radiosensitization, studied both in vitro and in vivo in showed that isoflavones targeted signaling survival pathways upregulated by radiation, including DNA repair and transcription factors, causing death of cancer cells. On the contrary, radioprotection of normal tissues and organs was mediated by SIF supplementation to radiation and was noted in the renal cell carcinoma model and lung preclinical models, and in a prostate cancer clinical trial.

The glucosides GEN administered singly 2 h before irradiation with 60 mJ/cm² of UVB, did not significantly prevent UVB-induced DNA injury [17]. The outcomes of the current study showed that the dose of 10 μ M/ml of GEN applied both prior as well as after irradiation enhanced damage to DNA of human peripheral lymphocytes induced by irradiation alone. In contrast, the low dose of GEN not significantly decreased the level of induced by irradiation DNA injury when applied before irradiation, especially 1 h prior to exposure.

GEN was effective against hematopoietic acute radiation syndrome when administered 48 to 12 h before irradiation with the maximal effect when applied 24 h prior radiation exposure [24]. Similarly to our current results, the study of *Song et al.* [39] showed that low concentration of GEN (1.5 μ M) protected L-02 cells against radiation damage via inhibition of apoptosis, alleviation of DNA damage and chromosome aberration, down-regulation of GRP78 and up-regulation of HERP, HUS1 and hHR23A, whereas high concentration of GEN (20 μ M) induced radiosensitization through the promotion of apoptosis and chromosome aberration, impairment of DNA repair, up-regulation of GRP78, and down-regulation of HUS1, SIRT1, RAD17, RAD51 and RNF8. Soy phytoestrogen was not mutagenic and reduced cyclophosphamide-induced DNA damage. The results

from the comet assay revealed a reduction of DNA damage; however, phytoestrogen did induce genotoxic damage during the 24-h treatment [30].

CONCLUSIONS

Taking together the above previous and the current study, there is confirmed that GEN may under special conditions act both as a radioprotector and radiosensitizer. Only very low concentration GEN applied before irradiation, which may be considered as a potential candidate for radiomitigator/radioprotector. Contrary, high doses of GEN work as a radiosensitizer and may be useful to the potent effects of radiotherapy. Further investigations are necessary to clarify the above findings.

Conflict of interest

The authors declared that they had no conflicts of interest.

Funding statement

This work was supported by the National Institute of Public Health NIH - National Research Institute, Warsaw, Poland. Scientific projects No 12/ZŚ1/2018.

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Received: 25.10.2023

Accepted: 09.11.2023

Published online first: 20.11.2023

INFLUENCE OF *INULA HELENIUM* RHIZOMES AND *MATRICARIA CHAMOMILLA* INFLORESCENCES ON THE BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS IN MALE RATS FED A HIGH-FAT DIET

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ABSTRACT

Background. Pharmacological correction of a high-fat diet is of great interest to prevent the development of obesity and hypertension. More and more research is being done on the preventive use of medicinal herbs for excess caloric intake.

Objective. The aim of this study was to determine the general physiological effect of *I. helenium* rhizomes and *M. chamomilla* inflorescences used in the diet of male rats consuming excess amounts of fat and calories in the daily diet.

Material and methods. In a 30-day experiment, we determined the effect of *I. helenium* rhizome and *M. chamomilla* on the physiological activity and metabolic processes of laboratory rats consuming a high-fat diet. The physical activity was evaluated according to the mass gain of animals and change in the relative mass of the internal organs, and also the functional conditions of the central nervous system. The influence on the metabolic processes was revealed by biochemical and clinical blood analyses.

Results. In a laboratory experiment on male rats, it was found that the addition of dry crushed rhizomes of *Inula helenium* L. and inflorescences of *Matricaria chamomilla* L. to the diet caused opposite changes in body weight. In the control group, the animals slightly increased their body weight (up to 111.5% of the initial weight by the end of the experiment); the rhizomes of *I. helenium* caused a decrease in body weight gain (up to 105.5% on the 30th day of the experiment compared to the initial weight); rats fed *M. chamomilla* inflorescences gained 123.2% of their initial body weight during the month of the experiment. The rhizomes of *I. helenium* caused an increase in the stomach relative mass. A decrease in the thymus relative weight was observed when animals were fed *M. chamomilla* inflorescences. The rhizomes of *I. helenium* stimulated an increase of blood protein concentration (mainly due to globulins), an increase in the alkaline phosphatase activity and cholesterol, and a decrease in the triglycerides concentration. *M. chamomilla* inflorescences reduced the blood urea concentration and increased the activity of alkaline phosphatase, causing strong changes in fat metabolism. Under the influence of the diet with the addition of *M. chamomilla* inflorescences, the atherogenic index increased in animals by 6.5 times relatively to the control group (due to a decrease in the concentration of high-density lipoprotein cholesterol and an increase in the concentration of low-density lipoprotein cholesterol, an increase in the total cholesterol concentration). When *M. chamomilla* was added to the diet, the blood triglycerides concentration in animals decreased sharply and the concentration of leukocytes increased. The concentration of monocytes exceeded the limits of the physiological norm both in the control group and in the group of animals fed on *M. chamomilla* inflorescences.

Conclusions. The results of the studies show the promise for further research of *I. helenium* rhizomes in the prevention of hypertension and also indicate strong risks when using *M. chamomilla* inflorescences for preventive purposes during high-fat and hypercaloric diet.

Key words: relative organs' mass, increase in the bodyweight, high-fat diet, pharmaceutical chamomile, elecampane, phytotherapy, weight loss.

INTRODUCTION

Excessive fat intake is one of the major health problems at the global level [27, 52, 65]. High-calorie food causes metabolic changes, disturbances in the digestive and endocrine systems, and most importantly, stimulates the vascular diseases development [7, 19, 46]. Problems of excess dietary energy are often

addressed by adding spices, aromatics and medicinal herbs to human food [18, 50]. However, their use can not only cause positive health changes in the body but can also stimulate an increase in pathological changes caused by excess calorie intake [24, 33, 35, 37]. Some of the most widely used plants in traditional folk medicine and modern medical practice are elecampane and chamomile [70].

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

Elecampane (*Inula helenium* L.) has an expectorant and anti-inflammatory effect, reduces intestinal motility and secretion of gastric juice. Tavares and Seca [58] in their study with *Inula* L. identified the most promising secondary metabolites in the treatment of human diseases associated with oxidative stress. The most promising metabolite was β -caryophyllene, as it showed the highest antioxidant activity, even in comparison with ascorbic acid [62, 75].

The main biologically active substance of elecampane is believed to be alantolactone and associated terpenoids [32]. Studies have been conducted on alantolactone, obtained from the *I. helenium* rhizomes, which showed the effectiveness of this substance against tumor cells [3, 12, 67, 74]. Koc et al. [31] revealed antioxidant and antitumor activity of *I. helenium* extract. Alantolactone has also been shown to have anti-inflammatory activity by enhancing the phagocytic activity of macrophages [22, 49]. The essential oil of *I. helenium* rhizomes has bactericidal and fungicidal properties [26, 29]. In folk medicine, tinctures and rhizome's extracts are used for malaria, oedema, urolithiasis, hypoacid gastritis, migraine, whooping cough, bronchial asthma, as a haemostatic, diuretic, and anti-inflammatory agent for skin diseases [10, 19, 64].

Since ancient times, chamomile (*Matricaria chamomilla* L.) has been known as a valuable medicinal plant, which is used both in traditional medicine as well as in the official pharmacopoeia of 26 countries [57, 58]. Chamomile contains a number of phytochemicals: flavonoids and other phenolic compounds, essential oil, polysaccharides, amino acids, fatty acids and mineral elements [39, 44, 53].

Due to the diverse composition of active substances, chamomile preparations have numerous pharmacological effects. Chamazulene has anti-inflammatory, sedative, antiallergic and local anaesthetic properties [26], it activates the function of immune organs, and enhances regenerative processes. There have been shown antidiabetic and hypocholesterolemic effects of chamomile extract in animal studies [47, 71, 72]. Zand et al. [73] showed weak estrogenic and progestogenic effects of chamomile extract at high concentrations, and Kabiri et al. [28] compared the effect of chamomile syrup and cabergoline in reducing the blood prolactin concentration in patients with idiopathic prolactinemia.

Chamomile preparations have a pronounced effect on the nervous system [54]. It has been experimentally shown that chamomile essential oil enhances reflex activity, stimulates the medulla oblongata, strengthens and quickens breathing, increases heart rate, dilates the blood vessels of animals' brain, but in large doses can cause central nervous system depression and muscle tone decrease [16, 51].

Preparations made from chamomile are often used externally primarily as an anti-inflammatory agent, but the infusion of chamomile inflorescence inflorescence has a haemostatic, antiseptic, mild astringent, analgesic, sedative, anticonvulsant, diaphoretic, and choleric effect [25]. Research with animals suggests antispasmodic, anxiolytic, anti-inflammatory and some antimutagenic and cholesterol-lowering effects for chamomile [45].

