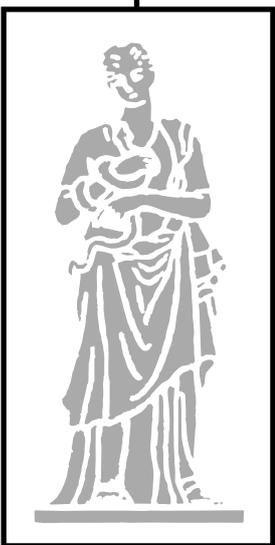


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# DIETARY INTERVENTIONS IN THE TREATMENT OF METABOLIC SYNDROME AS A CARDIOVASCULAR DISEASE RISK-INDUCING FACTOR. A REVIEW

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## ABSTRACT

Metabolic syndrome (MetS) is a concept which refers to a simultaneous occurrence of clinically significant cardiovascular disease (CVD) risk factors that increase the risk of atherosclerosis and type 2 diabetes as well as their vascular complications. The metabolic syndrome is a complex disorder, therefore its treatment should be multifactorial and intensive. MetS occurs due to a combination of genetic and environmental factors. Each of MetS components is a well-known risk factor of atherosclerosis.

Such modifications to the lifestyle as increasing physical activity, introducing a well-balanced diet and reducing the body mass are associated with reduced occurrence of MetS and its individual components.

It is necessary to implement proper dietary processes, a physical training program and pharmacological treatment. The treatment of MetS should begin with weight loss, which affects the occurrence of abdominal obesity, as well as increased physical activity, leading to an increased tissue sensitivity to insulin. It is recommended to introduce a low-energy, individually balanced diet that will lead to a 7-10% weight loss over the course of 6-12 months. Patients are also advised to quit smoking and limit the consumption of salt and alcohol.

**Key words:** *metabolic syndrome, diet, dietary interventions, obesity*

## STRESZCZENIE

Pojęcie zespołu metabolicznego (ZM) odnosi się do wspólnego występowania istotnych klinicznie czynników ryzyka chorób sercowo-naczyniowych (ChSN), zwiększających ryzyko rozwoju miażdżycy i cukrzycy typu 2 oraz ich powikłań naczyniowych. Zespół metaboliczny jest zaburzeniem złożonym, dlatego jego leczenie powinno być wieloczynnikowe oraz intensywne. Na występowanie ZM składa się skojarzenie czynników genetycznych i środowiskowych. Każda ze składowych ZM jest dobrze poznanym czynnikiem ryzyka wystąpienia miażdżycy. Modyfikacje stylu życia, takie jak zwiększenie aktywności fizycznej, wprowadzenie prawidłowo zbilansowanej diety oraz redukcja masy ciała są związane ze zmniejszeniem częstości występowania ZM oraz jego poszczególnych składowych. Niezbędne jest wdrożenie prawidłowego postępowania dietetycznego, programu treningu fizycznego oraz leczenia farmakologicznego. Leczenie ZM należy rozpocząć od redukcji masy ciała, która wpływa na występowanie otyłości brzusznej i zwiększenia aktywności fizycznej, prowadzącej do zwiększenia wrażliwości tkanek na insulinę. Zalecane jest wprowadzenie diety niskoenergetycznej, indywidualnie zbilansowanej, która doprowadzi do redukcji masy ciała o 7-10% w ciągu 6-12 miesięcy. Pacjentom zaleca się również zaprzestania palenia papierosów oraz ograniczenia spożycia soli i alkoholu.

**Słowa kluczowe:** *zespół metaboliczny, dieta, interwencja żywieniowa, otyłość*

## INTRODUCTION

Metabolic syndrome (MetS) is a concept which refers to a simultaneous occurrence of clinically significant cardiovascular disease (CVD) risk factors that increase the risk of atherosclerosis and type 2 diabetes as well as their vascular complications. Epidemiological data indicate a significant and growing spread of MetS in the United States and

Europe, including Poland. At 30-40%, incidence of MetS is particularly high among the middle-aged and elderly population [21, 38]. It should be emphasised that the incidence of MetS is dependent on the applied criteria of diagnosis, according to the established definitions of MetS and the characteristics of the study group, especially in terms of age [17].

It is estimated that MetS occurs in 15-30% of adult inhabitants in developed countries. In the United

States, MetS is believed to affect approximately 50 million Americans, which constitutes 38% of adult females and males [13]. In Europe, it is believed to occur in 25% of the adult population [11]. Based on various epidemiological studies, MetS in Poland has been estimated to occur in approximately 20-25% of the adult population. The risk of developing MetS increases with age, especially in women. Moreover, MetS increases the risk of death due to CVD by 2.2 times in men and 2.7 times in women [27].

## DEFINITIONS OF THE METABOLIC SYNDROME

Several definitions MetS along with diagnostic criteria have been proposed in the last decade. In 1998, the World Health Organization (WHO) announced a definition of MetS according to which a proper diagnosis required evidence of carbohydrate metabolism disorders [19, 28].

According to the NCEP-ATP III (The National Cholesterol Education Program Adult Treatment Panel III) definition from 2005, it was necessary to identify at least three disorders in order to diagnose MetS. In turn, the definition created by IDF (International Diabetes Federation) recognizes that the basic components of MetS consist of abdominal obesity, assessed on the basis of waist circumference measurements, and any two MetS criteria which evaluate lipid parameters, carbohydrate parameters and arterial blood pressure. The currently applied criteria for MetS diagnosis are the ones defined by IDF as well as the modified NCEP ATP III and AHA/NHLBI (American Heart Association and the National Heart, Lung, and Blood Institute) criteria from 2009 [11, 19, 28].

## MetS COMPONENTS CONTRIBUTING TO THE DEVELOPMENT OF METABOLIC CHANGES

MetS occurs due to a combination of genetic and environmental factors. Studies have shown that the basic components of MetS, such as insulin resistance with hyperinsulinaemia, obesity, arterial hypertension, atherogenic dyslipidemia, hyperglycaemia and diabetes, are often accompanied by other disorders. These include: hyperuricemia, microalbuminuria, endothelial dysfunction, prothrombotic and proinflammatory states. Each of MetS components is a well-known risk factor of atherosclerosis [11, 17].

The most important aetiological factors of MetS include: abdominal obesity and insulin resistance, which are closely related to each other and constitute the core of MetS, thus increasing cardiometabolic risk in patients. Their occurrence, and the

resulting occurrence of MetS, is driven by: genetic predispositions, incorrect lifestyle comprising a high-calorie and atherogenic diet as well as low physical activity [17].

Abdominal obesity is not a mere derivative of the BMI (Body Mass Index), therefore a measurement of waist circumference better reflects the amount of adipose tissue in the abdominal cavity [15]. Excess fat located in the abdominal cavity is particularly dangerous in terms of potential MetS development. High accumulation of intraperitoneal fat is associated with increased levels of hormones secreted by this tissue in addition to proinflammatory factors - adipocytes (interleukin 6, C-reactive protein, leptin, resistin, tumor necrosis factor alpha, plasminogen activator inhibitor-1) and reduced levels of the adiponectin hormone which prevents the development of MetS [11].

The occurrence of abdominal obesity and excessive visceral fat also leads to the activation of neurohormonal systems, especially the sympathetic nervous system and the renin-angiotensin-aldosterone system. The role of adipose tissue in the postprandial period as a lipid buffer which drains the fatty acids originating mainly from TG (triglycerides)- and VLDL (very low density lipoprotein) -rich lipoproteins, is also emphasised. Their high concentration in the blood serum, and the variations of lipase and lipid transfer proteins which accompany obesity and insulin resistance, modulate the metabolism of plasma lipoprotein, leading to the formation of highly atherogenic lipoproteins [7].

MetS is often associated with cardiovascular complications. Vascular endothelial dysfunction, which is one of the earliest disorders leading to premature atherosclerosis, develops as a result of both hyperglycaemia and insulin resistance. Insulin resistance is considered a key pathogenetic mechanism of MetS. In a study conducted by *Rodriguez et al.* [32], 579 people, aged 66 years on average, who were not affected by cardiovascular diseases, were examined in terms of their health. Elevated fasting blood glucose levels were found in approximately 16% of the patients. *Thomas et al.* [35] found that along with elevated fasting glycemia levels, the intima-media thickness (IMT), considered a subclinical marker of carotid artery atherosclerosis, also increases. Apart from obesity, hypertension and age, hyperglycaemia was the most important factor determining the dysfunction of endothelial functions in both studies. Investigating the changes in vascular reactivity in patients at risk of type 2 diabetes, other authors [5] determined that both the people with a burdened family history and those with elevated levels of fasting glycemia showed clear signs of vascular endothelial dysfunction.

One of the mechanisms explaining the influence of insulin resistance on the endothelial function lies

in the reduction of vasodilatation and the associated impaired distribution of glucose to insulin-sensitive tissues. In addition, insulin resistance leads to increased release of free fatty acids from the tissues, resulting in increased production of free oxygen radicals [29].

Lifestyle and stress have the most significant impact on rapid development of insulin resistance. Excessive consumption of food products combined with insufficient physical activity creates favourable conditions for the development of overweight and obesity, which consequently induces a decreased peripheral tissue sensitivity to insulin and impairs the endothelial function, leading to hyperglycaemia.

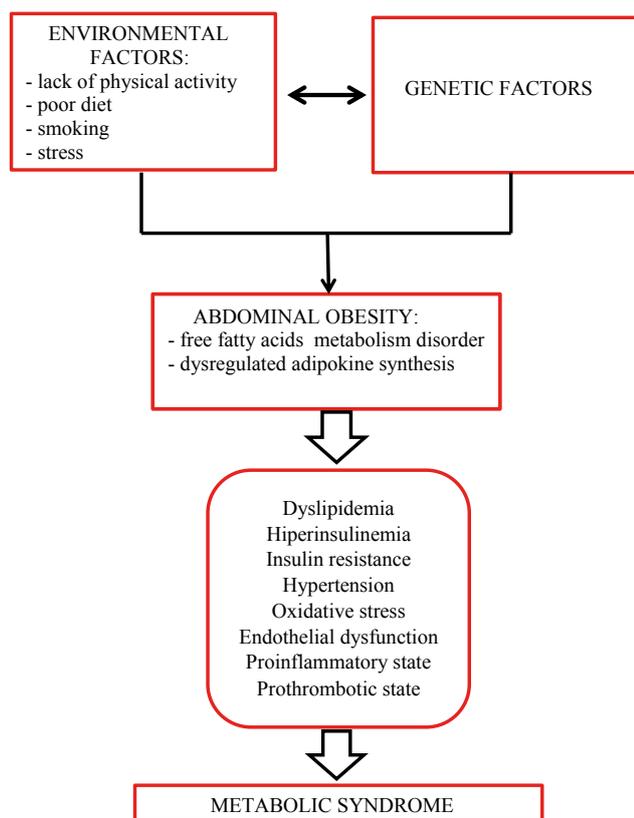


Figure 1. Causes and consequences of the metabolic syndrome (MetS) [11, 15, 17].

In the view of both the IDF and the NCEP ATP II definitions, two criteria for diagnosing MetS are associated with lipid disorders. A characteristic lipid disorder in MetS is atherogenic dyslipidemia (AD), defined as an increased level of triglycerides (TG) and a low level of HDL cholesterol in the blood serum. According to the WOBASZ study, elevated levels of TG occur in 32% men and 20% women, while decreased levels of HDL cholesterol can be observed in 20% men and 26% women [29].

Low HDL cholesterol constitutes a risk factor for CVD. The beneficial effect of HDL cholesterol involves it capturing free cholesterol from the artery wall macrophages and transporting it to the liver.

Apart from enterohepatic transport by HDL particles, it also shows has antioxidant, anti-inflammatory and anti-atherosclerotic effects [20].

European studies confirmed the concurrence of lipid and carbohydrate disorders with hypertension. Data obtained from the NATPOL PLUS 2002 study show that hypertension is the most frequent component of MetS. An important part in its development is played by increased activity of the sympathetic nervous system as well as: stimulation of the renin-angiotensin-aldosterone system, increased sodium reabsorption in the renal tubules and endothelial dysfunction, all mainly associated with obesity and insulin resistance [40].

In the NHANES III study, it was found that the most commonly observed disease connected to overweight and obesity was hypertension, the incidence of which increased with age and depended on the type of obesity [26]. Visceral tissue adipocytes are extremely active metabolically and release to the blood a number of substances which increase insulin resistance and create favourable conditions for chronic inflammatory and prothrombotic states, at the same time taking part in pathogenesis of hypertension [31, 33].

There are several key mechanism which link obesity with hypertension, one of which is increased activity of the sympathetic nervous system. Reasons for hyperactivity of the sympathetic nervous systems in obesity include: hyperleptinemia, frequent occurrences of obstructive sleep apnea (OSA), hyperinsulinaemia and insulin resistance, hyperactivity of the renin-angiotensin-aldosterone system (RAAS), increased inflammation and hiperadiponektynaemia. Fat tissue reduction significantly reduces the activity of the sympathetic nervous system in hypertension pathogenesis [12, 18, 39].

Authors of numerous epidemiological studies have shown that hypertension occurs from 1.5 to 7 times more often in patients suffering from obesity as compared to people with correct body mass [25, 36]. In addition, NT is much more common in patients with abdominal obesity. Increasing the waist circumference by 2.5 cm in women and 4.5 cm in men leads to a systolic blood pressure (SBP) increase of 1 mmHg [9]. Moreover, increasing the body weight by 10% increases the risk of hypertension by 70% [14].

The basic strategy of hypertension treatment in obese patients is to reduce the fat body mass and maintain a healthy body mass. In the TOPH I and II study [6], low-calorie diet and properly selected physical activity were introduced in patients with BMI= 33 kg/m<sup>2</sup> and prehypertension. Only 13% of those studied managed to maintain a 4.5 kg lower body mass for 3 years. In those patients who achieved the goal, blood pressure values decreased and the risk of hypertension fell by 65%.

## DIETARY INTERVENTIONS AND CHANGING THE LIFESTYLE

Such modifications to the lifestyle as increasing physical activity, introducing a well-balanced diet and reducing the body mass are associated with reduced occurrence of MetS and its individual components.

Authors of the Greek ATTICA study [30] noted that the application of the Mediterranean diet is associated with a 20% reduction of MetS risk. The Mediterranean diet is considered one of the healthiest dietary patterns. It has been shown that its use is beneficial to patients with cardiovascular diseases as well as for the prevention and treatment of: diabetes, hypertension and metabolic syndrome.

In the PREDIMED study [4], the Mediterranean diet was recommended to patients with a high risk of CVD or MetS. It was found that the use of this diet had a positive effect on reducing the occurrence of MetS, abdominal obesity, hyperglycemia and type 2 diabetes. In addition to monounsaturated fatty acids, the Mediterranean diet also consists of nutrients which reduce inflammation, oxidative stress, insuline resistance and production of pathogenic factors which lead to obesity, type 2 diabetes and MetS.

*Giuliano et al.* [16] pointed out that probably none of the individual nutrients found in a diet seems to be responsible for the association between diet and the occurrence of MetS. However, there might be a correlation between multiple components of the diet or its overall quality and the occurrence of MetS. The authors suggest that lower incidence of MetS is observed in patients whose diet is rich in fruit and vegetables, wholegrain products, low-fat dairy products and unsaturated fats.

Patients diagnosed with hypertriglyceridemia should avoid excessive use of alcohol or stop its consumption altogether due to increased synthesis of TG in the liver caused by alcohol consumption. In these patients, additional supplementation of polyunsaturated *omega-3* acids at a dose of 2-4 g/day may be considered.

Reduction of carbohydrate consumption in the treatment of MetS, especially in relation to those carbohydrates which are easily absorbed and have a high glycemic index (GI), is dictated by their hyperinsulinemia- and hypertriglyceridemia-inducing effects as well as glucose intolerance. Excessive amounts of carbohydrates in the diet also contribute to the reduction of HDL cholesterol levels in the blood serum. Decreasing the intake of carbohydrates in the treatment of MetS leads to a fats intake increase to the point of 35% of the energy from DFI while maintaining an appropriate food energy level in the diet.

A special role in MetS and CVD prevention is attributed to the essential unsaturated fatty acids

belonging to the polyunsaturated *omega-6* and *omega-3* groups. Deficiencies of these fatty acids in the diet lead to irregularities in the lipid profile of the blood serum. Oily sea fish caught in cold waters are the main source of *omega-3* fatty acids. Their effect is a result of beneficial interaction with metabolic processes, and in particular the systems which regulate biochemical transformations in cells and tissues [8].

Saturated fatty acids (SFA) on the other hand, are associated with hypercholesterolemic and prothrombotic effects. It is therefore beneficial to replace them with food products which are source of monounsaturated fatty acids (MUFA) in the diet [22].

The main lifestyle changes in patients with MetS where hypertension is one of the components include: giving up on smoking, reduction of excessive body weight, limited consumption of alcohol, increased physical activity, limited consumption of salt, increased consumption of fruit and vegetables, reduced consumption of fats in general as well as food product which are the source of *omega-6* polyunsaturated fatty acids.

The DASH (Dietary Approaches to Stop Hypertension) study [1] constituted a significant contribution to the formulation of comprehensive dietary recommendations for patients with hypertension. The study was conducted among patients with prehypertension and patients with hypertension who did not use pharmacological treatment. Over a period of 11 weeks, 3 groups of participants received diets of different compositions. Patients from the first group consumed a typical American diet of low calcium, potassium and magnesium. The second group received a similar diet, however it added fruit and vegetables. The DASH diet was used in the third group of patients. The main ingredients of this diet are fruit and vegetables, low-fat dairy products, wholegrain cereals, poultry, fish and nuts. All participants consumed a similar amount of sodium ( $\approx 3\text{g/d}$ ) and limited their alcohol consumption. The authors of the study noted a decrease in blood pressure by 5.5/2.7 mmHg in patients with prehypertension as well as those with hypertension. The anti-hypertensive effect of the DASH diet was significantly higher (11.4/5.5 mmHg) in patients with hypertension compared with other study groups.

The DASH-Sodium study additionally assessed the influence of excessive sodium consumption on blood pressure [34]. The best anti-hypertensive effect was observed in patients who used the DASH diet in combination with low sodium consumption (50 mmol/d). Apart from the anti-hypertensive effect of the DASH diet, the authors also observed its beneficial contribution to lowering the level of cholesterol in the blood serum and weight loss [3].

The DEW-IT study [23] went on to confirm the beneficial impact of the DASH diet on blood pressure. In the studied persons, a non-pharmacological intervention was introduced in addition to pharmacological treatment. The intervention involved the use of the DASH diet combined with reduced consumption of calories and sodium (100 mmol/d) and introduction of daily physical activity. As a result of dietary modification, a significant decrease of 9.5/5.3 mmHg was achieved in the arterial blood pressure.

*Azadbakht et al.* [2] evaluated the effects of a DASH-based nutritional plan on the development of risk factors for cardiovascular diseases in patients suffering from type 2 diabetes. For 8 weeks, patients received the DASH diet, while the control group received a typical diet comprising 50-60% of energy (E) from carbohydrates, 15-20% of E from proteins and <30% of E from fats, while simple sugars accounted

for <5% of E from DFI. It was noted that the DASH-based plan had a positive effect on the improvement of cardiometabolic parameters in patients affected by type II diabetes in comparison with the control group.

Recommendations regarding the amounts of individual nutrients and portions of different food groups in the DASH diet are presented in Table 1.

The type of dietary intervention applied according to the occurring MetS components is an important element of nutritional therapy. *Esposito et al.* [10] applied the Mediterranean diet in MetS patients. After two years of research, it was found that the application of the Mediterranean diet was associated with MetS subsidence in 56% of the patients. Lipid profiles of these patients improved significantly. In another study, the authors used the DASH diet for MetS therapy in the patients. After 6 months of observation, the incidence of MetS decreased by 35% in comparison with the control group.

Table 1. Nutritional recommendations in the DASH diet [24, 37]

Amount of nutrients in the DASH diet	27% of E from fats in DFI 6% of E from unsaturated fatty acids in DFI 18% of E from protein in DFI 55% of E from carbohydrates in DFI *Dietary cholesterol 150 mg/d *Sodium 1500 - 2300 mg/d *Potassium 4700 mg/d *Calcium 1250 mg/d *Magnesium 500 mg/d *Dietary fiber 30 g/d		
Number of individual food product portions per day or week in the DASH diet according to calorie supply			
Food groups	1600 kcal	2100 kcal	3100 kcal
Grain products	6 p/d	7-8 p/d	12-13 p/d
Vegetables	3-4 p/d	4-5 p/d	6 p/d
Fruit	4 p/d	4-5 p/d	6 p/d
Low-fat dairy products	2-3 p/d	2-3 p/d	3-4 p/d
Lean meat, poultry and fish	1-2 p/d	2 p/d	2-3 p/d
Nuts, seeds, legume seeds	3 p/week	4-5 p/week	1 p/d
Fats and oils	2 p/d	2-3 p/d	4 p/d
Sweets	0	5 p/week	2 p/d

DASH- Dietary Approaches to Stop Hypertension; E- energy; DFI- daily food intake; \*Nutrient content in a 2100 kcal; p/d - portions per day; p/week - portions per week

## SUMMARY

The prevalence of MS depends on the criteria used to diagnose the disease, in accordance with the definitions of MS and the characteristics of the study group, especially its age.

The most important etiological factors of MS are abdominal obesity and insulin resistance, which are closely related and constitute the central link of MS, increasing the cardiometabolic risk of the patient.

Excess fat located inside the abdominal cavity is particularly dangerous for the development of MS in the future. A large accumulation of intraperitoneal fat is associated with an increase in hormone levels secreted by this tissue and proinflammatory factors -

adipocytes and lowering of the hormone adiponectin, protecting against the development of MS.

Insulin resistance is considered to be the key pathogenetic mechanism of MS. One of the mechanisms explaining the effect of insulin resistance on endothelial function is the reduction of vasodilatation and the associated disruption of glucose distribution to tissues susceptible to insulin. In addition, insulin resistance leads to increased release of free fatty acids from the tissues, resulting in increased production of free oxygen radicals.

Metabolic syndrome is a complex disorder, therefore its treatment should be multifactorial and intense. It is necessary to implement proper dietary management, physical training program and

pharmacological treatment. Treatment with MS should begin with weight reduction, which affects the occurrence of abdominal obesity and increase physical activity, leading to increased sensitivity of tissues to insulin.

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## DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN MATERIALS FROM POLYSTYRENE INTENDED FOR CONTACT WITH FOOD: COMPARISON OF HS-GC/MS AND SPME-GC/MS TECHNIQUES

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### ABSTRACT

**Background.** Plastic materials intended for contact with food sometimes exhibit unfavorable organoleptic properties which is related to the presence of volatile organic compounds. These substances not only worsen organoleptic properties, but can be very harmful to humans health. For the sake of consumer safety, it is necessary to control such materials for the content of substances harmful to health, as well as the possibility of their migration to food. Therefore, there is a need to have an appropriate and verified analytical method that could be used in the routine analysis of volatile organic substances present in food contact materials.

**Objective.** In this study, the possibilities of the application of HS-GC/MS and SPME-GC/MS analytical techniques for analyses of volatile organic compounds present in polystyrene food contact materials, demonstrating disadvantageous organoleptic properties were evaluated.

**Materials and methods.** On the basis of sensory tests four types of food contact materials (plastic dishes) were selected for the study. The analytical measurement was performed by HS-GC/MS and SPME-GC/MS techniques parallel.

**Results.** In quality examinations of samples the aliphatic and aromatic, saturated and unsaturated hydrocarbons, as well as other compounds, e.g. ketones contamination was estimated. For all the samples a quantitative analysis of the content of styrene, ethylbenzene and cyclohexane was carried out. Additionally, the optimization of SPME analysis parameters was carried out. It was assumed that the optimal SPME extraction conditions for this purposes are: extraction time of 15 - 30 min, extraction temperature of 80°C, CAR/PDMS fibre.

**Conclusions.** The results of this study indicated that from two chosen analytical methods, definitely HS-GC/MS technique was more universal, as well as more comfortable and faster. Sometimes, however additional studies should be undertaken and then it is recommended to use the SPME-GC/MS technique optimized for our purposes.

**Key words:** *materials intended for contact with food, gas chromatography, mass spectrometry, SPME*

### STRESZCZENIE

**Wprowadzenie.** Opakowania i materiały z tworzyw sztucznych przeznaczone do kontaktu z żywnością niekiedy wykazują niekorzystne właściwości organoleptyczne. Jest to związane z obecnością lotnych związków organicznych. Substancje te nie tylko pogarszają właściwości organoleptyczne, ale mogą być bardzo szkodliwe dla zdrowia człowieka. Ze względu na bezpieczeństwo zdrowotne konsumenta konieczna jest kontrola takich materiałów pod kątem zawartości substancji szkodliwych dla zdrowia, a także możliwości ich migracji z wyrobu do żywności. Istnieje zatem potrzeba dysponowania odpowiednią, sprawdzoną metodą analityczną, która mogłaby być zastosowana w rutynowej analizie lotnych substancji organicznych obecnych w materiałach do kontaktu z żywnością.

**Cel badań.** W niniejszej pracy podjęto się oceny możliwości zastosowania technik HS-GC/MS i SPME-GC/MS do badania lotnych związków organicznych zawartych w wyrobach przeznaczonych do kontaktu z żywnością wykonanych z polistyrenu, a wykazujących niekorzystne właściwości organoleptyczne.

**Materiał i metody.** Na podstawie analizy sensorycznej wytypowano do badań cztery rodzaje wyrobów (naczyni z tworzyw sztucznych) przeznaczonych do kontaktu z żywnością. Badania prowadzono równolegle przy zastosowaniu dwóch technik analitycznych, a mianowicie HS-GC/MS i SPME-GC/MS.

**Wyniki.** W badaniach jakościowych stwierdzono zanieczyszczenie próbek nasyconymi i nienasyconymi węglowodorami alifatycznymi, węglowodorami aromatycznymi, a także innymi jeszcze związkami organicznymi, takimi jak np. ketony. Dla wszystkich próbek wykonano analizę ilościową zawartości styrenu, etylobenzenu i cykloheksanu w oparciu o zmierzone

krzywe kalibracyjne. Ponadto przeprowadzono optymalizację parametrów analizy techniką SPME i na tej podstawie stwierdzono, że ekstrakcję SPME należy prowadzić przez 15 do 30 min. w temperaturze 80°C stosując włókno CAR/PDMS.

**Wnioski.** Na podstawie przeprowadzonych badań wykazano, że z pośród dwóch wybranych metod analitycznych, zdecydowanie bardziej uniwersalna, a także wygodniejsza w użyciu i szybsza jest technika ze statyczną analizą fazy nadpowierzchniowej, HS-GC/MS. Niekiedy jednak może zajść konieczność przeprowadzenia dodatkowych badań i wtedy warto sięgnąć po zoptymalizowaną dla naszych celów technikę SPME-GC/MS.

**Słowa kluczowe:** *materiały do kontaktu z żywnością, chromatografia gazowa, spektrometria mas, SPME*

## INTRODUCTION

Materials which are intended to be in contact with food are all materials and articles that come into contact with food products, namely packaging, containers, kitchen equipment, dishes and cutlery. For the sake of consumer safety, it is necessary to control such materials for the content of harmful and toxic substances, as well as the possibility of their migration from material to food. Most often the test is subjected to the packaging material itself, not the food or food simulants [12, 15]. Plastic materials intended to be in contact with food, in addition, sometimes exhibit unfavorable organoleptic properties. It is related to the presence of volatile organic compounds [13]. These substances not only worsen organoleptic properties, but can be very harmful to humans. Their source in the materials and articles can be printed ink, but they can also be a residue after the production process of packaging material (monomers, plasticizers and others) [10]. An example of residual monomer contaminating packaging is styrene [8], classified as harmful, irritating to eyes, respiratory system and skin, and can cause lung damage if ingested and posing a serious risk to human health as a result of prolonged exposure [9]. Therefore, prior to the material being released for use, its sensory control and the identification of volatile substances potentially posing a risk to the consumer are extremely important.

The determination of volatile substances present in food contact material is currently conducted primarily based on gas chromatography (GC) [12], using different detection techniques. Furthermore, various techniques at the stage of taking volatile compounds from the tested material can be used. The most frequently chosen detection techniques are mass spectrometry (MS) [10, 12, 13] and flame ionization detection (FID) [14]. At the stage of taking volatile organic substances contained in the material, techniques such as static headspace (HS) analysis [1, 7], direct injection of water sample (DAI) [18], inside needle capillary adsorption trap (INCAT) [19], microextraction to the solid phase (SPME) [11, 17], and particularly, microextraction to the solid phase from the headspace (HS-SPME) [2, 10, 13], including multiple HS-SPME [4, 5] are used. The HS-SPME technique (hereafter referred to as SPME) consists of adsorption on a quartz fiber (covered with a suitable sorption material)

of volatile substances in the gas phase above the surface of the sample, which are then desorbed under the influence of high temperature directly into the gas chromatograph injector.

The aim of the study was to compare two analytical techniques, HS-GC/MS and SPME-GC/MS, in terms of their suitability for testing volatile organic compounds contained in polystyrene packaging, exhibiting unfavorable organoleptic properties. The results obtained were used to determine the optimal analytical conditions for volatile compounds found in packaging and packaging materials intended for contact with food.

## MATERIAL AND METHODS

### *Samples for testing*

Four types of packaging materials (plastic dishes) for contact with food, available on the domestic market, were selected for the study. The selection was made on the basis of sensory tests. All selected samples showed a distinct chemical odor. The test samples were finely chopped and then 2 g of the sample (with accuracy 0.1 mg) were placed into 20 ml vial and immediately sealed with the teflon-lined/silicone septum crimp cap. The samples thus prepared were directly subjected to HS-GC/MS measurements (the samples were thermostated in the headspace sampler oven for 1 hour at 80°C) or conditioned for 1 hour at 80°C in the heating block and then subjected to SPME extraction.

### *Calibration reagents and solutions*

This work uses commercially available reagents of analytical purity. All standard solutions used to measure of the calibration curves were prepared in methanol.

### *Calibration curve of styrene and ethylbenzene for HS-GC/MS measurements*

To prepare the calibration curves (Figure 1, above), standard solutions of styrene and ethylbenzene with concentrations of 304 and 336 mg/l respectively, were used. After mixing in appropriate proportions and dilution, intermediate solutions of: 20, 40, 79, 149, 249 mg/l of styrene and 4, 8, 16, 30, 50 mg/l of ethylbenzene were obtained. After injection of 150 µl into the sealed 20 ml vials, points of calibration curves were obtained with the amount of styrene and ethylbenzene in the vial: 3, 6, 12, 22.5, 37.5 µg and 0.6, 1.2, 2.4, 4.5, 7.5 µg.

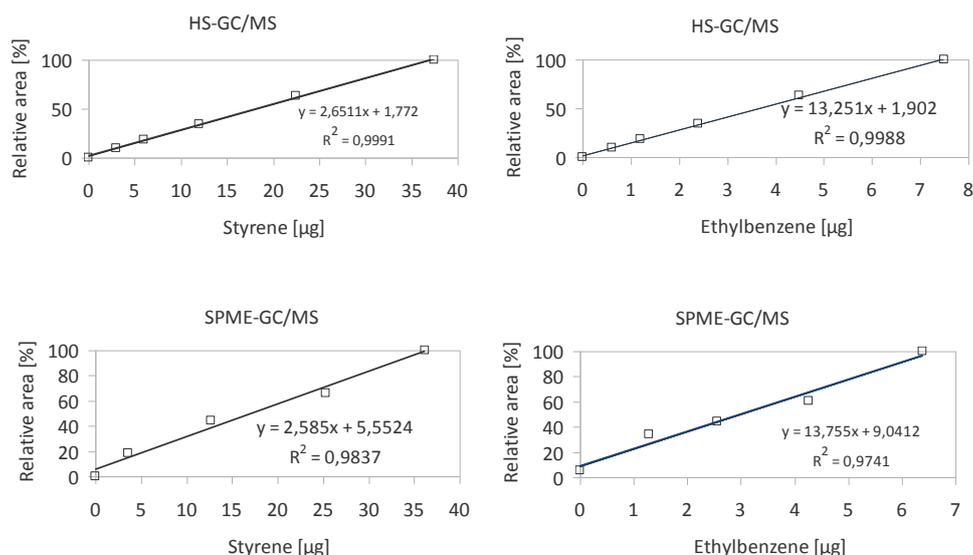


Figure 1. Calibration curves obtained for styrene and ethylbenzene in the HS-GC/MS (top) and SPME-GC/MS (bottom) measurements

#### Calibration curve of styrene and ethylbenzene for SPME-GC/MS measurements

For SPME measurements, more concentrated solutions were used for preparation of the calibration curves to avoid methanol displacement of reference substances from the microfilter fiber. Standard solutions of styrene and ethylbenzene at concentrations of 9050 and 4260 mg/l were used. After mixing in appropriate proportions and dilution, intermediate solutions with concentrations: 362, 1267, 2534, 3620 mg/l of styrene and 128, 256, 426, 639 mg/l of ethylbenzene were obtained. After injection of 10 µl into the sealed vials (20 ml capacity), the calibration solutions with the amount of styrene and ethylbenzene in the vial, respectively: 3.6, 12.7, 25.3, 36.2 µg and 1.3, 2.6, 4.3, 6.4 µg were finally prepared. In the case of styrene, a calibration curve was obtained with a regression coefficient of 0.9837 and for ethylbenzene of 0.9741 (Figure 1, bottom). These was satisfying result for as a rule, the SPME technique gives less repeatable results due to the additional variable, which is the process of fiber adsorption and fiber desorption in the apparatus.

#### SPME fibers

The SPME holder for manual sampling and fibers used were purchased from Supelco. Three different fibers were tested for our purpose: polydimethylsiloxane (PDMS; red fiber), polydimethylsiloxane/divinylbenzene (PDMS/DVB; blue fiber) and carbowax/divinylbenzene (CAR/PDMS; black fiber). All the fibers were preconditioned in the hot injector of the gas chromatograph according to supplier's manual.

#### Apparatus and measurement conditions

**HS-GC/MS technique.** Samples prepared in the test vials were placed directly into the Headspace and subjected to GC/MS analysis. Agilent Technologies 6890N gas chromatograph with MS 5973 detector and Headspace Sampler G1888 were used for analysis. Agilent Technologies HP-Plot/q capillary column (30 m, 0.32 mm, 20 µm) was used to separate the compounds. The conditions for chromatographic analysis are given in Table 1. Mass spectra were recorded in the mass range  $m/z = 30 - 300$  Da. The measurement was carried out each time in SCAN mode. The identification of compounds was performed by comparing the recorded mass spectra with the standard mass spectral libraries - Wiley7n.1 or by comparison with the reference substance.

Table 1. Conditions of chromatographic analysis

	HS-GC/MS	SPME-GC/MS	
		Method I	Method II
headspace oven	80°C, 1 hour	-	-
injector/detector temperature	240°C	240°C	240°C
carrier gas	helium	helium	helium
carrier gas flow	0.8 ml/min.	0.95 ml/min	0.95 ml/min
oven temperature	110-220°C (3°C/min), 220°C (17 min)	50°C (5 min), 50-250°C (8°C/min), 250°C (5 min)	50°C (5 min), 50-150°C (3°C/min), 150-250°C (8°C/min), 250°C (5 min)

**SPME-GC/MS technique.** The SPME-GC/MS analysis was carried out using a Hewlett Packard 5890 Series II gas chromatograph with an MS 5972 detector equipped with an HP-FFAP Agilent Technologies capillary column (30 m, 0.25 mm, 0.25  $\mu$ m). The conditions of the chromatographic analysis are shown in Table 1. The mass spectra were recorded in the mass range  $m/z = 50 - 250$  Da. The measurement was carried out each time in SCAN mode. Two similar measurement methods were used (Table 1). Method I was used for qualitative analysis and was longer (55 min) than method II (35 min) used in the quantitative analysis. The identification of compounds was performed by comparing the registered mass spectra with the standard mass spectrum library - Wiley 138.1 or by comparison with the reference substance.

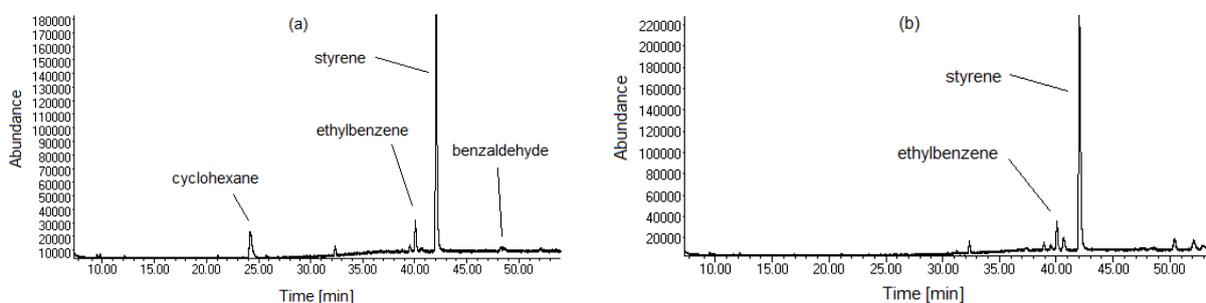


Figure 2. Total ion chromatograms (TIC) registered by HS-GC/MS: (a) PS1 sample, (b) PS4 sample.

A very similar result was obtained in the GC/MS measurement of the PS2 sample. The differences between the PS1 and PS2 dishes were rather quantitative than qualitative. The presence of styrene and ethylbenzene was also demonstrated for other packaging materials, namely PS3 and PS4. In PS2 - PS4 samples, as in the case of PS1 samples, other aromatic hydrocarbons such as toluene, dimethylbenzene isomers and (1-methylpropyl) benzene were found. Cyclohexane was found only in PS1 and PS2 samples. Figure 2b shows a chromatogram of the PS4 sample with clear peaks from ethylbenzene and styrene, but no cyclohexane signal.

The contamination of the packaging materials with residual styrene monomer, ethylbenzene, cyclohexane

## RESULTS

### Studies using the HS-GC/MS technique

**Qualitative analysis.** In the first stage of the HS-GC/MS investigation a qualitative analysis of the available samples was performed. The obtained total ion current (TIC) chromatograms were analyzed based on the mass spectral basis. Figure 2a is an exemplary chromatogram obtained by the HS-GC/MS technique for a PS1 sample. Dominant peak with retention time 42.3 min comes from styrene - residual monomer. The next largest peaks are derived from ethylbenzene (retention time, RT = 40.2 min) and cyclohexane (RT = 24.2 min). In addition, PS1 samples were found to contain various aromatic hydrocarbons, such as toluene, dimethylbenzene isomers and (1-methylpropyl)benzene.

and benzaldehyde was confirmed in the measurement of standard substances (retention times: styrene, 42.28 min, ethylbenzene, 40.17, cyclohexane, 24.23, benzaldehyde, 47.02 min).

**Quantitative analysis.** Quantitative studies of volatile impurities of selected dishes included determination of styrene, ethylbenzene and cyclohexane content. It was found that the content of the styrene monomer was the highest in PS4 samples, slightly smaller in PS3 and PS1 samples (see Table 2). The content of ethylbenzene was the highest for PS3 samples, while cyclohexane was detected only in PS1 and PS2 samples in amount of only about 0.1 - 0.2 mg/kg (Table 2).

Table 2. List of the results of quantitative analysis of styrene, ethylbenzene and cyclohexane content in the tested dishes, expressed in mg of the substance determined per kg of the tested sample, obtained by the HS-GC/MS technique

	Sample (*)	PS1 (10)	PS2 (8)	PS3 (5)	PS4 (10)
		mg/kg			
styrene	mean	10.8	10.3	14.3	15.7
	RSD%	6	5	12	6
ethylbenzene	mean	1.2	3.1	5.9	1.8
	RSD%	5	8	14	11
cyclohexane	mean	0.2	0.1		
	RSD%	17	15		

\* The number in parenthesis represents the total number of samples tested.

### Studies using the SPME-GC/MS technique

*Optimization of the solid-phase microextraction (SPME) process.* The SPME method optimization parameters include type of fiber, extraction time and extraction temperature. The optimization of SPME analysis parameters was carried out for PS1 samples, and the selected extraction conditions were then applied in the analysis of all tested products.

First task was the fiber selection. For our research purposes, three fibers were chosen, each of which could potentially be used for extraction of volatile organic compounds, namely: PDMS, PDMS/DVB, and CAR/PDMS [16]. Based on our research, we have determined that the most efficient in the headspace analysis of polystyrene packaging is CAR/PDMS fiber, as illustrated in Figure 3, showing the intensity of the SPME-GC/MS peak obtained using different fibers.

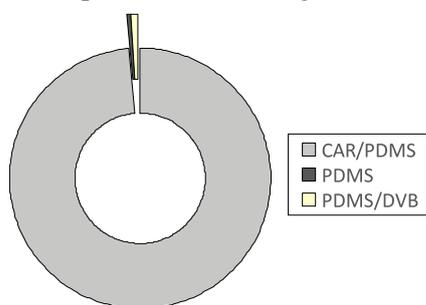


Figure 3. Results of SPME-GC/MS analysis of SP1 samples: dependence of the peak area of styrene on the type of fiber used. Extraction temperature and extraction time: 80°C, 15 min.

Due to the obtained results, further optimization of the SPME process was carried out by the use of the SPME fiber with CAR/PDMS coating. Using our previous work and literature data [3, 6, 9, 14] we selected two extraction temperatures: 60°C and 80°C. It is known that the increase in temperature causes an increase in the extraction rate due to the increase of the diffusion rate of molecules towards the

fiber, at the same time worsening the partition coefficients, as the adsorption is a strongly exothermic process [3, 6]. Additionally, the increase in the temperature can lead to the decomposition of unstable substances. Finally, based on the measurements made (Figure 4), the extraction temperature of 80°C was selected for further testing.