Thus, both medicinal plants – elecampane and chamomile – are recommended for the treatment of certain pathological changes amid hypertension, are widely used in folk and official medicine, and are used by people in everyday life. However, comprehensive studies of changes in the body against the background of excessive fat consumption have not yet been carried out for the herbal preparations *I. helenium* and *M. chamomilla*.

The objective of this study was to determine the general physiological effect of *I. helenium* rhizomes and *M. chamomilla* inflorescences used in the diet of male rats consuming excess amounts of fat and calories in the daily diet.

MATERIAL AND METHODS

Ethics. The choice of animals for the experiment, research protocols, and withdrawal of animals from the experiment was approved by the local ethical committee of Dnipro State Agrarian and Economic University (Decision No. 3/20-21 of 20.09.2020). The maintenance, nutrition, care of animals and their withdrawal from the experiment were carried out in accordance with the principles set forth in the "European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes" (Strasbourg, France, 18th March, 1986, ETS No. 123) and in Law of Ukraine "On protection of animals from cruel treatment" (Kyiv, 21st February, 2006, No. 3447-IV).

Animals. The experiment used 24 adult white outbred male laboratory rats, weighing 200 ± 10 g. The rats were divided into a control group and two experimental groups ($n = 8$). The rats were kept in groups of 4 in polycarbonate cages with steel mesh lids and a food recess. The temperature in the room where the experimental animals were located was 20–22 °C, relative air humidity was 50–65%, and the light regime was 12 hours light/12 hours dark.

Diet. Animals received water and food ad libitum. The diet of all animals had an excess fat content (3600 kcal/kg). The high-fat diet was based on a standard diet (75% grain mixture (corn, sunflower grain, wheat, barley, soybeans), 8% root vegetables (potatoes, carrots), 2% meat-and-bone meal, 2% mineral-vitamin complex) with the introduction of 15 % sunflower oil.

The control group of animals received a high-fat diet, and the experimental group received a semi-synthetic diet, to which a dry medicinal plant of 5% of the feed weight was added. In the first experimental group, 5% of dry *I. helenium* crushed rhizomes of the second year of life were additionally added to the high-fat diet. The raw materials were collected in mid-August. The rhizomes were washed in water, cut into pieces 5–7 mm thick, and dried at room temperature without direct sunlight. The second experimental group had 5% of crushed dry chamomile inflorescences to the high-fat diet, which were collected manually as the flowers bloomed on the plants. They were dried without direct sunlight at room temperature.

The main ingredients of the diet were ground in a mill (grain, meat-and-bone meal, mineral and vitamin complex, dry rhizomes and inflorescences of medicinal plants) and mixed, then sunflower oil was added and granules were made at the rate of 4,200 g for each group for the entire period of the experiment (30 days). Root vegetables in appropriate quantities were additionally given fresh every day. During the experiment, we took into account the amount of food consumed by each group per day and the total amount of food consumed during the entire period of the experiment.

Morphometric parameters (live weight, abdominal volume) were determined on days 1–4 and on days 26–30 of the experiment. The total increase in animal weight and daily increase in live weight were calculated. Orientation-physical activity and the emotional state of experimental animals were studied in the “open field” test [55]. We used an installation consisting of 1 m² square area, divided into 16 squares and limited by an opaque wall 20 cm high. The experiment was carried out in complete silence with intense lighting of the field itself. The animal, taken from a cage in a previously darkened room, was placed in the centre of the field. The exposure time was 2 minutes. Animals were tested for four days (days 1–4) at the beginning of the experiment and four days at its end (days 26–30). The number of crossed squares was counted: peripheral and central – physical activity was assessed; peripheral (with support on the wall) and central (without support on the wall) racks – orientation activity; the number of grooming acts, defecation and urination – emotional state [34, 36].

Animals were euthanized on the 30th day of the experiment under anaesthesia (80 mg/kg ketamine and 12 mg/kg xylazine, intraperitoneally) by total bleeding from the heart. After the autopsy, the condition of the internal organs was visually assessed for the presence of pathological changes, organs were selected and weighed (heart, liver, lungs, thymus, spleen, stomach, small and large intestines, kidneys). The mass of

internal organs was determined with an accuracy of 10 mg [8].

Biochemical studies of blood serum were carried out on an automatic analyser Miura 200 (Italy) using reagent kits High Technology (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). Total protein was determined using the biuret method; globulins and protein coefficient – by calculation; albumin concentration – by reaction with bromocresol green; activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – by a kinetic method based on the *Warburg* optical test; alkaline phosphatase – enzymatically by reaction with p-nitrophenyl phosphate; glucose – using the glucose oxidase method [11].

Characteristics of lipid metabolism. The concentration of total cholesterol was determined enzymatically using cholesterol oxidase; triglycerides – after cleavage by lipoprotein lipase with detection by the Trinder reaction; HDL and LDL – using selective detergents with subsequent staining of the enzymatic reaction products, the atherogenic index was calculated.

On the biochemical markers of inflammation, C-reactive protein was determined by immunoturbidimetry.

The number of red and white blood cells, haematocrit and haemoglobin content were determined in the rats' blood after the addition of K3EDTA using a PCV-80 Vet automatic haematology analyser. For leukogram, blood smears were prepared according to *Pappenheim*.

The data were analyzed using Statistica 8.0 program (StatSoft Inc., USA). The tables demonstrate the results as $\bar{x} \pm SD$ (\bar{x} – standard deviation). Differences between the values of the control and experimental groups were determined using the Tukey test (with consideration of Boniferroni's correction), where the differences were considered significant at $P < 0.05$.

RESULTS

The addition of dry crushed rhizomes of *Inula helenium* L. and inflorescences of *Matricaria chamomilla* L. to the diet of rats caused opposite changes in the dynamics of the animals' body weight. If in the control group the weight of male rats increased up to 110.0% of the weight on the first day of the experiment, slightly increasing to 111.5% by the end of the experiment, then the rhizomes of *I. helenium* caused a decrease in the body weight of animals to 95.0% of the initial weight on the 13th day of the experiment (Figure 1a). On the 26th day, the weight of the rats reached a maximum (107.9% from the beginning of the experiment), decreasing to 105.5% by the 30th day. Rats fed *M. chamomilla* inflorescences increased their body weight relatively evenly, reaching 123.2% of

their body weight at the beginning of the experiment (Fig. 1b). Thus, the addition of *I. helenium* rhizomes to the diet reduced the weight of animals by 6.0% by the end of the experiment compared to the control, and the addition of *M. chamomilla* inflorescences increased it by 11.7% compared to the control group (Figure 1).

Probably, the bitter taste of the rhizomes of *I. helenium* caused a decrease in food consumption in male rats to the level of 81.9% of the control group, but we did not observe any changes in fluid consumption

by the animals (Table 1). It is interesting that with a significant increase in body weight in animals fed on *M. chamomilla* inflorescences (1505 ± 198 compared to 700 ± 271 mg/day in the control group), their food consumption did not increase (96.0% of the control group), there was only slightly increased water consumption (105.1% of the control group), there was only slightly increased water consumption (105.1% of the control group, Table 1). Abdominal volume also increased when rats ate *M. chamomilla* inflorescences and amounted to 111.2% of the control group, remaining unchanged on the diet