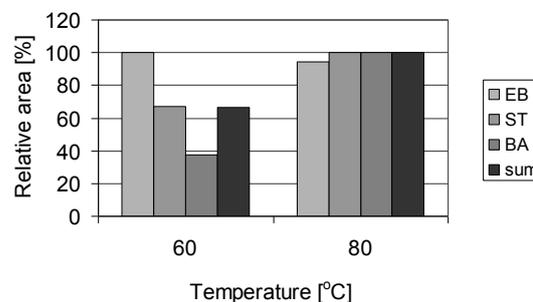


Figure 4. Results of SPME-GC/MS analysis of SP1 samples: dependence of the peak area of styrene (ST), ethylbenzene (EB) and benzaldehyde (BA) on the extraction temperature. Fiber and extraction time: CAR/PDMS, 15 min.

The final step in the process of SPME optimization was the selection of the appropriate time for extraction of volatile substances from the headspace of the samples. All measurements were performed using CAR/PDMS fiber and an extraction temperature of 80°C. Various publications on the applications of SPME technique addressing the optimization of the extraction time usually take into account time from 15 to 60 minutes, although it may also be encountered with the extraction lasting even above 2 hours [4, 10, 13]. In this work the extraction time from 5 to 120 minutes was used. The results of the measurements performed are shown in Figure 5. It was found that the maximum absorption in most cases is obtained already after 15 minutes and this extraction time was used in all quantitative determinations. In qualitative studies, the extraction time was extended to 30 minutes.

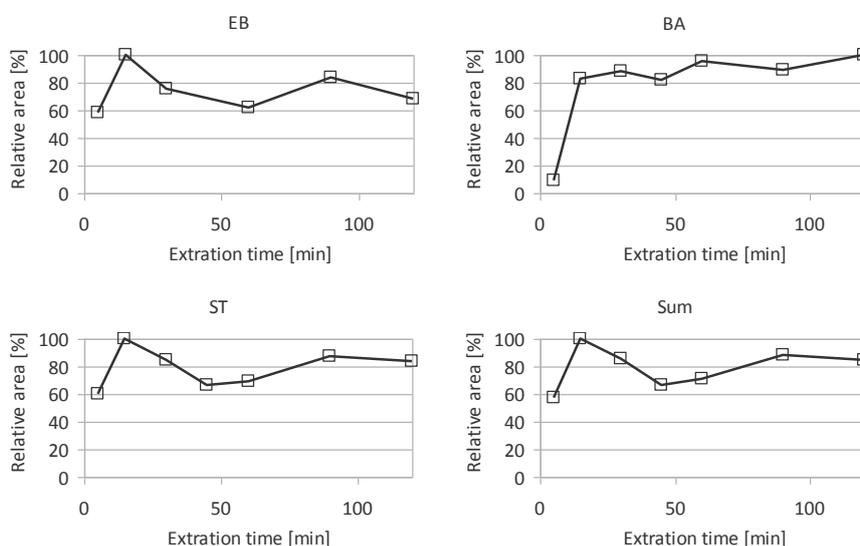


Figure 5. Results of SPME-GC/MS analysis of SP1 samples: dependence of the peak area of ethylbenzene (EB), benzaldehyde (BA) and styrene (ST) on the extraction time. Fiber and extraction temperature: CAR/PDMS, 80°C.

**Qualitative analysis.** All analyzed packaging materials were subjected to SPME extraction under optimized conditions, i.e. 30 minutes at 80°C. The measurements were made using method I (Table 1). The presence of residual styrene, ethylbenzene and benzaldehyde was found in the dishes (Figure 6), while in none of the samples tested was observed the presence of cyclohexane (RT = 2.88 min), which gave a clear peak when analyzed by the HS-GC/MS technique. The presence of benzaldehyde (RT = 21.95 min), styrene (RT = 11.04 min) and ethylbenzene (RT = 6.68 min) in the packaging materials was confirmed by chromatography of the standard samples. In addition, in PS1 - PS4 samples, we have detected less volatile aromatic hydrocarbons and their derivatives such as naphthalene, methylnaphthalene, acetophenone, and benzoic acid.

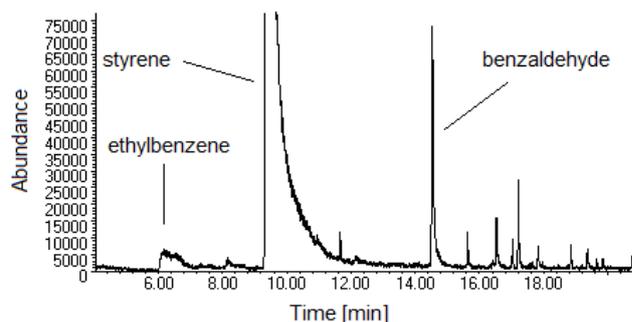


Figure 6. A fragment of the SPME-GC/MS total ion chromatogram (TIC) of a PS1 sample. Extraction temperature and extraction time: 80°C, 15 min.

Table 3. Comparison of the results of quantitative analysis of the styrene and ethylbenzene content in the tested dishes expressed in mg of the substance determined per kg of the tested sample, obtained by the SPME-GC/MS technique

	Sample (*)	PS1 (5)	PS2 (6)	PS3 (5)	PS4 (5)
		mg/kg			
styrene	mean	12.9	13.2	11.3	7.9
	RSD%	4	5	8	9
ethylbenzene	mean	0.6	1.2	5.1	
	RSD%	20	15	11	

\* The number in parenthesis represents the total number of samples tested

**Quantitative analysis.** Based on the calibration curves obtained using the SPME-GC/MS technique, quantitative determinations of styrene and ethylbenzene content were carried out. Analysis showed a similar amount of styrene in PS1 - PS3 samples, with the observed styrene content in samples PS1 and PS2 being the same in the limit of measurement error (Table 3). Unlike HS-GC/MS measurements, this time a clearly smaller amount of residual monomer was determined in PS4 samples, however, this result is not entirely reliable, as the tailing part of the peak was not included in the quantitative analysis due to the overlap of other signals. The presence of ethylbenzene was found in three dishes: PS1 - PS3. In PS1 sample it was the smallest and in PS3 sample the largest (Table 3). In the PS4 samples, ethylbenzene, which was visible in the HS-GC/MS measurements, could not be determined. Presumably, a large amount of other volatiles competed with ethylbenzene at the fiber extraction stage and, as a result, ethylbenzene was not adsorbed.

## DISCUSSION

This study compares the SPME-GC/MS and HS-GC/MS techniques with respect to their suitability for the analysis of volatile organic compounds in polystyrene packaging. The main difference between the analytical techniques chosen was the use of another

method at the stage of collecting volatiles contained in the samples. Both in SPME-GC/MS and HS-GC/MS, substances were removed from the headspace of the sample, not using solvents for this purpose. In both cases, the sample was heated for 1 hour at 80°C, however, in the SPME technique, an additional step took place, which is the extraction on the capillary fiber.

This is obvious that the SPME extraction process can theoretically reduce or increase both the sensitivity and selectivity of the assay. For this reason, it is important to choose the SPME conditions, so that they are the most favorable for the assays of all compounds from the test group. In our case, the conditions of 30 min/80°C for the qualitative determinations were considered as optimal, whereas in the quantitative determinations of styrene and ethylbenzene, the extraction time of 15 minutes was sufficient. In the case of quality determinations, a slightly longer extraction time is indicated, since, as noted, for the determination of certain substances, such as benzaldehyde (Figure 5), it would be advisable to extend the extraction time even up to 1 hour.

When comparing the SPME-GC/MS and HS-GC/MS techniques, we also noticed, that the thresholds for individual analytes could be significantly different. The possibility of competition of certain substances at the fiber extraction stage, which may lead to erroneous

determinations, should be taken into account, as can be seen from the example of PS4 sample in which ethylbenzene was determined by the HS-GC/MS method only. Certain substances, due to the unfavorably selected SPME conditions, may not be observed at all, as was in the case of cyclohexane. On the other hand, however, less volatile substances are easier to determine using the SPME technique than the static headspace method. In PS1-PS4 dishes, less volatile aromatic hydrocarbons such as naphthalene and methylnaphthalene were detected by the SPME-GC/MS method and were not recorded in the HS-GC/MS measurements.

It remains to pay attention to the labor intensity of the SPME method with manual injection compared to the automated HS technique.

## CONCLUSIONS

1. From the studies and the obtained results it could be concluded that two applied analytical methods, HS-GC/MS and SPME-GC/MS, are appropriate for the determination of volatile organic compounds present in polystyrene materials intended to come into contact with food. However, the HS-GC/MS technique is more convenient and faster in the quantitative routine analysis.
2. If the static headspace analysis will not give an explanation of unfavorable organoleptic properties, it is worth to use the alternative SPME-GC/MS technique. It was found out that the less volatile substances are easier to determine using the SPME-GC/MS method than the HS-GC/MS one.
3. Based on our research, CAR/PDMS fiber is the most efficient in the SPME analysis of polystyrene packaging. The extraction conditions of 30 min/80°C were considered as optimal but in some applications the extraction time can be reduced to 15 minutes.

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## Conflict of interest

*The authors declare no conflict of interest.*

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## ASSESSMENT OF THE NUTRITIONAL SAFETY OF NEW POTATOES IMPORTED TO POLAND USING AN ASCORBATE-NITRATE INDEX

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### ABSTRACT

**Background.** New potatoes are imported to Poland mainly from the Mediterranean countries. In climate of the Mediterranean Basin potatoes can be grown twice a year. The different environment conditions during plant growth have effect on the tuber quality.

**Objective.** The aim of the study was to assess the nutrition safety of new potatoes imported to Poland in the winter period from Mediterranean countries on the basis of the ascorbate-nitrate index.

**Material and methods.** The study material included potatoes imported from Cyprus, Egypt and Israel, purchased in the Siedlce city, from the beginning of February to the end of March 2015. Laboratory tests were performed on a total of 54 potato samples. The contents of L-ascorbic acid was determined by titration method with the 2,6-dichlorophenolindophenol according to Tillmans and nitrate by spectrophotometric method based on the Griess reaction. The ascorbate-nitrate index ( $I_{AN}$ ) as the ratio of L-ascorbic acid amount-to-nitrate amount in potato tubers was calculated.

**Results.** The L-ascorbic acid content in imported new potatoes ranged from 102.7 to 131.0 mg kg<sup>-1</sup> and nitrate content from 22.70 to 64.74 mg N-NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers. The L-ascorbic acid content in potatoes imported from Cyprus was similar to potatoes of the same cultivar imported from Egypt. The L-ascorbic acid content in potatoes imported from Israel was at a similar or higher level than in potatoes originating from Cyprus or Egypt. The lowest nitrate were in potatoes imported from Egypt. The nitrate content determined in potatoes imported from Cyprus was almost 2.5-times higher than for potatoes of the same cultivar imported from Egypt, whereas the nitrate contents in potatoes imported from Israel was 1.6-2-times higher than in potatoes originating from Egypt. The  $I_{AN}$  was from 1.68 to 5.73. The  $I_{AN}$  for the potatoes imported from Egypt was above 2.5-times higher than for potatoes of the same cultivar imported from Cyprus, and almost 2-times higher than for potatoes imported from Israel.

**Conclusion.** The nitrate content in tested potato samples did not exceed the permissible content of 200 mg NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers. The  $I_{AN}$  value calculated for all tested potato samples was higher than 1, which indicates that new potatoes imported to Poland in the winter period from Mediterranean countries are safe for human health regarding the nitrate content.

**Key words:** food safety, new potatoes, L-ascorbic acid, nitrate, ascorbate-nitrate index

### STRESZCZENIE

**Wprowadzenie.** Młode ziemniaki są importowane do Polski głównie z krajów śródziemnomorskich. W klimacie basenu Morza Śródziemnego ziemniaki można uprawiać dwa razy w roku. Różne warunki środowiska w okresie wzrostu roślin mają wpływ na jakość bulw.

**Cel badań.** Celem badań była ocena bezpieczeństwa żywnościowego młodych ziemniaków importowanych do Polski w okresie zimowym z krajów śródziemnomorskich na podstawie indeksu askorbinowo-azotanowego.

**Material i metody.** Materiał do badań stanowiły ziemniaki importowane z Cypru, Egiptu i Izraela, zakupione w Siedlcach w okresie od początku lutego do końca marca 2015 roku. Badania laboratoryjne wykonano na 54 próbkach ziemniaków. Oznaczono zawartość kwasu L-askorbinowego metodą miareczkowania za pomocą 2,6-dichlorofenolindofenolu według Tillmansa i azotanów (V) metodą spektrofotometryczną w oparciu o reakcję Griessa. Indeks askorbinowo-azotanowy ( $I_{AN}$ ) wyliczono jako stosunek ilości kwasu L-askorbinowego do ilości azotanów (V).

**Wyniki.** Zawartość kwasu L-askorbinowego w młodych ziemniakach z importu wahała się od 102,7 do 131,0 mg kg<sup>-1</sup>, a zawartość azotanów (V) od 22,70 do 64,74 mg N-NO<sub>3</sub> kg<sup>-1</sup> świeżej masy bulw. Zawartość kwasu L-askorbinowego w ziemniakach importowanych z Cypru była podobna jak w ziemniakach tej samej odmiany importowanych z Egiptu. Zawartość kwasu L-askorbinowego w ziemniakach importowanych z Izraela była podobna lub wyższa niż w ziemniakach pochodzących z Cypru lub Egiptu. Najmniej azotanów (V) zawierały ziemniaki importowane z Egiptu. Zawartość

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azotanów (V) w ziemniakach importowanych z Cypru była prawie 2,5 raza większa niż w ziemniakach tej samej odmiany importowanych z Egiptu, natomiast zawartość azotanów (V) w ziemniakach importowanych z Izraela była 1,6-2 razy większa niż w ziemniakach pochodzących z Egiptu.  $I_{AN}$  wynosił od 1,68 do 5,73.  $I_{AN}$  ziemniaków importowanych z Egiptu był ponad 2,5 raza wyższy niż ziemniaków tej samej odmiany importowanych z Cypru i prawie 2 razy wyższy niż ziemniaków importowanych z Izraela.

**Wnioski.** Zawartość azotanów (V) w badanych próbkach ziemniaków nie przekraczała dopuszczalnej zawartości 200 mg  $\text{NO}_3 \text{ kg}^{-1}$  świeżej masy bulw. Wartość  $I_{AN}$  obliczona dla wszystkich badanych próbek ziemniaków była wyższa niż 1, co wskazuje, że młode ziemniaki importowane do Polski w okresie zimowym z krajów śródziemnomorskich są bezpieczne dla zdrowia człowieka pod względem zawartości azotanów (V).

**Słowa kluczowe:** bezpieczeństwo żywności, młode ziemniaki, kwas L-askorbinowy, azotany (V), indeks askorbinowo-azotanowy

## INTRODUCTION

Potatoes provide significant amounts of beneficial bioactive components in the human diet, such as vitamin C, protein of high biological value, phenolic compounds, mineral compounds and others [3, 7, 9]. New potatoes contain higher amounts of some phytonutrients than mature potato tubers. In addition to, new potatoes are typically cooked and eaten with their skin on, thereby avoiding nutrient losses due to peeling and reducing leaching [20, 28]. Apart from nutrients, potatoes also contain bioactive non-nutrient components such as nitrates or glycoalkaloids [3, 9].

Potatoes are an important source of vitamin C (mainly L-ascorbic acid and smaller amounts of dehydroascorbic acid), contributing about 20% of the dietary intake in Europe. Ascorbic acid plays an important physiological function in the human body, assisting in the prevention of some diseases and the maintenance of good health [22]. A typical meal with 150-200 g boiled or baked potatoes (at least three medium-sized tubers) can provide about 45-50% of the recommended daily intake (RDI) of vitamin C [9].

Potatoes are not classified as a high nitrate-containing vegetable [34], however because of the large amounts consumed, they can make a significant contribution to the daily intake of nitrate in the human diet [3]. According to *Matin* et al. [23], up to 27% of the daily nitrate with food can be derived from potatoes. Nitrates are non-toxic, but their metabolites (nitrites, nitrosamine) can produce a number of health effects [5, 12, 34]. The accumulation of nitrates in potato tubers depends on environmental conditions, agricultural practices, cultivar as well as physiological maturity of the tubers [32, 37, 40]. Immaturity in potato tubers has been connected with high nitrate levels, but the relationship between nitrate concentration and maturity is not clear and differs between genotypes [13].

Early potato is a typical crop grown in most of the Mediterranean countries. New potatoes are one of the most important crops exported from the Mediterranean area to the central and northern European markets. In climate of the Mediterranean Basin potatoes are mainly grown in off-season crops: winter-spring (planting from December to January

and harvesting from March to early June) or summer-autumn (planting in early September and harvesting from November to the end of January). Cultivation is often carried out twice a year [13, 14]. The different climate conditions can have effect on the tuber quality. When tuber harvest time was delayed, the ascorbic acid content increased in winter-spring crops and decreased in summer-autumn crops, whereas the nitrate contents significantly decreased in the winter-spring crop and increased in the summer-autumn crop [13, 16].

There was a significant correlation found between the ascorbic acid content and nitrate level in potato tubers [4, 25]. The relative levels of these components may be expressed using the ascorbate-nitrate index ( $I_{AN}$ ) [18]. The  $I_{AN}$  is one of the indicator of food safety. A higher index value reflects higher food safety. Thus, vegetables can be classified into three groups based on the ascorbate-nitrate index value according to the health risk;  $<0.5$  indicates a risk,  $0.5-1.0$  is considered harmless while  $>1.0$  is absolutely safe. The ascorbate-nitrate index was significantly influenced by the species of vegetable, the cultivar and the year of cultivation. Many vegetables have an ascorbate-nitrate index over 1.0 and can be regarded as nutritionally safe [30]. It has been shown that ascorbic acid may inhibit nitrosamine synthesis [26, 35]. In recent years, there has been an increase demand of new potatoes imported from Mediterranean area, which prompts a need to estimate their nutrition safety.

The aim of the study was to assess the nutrition safety of new potatoes imported to Poland in the winter period from Mediterranean countries on the basis of the ascorbate-nitrate index.

## MATERIAL AND METHODS

The study material included imported new potatoes purchased in small local fruit and vegetable shops and in supermarkets once a week in the city of Siedlce (Poland) from the beginning of February to the end of March. Each time, three samples of each cultivar of potato, each weighing from 1 to 1.5 kg, were purchased. A total of 54 potato samples were purchased for the study. Four potato cultivars were analysed imported from three countries (Table 1).

Table 1. Characteristics of potato cultivar [6]

Cultivar	Country of origin	Potato imported from	Tuber shape	Tuber flesh colour	Cooking type*
Spunta	Netherland	Cyprus, Egypt	long to oval	light yellow	B
Nicola	German	Israel	oval to long	light yellow	A
Maris Peer	United Kingdom	Israel	oval	cream	A
Orchestra	Netherland	Israel	round-oval	light yellow	AB

\*Cooking type: A – salad, AB – salad to general purpose, B – general purpose

The potatoes purchased in February were imported from Cyprus (n=15), and in March from Cyprus (n=15), Egypt (n=9) and Israel (n=15). Only potatoes imported from Cyprus exhibited the characteristics of early potatoes, consistent with United Nations Economic Commission for Europe Standard FFV-52 concerning the marketing and commercial quality control of early and ware potatoes, i.e. were harvested before they are completely mature, marketed immediately after their harvesting, and whose skin can be easily removed without peeling [38]. The other potatoes introduced to the market as new potatoes were harvested after completely mature, had corky skins and were washed and packed.

Potatoes were washed, blotted dry and homogenized. Ten grams samples of homogenized potatoes were used for laboratory analyses. The content of L-ascorbic acid was determined using the titration method with 2,6-dichlorophenolindophenol (DCPIP) according to Tillmans after extraction with 2% oxalic acid ( $C_2H_2O_4 \cdot H_2O$ ) [31]. The content of nitrate was determined with the spectrophotometric method based on the Griess reaction after reduction

of the nitrates to nitrites with cadmium dust. The absorbance was measured at a wavelength of 538 nm [17]. All laboratory analyses were in duplicate and the mean results were expressed in mg per 1 kg of the potatoes fresh weight. The ascorbate-nitrate index as the ratio of L-ascorbic acid amount-to-nitrate amount in potato tubers was calculated [18].

The results of the study were analysed statistically using *Student's t*-test. Statistical analysis was performed for three replication of each potato cultivar. The significance of differences was verified using *Tukey's test* at  $p = 0.05$ .

## RESULTS AND DISCUSSION

The L-ascorbic acid content in tested new potatoes samples ranged from 102.7 to 131.0 mg kg<sup>-1</sup> fresh weight (Table 2). Potatoes of the same cultivar, originating from the same country, differed in terms of the L-ascorbic acid content. The L-ascorbic acid content in 'Spunta' potatoes imported from Cyprus was similar to the potatoes of this cultivar originating from Egypt.