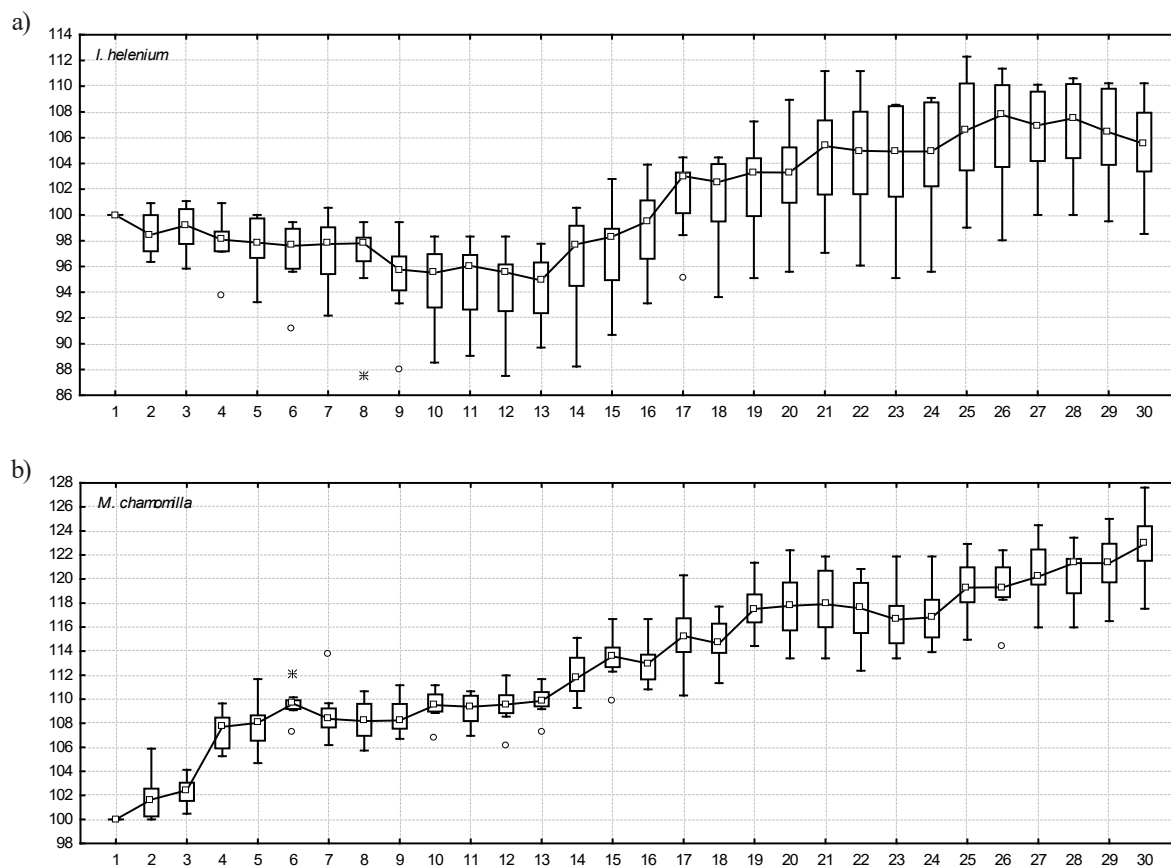


Figure 1. Changes in the rats' body weight when adding crushed rhizomes of *Inula helenium* L. (a) and *Matricaria chamomilla* L. inflorescences (b) to the diet of animals: the x-axis is the day of the experiment, the y-axis is the animals' body weight (% relative to the initial body weight of each animal, taken as 100% at the beginning of the experiment); small square – median, upper and lower borders of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n=8

Table 1. Change in the body weight and fodder consumption of young male rats under the impact of *Inula helenium* L. and *Matricaria chamomilla* L supplementation to their diet ($\bar{x} \pm SD$, n = 8, duration of experiment – 30 days)

Parameter	Control	<i>I. helenium</i>	<i>I. helenium</i> compared to control, %	<i>M.</i> <i>chamomilla</i>	<i>M.</i> <i>chamomilla</i> compared to control, %
Consumption of food by animals, g/day	20.09	16.46	81.9	19.29	96.0
Consumption of water by animals, g/day	18.42	17.27	93.8	19.36	105.1
Change in body weight, µg/day	700 ± 271	$325 \pm 208^*$	46.4	$1505 \pm 198^{***}$	215.0
Change in body weight, %/30 day	13.6 ± 5.9	$5.3 \pm 3.5^*$	38.9	$22.9 \pm 2.9^{**}$	167.6
Abdominal volume, cm	14.00 ± 0.46	14.50 ± 0.56	103.6	$15.57 \pm 0.68^{**}$	111.2

Note: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ probability of differences compared to control within one line of the table according to the results of comparison using the ANOVA with Bonferroni correction.

supplemented with *I. helenium* rhizomes (103.6% of the control group, Table 1).

The rhizomes of *I. helenium* caused an increase of up to 138.3% of the control group in the relative weight of the stomach (Table 2). A significant decrease in the relative weight of the thymus to 42.7% of the control group was observed when animals were fed

M. chamomilla inflorescences (Table 2). The relative weight of other organs did not change statistically significantly compared to the control group.

The rhizomes of *I. helenium* in animals' diet increased the blood total protein concentration to 117.0% (mainly due to globulins, which reached a level of 123.6% of the control group, Table 3). *I. helenium*

Table 2. Change in relative organ mass (%) of male rats under the influence of *Inula helenium* L. and *Matricaria chamomilla* L. addition to their diet ($\bar{x} \pm SD$, n = 8, duration of experiment – 30 days)

Organ	Control	<i>I. helenium</i>	<i>I. helenium</i> compared to control, %	<i>M. chamomilla</i>	<i>M. chamomilla</i> compared to control, %
Heart	0.352 \pm 0.023	0.345 \pm 0.031	98.0	0.355 \pm 0.012	101.0
Liver	4.08 \pm 0.17	4.23 \pm 0.24	103.7	4.15 \pm 0.47	101.7
Lungs	0.979 \pm 0.169	0.846 \pm 0.153	86.4	0.952 \pm 0.327	97.3
Thymus	0.285 \pm 0.046	0.210 \pm 0.076	73.7	0.122 \pm 0.065***	42.7
Spleen	0.370 \pm 0.036	0.353 \pm 0.050	95.6	0.334 \pm 0.035	90.4
Stomach	0.699 \pm 0.060	0.966 \pm 0.126***	138.3	0.599 \pm 0.069	85.6
Small intestine	2.58 \pm 0.52	2.60 \pm 0.31	100.7	2.17 \pm 0.24	83.9
Cecum	0.509 \pm 0.176	0.458 \pm 0.124	90.0	0.392 \pm 0.084	77.0
Colon	0.374 \pm 0.085	0.388 \pm 0.065	103.8	0.315 \pm 0.050	84.3
Rectum	0.398 \pm 0.073	0.381 \pm 0.109	95.6	0.368 \pm 0.089	92.4
Right kidney	0.358 \pm 0.031	0.313 \pm 0.030	87.4	0.341 \pm 0.035	95.2
Left kidney	0.372 \pm 0.040	0.303 \pm 0.032	81.4	0.333 \pm 0.026	89.5
Brain	0.867 \pm 0.052	0.829 \pm 0.065	95.7	0.613 \pm 0.253	70.7

Note: see Table 1

Table 3. Changes in blood biochemical parameters of male rats under effect of *Inula helenium* L. and *Matricaria chamomilla* L. supplementation ($\bar{x} \pm SD$, n = 8, duration of experiment – 30 days)

Parameters	Control	<i>I. helenium</i>	<i>I. helenium</i> compared to control, %	<i>M. chamomilla</i>	<i>M. chamomilla</i> compared to control, %
Total protein, g/L	77.0 \pm 4.9	90.1 \pm 6.2**	117.0	67.6 \pm 27.7	87.8
Albumins, g/L	39.6 \pm 2.6	43.9 \pm 2.6	110.9	41.2 \pm 2.0	104.0
Globulins, g/L	37.4 \pm 3.9	46.3 \pm 4.5*	123.6	37.7 \pm 2.1	100.6
Albumin/Globulin ratio, U	1.10 \pm 0.15	0.83 \pm 0.32	75.0	1.10 \pm 0.10	100.0
Urea, mmol/L	6.84 \pm 1.02	6.06 \pm 1.24	88.6	3.97 \pm 0.40***	58.0
Creatinine, μ mol/L	63.0 \pm 4.4	65.5 \pm 7.3	104.0	69.5 \pm 5.3	110.3
AST, U/L,	186 \pm 61	183 \pm 54	98.4	171 \pm 31	91.6
ALT, U/L	131 \pm 41	186 \pm 77	142.0	111 \pm 16	84.7
Alkaline phosphatase, U/L	129 \pm 64	330 \pm 90***	255.6	525 \pm 101***	407.0
Total bilirubin, μ mol/L	6.07 \pm 1.67	6.96 \pm 1.91	114.7	4.00 \pm 1.42	65.9
Glucose, mmol/L	7.39 \pm 1.04	6.88 \pm 0.85	93.1	7.43 \pm 0.88	100.6
Total calcium (Ca), mmol/L	2.53 \pm 0.09	2.38 \pm 0.19	93.9	2.43 \pm 0.11	96.2
Inorganic phosphorus (P), mmol/L	3.07 \pm 0.58	2.99 \pm 0.84	97.3	2.78 \pm 0.35	90.6
Gamma-glutamyl-transferase, U/L	9.29 \pm 2.60	9.38 \pm 2.60	101.0	7.33 \pm 0.94	79.0
Cholesterol, mmol/L	1.27 \pm 0.14	1.55 \pm 0.17*	121.9	1.57 \pm 0.21*	123.2
Blood triglycerides, mmol/L	2.13 \pm 0.55	0.98 \pm 0.37***	45.8	0.65 \pm 0.11***	30.5
HDL cholesterol, mmol/L	0.650 \pm 0.128	0.575 \pm 0.348	88.5	0.212 \pm 0.047***	32.6
LDL cholesterol, mmol/L	0.517 \pm 0.290	0.733 \pm 0.305	141.6	1.460 \pm 0.158***	282.3
C-reactive protein, mg/L	12.5 \pm 5.4	19.0 \pm 10.6	151.6	8.8 \pm 1.9*	70.2
Atherogenic index of plasma	1.04 \pm 0.45	2.69 \pm 1.88	258.0	6.76 \pm 1.92***	648.9

Note: see Table 1.

rhizomes also greatly increased alkaline phosphatase activity (up to 255.6% of the control group), cholesterol concentration (up to 121.9% of the control group) and decreased triglyceride concentration (up to 45.8% of the control group). Despite the increase in the average atherogenic index value by 2.58 times (Table 3), but this change was statistically insignificant, since it was not observed in all animals in this group.

M. chamomilla inflorescences (Table 3) had little effect on blood albumins and globulins and their ratio, but reduced the blood urea and urea nitrogen concentrations in animals by up to 58.0% of the control group. Under the influence of *M. chamomilla*, alkaline phosphatase activity increased to 407.0% of the control group. However, the most dramatic changes occurred in fat metabolism: under the influence of a diet with *M. chamomilla* inflorescences supplementation (Table 5), the atherogenic index increased in male rats up to 648.9% relatively to the control group (both due to a decrease in the concentration of high-density lipoprotein cholesterol (HDL) to 32.6%, and due to an increase up to 282.3% in low-density lipoprotein cholesterol concentration (LDL)). The cholesterol concentration also significantly increased (up to 123.2% of the control group level). When *M. chamomilla* was added to the diet, the blood triglycerides concentration in animals sharply decreased – to 30.5% of the level of the control group (Table 3).

When supplementing with *I. helenium* rhizomes, we did not observe significant changes in the rats' blood cytological composition (Table 4). The addition of *M. chamomilla* inflorescences to the diet (Table 4) also

generally did not cause significant changes in complete blood count and leukogram, except for a sharp increase in the leukocytes concentration up to 162.5% of the control group level (however, the concentration of leukocytes was still within the physiological norm). It should be noted that being on a hypercaloric diet with the addition of *I. helenium* rhizomes caused a decrease in haematocrit. The number of monocytes under the *I. helenium* rhizomes influence tended to decrease, however, it was not considerable due to the significant individual indicator variability (Table 4).

The physical activity (Figure 2a) of the animals did not change significantly by the end of the experiment when fed crushed rhizomes of *I. helenium* and inflorescences of *M. chamomilla*. Under the influence of the same plants, the orientation activity of male rats did not change significantly (Figure 2b). Important changes in emotional state were also not observed during the experiment, however, in the animals group fed the diet supplemented with *I. helenium* crushed rhizomes, a tendency towards a decrease in emotional state was recorded, especially noticeable towards the end of the experiment (Figure 2c). No significant changes in the open field test were observed between the groups of rats with *I. helenium* and *M. chamomilla* in the diet and the control group at the beginning and end of the experiment (Table 5).

DISCUSSION

The use of elecampane rhizome ethanol extract (*Inula helenium* L.) in doses from 0 to 1000 mg/kg of

Table 4. Change in complete blood count and leukogram of male rats under effect supplementing with *Inula helenium* L. and *Matricaria chamomilla* L. ($\bar{x} \pm \text{SD}$, $n = 8$, duration of experiment – 30 days)

Parameter	Control	<i>I. helenium</i>	<i>I. helenium</i> compared to control, %	<i>M. chamomilla</i>	<i>M. chamomilla</i> compared to control, %
Hemoglobin, g/L	127 \pm 7	117 \pm 13	92.4	116 \pm 14	91.8
Hematocrit, %	40.5 \pm 2.7	36.5 \pm 4.3	90.2	40.6 \pm 6.8	100.3
Erythrocytes, 10 ¹² /L	6.93 \pm 0.29	6.48 \pm 0.73	93.5	6.88 \pm 0.79	99.3
Erythrocyte sedimentation rate (ESR), mm/h	1.17 \pm 0.37	1.00 \pm 0.00	85.7	1.00 \pm 0.00	85.7
Thrombocytes, 10 ⁹ /L	339 \pm 66	272 \pm 93	80.3	298 \pm 67	88.1
Leukocytes, 10 ⁹ /L	8.6 \pm 1.6	8.6 \pm 4.3	100.1	13.9 \pm 3.3***	162.5
Leukocytic formula	–	–	–	–	–
Basophils, %	0.0 \pm 0.0	0.0 \pm 0.0	–	0.0 \pm 0.0	–
Eosinophils, %	1.50 \pm 0.76	0.50 \pm 0.50	33.3	0.60 \pm 0.49	40.0
Neutrophils, %:	–	–	–	–	–
– band	1.17 \pm 0.69	1.13 \pm 0.78	96.4	0.60 \pm 0.80	51.4
– with segmented nuclei	23.0 \pm 8.2	22.8 \pm 7.3	98.9	18.6 \pm 3.2	80.9
Lymphocytes, %	68.8 \pm 8.6	72.9 \pm 7.7	105.9	71.0 \pm 8.1	103.1
Monocytes, %	5.5 \pm 1.3	2.8 \pm 2.2	50.0	5.6 \pm 2.1	101.8

Note: see Table 1.

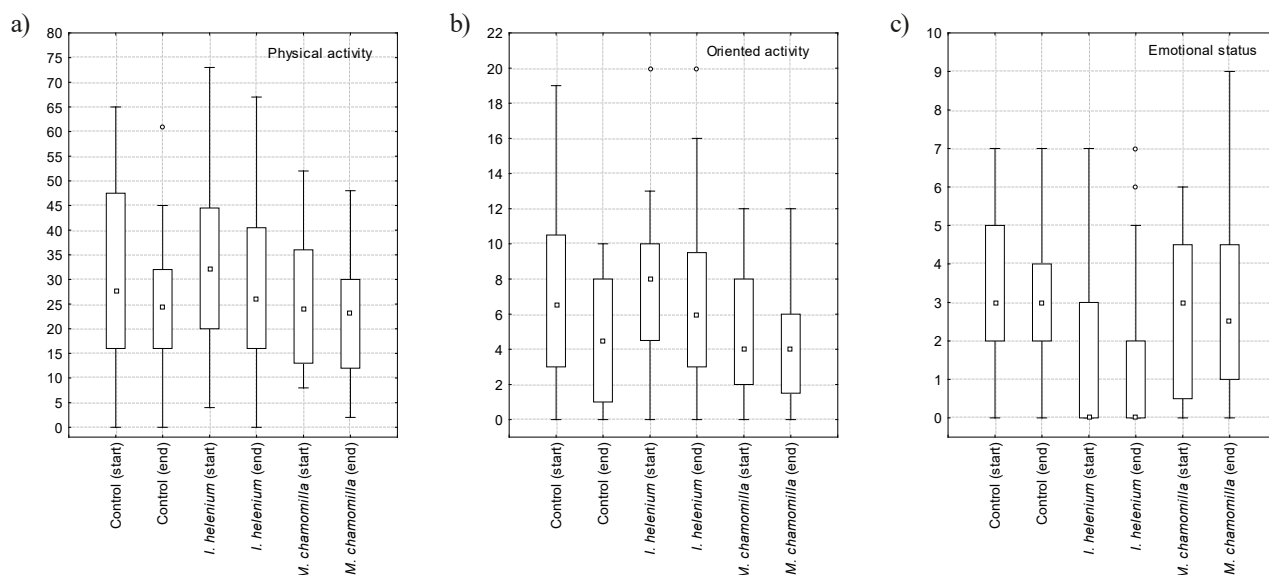


Figure 2. Changes in physical (a) and orientation activity (b), as well as emotional state (c) of male rats when crushed rhizomes of *Inula helenium* L. and inflorescences of *Matricaria chamomilla* L. were added to their diet: along the x-axis – animals' group ($n = n1 * n2 = 32$: $n1 = 8$ animals, $n2 = 4$ experiments with each animal), on a diet with excess fat and the addition of plants (the days after the start of the experiment are indicated in parentheses: 1–4th – start or 26–30 – end), along the ordinate - the absolute number of markers of this behavior type during 120 seconds of the experiment: for physical activity - the number of visited squares of the “open field”, for orientation activity - the number of racks, for emotional state - the number of grooming, defecation and urination acts; small square – median, upper and lower edges of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; Tukey's test results showed no significant ($P < 0.05$) differences between animal's groups.