Table 2. L-ascorbic acid content in potato tuber (mg kg<sup>-1</sup> of the fresh weight of tubers)

Potato imported from	Cultivar	No of samples	Range min – max	Mean*
Cyprus	Spunta	30	105.0-131.0	113.5 b
Egypt	Spunta	9	102.7-130.0	119.7 ab
Israel	Nicola	9	103.7-118.0	110.1 b
Israel	Maris Peer	3	117.0-118.0	117.7 ab
Israel	Orchestra	3	125.0-127.0	126.3 a

\*Means followed by the same letters do not differ significantly at  $p=0.05$

The L-ascorbic acid content in tubers of 'Nicola' and 'Maris Peer' was similar, and in tubers of 'Orchestra' was higher than in potatoes originating from Cyprus and 'Nicola' from Israel. The differences in the L-ascorbic acid content in potatoes imported from Israel could be due to the biological characteristics of plants and date of import, and number of tested samples. 'Nicola' was imported in the first half of March and 'Maris Peer' and 'Orchestra' at the end of March. Early potato production in Mediterranean countries (e.g. Egypt, Cyprus, Israel, Morocco or in

southern Italy) twice a year requires cultivars that can adapt to contrasting climate conditions. Cultivation is carried out in a winter-spring crop utilising seed-tubers of foreign cultivars (from northern European countries) and in summer-autumn crop seed-tubers produced locally [14]. 'Spunta' is the most common cultivar grown in the Mediterranean area in both the winter-spring and the summer-autumn seasons [1]. In the present study, the L-ascorbic acid content determined in 'Spunta' potatoes imported from Cyprus and Egypt was almost two times lower than that grown

in southern Italy in a region with a typical maritime-Mediterranean climate [1]. Considering its good tuber quality characteristics, this cultivar is recommended in areas with a good water supply [15].

The L-ascorbic acid content in 'Spunta' potatoes grown in Cyprus and 'Nicola' grown in Israel did not differ significantly, which was confirmed by a study carried out in southern Italy [16]. The ascorbic acid content in early potato crops in Mediterranean countries depends on the growing season, cultivar and harvest date. When harvest time is delayed, ascorbic acid content increases in winter-spring and decreases during the summer-autumn growing season [16]. This opposite trend could be caused by a reduction of sunlight intensity and day-length during potato growth period. The higher the light intensity during the plant growth period, the higher is ascorbic acid content in plant tissues [19]. Because monosaccharides are necessary for the synthesis of ascorbate acid, the content of this compound is closely related to the intensity of photosynthesis. Ascorbic acid is translocated from the leaves to the potato tubers via phloem flow [36]. Other studies have associated increased levels of

L-ascorbic acid in potatoes with higher temperatures, lower rainfall and sandy soil [10, 24] and higher L-ascorbic acid levels were found in potatoes grown in more basic soil [2]. In conditions of southern Italy, the ascorbic acid contents in tubers of 'Spunta' and 'Nicola' were very similar in the winter-spring and summer-autumn growing season [16]. In the present study, the L-ascorbic-acid content in 'Nicola' potatoes was lowest with all tested cultivars, whereas Valcarcel et al. [39] reported that the L-ascorbic acid content in mature tubers of this cultivar was the highest among the sixty tested cultivars of potato grown in Ireland.

The nitrate content in tested new potatoes samples ranged from 22.70 to 64.72 mg N-NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers (Table 3) and did not exceed the permissible content of 200 g NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> of the fresh weight of tubers [38]. The nitrate content in tested potatoes samples was more differ than the L-ascorbic acid content. Accumulation of nitrate in potato tubers depends on environmental conditions, agricultural practices, cultivar properties and tuber physiological maturity [11, 32, 40]. The lowest nitrate content was determined in potatoes imported from Egypt.

Table 3. Nitrate content in potato tuber (mg N-NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers)

Potato imported from	Cultivar	No of samples	Range min – max	Mean*
Cyprus	Spunta	30	49.69-64.72	56.99 a
Egypt	Spunta	9	22.70-24.04	23.18 d
Israel	Nicola	9	35.76-38.94	37.27 c
Israel	Maris Peer	3	47.54-48.02	47.77 b
Israel	Orchestra	3	45.44-46.02	45.65 b

\*Means followed by the same letters do not differ significantly at  $p=0.05$

The nitrate content determined in 'Spunta' potatoes imported from Cyprus was almost 2.5-times higher than for tubers of the same cultivar imported from Egypt. The nitrate content in potatoes imported from Israel was 1.6-2-times higher than in potatoes imported from Egypt. The nitrate content in potatoes of the 'Maris Peer' and 'Orchestra' imported from Israel was at a similar level, and was higher, on average, by about 10 mg N-NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers compared to 'Nicola' imported from the same country. In a study carried out by other authors, the nitrate content in 'Nicola' potatoes in winter-spring crop in southern Italy amounted 670 mg NO<sub>3</sub> kg<sup>-1</sup> of the dry matter of tubers in conventional cultivation system and 440 mg NO<sub>3</sub> kg<sup>-1</sup> of the dry matter of tubers in an organic cultivation system [21]. Nitrates are accumulated in potato tubers when their uptake is greater than the possibility of the plant to its utilise in organic nitrogenous compounds. The level of nitrate determined in 'Spunta' potatoes imported from Cyprus was at a similar level than for tubers of the same cultivar cultivated in Sicily [13]. In early potato production in the Mediterranean region,

the nitrate content in tuber depends on the growing season. In general, a higher tuber nitrate content in winter-spring than in summer-autumn crop was found. This may be explained with sunlight intensity and day-length. Low intensity of sunlight cause an increase in the accumulation of nitrates due to the lower activity of nitrate reductase. By delaying potatoes harvest, nitrate contents significantly decreased in the winter-spring crop and increased in the summer-autumn crop, when the day is getting longer and shorter, respectively [13, 14]. In general, immaturity in potato tubers has been connected with high nitrate levels. The later the potatoes are harvested, the lower the nitrate contents are in tubers, but the relationship between nitrate content and tuber maturity differs between genotypes [11, 32]. In a study carried out in southern Italy, the nitrate content in 'Spunta' potatoes amounted to 173 mg NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers at 90 days after planting in the winter-spring crop and 42 mg NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers at 80 days after planting in the summer-autumn crop. Delaying potatoes harvest by 25-30 days caused decrease in the nitrate content by 64% in the

winter-spring crop and increased in the nitrate content by about 72% in the summer-autumn crop [14]. The Acceptable Daily Intake (ADI) of nitrates has been established as being 0-3.7 mg kg<sup>-1</sup> body weight per day by the European Commission's Scientific Committee on Food (CSF) [29] and the Joint Expert Committee of the Food and Agriculture (JECFA) organisation belonging to the United Nations/World Health Organization (FAO/WHO) [8]. Assuming that the daily consumption of potatoes is approximately 200 g (at last three medium-sized tubers), the nitrate intake with new potatoes ranged from 4.54 to 12.94 mg N-NO<sub>3</sub>, and did not exceed ADI for an adult person weighing 60 kg. On the other hand, the nitrate content in potatoes are significantly reduced during the culinary processes [27].

The ratio of L-ascorbic acid amount-to-nitrate amount in the tested new potatoes samples ranged from 1.68 to 5.73 (Table 4). In a study carried out by Mazurczyk and Lis [25], the ratio of Lascorbic acid

amount-to-nitrate amount for mature tubers of several of the twenty one tested potato cultivars ranged from 4.4 to 12.4. The higher content of vitamin C per unit was accompanied with the lower nitrate content by about five units. In the present study, the highest ascorbate nitrate index (I<sub>AN</sub>) value was obtained for the 'Spunta' potatoes imported from Egypt. The average I<sub>AN</sub> calculated for the 'Spunta' potatoes imported from Egypt was above 2.5 times higher than for tubers of the same cultivar imported from Cyprus. The I<sub>AN</sub> calculated for potatoes of the 'Nicola', 'Maris Peer' and 'Orchestra' imported from Israel was at the similar level, and was higher than for potatoes imported from Cyprus. The I<sub>AN</sub> value calculated for all tested potato samples was higher than 1, which indicates that the new potatoes imported to Poland in the winter period from Mediterranean countries were safe for human health regarding the nitrate content. A higher I<sub>AN</sub> value reflects a more potent action of ascorbic acid and less harmful nitrates in the plant [30, 33].

Table 4. Ascorbate-nitrate index for potato tuber

Potato imported from	Cultivar	No of samples	Range min – max	Mean*
Cyprus	Spunta	30	1.68-2.58	2.01 c
Egypt	Spunta	9	4.27-5.73	5.18 a
Israel	Nicola	9	2.90-3.03	2.95 b
Israel	Maris Peer	3	2.47-2.49	2.48 bc
Israel	Orchestra	3	2.75-2.79	2.77 b

\*Means followed by the same letters do not differ significantly at  $p=0.05$

## CONCLUSIONS

1. The L-ascorbic acid content in new potatoes imported from Cyprus was similar to potatoes of the same cultivar imported from Egypt. The L-ascorbic acid content in potatoes imported from Israel was at a similar or higher level than in potatoes originating from Cyprus or Egypt.
2. The lowest nitrate levels were in new potatoes imported from Egypt. The nitrate content determined in potatoes imported from Cyprus was almost 2.5-times higher than for potatoes of the same cultivar imported from Egypt, whereas the nitrate contents in potatoes imported from Israel was 1.6-2-times higher than in potatoes originating from Egypt.
3. The highest I<sub>AN</sub> value obtained for the potatoes imported from Egypt was above 2.5-times higher than for potatoes of the same cultivar imported from Cyprus, and almost 2-times higher than for potatoes imported from Israel.
4. The nitrate content in tested potato samples did not exceed the permissible content of 200 mg NO<sub>3</sub> kg<sup>-1</sup> of the fresh weight of tubers. The I<sub>AN</sub> value calculated for all tested potato samples was higher than 1, which indicates that new potatoes imported

to Poland in the winter period from Mediterranean countries are safe for human health regarding the nitrate content.

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## Conflict of interest

*The authors declare no conflict of interest.*

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## THE LINK BETWEEN THE CONSUMPTION OF SWEETENED BEVERAGES AND THE DEVELOPMENT OF OVERWEIGHT AND OBESITY AMONG STUDENTS OF THE UNIVERSITY OF THE WEST INDIES, ST AUGUSTINE CAMPUS IN TRINIDAD AND TOBAGO

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### ABSTRACT

**Background.** Many risk factors, which contribute to the development of overweight and/or obesity have been investigated and identified. However, one of the largest independent contributors to the obesity epidemic, which is often overlooked, is the increased consumption of sweetened beverages, particularly among the adolescents and young adults.

**Objective.** The purpose of this study was to investigate the relationship between sweetened beverage consumption and the development of overweight and obesity among the students of The University of the West Indies, St. Augustine campus in the Republic of Trinidad and Tobago.

**Material and methods.** A cross-sectional study design was used, with a non-probability sampling method. The target population were the students of the University of the West Indies, St Augustine aged  $\geq 18$  years of age. The research data were collected through the distribution of a self-administered questionnaire, which was completed by each participant while anthropometric and clinical measurements were completed by the researchers. A total of 161 questionnaires were distributed to the students of participants. Out of 161 questionnaires distributed, 137 were collected, but only 133 were fully completed, which gave a response rate of 83%. Statistical analyses were conducted using IBM SPSS Statistics 19.0 (IBM Corporation, Chicago, IL, USA).

**Results.** 97.1% of the participants stated that they consumed sweetened beverages while only 2.9% said that they did not. Based on the results generated from chi-squared statistic test, there was no association between BMI and gender, age group, ethnicity, and the frequency of the consumption of sweetened beverages and their quantities.

**Conclusion.** No association between the intakes of sweetened beverages and overweight and/or obesity was found among the participants, but a very high prevalence of the consumption of these beverages was observed in the subjects.

**Key words:** *sweetened beverages, overweight, obesity, students, anthropometrics*

### INTRODUCTION

Obesity, defined as an excess of adipose tissue when body mass index is  $>30$  kg/m<sup>2</sup>, is due to an imbalance between energy intake and energy expenditure. Obesity is a major health problem in the industrialized world and it has reached epidemic proportions globally. The World Health Organization estimates that worldwide, obesity has more than tripled since 1975. In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these, over 650 million were obese. 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese. Overall, about 13% of the world's adult populations (11% of men and 15%

of women) were obese in 2016. 41 million children under the age of 5 were overweight or obese in 2016. Over 340 million children and adolescents aged 5-19 were overweight or obese in 2016. Most of the world's populations live in countries where overweight and obesity kills more people than underweight [16].

Obesity, once developed, is difficult to reverse. This is caused not only by biological resistance to weight loss in the form of decrease in energy expenditure [6] and increase in appetite, but also because the large behavior changes needed to sustain weight loss [15] are difficult for most people to sustain in what is termed a toxic environment, that is, where food is abundant, and where little physical activity is required in daily living [10].

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Many risk factors which contribute to the development of overweight and/or obesity have been investigated and identified. However, one of the largest independent contributors to the obesity epidemic, which is often overlooked, is the increased consumption of sugar sweetened beverages (SSBs), particularly among adolescents and young adults. Although argued that sweetened beverages do not contribute to weight gain and thus the obesity epidemic, a calorie, regardless of the source has the same effect and the extra calories that these beverages add to the diet might be a significant link to overweight and obesity [1, 13]. Thus, the purpose of this study was to investigate the relationship between sweetened beverages consumption and the development of overweight and obesity among the students of the University of the West Indies, St. Augustine campus.

## MATERIAL AND METHODS

The scopes of this study were: a) to assess the frequency and quantity of the consumption of SSBs among the subjects; b) to examine the relationship between SSBs consumption and BMI; c) to compare the consumption of SSBs between males and females.

A cross-sectional study design was used, with a non-probability sampling method. The target population were the students of the University of the West Indies, St Augustine aged  $\geq 18$  years of age.

The sample size of the study was determined based on the precision of the study as follows:

$$n = Z^2 pq/e^2, \text{ where:}$$

$n$  = sample size;  $z$  = standard normal value of Z-Score which has a value of 1.96 and 2.58 at the 95% and 99% confidence level respectively;  $p$  = the proportion of the population with the attribute of interest (expressed as a decimal);  $e$  = level of precision or confidence interval. The sample size which was calculated with a margin of error of 5% came to a sum of 322 students as the target population.

The research data were collected through the distribution of a self-administered questionnaire, which was completed by each participant and anthropometric and clinical measurements, which were performed by the researcher. The questionnaire was pre-tested, where 15 questionnaires were distributed to students of The University of the West Indies, St Augustine to obtain a feedback aimed at improving the study design. After the pre-test, adjustments were made to the structure of the questions and a total of 161 questionnaires were distributed to the participants. Out of 161 questionnaires distributed, 137 were collected, but only 133 were fully completed, which gave a response rate of 83%.

Food frequency questionnaire (FFQ) for the assessment of habitual dietary intake was used. Food consumption was estimated in 2 ways: as energy intake in kilocalories and amount of beverages consumed in ounces (0.035 oz = 1.0 g). The questionnaire comprised of twenty-six (26) questions the majority of which were in close-ended format. The questions were categorized into five (5) sections: Section 1 - demographics, Section 2 - behaviours/habits, Section 3 - access/availability of beverages, Section 4 - frequency of beverage consumption and Section 5 - 24-hour recall.

After the questionnaire was completed, anthropometric and clinical measurements, including body composition with the use of bioimpedance analyzer (BIA) were conducted.

The height of each participant was taken using a stadiometer. Body mass index (BMI) and percentage body fat were computed by BIA. Waist circumference (WC) was measured to the nearest 0.5 inch (1 cm) with a stretch resistant cloth tape measure. A standardization protocol was used; observing waist as the midpoint between the lowest rib and the top of the iliac crest [14]. This procedure was repeated for all participants. The 'adult' cut-offs for high WC circumference based on classifications were 35 inches (88 cm) for females and 44 inches (102 cm) for males.

Statistical analyses were conducted using IBM SPSS Statistics 19.0 (IBM Corporation, Chicago, IL, USA). Descriptive statistics such as ANOVA were used to calculate the frequencies, percentages and mean values of data collected. Chi-squared tests, independent t-tests and regression analysis were used to compare variables and to find associations between variables. The statistical significance for all tests was  $p < 0.05$ .

## RESULTS

Table 1 shows the frequency distribution of selected demographic characteristics of the respondents. As can be seen, participants were predominantly female ( $n=100$ ; 73%); aged between 20-22 years ( $n=72$ ; 52.6%) and Afro-Trinidadian ( $n=64$ ; 46.7%).

A total of 133 students (97.1 %) said that they consumed SSBs and only 2.9% ( $n=4$ ) said that they did not. 130 respondents (94.9%) declared that they drank water at least 'Once per Week while 6 (4.1%) said they 'Rarely or Never' drank water and only 1 student (0.7 %) did not respond to this question. Figure 1 below shows the prevalence of water and SSBs consumption among the participants of the study. As seen among the beverages, the prevalence is highest for 100% fruit Juice ( $n = 113$ ; 84.3%) while energy drinks had the lowest prevalence ( $n = 20$ ; 14.7%).

Table 1. Selected demographic characteristics and anthropometrics of the respondents

Variable	n	%
<i>Gender</i>		
Male	37	27
Female	100	73
<i>Age Group</i>		
17-19	23	16.8
20-22	72	52.6
23-25	23	16.8
>25	19	13.9
<i>Ethnicity</i>		
Afro-Trinidadian	64	46.7
Indo-Trinidadian	28	20.4
Mixed	45	32.8
<i>Anthropometrics</i>		
Body weight (pounds)	145 ± 35	
BMI	24.04 ± 4.5	
Mean body fat (%)	31.34 ± 9.8	
Mean muscle mass (%)	24.92 ± 6.5	

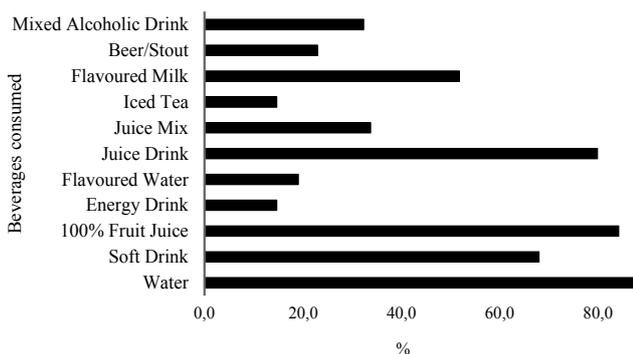


Figure 1. Prevalence of the consumption of different beverages

Independent statistical samples t-tests revealed that male students consumed more soft drinks than their female counterparts on any given occasion (p=0.025). There was no statistically significant difference in the ingestion of other beverages between males and females on any given occasion. Table 2 shows the p-values associated with comparisons of male-female frequency of consumption of different beverages. As seen, males consumed soft drinks, 100% fruit drinks, beer or stout and mixed alcoholic drinks more often than females.

Based on results generated from linear regressions, no associations between the potential risk factors and BMI were found as illustrated in Table 3.

As can be seen from Table 3 above, the consumption of SSBs was not associated with BMI. However, a trend towards statistical significance was observed between the frequency of the consumption of SSBs and BMI.

Table 2. Amount of beverages consumed stratified by gender

Variable	Gender	n	Mean	Standard Deviation	p-value
Water	Male	36	2.17	1.540	0.409
	Female	100	2.49	2.149	
Soft drink	Male	36	2.08	1.977	<b>0.025</b>
	Female	98	1.34	1.566	
100% Fruit juice	Male	36	1.94	1.492	0.269
	Female	100	1.61	1.569	
Energy drinks	Male	36	0.92	1.628	0.647
	Female	100	0.76	1.799	
Flavoured water	Male	36	0.97	1.630	0.823
	Female	100	0.89	1.974	
Juice drinks	Male	36	1.69	1.508	0.755
	Female	100	1.58	1.996	
Juice mix	Male	36	1.17	1.648	0.986
	Female	100	1.16	2.009	
Sweetened ice-tea	Male	36	0.89	1.635	0.977
	Female	100	0.90	2.130	
Flavoured milk	Male	36	1.39	1.554	0.844
	Female	100	1.31	2.205	
Beer/Stout	Male	36	1.42	1.826	0.476
	Female	100	1.14	2.045	
Mixed alcohol drinks	Male	36	1.92	2.130	0.093
	Female	100	1.24	2.036	

Table 3. Factors affecting BMI

Variable	Statistical significance
Age	0.108
Gender	0.612
Age Group	0.828
Ethnicity	0.565
Frequency of beverage consumption	0.087
Quantity of beverage consumption	0.780

## DISCUSSION

The ingestion of 100% fruit juice was found to be the highest (n = 113; 84.3%) while energy drinks have the smallest prevalence (n = 20; 14.7%). The results of the present studies are partially in concordance with the data obtained by many investigators. A multitude of demographic factors have been examined in relationship to sugar-sweetened beverages (SSB) consumption patterns. Findings suggest multifactorial factors may relate to consumption patterns, particularly among younger adults. Overweight men aged 18-24 and individuals with less than a college degree have been found more likely to be frequent SSBs consumers [11].

Gender differences were observed in the consumption trends – significantly more number of male students consumed sodas and sports drinks than

female students – which conforms to findings from studies by *Park et al.* [9]. However, these data are not in full agreement with the results obtained by *O'leary et al.* [7], who found that males and females drank similar amounts of sugary drinks, with carbonated soft drinks and fruit-based drinks most popular. Females preferred sweetened milk- and fruit-based beverages. The measured drink records showed both genders drank most of their sugary beverages at home followed by social settings. The differences between males and females in types of sugary drinks chosen and the settings in which these are consumed have important implications for developing targeted and effective interventions. Caloric carbonated soft drinks continue to represent a significant proportion of sugary drink intake among males, while young females prefer sweetened milk-based drinks, 100% fruit juice and fruit-based drinks. Fruit-based drinks, particularly 100% fruit juice, offer some nutritional value, but they have the potential to contribute significantly to energy intakes and weight gain [12].