Table 5. Changes in behavioral characteristics of three rats groups over a 2-minute experiment, to whose diet *Inula helenium* L. rhizomes and *Matricaria chamomilla* L. inflorescences were added ($x \pm SD$, $n = 32$, duration of the experiment was 30 days)

Характеристика	Control, 1–4th days	Control, 26–30th days	<i>I. helenium</i> , 1–4th days	<i>I. helenium</i> , 26–30th days	<i>M. chamomilla</i> , 1–4th days	<i>M. chamomilla</i> , 26–30th days
Number of visited peripheral squares	28.1 ± 18.0	24.3 ± 14.5	31.9 ± 15.0	27.3 ± 15.9	24.8 ± 12.8	22.8 ± 11.9
Number of visited central squares	1.00 ± 2.34	0.29 ± 1.04	1.00 ± 1.50	1.34 ± 2.50	0.50 ± 1.17	0.25 ± 0.70
Number of racks in peripheral squares	5.58 ± 4.53	3.79 ± 3.13	5.56 ± 2.90	4.44 ± 3.22	3.61 ± 2.67	2.86 ± 2.19
Number of racks in central squares	1.29 ± 1.43	0.71 ± 1.00	2.22 ± 1.72	2.22 ± 2.11	1.50 ± 1.86	1.29 ± 1.63
Number of grooming acts	0.583 ± 0.830	0.583 ± 0.929	0.438 ± 0.801	0.375 ± 0.907	0.714 ± 1.150	0.679 ± 1.020
Number of faecal boluses	2.25 ± 2.03	2.38 ± 1.56	1.16 ± 1.73	1.00 ± 1.74	1.93 ± 1.72	2.25 ± 1.96
Number of urination	0.333 ± 0.482	0.375 ± 0.495	0.063 ± 0.246	0.031 ± 0.177	0.071 ± 0.262	0.000 ± 0.000

Notes: No significant differences ($P < 0.05$) were found between the groups for all studied parameters according to the results of the Tukey test.

diet causes a linear increase in broiler chickens' weight gain due to an increase in the digestibility of feed dry matter, but the level of its consumption remains unchanged [1, 2]. Our studies noted a decrease in food consumption and, accordingly, a decrease in body weight in rats. This is likely due to the bitter and pungent aftertaste characteristic of the *I. helenium* rhizomes

[38], which could cause a decrease in animals' appetite. *Inula helenium* contains sesquiterpene lactones, which, depending on the dose, can cause both an anti-inflammatory and a local irritant effect [14]. This may probably explain the increase in stomach weight in rats fed the roots and rhizomes of *I. helenium* (considering that C-reactive protein level both on average in the

group and in individual animals was higher than in the control group, although changes were reliable). At the same time, the WBC number under the influence of *I. helenium* did not change, which also indicates the absence of a pronounced inflammatory process.

The RBC number, as well as hemoglobin concentration and hematocrit in the animals' blood of all groups were within the reference values [20]. At the same time, the use of *M. chamomilla* inflorescences led to a significant increase in the WBC number, which was also noted by *Alsaadi et al.* [4] in rabbits when they used an aqueous solution of chamomile inflorescences, as well as when they injected a methanolic extract of this plant into mice [21]. The noted changes were accompanied by a trend towards an increase in the concentration of lymphocytes, which was also noted by *Shwaikh et al.* [56]. It is possible that stimulation of the humoral immune system by the chamomile inflorescences active substances also caused a decrease in thymus mass in these animals, although this effect requires further study.

The number of monocytes in the rats' blood of the control group and animals that received *M. chamomilla* inflorescences did not differ significantly. Although the number of these blood cells was within the reference level [15], it was close to its upper limit. At the same time, the noted tendency towards a decrease in the concentration of monocytes under the influence of *I. helenium* rhizomes can be regarded as a positive phenomenon for health due to a possible reduction in the risk of developing atherosclerosis. The high monocytes concentration in the pigs' blood [30] correlates with their adhesion level to the endothelium of large arterial vessels, which subsequently leads to the appearance of atherosclerotic changes in the circulatory system.

Changes in the blood cellular composition and organs morphological features of rats on a high-fat diet should be assessed taking into account biochemical parameters, which are largely the first to undergo changes.

Total protein concentration was higher in rats fed *I. helenium*. Significant changes were due to a significant increase in the content of globulin protein fractions. According to *Petterino and Argentino-Storino* [43], the total protein and albumin blood concentration in rats is 58–81 and 24–41 g/L, respectively. The noted increase in globulin concentration under the *I. helenium* influence may be associated with the immunostimulating effect of phenolic compounds and allantolactone, which are part of elecampane rhizomes [23].

Transamination enzymes, AST and ALT, are considered to be biomarkers of liver damage. An increase in their blood serum concentration is characteristic of the hepatocyte cytolysis process [66].

Our studies showed high activity of both enzymes compared to reference values [66], with no significant differences between animals' groups. Obviously, the reason for this is the development of non-alcoholic fatty liver disease (NAFLD), which is characteristic of animals and humans on a high-fat diet [5, 41, 63]. NAFLD is either a cause or a consequence of the metabolic syndrome development, which is characterized by a combination of risk factors for cardiovascular disease and type 2 diabetes [48].

Cholesterol is an essential component of cell membranes, ensuring their permeability and fluidity. In addition, cholesterol is a precursor to steroid hormones, bile acids, and vitamin D [69]. Our work noted an increase in the concentration of cholesterol in the blood serum both under the *I. helenium* rhizomes and *M. chamomilla* inflorescences influence. More importantly, there was an increase in the cholesterol level that is part of low-density lipoproteins amid a decrease in the content of high-density lipoprotein cholesterol. A relationship has been established between an increase in LDL-C levels, a decrease in HDL-C and the risk development of cardiovascular diseases due to the development of vascular atherosclerosis [13, 40], which is confirmed by a significant increase in the atherogenic index in experimental animals. Reducing serum LDL cholesterol concentrations is not always prophylactically effective due to high triglyceride concentrations [9]. Therefore, the decrease we noted in the concentration of triglycerides under the influence of the elecampane and chamomile active ingredients can be regarded as a positive factor, since triglycerides and their metabolites can contribute to the development of atherosclerosis by modulating inflammation, oxidative stress and foam cells formation [68].

Some chamomile components like flavonoids have effects on the central nervous system through neurotransmitter (GABAergic, serotonergic, noradrenergic and dopaminergic) and have sedative, anxiolytic and antidepressant effects [6, 42, 61]. In our experiment, the consumption of the studied plants amid a high-fat diet did not affect the physical and orientation activity of the animals. In the group of animals that received *I. helenium* in addition to a high-fat diet, by the end of the experiment a tendency towards a decrease in emotional state was revealed, which indicates the need to continue further research about the elecampane and its active substances effects on behavioural reactions and the nervous system as a whole.

CONCLUSIONS

When rats were kept on a hypercaloric diet, a number of characteristic features were noted indicating the development of metabolic syndrome in them,

accompanied by an increase in body weight, as well as an increase in the transamination enzymes activity, indicating the manifestation of non-alcoholic fatty liver disease syndrome. The introduction of elecampane rhizomes into their menu leads to a decrease in the body weight gain intensity with a simultaneous increase in the stomach size, apparently due to the specific taste qualities and the presence of irritating substances in the plant composition itself. The active substances of elecampane rhizomes stimulate an increase in the concentration of total protein globulin fractions in the blood serum, which may be a consequence of the plant immunomodulatory effect.

The active ingredients of chamomile inflorescences have the most pronounced effect on the rats' body receiving a high-fat diet, which is manifested by an increase in rats' body weight against the background of an increase in abdominal volume, nitrogen retention in the body, and an increase in the activity of bone tissue cellular components. In addition, we have established an increase in the WBC concentration due to stimulation, presumably, of the immune system's humoral component.

A number of morphological and physiological-biochemical changes that we noted, together with a positive effect on the body of rats, indicate the possibility of increasing the risk of cardiovascular diseases amid the long-term oral use of chamomile inflorescences and elecampane rhizomes due to the development of dyslipidaemia. However, this requires additional research, given the decrease in triglyceride concentrations under the influence of herbal remedies from both plant species.

Funding

This research was funded by the Ministry of Education and Science of Ukraine within the topic "Modelling of metabolic processes and immune status of animals by drugs that are based on medicinal herbs with a high-calorie diet", grant number 0122U000975.

Conflict of interest

The authors declare no conflict of interest.

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Received: 05.10.23

Accepted: 08.11.23

Published online first: 21.11.2023

ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OF *SALVIA OFFICINALIS* GROWING IN MOROCCO

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ABSTRACT

Background. The bacterial infections treatment is complicated by antibiotic resistance. In this fact, the need for new therapeutic approaches to control bacterial infections is crucial. Therefore, discovering new antibiotics from medicinal plants, able to kill drug-resistant bacteria, is essential to saving modern medicine.

Objective. This study was to evaluate the *in vitro* antibacterial activity of *Salvia officinalis* essential oil (SoEO) growing in Morocco.

Material and methods. The essential oil was extracted by hydro distillation, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by agar dilution method. The essential oil was analyzed by Fourier-transform infrared spectroscopy (FTIR) and fractionated/purified using column chromatography followed by thin-layer chromatography (TLC).

Results. The results revealed that SoEO showed higher antimicrobial activity against *Enterococcus faecalis* and *Citrobacter freundii*. Fourier-transform infrared spectroscopy (FTIR) analysis, and purification/fractionation of SoEO, indicates that the most polar fraction F6 is the active fraction of SoEO. This finding can be explained by the existence of polar compounds in this fraction including alcohols, and phenols as thymol, eugenol, globulol, and spathulenol.

Conclusions. It can be conclude that alcohols and phenols from *Salvia officinalis* essential oil (SoEO) have promising antibacterial activity. This action can offer a great possibility of the application of SoEO in the treatment of bacterial diseases.