According to *Odegaard et al.* [8], participants with greater SSBs intake were more likely to be male and with increasing frequency of SSBs intake, they observed increases in waist circumference (WC) and the proportion of visceral to subcutaneous abdominal adipose tissue, with no change in total body fat or BMI, which is not in agreement with the results of the present study, where we demonstrated that the consumption of sweetened beverages was not associated with BMI. However, a trend towards statistical significance was observed between the frequency of the consumption of sweetened beverages and BMI. This is in concordance with the study by *Forshee et al.* [2], who on the basis of a quantitative meta-analysis and qualitative review found that the association between sweetened beverages consumption and BMI was near zero, based on the current body of scientific evidence. Certain studies have had similar findings in showing no association between SSBs and weight gain in children and adolescents [2, 3]. There could be several reasons for the lack of association between SSBs consumption and adiposity in this study. A recent review of systematic literature reviews reported that all reviews that showed a direct association between SSBs consumption and adiposity in children and adolescents involved longitudinal studies and only a few involved cross-sectional studies [5]. Although a majority of reviews showed a positive association between SSBs consumption and weight gain, the authors concluded that discrepancies in results in current literature could be due to methodological differences in study design, measures or analyses [5].

The overall effect of sweetened beverages on overweight and obesity depends primarily on 2 factors: the current distribution of consumption of

these beverages and the magnitude of the effect their ingestion on BMI. It is worth mentioning that many studies, including those of a cross-sectional nature have been carried out in an attempt to find the association between SSBs consumption and obesity but still the association remains controversial.

## CONCLUSIONS

No association between the intakes of sugar sweetened beverages (SSBs) and overweight and/or obesity was found among the participants, but a very high prevalence of the consumption of these beverages was observed in the subjects. Although this study does not find a significant association between SSBs intake and adiposity, possibly due to the limited range of SSBs taken into account and the study design, we should consider the totality of evidence about the harmful effects of SSBs in youth. This study's findings can provide useful considerations while developing and implementing programs or policies that target reducing SSBs consumption among adolescents. These findings about SSBs consumption trends across gender, ethnicities and physical activity groups can help guide targeted strategies to reduce SSBs consumption among high-risk populations. Ideally, a combination of measures including curbing availability, reducing targeted marketing, increasing SSBs cost, simultaneous reduction in costs of healthier alternatives, educative and informational measures through mass media, school-based policies and interventions targeted at adolescents and parents will together prove most effective in reducing SSBs consumption.

## LIMITATIONS

For better detection of statistical significance, the sample size was determined to be 322 students, with 80% power and 5% significance level, which is considered to be a representative of all students, to whom results would have been generalized or transferred. In the time allowed, we could only obtain full data from 133 students, which made it difficult to find significant relationships.

### Conflict of interest

*The authors declare no conflict of interest.*

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## COMPARISON OF SELECTED PARAMETERS OF BODY COMPOSITION IN A GROUP OF SPORTING AND NON-SPORTING WOMEN

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### ABSTRACT

**Background.** Physical activity is bodily movement produced by skeletal muscle that requires energy expenditure and promotes health benefits. Appropriate physical activity is important in the prevention of cardiovascular disease, coronary heart disease, type 2 diabetes mellitus, obesity, metabolic syndrome, breast cancer, and others.

**Objective.** The aim of the study was to compare the body composition of the selected group of women in relation to physical activity (group of sporting women (S) versus group of non-sporting women (N-S) using an InBody 230 instrument based on the principle of bioelectric impedance.

**Material and Methods.** The group consisted of 140 women ( $n_s = 70$  vs  $n_{n-s} = 70$ ) aged 20-63 years (the average age of women doing sport was  $41.1 \pm 11.9$  and  $42.3 \pm 10.87$  for non-sporting women). Anthropometric measurements were made using the InBody 230 (Biospace Co. Ltd., Seoul, Republic of Korea). The Lookin'Body 3.0 software to process the results was used. The collected data concerning the anthropometric measurements were evaluated statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA).

**Results.** The average value of BMI (*Body Mass Index*) of sporting women was  $24.20 \pm 3.54$  kg.m<sup>-2</sup> and non-sporting women  $27.30 \pm 5.97$  kg.m<sup>-2</sup> ( $P < 0.5$ ). Average values of WHR (*Waist hip ratio*) were higher than 0.85 for both groups. Average BMR (*Basal metabolic rate*) values of women doing sport ranged from 1364-1585 kcal. The higher percentage of TBW (*Total body water*), ICW (*Intracellular water*) and ECW (*Extracellular water*) from the average body weight was achieved by the group of women doing sport, where the average TBW was 51.51% of body weight, ICW 31.93% and ECW 19.58% of body weight. Higher values of FFM (*Fat free mass*) were achieved by a group of sporting women. The average BFM in the group of women doing sport was  $20.10 \pm 6.73$  kg, in the non-sporting group  $27.60 \pm 12.73$  kg. The minimum PBF in the sporting group of women was 16.40% and a maximum of 43.30%; the minimum value in the group of women doing not sport was 19.30% and a maximum of 50.40% ( $p < 0.01$ ). The average VFA (*Visceral fat area*) in the group of women doing sport was  $86.70 \pm 28.79$  cm<sup>2</sup> and in the group of non-sporting women  $113.90 \pm 44.95$  cm<sup>2</sup> ( $p < 0.01$ ).

**Conclusions.** The results of the measurements show the positive influence of physical activity on components of body composition in all age categories. Physical activity, along with rational nutrition, should be part of a healthy lifestyle for each individual.

**Key words:** *body composition, total body water, fat free mass, body fat mass, physical activity*

### STRESZCZENIE

**Wprowadzenie.** Aktywność fizyczna to każda praca, ruch wykonany przez mięśnie szkieletowe organizmu, który charakteryzuje się ponad spoczynkowym wydatkiem energetycznym. To składowa ogólnego wydatku energetycznego, niezbędna nie tylko do utraty nadmiaru masy, ale w ogóle funkcjonowania organizmu. Właściwie dobrana aktywność fizyczna odgrywa istotną rolę w profilaktyce chorób układu sercowo - naczyniowego, cukrzycy typu 2, zespołu metabolicznego, nowotworów oraz innych schorzeń określanych jako niezakaźne choroby przewlekłe.

**Cel.** Celem badań było porównanie składu ciała wybranych grup kobiet w aspekcie aktywności fizycznej (kobiety aktywne fizycznie (S) vs kobiety nieaktywne fizycznie (NS)). Do badań wykorzystano analizator składu ciała InBody 230.

**Material i metody.** Badaną grupę stanowiło 140 kobiet (S – n=70, NS – n=70) w wieku 20 – 63 lat (średni wiek kobiet uprawiających aktywność fizyczną wynosił  $41,1 \pm 11,9$  lat i  $42,3 \pm 10,87$  – dla kobiet nieaktywnych fizycznie). Pomiar antropometryczny zostały wykonane przy użyciu InBody 230 (Biospace Co. Ltd., Seoul, Republic of Korea). Do

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opracowania wyników wykorzystano oprogramowanie Lookin'Body 3.0. Analiza statystyczna oraz opracowanie graficzne zostało przeprowadzone z wykorzystaniem programu Microsoft Office Excel 2010 (Los Angeles, CA, USA).

**Wyniki.** Średnia wartość wskaźnika masy ciała (*Body Mass Index- BMI*) kobiet aktywnych fizycznie wynosiła  $24,20 \pm 3,54$  kg/m<sup>2</sup>, a kobiet nieaktywnych fizycznie  $27,30 \pm 5,97$  kg/m<sup>2</sup> ( $p < 0,5$ ). Średnie wartości wskaźnika WHR (*Waist Hip Ratio*) były wyższe niż 0,85 w obu grupach. Wartość wskaźnika podstawowej przemiany materii (BMR) kobiet aktywnych fizycznych mieściła się w zakresie 1364 – 1585 kcal. W ocenie składu ciała kobiet uprawiających sport stwierdzono wyższe wartości wskaźników: TBW (*Total body water*), ICW (*Intracellular water*) i ECW (*Extracellular water*), gdzie średnie wartości wynosiły odpowiednio 51,51% masy ciała, 31,93% i 19,58%. Podobnie w tej samej grupie kształtował się wskaźnik FFM (*Fat free mass*) wynosił on w grupie aktywnej fizycznie  $20,10 \pm 6,73$  kg, natomiast w grupie nieaktywnej fizycznie -  $27,60 \pm 12,73$  kg. Minimalna wartość wskaźnika PBF w grupie aktywnej sportowo wynosiła 16,40% i nie przekraczała 43,30%, natomiast w grupie kobiet nieaktywnych wskaźnik ten był wyższy i wynosił 19,30%, przy wartości maksymalnej 50,40% ( $p < 0,01$ ). Parametr VFA (*Visceral fat area*) wynosił średnio  $86,70 \pm 28,79$  cm<sup>2</sup> w grupie kobiet aktywnych, natomiast w grupie nieaktywnej fizycznie -  $113,90 \pm 44,95$  cm<sup>2</sup> ( $p < 0,01$ ).

**Wnioski.** Wyniki uzyskane w ocenie parametrów antropometrycznych wskazują na pozytywny wpływ aktywności fizycznej na funkcjonowanie organizmu, niezależnie od wieku. Aktywność fizyczna wraz z racjonalnym sposobem żywienia powinna być nieodzownym elementem zdrowego stylu życia każdego człowieka.

**Słowa kluczowe:** skład ciała, zawartość wody w organizmie, masa beztłuszczowa, tkanka tłuszczowa, aktywność fizyczna

## INTRODUCTION

Body building, body dimensions and body composition are among the essential factors of motoric performance and physical fitness. The composition of the body can be understood as the proportion of individual tissues in the total body weight, with the weight of the individual segments being evaluated. Muscle, fat and bone components are involved in their weight. When evaluating the correct development of the individual, the information about the proportionality of the body is very important [32]. The human body is composed of fatty and non-fatty mass, which together create the total body weight. The fat free mass consists of water, minerals, skeleton and muscles. We also refer to fat free mass as an active body mass because it needs energy. Body composition information is important for understanding human health, body functions, and disease dynamics. Research in this field deals with methods of evaluation and descriptive body composition depending on development and aging, from changes occurring in diseases and changes manifesting in movement activity [14]. Optimal body composition should be considered as an adequate indicator of the functional status of the organism and its fitness. In order to prove the change of the somatic state, a change in the body composition is a suitable indicator, the ratio of its fractions – the fat component, the fat free mass and the health indicators [28].

The basic component of the living organism is water. Its amount in the body depends on age (with age decreasing), sex and weight. In the adult human, water is formed by 72-75% of the non-fatty matter. Most of the water is in the blood, in the muscle tissue and in the skin. Relatively little is present in bone tissue (22%) and fatty tissue (10%). The water content is therefore low in obese people, with only 45% of body

weight. The lowest water content the tooth enamel. Total body water (TBW) is the only component of the body capable of conducting electric current and is thus the basic variable measured by the bioelectric impedance method [32]. It is essential to take care of daily intake and discharge of water, as dehydration or hyperhydration can occur in the event of imbalance. Both cases lead to damage to the body and may result in the death of an individual [21]. Water is divided into two main spaces – intracellular and extracellular. Intracellular water (ICW) accounts for 40% of the adult body weight or 66% of all body water. Extracellular water (ECW) accounts for 20% of the total adult body weight.

In terms of body composition, the weight of the body can be expressed as the sum of fat and fat free mass (FFM). This includes muscle mass (60%), support and connective tissue (25%) and internal organs (15%). The water content in the fat free mass ranges from 72-74%. The total body weight does not have to change due to the increased or decreased physical activity, only the proportion of the fat free mass to the body is changed by either increasing or decreasing it. The share of FFM is about 75-80% for women, about 85% for men. For athletes, the FFM share can be higher than 90%. These data do not apply to extreme types of body building. Body fat is the most frequently observed parameter because it is an indicator of the health condition but also of the physical fitness of an individual. Its quantity can be influenced by nutrition and physical activity. The proportion of body fat mass (BFM) is sex-dependent and increases with increasing age. Boys have a lower body fat share, whereas for 18-year-old girls the upper body fat can be up to 30%. At the age of 20-24, the proportion of fat in women is approximately 25%, in males 20%. Health risk arises when the body fat content is higher than 30% for teenage girls and 25% for adolescent boys

[22]. For non-sporting women, the proportion of body fat is between 20-25%, for men it is about 15%. The ideal sporting standard is 14-18% for women and 5-10% for men [15]. For performance athletes, the average body fat fraction is lower, which is due to a higher proportion of muscle mass [22].

The aim of the work was to evaluate the body composition of the selected group of women in relation to the physical activity based on the bioelectric impedance through the InBody 230 instrument.

## MATERIAL AND METHODS

The study consisted of 140 women aged 20 to 63 years (average age of women doing sport was  $41.1 \pm 11.9$  and non-sporting women  $42.3 \pm 10.87$ ), for whom body composition measurements were made based on bioelectric impedance. BIA is proved to be a clinically valid tool for assessment of overweight and obesity [2]. Participants were divided into two basic groups based on exercise, respectively non-exercise activity. Each group consisted of 70 women. The group consisted of clients of the nutrition and consulting company Lean Enterprise Slovakia s.r.o.

Anthropometric measurements were made using the InBody 230 (Biospace Co. Ltd., Seoul, Republic of Korea). Each of the participants was informed with the measurement procedure, explained the possible risks of measuring in the case of pregnancy or having an electrical device at the heart. Before the measurement, participants were asked to excrete and refrain from drinking excessive amounts of water [41]. At the same time each participant signed informed consent for the measurement procedure and also agreed to the processing of personal data. We used the Lookin'Body 3.0 software to process the results. We evaluated the collected data from anthropometric measurements statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA). The changes in different groups were performed using *t* tests and the data were presented as mean  $\pm$  SD. The levels of statistical significance chosen for the comparisons were  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ .

Height was determined using a wall-fixed measuring device and body mass using a calibrated scale. From these BMI was calculated. The InBody device divides body composition into four components: total body water (TBW), intracellular and extracellular water, proteins, minerals and body fat mass (BFM). Intracellular water (ICW) shows the amount of water in the cell membrane. The extracellular water (ECW) expresses the amount of interspace fluid and blood. In the case of a healthy body, ICW and ECW ratios must be 3:2. Total body water, proteins and minerals create the fat free mass (FFM) in the body. FFM together with BFM create the total body weight of an individual. InBody can accurately determine how much of the total body mass is the skeletal muscle mass (SMM) and the body fat mass (BFM). Body fat mass expresses the total amount of lipids that can be extracted from fat and other cells. The fat mass can not be estimated directly by the BIA method, but is calculated by excluding fat free mass (FFM) from body weight by the formula: body fat mass = body weight – fat free mass [36]. Through the Inbody 230, we also received data such as Body Mass Index, PBF and WHR, which we evaluated the presence of overweight and obesity in the monitored groups. PBF is the percentage of body fat to body weight of the test person. The standard for PBF is 15% for males and 23% for females, while the reference values range between 10-20% for males and 18-28% for females. We also observed visceral fat area (VFA). Its excessive amount causes metabolic and cardiovascular diseases. In the examination, the visceral fat area is defined as the cross-sectional area of visceral fat found in the abdomen. If VFA is greater than 100 cm<sup>2</sup> we talk about abdominal obesity [39].

## RESULTS AND DISCUSSION

From the obtained individual values, we calculated basic statistical characteristics. We focused on the evaluation of total body water, intracellular and extracellular water in litres, and analysis of fat free mass in kg, skeletal muscle mass in kg, body fat mass in kg, and percentage of fat mass and visceral fat area in cm<sup>2</sup>. The basic descriptive characteristics of participants are shown in Table 1.

Table 1. Basic descriptive characteristics of participants in monitored groups (n = 140)

Parameters	Mean		$\pm$ SD		Min		Max		Median		Modus		Significance
	S	N-S	S	N-S	S	N-S	S	N-S	S	N-S	S	N-S	
Age (years)	41.1	42.3	11.9	10.87	20	23	63	61	38.5	43.5	33	29	0.16
Body height (cm)	165.6	167.9	5.36	7.1	154	152	180	180	165	166.5	164	164	0.4
Body weight (kg)	66.4	77.9	10.36	20.1	47.8	48.8	97.2	124.4	65.35	70.9	60	74.5	0.006
BMI (kg.m <sup>-2</sup> )	24.2	27.3	3.54	5.97	18.7	17.7	33.6	43	23.85	26.5	24.4	28.6	0.01
WHR	0.89	0.94	0.06	0.09	0.77	0.82	1.3	1.2	0.885	0.92	0.84	0.88	0.003
BMR (kcal)	1370	1446	110.49	176.95	1145	1186	1649	1867	1362.5	1374	1358	1580	0.08

Abbreviations:  $\pm$  SD – standard deviation; Min – minimum value; Max – maximum value; S – group of sporting women; N-S – group of non-sporting women; BMI – body mass index, [kg.m<sup>-2</sup>]; WHR – Waist to Hip ratio; BMR – basal metabolism

Table 2. Basic descriptive characteristics of selected parameters of participants body composition (n = 140)

Parameters	Mean		± SD		Min		Max		Median		Modus		Significance
	S	N-S	S	N-S	S	N-S	S	N-S	S	N-S	S	N-S	
TBW (l)	34.2	36.5	4.8	6.11	20.5	27.7	45	50.6	33.65	34.15	32.3	41	0.12
ICW (l)	21.2	22.6	2.96	3.85	12.7	17	27.6	31.7	20.9	21.1	22.8	21.2	0.13
ECW (l)	13	13.9	1.83	2.27	7.8	10.4	17.4	18.9	12.85	13	12.4	12.4	0.1
Proteins (kg)	9.2	9.8	1.26	1.65	5.6	7.4	12	13.6	9	9.2	9.5	9.2	0.11
ML (kg)	3.3	3.5	0.46	0.63	2	2.6	4.2	5.1	3.25	3.28	3.29	3.4	0.1
BFM (kg)	20.1	27.6	6.73	12.73	8.7	10.2	38	62.6	19.4	22.35	19.9	10.2	0.02
FFM (kg)	46.6	49.8	6.53	8.74	28.1	37.8	61.1	69.3	45.85	46.55	43.8	56	0.17
SMM (kg)	25.4	27.4	3.6	4.83	19	20.2	33.3	39.3	25.25	25.4	25.9	25.7	0.13
BCM (kg)	30.1	32.4	3.35	1.73	23.1	24.3	38.8	45.4	29.9	30.1	30.7	30.1	0.09
PBF (%)	29.6	34.2	5.99	8.21	16.4	19.3	43.3	50.4	29.8	34.55	29.8	28.9	0.01
VFA (cm <sup>2</sup> )	86.7	113.9	28.79	44.95	35.3	36.5	148.1	202.7	84.2	104.75	90.2	115.8	0.003
Degree of obesity (%)	112.8	127.1	16.47	27.71	86.9	82.4	156.5	200	111.31	121.1	111.67		0.014
Fitness score	74	69	5.15	7.13	62	50	85	82	74	71	73	72	0.0006

Abbreviations: ± SD – standard deviation; Min – minimum value; Max – maximum value; S – group of sporting women; N-S – group of non-sporting women; TBW – total body water, [l]; ICW – intracellular water, [l]; ECW – extracellular water, [l]; ML – minerals, [kg]; BFM – body fat mass, [kg]; FFM – fat free mass, [kg]; SMM – skeletal muscle mass, [kg]; BCM – body cell mass, [kg]; PBF – percentage of body fat, [%]; VFA – visceral fat area, [cm<sup>2</sup>].

The average BMI for women doing sport was  $24.20 \pm 3.54 \text{ kg.m}^{-2}$ , for non-sporting women  $27.30 \pm 5.97 \text{ kg.m}^{-2}$ . In the case of BMI, we found a statistically significant difference ( $P < 0.5$ ) among the monitored groups of participants, in the case of body weight up to a highly detectable difference ( $P < 0.01$ ). On the basis of BMI's average values, sporting women can be included in the normal weight category, where the incidence of health risk is minimal. Despite this fact, 25% of the respondents that were in the group of women doing sport were in the overweight group and 8% of the respondents we included into a group with obesity grade 1. The group of women without sport activity can be ranked according to the average BMI in the category of women with overweight when the incidence of health risk is higher. In this group, we have 11% of respondents with obesity in the first grade, 14% of respondents with obesity in the 2<sup>nd</sup> grade and 3% of respondents with obesity in the third grade, where the incidence of health risk is very high [26, 35]. Pelclová et al. [31] found in their study, that only 26.3% of women were of optimal weight, while 51.5% were overweight and 21.6% were obese. An analysis of the BMI indicator in one study showed significant differences between groups of people with no time spent walking, and those with the average amount of walking ca. 30 or 120 minutes [9]. Women who walked vigorously for greater than 150 min/wk had a 35% reduction in coronary heart disease events compared with those who walked infrequently. The most hopeful finding was that women who became active in middle age had a lower risk when compared with women who remained sedentary. By exceeding the minimal recommended physical activity, persons

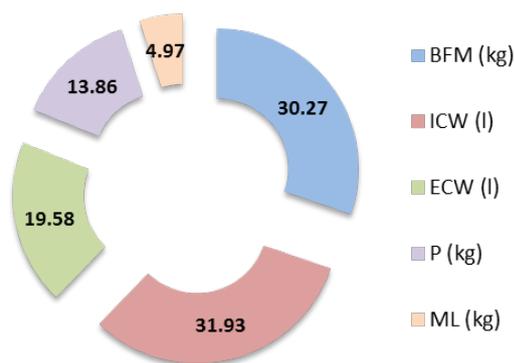
are more likely to improve their personal fitness and prevent unhealthy weight gain [16, 29]. The inverse relationship between walking and BMI values has already been documented in several studies [12, 28, 34, 36].