Key words: Gram-negative bacteria; bactericidal activity; FTIR analysis; alcohols; phenols

INTRODUCTION

Gram positive and Gram negative bacteria like *Citrobacter freundii*, *Escherichia coli*, *Pseudomonas* sp, *Staphylococcus aureus*, and *Enterococcus faecalis* are very common human pathogenic microorganisms which can trigger a variety of infectious diseases, including infections of the urinary tract, wounds, sepsis, neonatal meningitis, skin and soft tissue infections, endocarditis, osteomyelitis, lethal pneumonia, chronic bronchial infection, endodontic infection, bloodstream, surgical-site infections, and mixed bacterial infections [1, 2, 3, 4, 5, 6]. The

treatment of these bacterial infections is complicated by antibiotic resistance. For instance, this phenomenon kills at least 700 000 people each year. Within 30 years, it is predicted to kill 10 000 000 per year, and estimated to become the greatest challenge in healthcare by 2050 [7]. Indeed, *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus* are frequently resistant to several antibiotics, and they were published in the “critical and high” category of the WHO’s priority pathogens list for research and development of new antibiotics including *P. aeruginosa* carbapenem-resistant, *E. coli* carbapenem-resistant, and *S. aureus* methicillin-resistant, and vancomycin-resistant [8].

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

Moreover, It is worth mentioning that pathogenic *E. faecalis* has acquired extensive antibiotic resistance traits including resistance to “last-resort” antibiotics such as vancomycin, daptomycin, and linezolid [9, 10]. *C. freundii* has also showed strong resistance to β -lactam antibiotics [2, 7]. Thus, the immediate need for new therapeutic approaches to control bacterial infections is crucial.

The use of EOs may constitute an alternative solution to fight against multidrug-resistant bacteria. Many studies have focused on active substances from EOs, which contains natural bioactive substances used as alternative medicines, especially for their strong antibacterial activities [11]. One of them is the EOs obtained from *Salvia officinalis*, which has attracted the attention of microbiologists due to their widespread use against pathogenic bacteria [12].

Salvia officinalis, known in Morocco as “Salmya”, has been used since ancient times for medicinal and culinary use. Currently, the essential oil of *S. officinalis*, have shown several chemical and biological activities including anti-proliferative, antibacterial, anti-inflammatory, antioxidant, antiviral, and insecticidal activities [13, 14, 15, 16].

This study aims to investigate the antibacterial activity of *S. officinalis* essential oil (SoEO) against Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*), and Gram negative bacteria (*Citrobacter freundii*, *Escherichia coli*, *Pseudomonas* sp), it also aims to determine the chemical composition of active fraction of SoEO.

MATERIAL AND METHODS

Plants material

The fresh samples of *Salvia officinalis* were harvested in winter of 2020, at 23:00 nighttime, in El Jadida city in the Kingdom of Morocco, and located at the latitude of 33° 14' 0.0024'' N, and the longitude of 8° 30' 0.0000'' W). Then the leaves were removed and dried in a drying oven at 47°C, and stored in the shade prior to use.

Essential oil extraction

The extraction was carried out by hydrodistillation using a Clevenger-type apparatus, alongside samples of 120 g of Sage leaves dried. The crushed leaves were placed in a 500 ml flask which contains 300 ml of distilled water. The flask was attached to Clevenger apparatus for 4 hours. At the end of hydro-distillation, the pure essential oil was collected and stored in opaque glass bottles at a temperature of 4°C.

Antibacterial assay

Test microorganisms

Five bacteria species obtained from Collection of the Pasteur Institute in Paris (CIP) and American Type Culture Collection (ATCC) were used as the antimicrobial strains: *Citrobacter freundii* ATCC8090, *Escherichia coli* CIP54127, *Pseudomonas* sp ATCC 10145, *Enterococcus faecalis* ATCC19433, *Staphylococcus aureus* CIP 209. The bacterial strains were maintained on the *Mueller-Hinton* agar medium.

Disc diffusion method

Antibacterial activity was examined by the agar disc diffusion method [17]. The bacteria were first grown on *Mueller-Hinton* plates at 37 °C for 18–24 h prior to inoculation onto the gelose of *Mueller-Hinton* (MH). Then, the inoculums of bacteria were prepared from colonies in exponential growth phase at the concentration of 106 UFC / ml.

Petri dishes containing *Mueller-Hinton* (MHA) agar were inoculated with bacterial inoculums. The disc of *Whatman* paper of 6 mm in diameter was impregnated by essential oil EO) (20 μ l /disc). The Petri dishes were placed at 4 °C for 2–3 h and then incubated at 37 °C for 24–48 h. Antibacterial activity was evaluated by measuring the inhibition zone diameter after 48 h of incubation at 37 °C. The positive control was the antibiotic Ampicillin 30 μ g. Each experiment was conducted in triplicate and the mean zone diameter value was recorded.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of SoEO was performed out by micro-dilution method using the resazurin test [18] according to the following steps:

1. 100 μ l of *Mueller-Hinton* liquid culture medium are poured into each well;
2. 100 μ l of essential oil diluted in Tween 80 is taken to have a concentration of 33 % (v/v);
3. Dilution series of EO were prepared in Bouillon of *Mueller-Hinton*, to obtain a concentration range between 33 and 0.065 % (v/v);
4. Each well of the plate is inoculated with 40 μ l of bacterial strain (106 UFC/ml);
5. The plates were incubated at 37 C for 24 hours in the dark;
6. 15 μ l of resazurin 0.01 % (w/v) is added to each well.

The MIC is the lowest concentration at which the color changes of the resazurin from blue to fluorescent pink.

Minimal Bactericidal Concentration (MBC)

MBC was determined by inoculating 10 μ l aliquots taken from the wells in which the resazurin coloration is not changed. After the incubation of the dishes at

37 °C for 24 to 48 hours, the square without bacterial growth corresponds to MBC value.

• Antibacterial Effect Interpretation

MBC/MIC ratio was calculated to assess the bactericidal/bacteriostatic effect on the bacterial growth. If $MBC/MIC \leq 4$, then the effect is bactericidal, and if $MBC/MIC > 4$, the effect is bacteriostatic [19]. The tests were performed in triplicate.

FTIR analysis of *S. officinalis* essential oil

The chemical composition of SoEO was analyzed by an FTIR spectrometer of the JASCO 4000 type that is equipped with a detector (TGS), and a ceramic source. Michelson interferometer was also used for IR radiation analysis. The wavelength range used was between 400 - 4000 cm^{-1} and the resolution was 2 cm^{-1} .

Fractionation and purification of *S. officinalis* essential oil

• Column chromatography

The fractionation of SoEO was carried out using a Column chromatography, with a diameter of 1.2 cm, and a length of 23 cm. The adsorbent chosen was silica with a fine particle size of 0.063 mm to 0.2 mm. The fixed phase was prepared according to the following steps:

1. Add 10 g of silica to 100 ml of a mixture of hexane/ethyl acetate (95% + 5 %).
 2. Stirred the mixture was until the gel became homogeneous.
 3. Deposit the gel in the column and remove the excess solvent without drying the silica.
- The mobile fixed phase used was the hexane/ethyl acetate (95% + 5%).

• Thin-layer chromatography (TLC)

The migration tank was partially filled with the mixture hexane/ethyl acetate (95% + 5%). The samples were deposited by a glass capillary. After that, the plate was brought into contact with the mobile phase. The plates were then revealed by a UV lamp (254 nm), and by sulfuric vanillin.

After the determination of the fractions, the antibacterial activity of each fraction was tested by disc diffusion method to extract the active fraction(s). Moreover, the yield of the active fraction was calculated.

RESULTS

Antibacterial activity

The evaluation of the extraction parameters showed that extraction yield of SoEO recorded 2%. SoEO showed antibacterial activity against all the studied bacteria. This activity varies between

maximal activity against *E. faecalis* ($d = 21.33 \pm 5.69$ mm) and minimal activity against *E. coli* ($d = 10.67 \pm 0.58$ mm) (Figure 1). In the other hand, it is observed that the Gram-positive bacteria are more sensitive to the action of SoEO than the Gram-negative bacteria. It have recorded a zone of inhibition $d = 18.16$ mm. In contrast, the Gram-negative bacteria have shown more resistant to SoEO with a weaker zone of inhibition not exceeding $d = 12.78$ mm (Figure 2).