Average values of WHR were higher than 0.85 in both groups, it means that the participants are at risk for the occurrence of metabolic diseases. In the non-sporting women group the WHR values were higher than 0.85 to 79% of the respondents, in the group of sporting women 67%. Also with this parameter, we found a statistically highly significant difference between the observed groups ( $P < 0.01$ ). Cacek et al. [9] found significant differences in WHR values when comparing groups with different intensity of walking. Differences were confirmed between participants with no time spent walking and participants with the average amount of walking ca. 30 and 120 minutes, and between 30 and 60 minutes spent walking and 60 and 120 minutes. Analysis of prospective studies demonstrated that a 1 cm increase in waist circumference was associated with a 2% increase in the relative risk of a cardiovascular disease event [10, 27]. Even minor improvements in waist circumference resulting from walking interventions may have substantial public health gains.

BMR represents the amount of energy required for the essential functions of the organism. In our study the average BMR values ranged from 1364-1585 kcal for sporting women. The average value of this parameter is in this range for women doing sport ( $1370 \pm 110.49 \text{ kcal}$ ). The average BMR values for non-sporting women were between 1537 and 1796 kcal. The group of non-sporting women reached the average value of

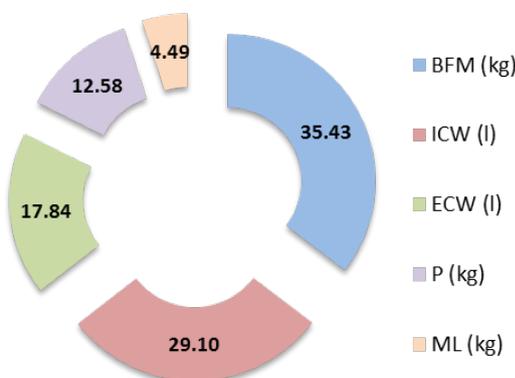
the observed parameter of  $1446 \pm 176.95$  kcal, and was below the average of the optimal range. We did not detect a statistically significant difference between the monitored groups.

The total body water is 53% of the body weight in an adult woman. The higher percentage of TBW, ICW and ECW from the average body weight was achieved by the group of women doing sport, where the average TBW was 51.51% of body weight, an average ICW 31.93% and an average ECW 19.58% of body weight. For non-sporting women the average TBW was 46.85% of body weight, an average ICW 29.10% and an average ECW 17.84% of body weight. The percentage of body fat mass (BFM) was 30.27% for women doing sport. The remainder consisted of proteins (13.86%) and minerals (4.97%). The average body weight of women without sport activities was  $77.90 \pm 20.10$  kg. The body fat mass accounted for 35.43% of the body weight of the participants, which is 5% (7.50 kg) more than for a group of women doing sport. Proteins accounted for 12.58% and minerals for 4.49% by weight. Percentage of individual components of the body composition of the participants of both groups is shown in Figure 1, 2.



Abbreviations: BFM – body fat mass; ICW – intracellular water; ECW – extracellular water; P – proteins; ML – minerals

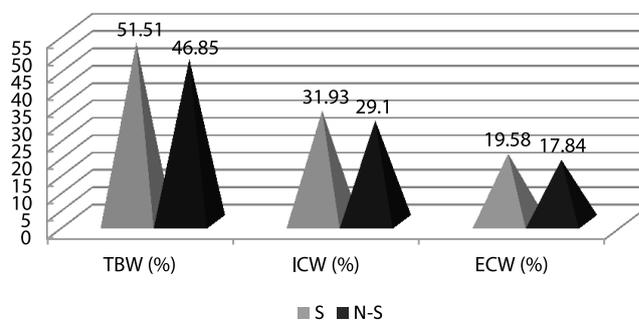
Figure 1. Percentage of individual body composition components of sporting women (n=70)



Abbreviations: BFM – body fat mass; ICW – intracellular water; ECW – extracellular water; P – proteins; ML – minerals

Figure 2. Percentage of individual body composition components of non-sporting women (n=70)

Abulmeaty [3] states that in the context of short-term changes in diet and physical activity the most significant changes in the composition of the body showed a decrease in TBW only in the obese group but not in the overweight group. This indicates that patients with obesity lose water and fat free tissues along with fat loss in early weeks of multimodal-lifestyle intervention for obesity management. The reference values for ICW in the group of women doing sport ranged between 18.50-22.60 l. The weighted average value of  $21.20 \pm 2.96$  l was within this optimal range. The average value of ICW in the non-sporting women group was  $22.60 \pm 3.85$  l and was also within the optimum range for the monitored group 19.20-23.50 l. The average ECW in the group of women doing sport was  $13.00 \pm 1.83$  l and in the non-sporting women group  $13.90 \pm 2.27$  l. Both average values were within the optimal range of values for ECW. Among the monitored women groups we did not confirm statistically significant differences in the evaluation of these parameters. Under physiological conditions a 2:1 ratio should be maintained between extracellular and intracellular fluid. This ratio was confirmed by the analysis in both groups of women. The amount of body water decreases with age, the decrease in age-related body water is due to physiological changes in the body. Percentage of TBW, ICW and ECW on the average body weight of the monitored groups is shown in Figure 3, the effect of physical activity of participants on TBW, ICW and ECW in Figure 4.



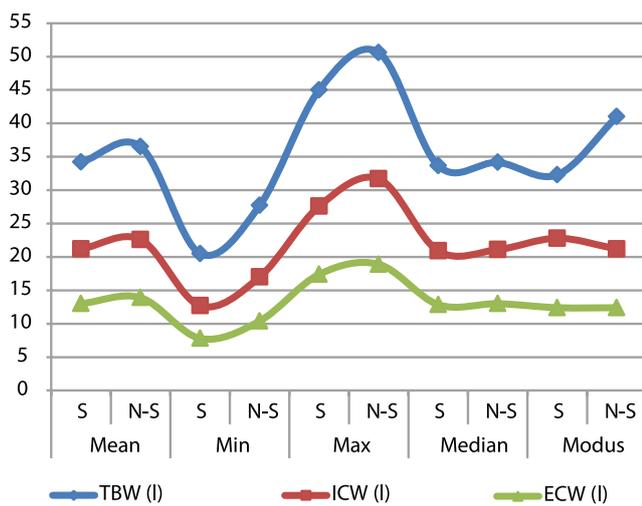
Abbreviations: TBW – total body water; ICW – intracellular water; ECW – extracellular water; S – group of sporting women; N-S – group of non-sporting women

Figure 3. Percentage of TBW, ICW and ECW on the average body weight of the monitored groups

Fat free mass (FFM) includes all components of body composition, excluding fat. Components of FFM, in addition to total body water, include proteins and minerals. The percentage of proteins and minerals on body weight of participants in the monitored groups is shown in Figure 5.

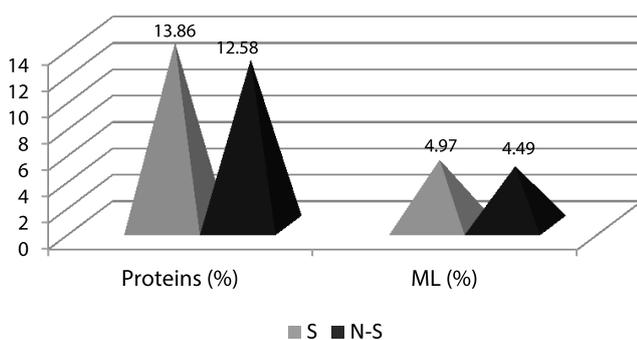
Higher FFM values were achieved in a group of sporting women but without statistical significance ( $P = 0.17$ ). The average FFM for this group was  $46.60 \pm 6.53$  kg representing 70.18% of the average body weight.

The average FFM for non-sporting women was  $49.80 \pm 8.74$  kg representing 63.93% of the average body weight. Even FFM values decrease with age gradually. Gradual decrease in FFM contributes to a decline in physical function, increased disability, frailty and loss of independence. Kyle et al. [24] found lower FFM in women older than 60 years and observed an accelerated loss in women older than 75 years. The skeletal muscle mass (SMM) is a significant part of the fat free mass. We found a higher proportion of skeletal muscle mass in a group of women doing sport, which has a great deal to do with exercise activity. The average value of SMM in this group was  $25.40 \pm 3.60$  kg representing 38.25% of the total body weight and it was 3.08% higher than the average value measured in the group of women without sport activities ( $27.40 \pm 4.83$  kg, 35.17%,  $P = 0.13$ ). Resistance exercise have the additional benefit of increasing FFM. Aerobic exercise decreases fat mass but have little effect on FFM [38]. Values of body fat mass, fat free mass, skeletal muscle mass and body cell mass in observed groups are shown in Figure 6.



Abbreviations: S – group of sporting women; N-S – group of non-sporting women; Min – minimum value; Max – maximum value; TBW – total body water; ICW – intracellular water; ECW – extracellular water

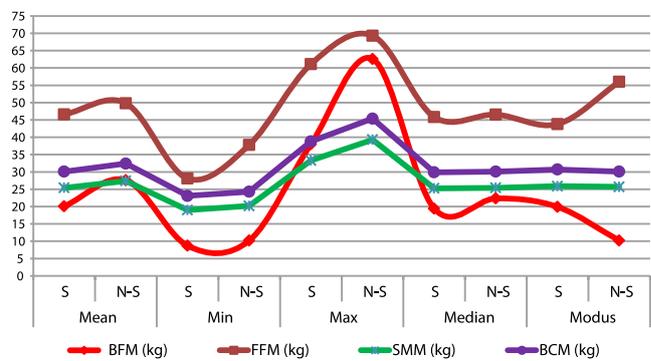
Figure 4. Effect of physical activity of participants on TBW, ICW and ECW



Abbreviations: S – group of sporting women; N-S – group of non-sporting women; ML – minerals

Figure 5. Percentage of proteins and minerals on the average body weight of the monitored groups

The body cell mass (BCM) is composed of muscle tissue cells and organs cells with the exception of nerve tissue and the amount of it form the metabolically active mass of the human body. The group of sporting women achieved higher BCM/body weight ratios than the second study group (45.33 vs. 41.59%). There were significant differences between the maximum and minimum measured values. For sporting women the minimum value was  $23.10 \pm 1.73$  kg and a maximum of  $38.8 \pm 2.13$  kg (average BCM  $30.10 \pm 3.35$  kg). For non-sporting women the minimum value was  $24.30 \pm 2.30$  kg and a maximum of  $45.40 \pm 2.81$  kg (average BCM  $32.40 \pm 1.73$  kg). However, the average BCM values in both groups ranged to the recommended values of the standard ranging from 24-33 kg. There was no statistically significant difference between groups ( $P = 0.09$ ) (Figure 6).



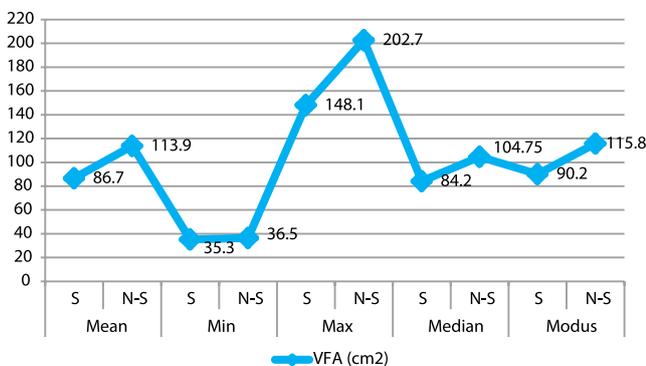
Abbreviations: S – group of sporting women; N-S – group of non-sporting women; Min – minimum value; Max – maximum value; BFM – body fat mass, [kg]; FFM – fat free mass, [kg]; SMM – skeletal muscle mass, [kg]; BCM – body cell mass [kg]

Figure 6. Values of body fat mass, fat free mass, skeletal muscle mass and body cell mass in observed groups

Obesity is a highly prevalent metabolic disorder that is characterized by excessive body fat mass (BFM) [1]. It is generally accepted that the total amount of body fat mass, as well as the level of visceral fat, is associated with the development of several diseases [17, 26]. The BFM value expresses the representation of the fat component in the human body. Together with the PBF is an important indicator of health risk. We can treat it as a variable part of the body composition that can be influenced by physical activity and proper eating habits [8]. The average BFM in the group of sporting women was  $20.10 \pm 6.73$  kg representing 30.27% of the average body weight and average BFM in the group of non-sporting women was  $27.60 \pm 12.73$  kg representing 35.43% of the total body weight (Figure 6). The proportion of body fat mass increases by age. The minimum value of PBF in the women doing sport was 16.40% and a maximum of 43.30%; the minimum value for the group of women without sport activities was 19.30% and a maximum of 50.40%. Among the monitored groups we found

a statistically highly significant difference ( $P < 0.01$ ) in this parameter. A clear inverse association had been found to exist between daily accumulated steps and PBF and WHR [18, 28, 37]. Progressive significant reduction of weight, BMI and PBF were observed throughout the study after multimodal-lifestyle intervention [3]. Beavers et al. [6] reported significant loss of FFM together with FM loss among a sample of elderly overweight subjects and those with obesity after 18 months of dietary weight loss program combined with physical activity plan.

We consider VFA as another indicator of health status and obesity. tis one of the important factors used in the assessment of cardiometabolic risk, which is correlated with the metabolic syndrome components in men and women, even at the normal BMI indicating the absence of obesity [4, 23]. The recommended amount of visceral fat should be 100 cm<sup>2</sup>. If VFA values are greater than 100 cm<sup>2</sup>, we are talking about abdominal obesity that is closely related to the occurrence of cardiovascular diseases [5, 11, 40, 42]. In the group of women doing sport, 27.10% of women had the value of VFA higher than 100 cm<sup>2</sup>, in the group of non-sporting women up to 55.7%. The average measured value in the group of women doing sport was 86.70 ± 28.79 cm<sup>2</sup> (Figure 7). The average VFA measured in the group of non-sporting women was 113.90 ± 44.95 cm<sup>2</sup> and indicates the incidence of abdominal obesity in this group. The lowest measured average values were 35.30 cm<sup>2</sup> in the group of women doing sport and 36.50 cm<sup>2</sup> in the group of non-sporting women. The highest measured average values were 148.10 cm<sup>2</sup> in the group of sporting women and 202.70 cm<sup>2</sup> in the group of women without sport activities. When compared to measured visceral fat values, a statistically highly detectable difference ( $P < 0.01$ ) was confirmed in the monitored groups. In the group of women doing sport VFA increased significantly with age [13, 43, 44]. Gába et al. [12] described differences in VFA dependent on the physical activity levels experienced by women.

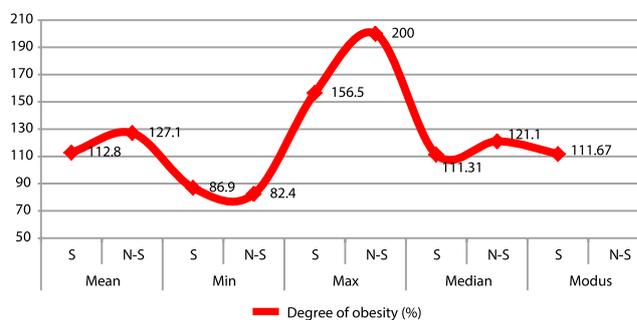


Abbreviations: S – group of sporting women; N-S – group of non-sporting women; Min – minimum value; Max – maximum value; VFA – visceral fat area [cm<sup>2</sup>]

Figure 7. Visceral fat area values in monitored groups

An increased number of steps per day that means increased physical activity contribute to an improvement in human anthropometric parameters. The team of authors examined the effect of physical activity in the number of steps on the amount of visceral fat in women. In steps of less than 7500 per day we found the VFA value 162.7 cm<sup>2</sup>, in steps of 7500-9999 per day it was 142.2 cm<sup>2</sup> and at 10000-12000 per day the VFA value was 128.5 cm<sup>2</sup> [31]. Study of Lee revealed that older women are significantly less active in comparison with older men. Many studies confirmed the beneficial effects of regular and moderate levels of physical activities on health status not only of older adults [19, 20, 28, 30].

The degree of obesity is based on the relationship of the actual weight to the ideal weight. According to InBody, the 90-100% range is optimal. Values ranging from 110-120% point to overweight and values above 120% for obesity. The average degree of obesity in the group of sporting women was 112.8 ± 16.47%. Based on this value we rank this female group as overweight. In the group of women without sport activities, the average degree of obesity was 127.1 ± 27.71%, which is why we rank this group of women as obese (Figure 8). Differences in degree of obesity were statistically significant among groups ( $P < 0.05$ ).



Abbreviations: S – group of sporting women; N-S – group of non-sporting women; Min – minimum value; Max – maximum value

Figure 8. Degree of obesity in monitored groups

We qualify Fitness Score (FS) as a fitness index. The index determines the typology of the individual according to the fat and muscle parts relative to the weight. The limit of the standard is according to InBody 70-90. In the group of non-sporting women the average FS was 69 ± 7.13. This value is just below the norm, we judge the fitness rate as underweight. In the group of sporting women the average FS was 74 ± 5.15. This value is within optimal range and determines physical fitness as an average. We have found a statistically very high level of difference between women doing sport and women without sport activities ( $P < 0.001$ ). When monitoring the intensity of walking effect on the fitness

score no significant differences were observed between the men and women (the amount of walking: 0, 30, 60 or 120 minutes) [9]. Individuals with a high level of fitness exhibited a lower risk of death than low-fit individuals [7]. Improvements in metabolic function, body composition, musculoskeletal and psychologic functioning are a few of the myriad benefits of increased physical activity. Even small increments in physical activity via reductions in sedentary behavior are beneficial [33].

The analysis of the anthropometric parameters of a group of sporting and non-sporting women brought as a result a difference in individual values, that if benefiting the group of women doing sport in lower values of body weight, BMI, WHR and BMR. In both groups, WHR values were higher than 0.85, which is a prerequisite for the development of metabolic diseases; however the group of sporting women scored lower values than the group of non-sporting women. Average basal metabolism values were found within the optimal range of values in the group of women doing sport, whereas for non-sporting women these values were below the optimum range. The group of non-sporting women had a lower percentage of proteins, minerals, total body water and skeletal muscle compared to the group of women doing sport. At the same time, this group reached higher body fat values compared to the group of women doing sport. The health status, we can include the percentage of body fat, visceral fat area and the degree of obesity. The group of women without sport activities had the above values higher than the group of sporting women. On the basis of the obtained results of fitness scores, the body performance of the group of non-sporting women was rated as undervalued, while the fitness score of the group of women doing sport as average. The results of the measurements show the positive influence of physical activity on components of body composition in all age categories. Physical activity, along with rational nutrition, should be part of a healthy lifestyle for each individual. Even a person who does not like to sport should include at least 30-60 minutes walk a day.

## CONCLUSION

The results of the measurements show the positive influence of physical activity on components of body composition in all age categories. Physical activity, along with rational nutrition, should be part of a healthy lifestyle for each individual.

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### Conflict of interest

*The authors declares no conflict of interest.*

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## COMPARISON OF VEGETABLES AND FRUIT CONSUMPTION FREQUENCY BY ATHLETES BEFORE AND AFTER MARATHON

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### ABSTRACT

**Background.** One of the prerequisites to achieve high sports scores is to ensure the proper supply of nutrients. Both deficiency and excess of these components can cause malfunctions of bodies in athletes, which adversely affects their health and performance.

**Objective.** Comparison of the frequency of intake of groups of vegetables and fruits, being sources of vitamins and minerals, by marathon runners in periods before and after the long-distance run.

**Material and Method.** The frequency of fruit and vegetable consumption by marathoners was analyzed among women (n = 105) and men (n = 87) aged between 19 and 73 years, before and after running competition. The Block's questionnaire was used to evaluate the consumption of groups of vegetables and fruits (leafy, root and cruciferous vegetables, as well as stone, berry and tropical fruits). Their consumption was assessed on a point scale.

**Results.** Sufficient vegetable and fruit intake was found in about 55% of the marathon runners. Diets of about 20% of the respondents were poor in fruits and vegetables. Higher fruit and vegetable intake was reported in both men and women after the marathon compared to the pre-run period.

**Conclusion.** Daily food rations of about 1/4 of the surveyed marathoners provided insufficient amounts of fruits and vegetables, and daily food rations of about 20% of the athletes were poor in these products. After the marathon, the consumption of vegetables and fruits improved in 15% of the surveyed women and in 10% of the men compared to the pre-marathon period.

**Key words:** *marathon runners, dietary behaviors, vegetables, fruits*

### STRESZCZENIE

**Wprowadzenie.** Jednym z warunków osiągnięcia wysokich wyników sportowych jest zapewnienie odpowiedniej podaży składników odżywczych. Zarówno niedobry, jak i nadmiary tych składników pokarmowych mogą powodować zaburzenia funkcjonowania organizmu sportowca, co niekorzystnie wpływa na zdrowie i osiągnięte wyniki sportowe.

**Cel.** Porównanie częstotliwości spożycia warzyw i owoców, stanowiących źródło witamin i składników mineralnych, przez maratończyków w okresach przed i po biegu długodystansowym.

**Materiały i metody.** Analizowano częstotliwość spożycia warzyw i owoców przez maratończyków: kobiety (n=105) i mężczyzn (n=87) w wieku od 19 do 73 lat w okresach przed i po biegu. Użyto kwestionariusza Block'a do oceny spożycia grup warzyw i owoców (warzyw liściastych, korzeniowych, krzyżowych, owoców pestkowych, jagodowych i tropikalnych). Oceniono spożycie tych produktów w skali punktowej.

**Wyniki.** Wystarczające spożycie warzyw i owoców stwierdzono u około 55% badanych maratończyków. Diety około 20% badanych osób były ubogie w warzywa i owoce. Wyższe spożycie warzyw i owoców wykazano zarówno u kobiet, jak i u mężczyzn w okresie po maratonie w porównaniu do okresu przed biegiem.

**Wnioski.** W około 1/4 badanej grupy maratończyków stwierdzono niewystarczające spożycie warzyw i owoców w całodziennej racji pokarmowej, a całodzienne racje pokarmowe około 20% sportowców były ubogie w te produkty. Po maratonie stwierdzono poprawę spożycia warzyw i owoców u 15% badanych kobiet i 10% mężczyzn w porównaniu do okresu przed biegiem długodystansowym.

**Słowa kluczowe:** *maratończycy, zachowania żywieniowe, warzywa, owoce*

## INTRODUCTION

One of the prerequisites to achieve high sport scores is the appropriate supply of nutrients, i.e. protein, fats, carbohydrates, vitamins, macro- and micronutrients. Both their deficiency and excess can cause malfunctions of the bodies in athletes, which can have adverse effects on their health and sports performance [5, 9, 14].

Studies of nutritional patterns of groups of athletes have demonstrated that these patterns are usually poor and that common dietary mistakes include: too low energy intake and unproportionate contents of most nutrients, including vitamins and minerals. Systematic physical effort and multiple loads the athletes are exposed to while on unbalanced diet may induce dietary deficiencies. This may in turn cause micro-damages to muscles as well as adverse metabolic transformations in the body, leading to among others, incorrect ratio between fatty tissue and lean tissue mass that affect athlete's tolerance to physical strain. Dietary deficiencies may diminish motor and concentration capabilities, disturb regeneration processes, and cause higher susceptibility of an athlete to fatigue and body overload. In addition, inappropriate nutrition increases the risk of malnutrition, dehydration, and the likelihood of mild and serious injuries [7, 8, 11, 12].

The aim of this study was to compare the frequency of intake of groups of vegetables and fruits, being sources of vitamins and minerals, by marathoners in the periods before and after the long-distance run.

## MATERIAL AND METHODS

The study was conducted in 192 persons, who participated in a marathon in Wrocław. It was a cross-sectional study with convenience sampling. The participants were asked to complete a questionnaire regarding the frequency of intake of vegetables and fruits being the main sources of vitamins and minerals in their diet. The survey was conducted in the months of May to August 2013. Women represented 54.7% and men 45.3% of the group. The mean age of women was 32.7 years (range: 20–57 years), whereas that of men was 35.1 years (range: 19–73 years). The frequency of intake of groups of fruits and vegetables was determined using a Block's questionnaire developed for the needs of the NHANES II survey and used in many countries [1, 13]. The questionnaire allowed collecting data about the usual frequency of consumption of leafy, root and cruciferous vegetables as well as stone, berry and tropical fruits. The frequency of intake of these products was evaluated using a 5-point descriptive scale with numerical values assigned:

- less than once a week - 0 pts., about once a week - 1 pt., 2-3 times a week - 2 pts., 4-6 times a week - 3 pts., every day - 4 pts. [1].

The frequency of intake of the group of fruits and vegetables was expressed in a point scale (0-24 pts.).

Three groups of respondents were distinguished based on the sum of points achieved:

- persons with sufficient intake of vitamins and minerals (>18 pts.);
- persons with insufficient intake of vitamins and minerals (6-18 pts.);
- persons whose diet is poor in vitamins and minerals (< 6 pts.).

The statistical analysis of results was conducted using Statistica 10.0. package. Mean values of traits assigned to categories of the frequency of intake were compared with *Kruskal-Wallis* test. Letters a – b in tables denote significant differences between mean values in rows at  $p \leq 0.05$ , whereas ns – denotes insignificant differences.

## RESULTS

The mean body mass of women before the marathon accounted for 62.4 kg (range: 50–95 kg), whereas after the marathon – for 60 kg (range: 48–86 kg). In the case of men, the respective values were as follows: 81.7 kg (range 63–112.7 kg) and 78.6 kg (range 55–92.6 kg).

Table 1 summarizes the frequency of intake of 6 groups of vegetables and fruits by the surveyed women (n=105) and men (n=87). Statistically significant differences were found in the frequency of intake of most of the analyzed food products being the main dietary sources of vitamins and minerals in both women and men between the pre- and post-marathon periods. A higher frequency of consumption of root and cruciferous vegetables and of stone and berry fruits by women was demonstrated in the post-marathon than in the pre-marathon period. After the long-distance run, the number of women declaring consumption of root and cruciferous vegetables 4-6 times a week increased by ca. 13% compared to the pre-marathon period. The frequency of intake of stone fruits increased after the marathon (to more than 4 times a week) in ca. ¼ of the surveyed women. Before the marathon, everyday consumption of berry fruits was declared by 11% of the women, whereas after the run – by 31% of the women. Before the marathon, the consumption of berry fruits 4-6 times a week was declared by 29% whereas after the marathon – by 38% of the women. No changes were found in the frequency of intake of leafy vegetables and tropical fruits by the surveyed women depending on the training period (before or after marathon).

After the marathon, the number of men who declared consumption of leafy vegetables 4-6 times a week increased statistically significantly (by 15%) compared to the pre-marathon period. Before the long-distance run, a low intake of stone and berry fruits (once a week or less frequently) was declared by 36% and 29% whereas after the marathon by only 17% and 9% of the surveyed men, respectively. After the marathon, an increase (by

12%) was noted in the number of men consuming stone fruits 2-3 times a week, compared to the pre-marathon period. Before the marathon, the consumption of berry fruits 4-6 times a week was declared by 24% while after the marathon by 37% of the male marathon runners.

No changes were found in the frequency of intake of cruciferous and root vegetables and tropical fruits by the surveyed men depending on the training period (before or after marathon).

Table 1. Frequency of intake of vegetables and fruits by the surveyed athletes (n=192) in the pre- and post-marathon periods

Frequency of intake of vegetables and fruits	Women (n=105)			Men(n=87)		
	before marathon	after marathon	p≤0.05	before marathon	after marathon	p≤0.05
Leafy vegetables						
less than once a week	5	2	ns	3	3	ns
about once a week	8	5	ns	9	7	ns
2-3 times a week	12	8	ns	25	15	a-b
4-6 times a week	45	51	ns	24	37	a-b
every day	35	39	ns	26	25	ns
Root vegetables						
less than once a week	10	6	ns	1	1	ns
about once a week	22	12	a-b	3	1	ns
2-3 times a week	14	17	ns	18	15	ns
4-6 times a week	33	47	a-b	26	28	ns
every day	26	23	ns	39	42	ns
Cruciferous vegetables						
less than once a week	8	2	ns	6	3	ns
about once a week	20	9	a-b	10	5	ns
2-3 times a week	26	28	ns	17	20	ns
4-6 times a week	31	43	a-b	24	29	ns
every day	20	23	ns	30	30	ns
Stone fruits						
less than once a week	5	3	ns	11	5	a-b
about once a week	15	4	a-b	20	10	a-b
2-3 times a week	31	18	a-b	20	30	a-b
4-6 times a week	32	49	a-b	24	30	ns
every day	22	31	a-b	12	12	ns
Berry fruits						
less than once a week	4	3	ns	12	3	a-b
about once a week	20	12	a-b	13	5	a-b
2-3 times a week	38	17	a-b	29	32	ns
4-6 times a week	31	40	a-b	21	32	a-b
every day	12	33	a-b	12	15	ns
Tropical fruits						
less than once a week	15	12	ns	12	10	ns
about once a week	29	32	ns	37	36	ns
2-3 times a week	31	28	ns	21	24	ns
4-6 times a week	19	21	ns	12	13	ns
every day	11	12	ns	5	4	ns

a-b statistically significant differences at p≤0.05

ns- not statistically significant differences

Table 2. Evaluation of the intake of vegetables and fruits in the surveyed groups of athletes (n=192) in the pre- and post-marathon periods

Intake of vegetables and fruits	Women (n=105) %			Men (n=87) %		
	before marathon	after marathon	p≤0.05	before marathon	after marathon	p≤0.05
sufficient	50.5	65.7	a-b	48.3	58.6	a-b
insufficient	23.8	18.0	ns	25.3	26.5	ns
poor	25.7	16.2	a-b	26.4	14.9	a-b

a-b statistically significant differences at  $p \leq 0.05$

ns- not statistically significant differences

Table 2 presents results of the assessment of vegetable and fruit consumption by the athletes. In diets of ca. 21% of the women and 26% of the men, on average, the intake of vegetables and fruits was insufficient irrespective of the training period (before and after the marathon). After the marathon, sufficient intake of these products was noted in diets of 65.7% of the women and 58.6% of the men, whereas in the period of intensive training before the long-distance run – in diets of only ca. 50% of the marathon runners. Before the marathon, poor intake of vegetables and fruits was demonstrated in over ¼ of the surveyed athletes, whereas after the marathon diets of only 15-16% of the women and men were poor in these products.

## DISCUSSION

Nutritional pattern and diet-related nutritional status may significantly affect the physical performance in endurance disciplines. An important element in nutrition of athletes and persons undertaking regular physical activity is the amount and type of ingested carbohydrates which may be provided with vegetables and fruits. Currently, the recommended amount of carbohydrates supplied with diet of long-distance runners should range from 6 to do 10 g/kg body mass/day and in the pre-competition period should provide 60-70% of energy. This allows increasing physical performance by 2-3% [2].

Improper intake of vegetables and fruits – being main sources of vitamins, minerals, and carbohydrates - by athletes was demonstrated in studies conducted by other authors. A survey carried out among 100 athletes, including 50 professional sportsmen (associated and representing sports clubs located in the Kujawsko-Pomorskie Province) and 50 amateur sportsmen, demonstrated that the consumption of vegetables few times a day was declared by 48% of the professionals and 41% of the amateurs, whereas the same frequency of fruit intake was declared by 68% of the professional athletes and by 72% of the amateurs. Improper practices were also demonstrated in the consumption of meals before and after trainings [8].

According to guidelines of rational nutrition for professional athletes and physically-active persons, vegetables should be provided in every meal, whereas fruits should be consumed 2-3 times a day as they provide vitamins, and minerals. In the study conducted by *Ostachowska-Gqsior* [11], the knowledge of the above guidelines was confirmed in ca. 30% of the 105 persons attending fitness clubs every day.

In turn, a survey carried out by *Szczepańska* and *Spałowska* [12] among professional volleyball and basketball players demonstrated appropriate intake (few times a day) of vegetables in 10% of volleyball players and in 30% of basketball players, whereas sufficient intake of fruits was declared by 55% and 71% of volleyball and basketball players, respectively. Ca. 70-80% of the athletes declared to consume vegetables and fruits in the fresh form. Similar results were reported by *Nowacka* et al. [10], who showed everyday consumption of vegetables and fruits in only ca. 40% of the surveyed athletes practicing shooting sport and canoe slalom.

Endurance sports have become highly popular even among recreational runners. An increasing number of persons compete in marathons, half-marathons, and other forms of long-lasting physical activity. Runs, especially the long-distance ones, are perceived as a measure of physiological capabilities of an athlete body. Many studies have demonstrated a positive effect of nutrition on athlete performance [3, 5, 9].

*Durkalec-Michalski* [5] evaluated the effect of diet balancing on body composition and performance capability of a selected group of persons practicing running disciplines. The balancing and rationalization of the nutritional pattern during intensive trainings had a positive effect on: reduced content of fatty tissue, increased aerobic adaptation, and physical performance of the surveyed runners.

Vegetables and fruits are the main and very important sources of vitamins and minerals in a diet of athletes. At trainings, athletes are at risk of high losses of macro- and microelements which are excreted with sweat. In endurance sports, high significance is ascribed to an appropriate sodium to phosphorus ratio

which helps maintaining the correct water-electrolyte balance in the body, work of the cardiac muscle and skeletal muscles, transport of the energetic material (glucose), and transmission of nervous stimuli in the muscles. Also calcium and magnesium are needed for muscle contraction. Proper body functions under conditions of physical effort are ensured by iron that takes part in oxygen transport. In the case of athletes, deficiencies of minerals may induce muscle cramps, body exhaustion and acidification as well as debilitation of the skeletal system. Appropriate intake of vitamins, including mainly vitamin C and B group vitamins, is indispensable during heavy physical effort to enhance glycogen deposition in the liver (B1), to aid respiratory enzymes (B2) or to ensure apt metabolism of proteins, fats, and carbohydrates (B3, biotin, folacin). Fat-soluble vitamins (A, D, E, K) regulate calcium metabolism, provide protection against free radicals, and determine proper functions of muscles. Ill-balanced, excessive supplementation may have adverse effects on the absorption and metabolism of various nutrients and lead to disruption of an athlete body homeostasis [4, 6].

Athletes demands for energy and nutrients are higher than these of an average person. This requires on the one hand increasing the mass of daily food rations, but on the other hand providing meals low in volume and easily digestible. An optimal solution is to skillfully use dietary supplements to accelerate regeneration and increase body performance. Nutritional needs of athletes differ as affected by the duration and intensity of training as well as by the type of sports discipline they compete in. The appropriate choice of dietary supplements is essential to ensure, e.g. muscle mass boost, fat burning acceleration, body immunity increase, joint regeneration, and covering losses of vitamin and minerals in order to restore the metabolic balance [4, 6]. It needs to be emphasized that ca. 80% of athletes surveyed in this study declared the intake of dietary supplements in the form of concentrated vitamins and macro- and microelements to enrich their diets being poor and insufficient in fruits and vegetables.

Abnormalities observed in the frequency of intake of vegetables and fruits among athletes competing in running disciplines point to the necessity of nutritional education of this population.

## CONCLUSIONS

1. After the marathon, an improvement in the intake of vegetables and fruits was observed in 15% of the women and in 10% of the men, compared to the pre-marathon period. Sufficient intake of vegetables and fruits was found in ca. 55% of the surveyed marathon runners. The intake of

vegetables and fruits was insufficient in daily food rations of ca. ¼ of the studied marathoners and poor in these of ca. 20% of the athletes.

2. A statistically significantly lower intake of root and cruciferous vegetables as well as stone and berry fruits by women was demonstrated in the pre- than in the post-marathon period.
3. A statistically significantly lower intake of leafy vegetables and stone and berry fruits by men was demonstrated before the marathon compared to the post-run period.
4. Nutritional mistakes demonstrated in the study in the case of persons competing in endurance disciplines point to the necessity of modifying their nutritional patterns by increasing the consumption of vegetables and fruits.

## Conflict of interest

*The authors declare no conflict of interest.*

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## PHYSICAL ACTIVITY OF KATOWICE URBAN AREA INHABITANTS WITH REGARD TO SELECTED PHYSICAL TRAITS AND SOCIAL FACTORS

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### ABSTRACT

**Background.** Modern research of health determinants and health-related behavior patterns places a particular emphasis on the assessment of physical activity levels in various social and professional groups. The deficit of physical activity has become a common cause of serious diseases. Following the ecological model of health behavior, physical activity as a biological and cultural phenomenon can be indirectly modified by such interpersonal factors as age, sex, somatic traits and education.

**Objectives.** The aim of the present study was the identification and assessment of habitual physical activity of adult inhabitants from the Katowice Urban Area with regard to selected physical traits and social determinants.

**Material and methods.** The study was carried out in the Katowice Urban Area in May and June, 2012-2014. The research material comprised 2,173 inhabitants, including 1,017 women and 1,156 men, aged 30 to 65 years. The respondents' habitual physical activity was assessed with the use of the International Physical Activity Questionnaire - Short Version (IPAQ-SF). The obtained data on the respondents' physical activity were compared with the American College of Sports Medicine (ACSM) recommendations

**Results.** The results of the study showed that the frequency and duration of high-intensity physical activity and duration of moderate-intensity physical activity were significantly greater in the male inhabitants. The age was also a significant factor affecting the levels of high-intensity physical activity in men. Body build was not a significant determinant of the frequency and duration of physical activity in both men and women from Katowice. The education level only affected the level of physical activity in men. Sex, age, and education of the respondents were statistically significant determinants of their level of fulfilment of the ACSM recommendations.

**Conclusions.** Propagation of physical activity among adults and the elderly, and women in particular, remains a continuing challenge facing health promotion professionals.

**Key words:** physical activity, urban population, physical traits, social status.

### STRESZCZENIE

**Wprowadzenie.** W badaniach uwarunkowań zdrowia i zachowań zdrowotnych współczesnych pokoleń, szczególnego znaczenia nabiera ocena aktywności fizycznej różnych grup społeczno-zawodowych. Niedobór wysiłków fizycznych jest bowiem coraz powszechniej przyczyną groźnych schorzeń. Zgodnie z modelem ekologicznym aktywność fizyczna jako zjawisko nie tylko biologiczne, lecz także kulturowe może być w sposób pośredni modyfikowane m.in. przez takie czynniki intrapersonalne, jak: wiek, płeć, cechy somatyczne, czy wykształcenie.

**Cel.** Celem pracy jest diagnoza i ocena parametrów nawykowej aktywności fizycznej osób dorosłych zamieszkujących aglomerację katowicką w świetle wybranych czynników somatycznych i społecznych.

**Material i metody.** W badaniach ankietowych przeprowadzonych na terenie aglomeracji katowickiej w latach 2012-2014 roku wzięły udział 2173 osoby, w tym 1017 kobiet i 1156 mężczyzn, w wieku od 30 do 65 lat. Do oceny nawykowej aktywności fizycznej wykorzystano kwestionariusz International Physical Activity Questionnaire Short Version (IPAQ-SF). Uzyskane informacje o aktywności fizycznej badanych pozwoliły na ich konfrontację z kryteriami zalecanymi dla uzyskania korzyści zdrowotnych przez ekspertów ACSM.

**Wyniki.** W badaniach wykazano u badanych znamienne różnicowanie płciowe w częstości i czasie trwania aktywności fizycznej o wysokiej intensywności oraz czasie trwania wysiłków umiarkowanych, na korzyść mężczyzn. Dostrzeżono również, że wiek znamienne różnicował częstość podejmowania wysiłków fizycznych o wysokiej intensywności przez

badanych mężczyzn. Wśród badanych mieszkańców aglomeracji katowickiej, parametry budowy somatycznej nie determinowały istotnie w obu grupach płci częstości oraz czasu trwania aktywności fizycznej. Wykształcenie okazało się czynnikiem modyfikującym poziom aktywności fizycznej wyłącznie u przedstawicieli płci męskiej. Płeć, wiek oraz poziom wykształcenia ankietowanych katowiczian znamienne determinowały także stopień wypełnienia przez nich zaleceń ACSM. **Wnioski.** Popularyzowanie aktywności fizycznej wśród osób dorosłych i starszych, szczególnie kobiet jest stale aktualnym wyzwaniem dla specjalistów promujących zdrowie.

**Słowa kluczowe:** *aktywność fizyczna, środowisko wielkomiejskie, cechy somatyczne, status społeczny*

## INTRODUCTION

Modern research of health determinants and health-related behavior patterns places a particular emphasis on the assessment of physical activity levels in various social and professional groups. The deficit of physical activity has become a common cause of serious diseases of the circulatory, respiratory and locomotive systems as well as metabolic diseases and cancer, which lead to the majority of premature deaths in adults [4, 28-30, 33, 42, 45, 48].

Regular and properly adjusted physical activity is one of the most recommended and desired human behaviors. An important aspect of health-related activities is their diagnosis both in individuals [6] and in populations [2, 7, 37]. Reliable evaluation of physical activity parameters is methodologically complex. One of the best measures is the assessment of energy expenditure associated with physical exercise [26, 40]; however, objective measurements of energy expenditure are possible only in laboratory conditions or with the use of expensive portable spirometers or multi-function and multi-sensory measuring devices, i.e. accelerometers [15].