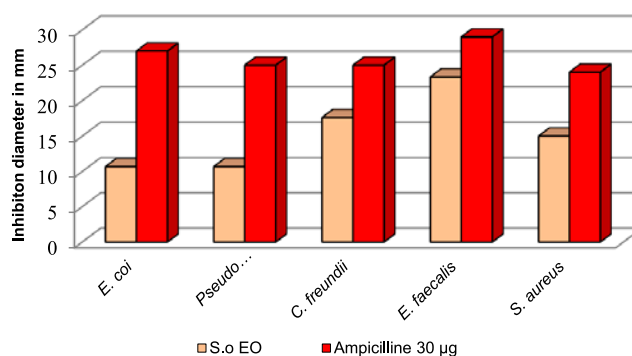


Figure 1. Inhibition diameter of *Salvia officinalis* essential oil (SoEO)

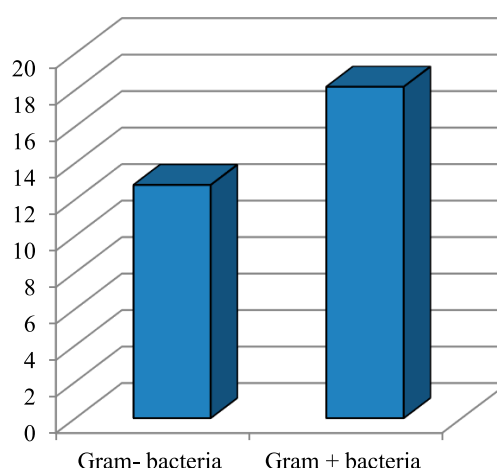


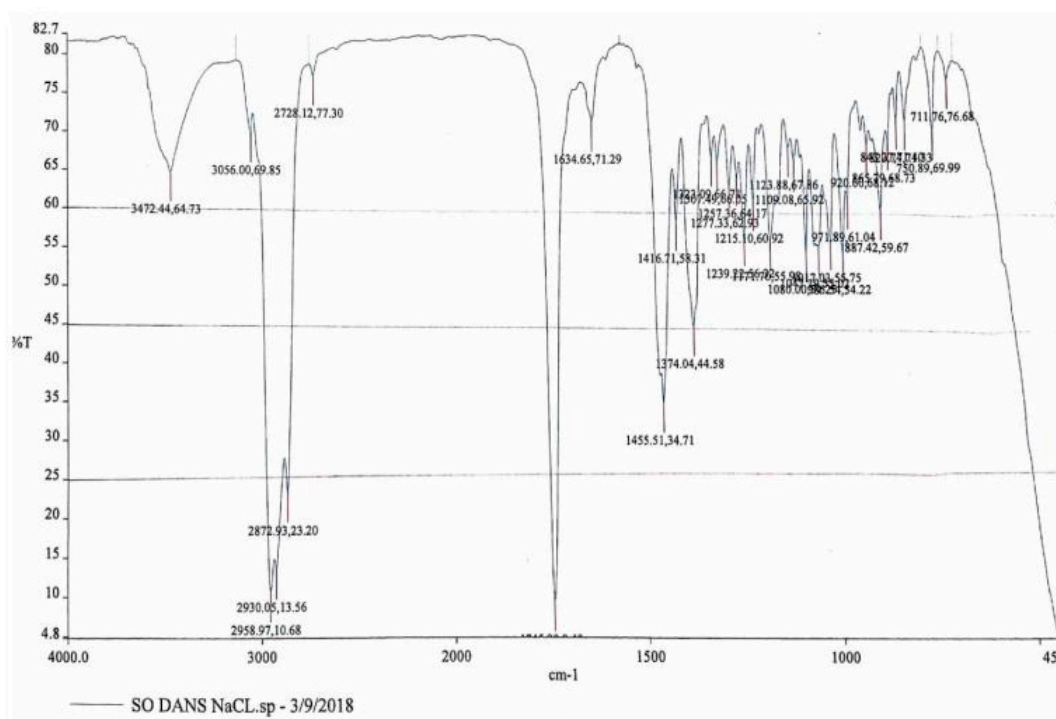
Figure 2. Antibacterial activity of SoEO against Gram-bacteria, and Gram+ bacteria

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

As indicated in Table 1, SoEO shows bactericidal activity against *Pseudomonas* sp, and *E. faecalis* ($MBC/MIC \leq 4$). However, it shows bacteriostatic activity against *E. coli*, *S. aureus*, and *C. freundii* ($MBC/MIC > 4$). The highest bactericidal activity of SoEO was observed against *Pseudomonas* sp which is a Gram-negative bacteria ($MBC/MIC = 1.00$). The weakest bactericidal effect was found against *E. faecalis* which is a Gram-positive bacteria ($MBC/MIC = 2.00$). Gram-negative bacteria were more sensitive to SoEO, with MIC values ranged from 0.13 % v/v to 4.13 % v/v, compared to Gram-positive bacteria which show MIC values between 4.13 % v/v, and 8.33 % v/v.

Table 1. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the MIC/MBC ratio of *Salvia officinalis* essential oil (SoEO)

Microorganisms		MIC (% v/v)	MBC (% v/v)	MBC/MIC		Bacteriostatic or Bactericidal
Gram -bacteria	<i>E. coli</i>	2.08	16.67	8.01	>4	Bacteriostatic
	<i>Pseudomonas sp.</i>	0.13	0.13	1.00	≤4	Bactericidal
	<i>C. freundii</i>	4.13	33.33	8.07	>4	Bacteriostatic
Gram+bacteria	<i>E. faecalis</i>	8.33	16.67	2.00	≤4	Bactericidal
	<i>S. aureus</i>	4.13	33.33	8.07	>4	Bacteriostatic

Figure 3. The FTIR spectrum of *S. officinalis* essential oilTable 2. FTIR absorption band for *Salvia officinalis* essential oil

σ (cm ⁻¹)	Standards	Intensity	Chemical bond	Functional groups	Interpretation
1634	1675-1645	Medium	C=C	Alkenes	Tricyclene, Myrcene, α -Phellandrene, α -Terpinene, Limonene, α -Copaene, γ and δ -Cadinene [20]
1715	1725-1670	Strong	C=O	Ketones	Thujone, camphor, trans-Pinocamphone, 3-octanone [21, 22]
2872	2900-2800	Strong	C-H	Aldehydes	Benzeneacetaldehyde, myrtenal [22]
1080	1150-1020	Medium to strong	C-O	Ethers	1,8-cineol [23]
1215	1300-1050	Medium	C-O	Esters	Bornyl acetate, trans-sabinyl acetate, myrtenyl acetate [21]
1455	1450-1650	Strong	C=C	Aromatic hydrocarbons	p -cymene, p -cymenene [20,21]
3056	> 3000	Medium	=C-H		
3472	3300-3600	Medium	O-H	Alcohols and phenols	Phenols: Thymol, carvacrol, eugenol [20, 21]
1239	1000-1300	Medium	C-O		Alcohols: Terpinene-4-ol, linalool, myrtenol, viridiflorol, trans-sabinol, borneol, globulol, terpineol, spathulenol [21]
1171	1200-1050	Medium	C-O		

The most sensitive Gram-negative bacterium was found to be *Pseudomonas* sp with MIC=0.13 % v/v, and the most sensitive Gram-positive bacterium was *S. aureus* with MIC value of 4.13 % v/v.

• *FTIR Spectroscopy of S. officinalis essential oil*

The Fourier Transform Infrared (FTIR) spectra for the investigated SoEO are depicted in Figure 3, and the wavelengths values (in the 400–4000 cm⁻¹ range) for all recorded peaks are presented in Table 2. The different chemical compositions of the investigated EOs samples led to obtaining major differences in the intensity of the peaks located at the following wavelengths: 1634 cm⁻¹, 1715 cm⁻¹, 2872 cm⁻¹, 1080 cm⁻¹, 1215 cm⁻¹, 1455 cm⁻¹, 3056 cm⁻¹, and 3472/1239/1171 cm⁻¹

Fractionation and purification of S. officinalis essential oil

Table 3 show that F6 fraction is active of SoEO; It is the most polar fraction (Mf = 0.2). It has a mass of 79.9 mg compared to the mass initial value of the essential oil injected into the column which is 271 mg, which gives a yield of 29.48%.

resistance to antibiotics. Indeed, this resistance can be attributed to the structure of Gram negative bacterial wall. Our results are in agreement with previous studies revealing the potential of EOs to exhibit strong antibacterial activity against other bacterial strains such as *S. mutans*, *P. fluorescens*, *A. bohemicus*, *K. marina*, *B. cereus* [14, 24].

The results of disk diffusion assay obtained in this study confirm our earlier observations of Gram negative bacteria being more resistant to the antibacterial effect of SoEO than Gram positive bacteria [25]. This finding could be explained by the fact that, at the membrane level, EOs can disrupt the permeability barrier of bacterial cell membrane structures and lead to the accompanying loss of chemiosmotic control [26]. Moreover, the SoEO can coagulate the cytoplasm and damage lipids and proteins at the cytoplasmic level, and lead to bactericidal action [27].

The FTIR analysis of SoEO (Figure 3) shows the existence of:

Two bands of medium intensity between 1050 and 1300 cm⁻¹ (1239 and 1171 cm⁻¹) associated with the C-O group of esters (Table 2), such as bornyl acetate, sabinyl acetate, and myrtenyl acetate [21].

Table 3. Fractionation of *S. officinalis* essential oil by column chromatography, and thin layer chromatography (TLC)

TLC mobile phase	Hexane/ethyl acetate					
Ratio	95%+5%					100%+00%
Fraction	F1	F2	F3	F4	F5	F6
M _r	0.92	0.78	0.64	0.5	0.42	0.2
Inhibition diameter against <i>Ent. faecalis</i>	0 mm	0 mm	0 mm	0 mm	0 mm	9.3±1.03 mm
Yield of active fraction	-	-	-	-	-	29.48%

DISCUSSION

The research of alternative and effective drugs from medicinal plants against antibiotic resistant bacteria has become a priority for the public health of people around the world. SoEO displayed highly varying antibacterial activity against tested bacterial strains. It showed a highly variable antibacterial activity, but the highest values were recorded against *E. faecalis* (d = 23.33 mm, MIC= 8.33 % v/v, MBC= 16.67 % v/v), followed by *C. freundii* (d = 17.61 mm, MIC = 4.13 % (v/v), MBC = 33.33 % (v/v) with bactericidal activity against *E. faecalis*, and only bacteriostatic effect against *C. freundii*. Moreover, it should be noted that SoEO showed a good antibacterial activity against *Pseudomonas* SP (d = 10.67 mm, MIC = 0.13% v/v, MBC = 0.13 % v/v (Figure 1), with considerable bactericidal effect. This is a very interesting result because *Pseudomonas* sp belongs to Gram negative bacteria which are characterized by their strong

The above mentioned compounds are known for their antibacterial power [28, 29].

A band of medium intensity, in the region of 1020-1150 cm⁻¹ (1080 cm⁻¹) (Table XI), indicates a C-O grouping of ethers as 1,8-cineol [23]. Several studies have shown the inhibitory effect of 1,8-Cineol, especially against *E. coli*, *M. catarrhalis*, and *S. aureus* [30, 31].

Two bands exist, one in a strong intensity (1455 cm⁻¹), and the other in a weak intensity (3056 cm⁻¹), which are associated with C=C and =C-H bonds of aromatic hydrocarbons such as p-cymene [20,21]. However, based on a recent study, the antibacterial activity of p-cymene was negligible on bacterial culturability [32].

Absorption bands are also found in the 1634, 1715 and 2872 cm⁻¹ regions, which are probably associated with the C=C bonds of alkenes, C=O of ketones and C-H of aldehydes, respectively. This observation is confirmed by the composition of SoEO in terpenes

(tricyclene, myrcene, α -terpinene, limonene), and ketones (thujone, dihydrocarvone, camphor, trans-pinocamphone, 3-octanone).

The SoEO also contains aldehydes especially acetaldehyde benzene, and myrtenal [20,21]. Indeed, the most commonly reported constituent in the literature which tested positive for antibacterial activity are camphor, thujone, and 1,8-cineole [33, 34]. Other studies revealed that high quantities of 3-octanone exhibited antibacterial activity against *E. coli* and *S. aureus* [35, 36]. The abundance of δ -cadinene, α -copaene, and caryophyllene displayed moderate antibacterial activity against *S. aureus* [37]. Moreover, trans- and cis-pinocamphone were responsible for the antibacterial activity of plant essential oil, demonstrating that they passed through the cell wall and the plasma membrane, disrupting their structure [38]. Benzene acetaldehyde, and myrtenal showed antibacterial activity against *S. aureus* [39,40]. In the other hand, Tricyclene, α -terpinene, myrcene, and limonene are the most terpenes compounds which are responsible for the antibacterial activity of several studied essential oils of the specimens against *B. subtilis*, *S. epidermidis*, *S. aureus*, and *E. coli* [41-45].

A broad band of medium intensity exists at 3472 cm⁻¹ alongside another medium band at 1239 cm⁻¹, which suggests the existence of the O-H and C-O groups of phenols, including thymol, carvacrol and eugenol [20, 21]. These compounds have demonstrated high antibacterial potential [46, 47].

The existence of another band at an average intensity of 1171 cm⁻¹, was associated with the C-O group of alcohols including terpinene-4-ol, linalool, myrtenol, viridiflorol, trans-Sabinol, borneol, globulol, and spathulenol [21, 23]. Indeed, the antibacterial studies focusing on these compounds have proven against several bacteria as *S. aureus*, *P. aeruginosa* and *E. coli* [48-56].

The combination of the results of the FTIR analysis and those of the purification / fractionation of SoEO allows us to conclude that the most polar compounds, which are responsible for the antibacterial activity of SoEO are the alcohols and the phenols. Indeed, the activity of alcohols and phenols from EO against Gram-negative and Gram-positive bacteria has been recently confirmed by several studies focusing on antibacterial activity of EO.

As regarding to phenols, the thymol (monoterpenoid phenol) exerts relevant antibacterial activity against *S. aureus*. These results may be justified by the hydrophobic nature and low solubility of thymol in the hydrophobic domain of the cytoplasmic membrane of bacterial cells [57]. In addition, the antimicrobial action of carvacrol provides evidence of its rapid antibacterial action. The most frequently reported mechanism of antibacterial action of carvacrol and

thymol involves the inhibition of efflux pumps, prevention in the formation and disruption of preformed biofilms, inhibition of bacterial motility, inhibition of membrane ATPases, and the disruption of bacterial membrane that leads to bacterial lysis and leakage of intracellular contents resulting in death. Thus, when carvacrol interacts with the lipid bilayer and aligns itself between fatty acid chains, it leads to the expansion and destabilization of the cytoplasmic membrane [58, 59]. Moreover, Eugenol is a phenolic monoterpenoid belonging to the phenylpropanoids class of natural products (2-Methoxy-4-(prop-2-en-1-yl) phenol). This compound demonstrated a strong antibacterial activity against *S. aureus* strain, by modifying the transmembrane electrochemical potential of the bacteria [60].

The second group of polar compound responsible for the antibacterial activity of SoEO is alcohols. Several previous studies confirm this finding, the growth of *P. aeruginosa* was inhibited by linalool and the results revealed that this monoterpene alcohol disrupted the normal morphology of the cell by the decrease of membrane potential as well as the release of nucleic acids; and the respiratory chain was also damaged [51]. Other studies show that myrtenol exhibit bactericidal activity against *S. aureus*, the results indicate that PBP2 (penicillin-binding protein 2) is a possible target for myrtenol to act against *S. aureus*. This compound interfered in bacterial cell wall synthesis and inhibited the production of major virulence factors, such as staphyloxanthin, lipase, and hemolysin. It also affected the eDNA production in *S. aureus* [53, 61].

In relation to the sesquiterpenoid viridiflorol, it have been reported that this compound has a strong antibacterial activity [62]. Its mechanism of action can be associated with its lipophilicity, which allows this compound to propagate through cell membranes and cause the death of bacteria by affecting their metabolic paths and organelles or inhibiting syntheses of DNA, RNA, proteins and polysaccharides in bacterial cells [62]. Other research has demonstrated that borneol attracts increasing attention due to its broad-spectrum antibacterial properties via membrane disruption mechanism [63]. In addition, borneol can induce drug accumulation in cells due to its interference with P-glycoprotein (Pgp) that is an efflux protein contributing of multidrug resistance to antibiotics drugs. This finding explains the synergistic effects between borneol and antibiotics [54]. On the other hand, spathulenol, the tricyclic sesquiterpene alcohol, present notable antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* [64]. Globulol which is a sesquiterpene alcohol alongside terpinene-4-ol (monoterpene alcohol) were screened to have greater inhibitory effect against *E. coli*, *P. aeruginosa* and *S.*

aureus [65, 66]. The specific mechanisms involved in the antimicrobial action of these compounds could be associated with their lipophilic character; monoterpenes are preferentially divided from an aqueous phase into bacterial membrane structures; thus causing structural and functional damage, increasing fluidity and permeability, disturbing of protein function, and inhibiting of ion transport [54].

Accordingly, it is important to note that the antimicrobial activity of active fraction was reduced after fractionation in comparison to the raw SoEO. This result suggests the possibility of synergism action between molecules presented in SoEO. Synergistic effects were found between thymol/eugenol, carvacrol/eugenol, and thymol/carvacrol. Moreover, the association between eugenol, and tetracycline indicates a potentiation of antibiotic activity, and a remarkable synergism [60].

Our promising findings provide evidence that phenols and alcohols from essential oil of *Salvia Officinalis* growing in El Jadida city of Morocco exhibit an antibacterial activity against many bacterial strains and it will be clinically valuable.

CONCLUSION

Based on our investigations, it can be concluded that alcohols and phenols from SoEO have a promising antibacterial activity, especially against *Pseudomonas* sp, *E. faecalis*, *E. faecalis*, *C. freundii*, and *S. aureus*. In addition, this contribution can offer a distinguished contribution of the application of SoEO in the field of medicines.

Conflict of interest

The authors declare no conflict of interest.

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Received: 11.09.2023

Accepted: 02.10.2023

Published online first: 09.10.2023

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ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

[ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE]

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