In epidemiological studies one of the most commonly used methods of physical activity assessment is the diagnostic questionnaire survey, and one of the most popular questionnaire instruments worldwide is the International Physical Activity Questionnaire (IPAQ) [3, 21]. Its accuracy and reliability of measurements of caloric cost of physical activity, heart rate, or total number of steps per day, have been confirmed by numerous authors [9, 17, 22]. The studies using the IPAQ were far more accurate regarding the assessment of low- and moderate-intensity exercise, since researchers noted respondents' tendency to overestimate the declared amount of physical activity of high intensity, in particular [30, 32, 47].

IPAQ-based studies in Poland have been mostly conducted on particular professional groups comprising relatively small numbers of respondents [1, 29-30, 32]. Some exceptions include representative studies on sixteen professional groups from Warsaw [2] and on working-age populations from Wrocław [37].

No diagnostic surveys regarding the physical activity of regional populations, including representative urban

areas, have been carried out in Poland, nevertheless authors have indicated the significant role of place of residence as a predictor of physical activity levels [11, 20, 25, 44].

The Upper Silesia Province of Poland is a geographical, cultural and industrial region of Poland with its own identifiable characteristics and the dynamically developing administrative center of the Katowice Urban Area. In 2017 the local and regional authorities granted Katowice the status of a metropolitan area, which is now inhabited by about 2.5 million inhabitants.

Following the ecological model of health behavior developed by Sallis et al. [41] physical activity, being a biological and cultural phenomenon, can be indirectly modified by such interpersonal factors as age, sex, somatic traits and education. Researchers have rarely considered the impact of all these factors together, and rather focused on identifying biological [12, 13, 16] or cultural [5, 36, 38] correlates of physical activity, separately.

The aim of this study was the identification and assessment of parameters of the habitual physical activity of adult inhabitants from the Katowice Urban Area with regard to selected physical traits and social determinants.

## MATERIAL AND METHODS

The study was carried out in the Katowice Urban Area in May and June, 2012-2014. The research material comprised 2,173 inhabitants, including 1,017 women and 1,156 men, aged 30 to 65 years. The average age of the respondents was 42.3 years. All participants were informed about the aim and the course of the study and gave their written consent to participate.

The respondents' habitual physical activity was assessed using the International Physical Activity Questionnaire - Short Version (IPAQ-SF) [21]. It contains items regarding the frequency and duration of inhabitants' physical activity in three intensity ranges: vigorous physical activity (VPA=8.0 METs), moderate physical activity (MPA=4.0 METs), and light physical activity (LPA=3.3 METs) at work, while getting from place to place, as part of house and yard work, and in one's leisure time during a typical week.

The obtained data on the respondents' physical activity were compared with the American College of Sports Medicine (ACSM) recommendations [14, 33] which stipulate that health-enhancing physical activity must consist of either:

1. Vigorous-intensity aerobic physical activity for a minimum of 20 min on three days each week; or
2. Moderate-intensity aerobic physical activity for a minimum of 30 min on five days each week.

Individuals fulfilling one of the above criteria are regarded as meeting the recommendation of health-related physical activity.

Research data also included information on respondents' education, body height and body mass. The calculation of BMI allowed a categorization of respondents into normal (healthy weight), overweight, and obese.

The statistical analysis involved the calculation of arithmetic means ( $\bar{x}$ ), standard deviations (s), medians (Me), 25<sup>th</sup> and 75<sup>th</sup> centiles, and minimum (min) and

maximum (max) values. The differences in results between men and women were checked with the *Mann-Whitney U* test and *Kruskal-Wallis* test. The level of fulfillment of health-related PA recommendations with regard to respondents' sex, age, physical traits, and education was checked with the chi-squared test. The level of statistical significance was set *ex ante* at  $p < 0.05$ . All statistical calculations were made using the Statistica 9.1 software package (StatSoft).

## RESULTS

The mean ages of studied female and male respondents were similar ( $p=0.272$ ). Sex differences in physical traits of the Katowice Urban Area inhabitants were found. The men had a greater body height and body mass ( $p<0.001$ ) than the women. They also featured significantly higher BMI values ( $26.2\pm 3.2$  kg/m<sup>2</sup>) than the women ( $24.8\pm 3.6$  kg/m<sup>2</sup>) (Table 1).

Table 1. Differences in age and physical characteristics of men and women under study

Sex	Age [years]		Body height [cm]		Body mass [kg]		BMI [kg/m <sup>2</sup> ]	
	$\bar{x} \pm s$	p	$\bar{x} \pm s$	p	$\bar{x} \pm s$	p	$\bar{x} \pm s$	p
Women	42.4±8.5	0.272	166.3±6.3	<0.001*	64.0±9.8	<0.001*	23.1±3.3	<0.001*
Men	42.1±8.9		178.7±6.7		83.7±11.2		26.2±3.2	
Total	42.3±8.7		172.9±9.0		74.5±14.4		24.8±3.6	

\* $p < 0.05$

The respondents revealed statistically significant inter-sex differences in their level of physical activity. The men undertook high-intensity physical activities

more often, and the duration of their high- and moderate-intensity exercises was longer than in the female respondents ( $p<0.001$ ) (Table 2).

Table 2. Differences in physical activity frequency and duration of physical activity in men and women under study

Sex	HPA [days/week]		MPA [days/week]		HPA [min/day]		MPA [min/day]	
	$\bar{x} \pm s$	p	$\bar{x} \pm s$	p	$\bar{x} \pm s$	p	$\bar{x} \pm s$	p
Women	2.4±1.4	<0.001*	3.3±1.7	0.334	59.5±31.2	<0.001*	80.6±41.5	<0.001*
Men	3.0±1.7		3.4±1.7		80.3±42.0		94.0±48.3	
Total	2.8±1.6		3.4±1.7		71.7±39.3		87.8±45.7	

\* $p<0.05$ ; HPA [days/week] – high-intensity physical activity frequency; MPA [days/week] – moderate-intensity physical activity frequency; HPA [min/day] – high-intensity physical activity duration; MPA [min/day] – moderate-intensity physical activity duration.

Figure 1 shows differences in the frequency of undertaken physical activity with regard to the respondents' age. The results indicate that age was only a significant determinant of the frequency of high-intensity exercises undertaken by the male respondents ( $H=7.87$ ,  $p=0.019$ ). Those aged 30 to 49 years engaged in high-intensity PA significantly more

often than respondents aged 50 to 65 years. The mean frequency of high-intensity and moderate-intensity physical activity undertaken by female respondents, and the frequency of moderate-intensity PA by male respondents did not differ significantly between age categories (Figure 1).

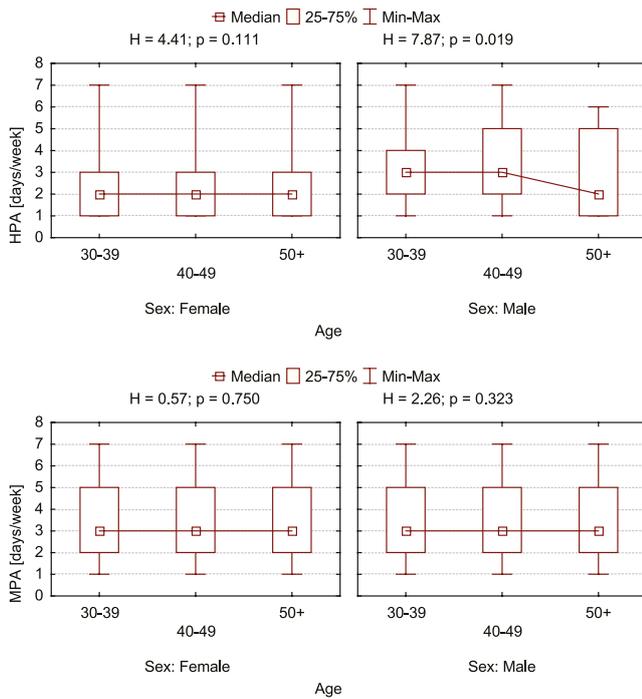


Figure 1. Differences in frequency of high-intensity physical activity (HPA) and moderate-intensity physical activity (MPA) with regard to the age of men and women under study

The duration of high-intensity physical activity of male Katowice inhabitants ( $H=7.72$ ;  $p=0.021$ ) was shown to decrease significantly with age. However, age was not a significant determinant of the duration of high- and moderate-intensity physical activity in women and of moderate-intensity physical activity in men (Figure 2).

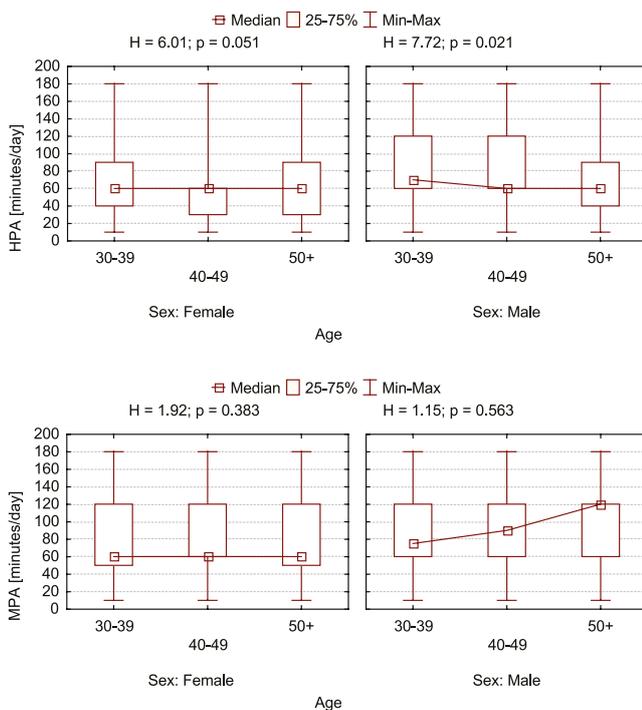


Figure 2. Differences in duration of high-intensity physical activity (HPA) and moderate-intensity physical activity (MPA) with regard to the age of men and women under study.

Body weight was not a factor that significantly affected the frequency and duration of physical activity at different levels of intensity in respondents from Katowice (Figures 3, 4).

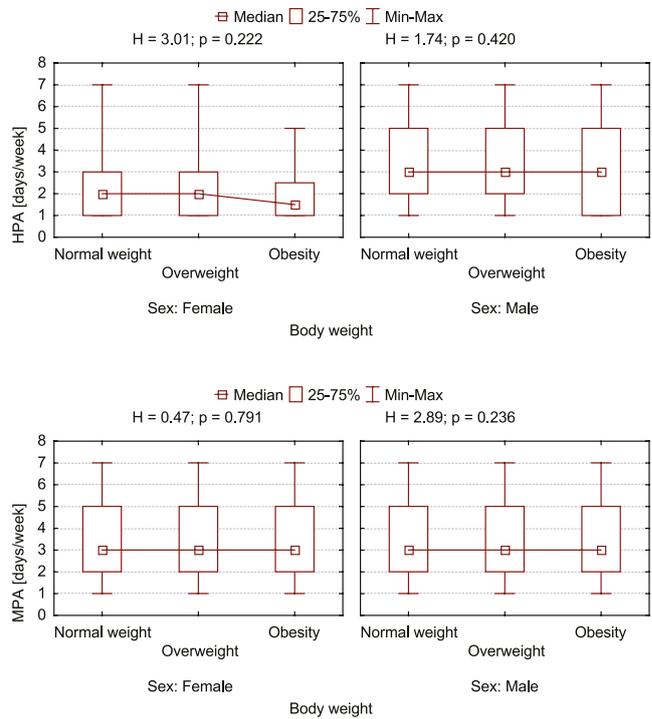


Figure 3. Differences in frequency of high-intensity physical activity (HPA) and moderate-intensity physical activity (MPA) with regard to the body weight of men and women under study.

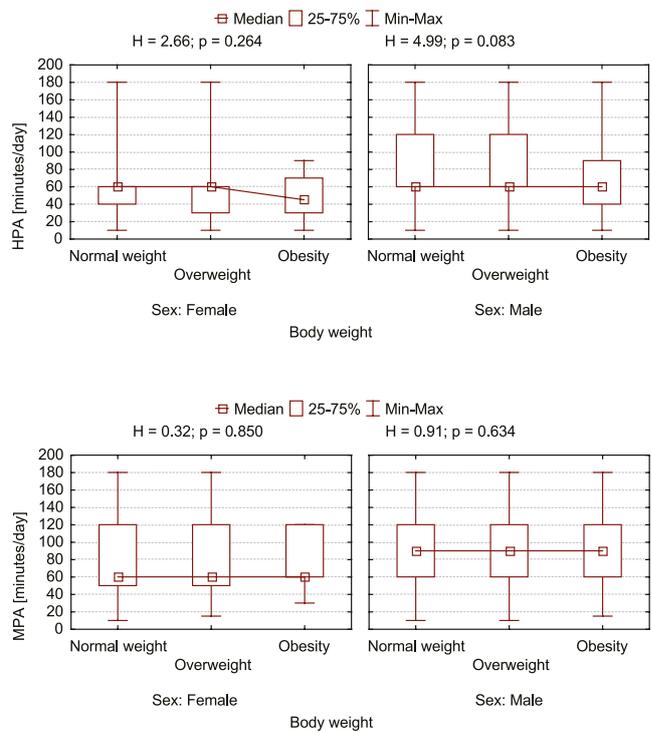


Figure 4. Differences in duration of high-intensity physical activity (HPA) and moderate-intensity physical activity (MPA) with regard to the body weight of men and women under study.

Education turned out to be a significant determinant of the level of physical activity only in male Katowice inhabitants. The mean frequency and duration of high- and moderate-intensity physical activity were significantly higher ( $p < 0.001$ ) in men with a primary and basic vocational education, than in men with a secondary and university education (Figures 5, 6).

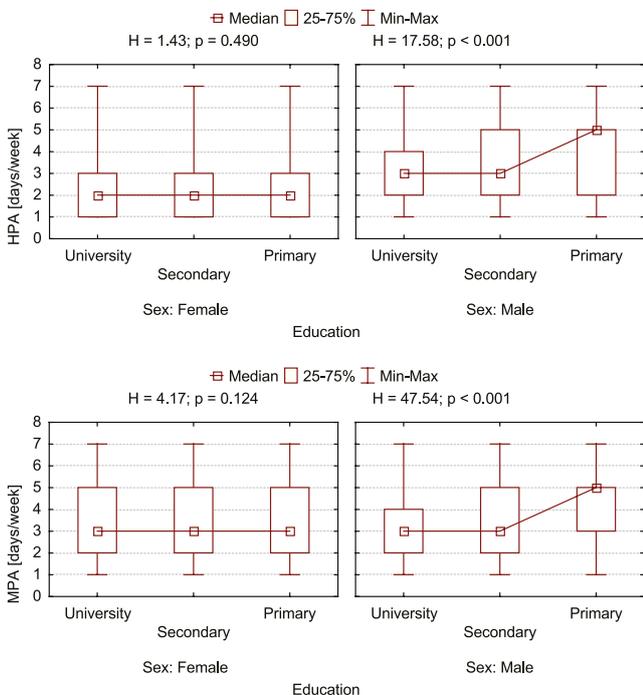


Figure 5. Differences in frequency of high-intensity physical activity (HPA) and moderate-intensity physical activity (MPA) with regard to the education level of men and women under study.

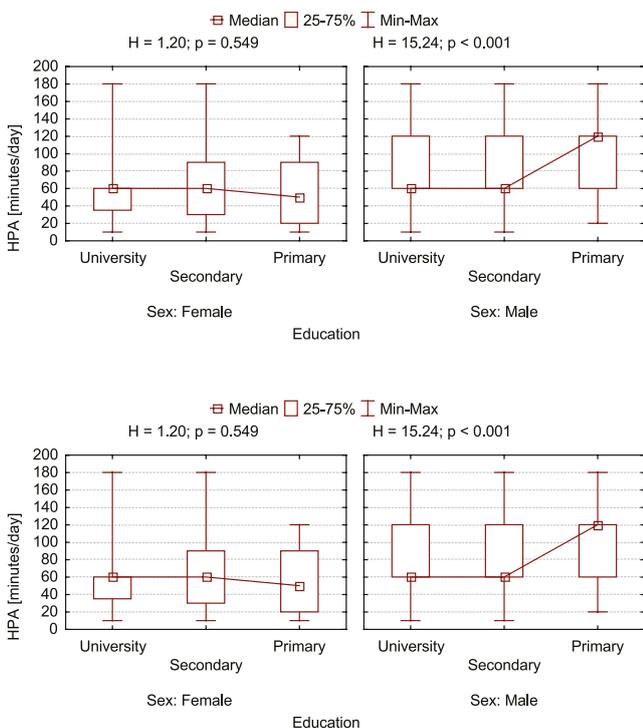


Figure 6. Differences in duration of high-intensity physical activity (HPA) and moderate-intensity physical activity (MPA) with regard to the education level of men and women under study.

The data on the frequency and duration of Katowice inhabitants' habitual physical activity were then used to calculate the percentage of respondents meeting the recommendations for health-related physical activity published by the American College of Sports Medicine (Table 3). Sex, age, and education were significant determinants of the levels of fulfilment of ACSM recommendations by the Katowice Urban Area inhabitants. Significantly more Katowice men (50%) than women (34%) met the ACSM recommendations for health-enhancing physical activity ( $\chi^2=57.58$ ;  $p < 0.001$ ). The highest percentage of respondents meeting the recommendations (46%) were found among the inhabitants aged 30 to 39 years. In the age group of 40 to 49 years, the ACSM recommendations were met by 43%, and in the oldest group under study – 34% ( $\chi^2=18.80$ ;  $p < 0.001$ ). A significantly higher percent (56%) of respondents engaged in health-enhancing physical activity were found among Katowice inhabitants with a primary and basic vocational education, than with a secondary education (45%) and higher education (37%) ( $\chi^2=23.04$ ;  $p < 0.001$ ). The body build measures were not significant determinants of the level of fulfilment of ACSM recommendations in the respondents under study ( $\chi^2=3.55$ ;  $p < 0.169$ ).

Table 3. Meeting ACSM recommendations and respondents' sex, age, body weight, and education

Category	ACSM recommendations				$\chi^2$	p
	yes		no			
	n	%	n	%		
<b>Sex</b>						
Women	342	33.6	675	66.4	57.58	<0.001*
Men	575	49.7	581	50.3		
<b>Age [years]</b>						
30-39	415	46.2	484	53.8	18.80	<0.001*
40-49	338	42.6	455	57.4		
50+	164	34.1	317	65.9		
<b>Body weight</b>						
Normal	489	41.5	689	58.5	3.55	0.169
Overweight	372	44.2	470	55.8		
Obese	56	36.6	97	63.4		
<b>Education</b>						
University	368	37.3	618	62.7	23.04	<0.001*
Secondary	478	45.1	582	54.9		
Primary	71	55.9	56	44.1		

\* $p < 0.05$

## DISCUSSION

The study assessed the levels of habitual physical activity of men and women from the Katowice Urban Area aged 30 to 65 years, in view of recommendations for health-related physical activity published by the American College of Sports Medicine [14, 33].

The results of the study showed that the frequency and duration of high-intensity physical activity and duration of moderate-intensity physical activity were significantly greater in male Katowice inhabitants. Age was a significant factor affecting the levels of PA only in men as well. These observations correspond to earlier study results on age and sex as significant correlates of physical activity levels in studied populations. The dominance of the male sex over the female sex and the young over the older with regard to the duration and frequency of physical activity had been also confirmed in some earlier research [9, 20, 39, 46].

Results of earlier studies had also indicated correlations between physical activity and body build. A higher level of physical activity was shown to be positively correlated with normal body weight, but not with overweight [10, 12, 13, 16, 19, 43]. Such correlations, however, were not found among the respondents from the Katowice Urban Area.

Numerous authors have also pointed out the significant impact of social factors on the level of physical activity [2, 3, 8, 18, 20, 22, 31, 34, 36-39]. The duration of habitual physical activity was usually shown to increase with the level of education [11, 36-37]. In the present study an opposite trend was noted. It can be most likely associated with the fact that respondents with a primary or basic vocational education usually performed physical work, often of high intensity. A relatively high level of physical activity among manual workers had been found before by *Puciato et al.* [39]. It should be noted that some researchers had also found negative correlations between education level and physical activity [2, 39].

The present study regarded mainly the subject of fulfilment of health-related physical activity parameters by studied men and women. In fact, one-third of female respondents and one-half of male respondents from the Katowice Urban Area met the physical activity recommendations by the American College of Sports Medicine.

These results correspond with results of earlier studies, e.g. the National Multicenter Health Survey in Poland program indicated that the levels of physical activity of 50-60% of Poles was not sufficient [7]. Another study of physical activity levels in various European countries showed that only 6-8% Poles were sufficiently physically active [11]. Research on top-level managers revealed that the most recent WHO and ACSM physical activity recommendations were only fulfilled by 27% of respondents [32]. The results of the present study regarding meeting the ACSM recommendations are very similar. We are, however, fully aware that the present study assessed only reported physical activity levels, which can be

overestimated by respondents [1, 3, 9, 17, 22, 27, 30, 35, 47]. The respondents' actual physical activity levels could be, therefore, lower.

The significance of the results of the present study indicates the necessity of their further practical application. The prospective research area must be wider and the research process more in-depth. Future studies should also cover populations of children and adolescents as well as senior citizens. They should also be extended to other regions of Poland and employ tools measuring physical activity levels in leisure time, at work, while getting from place to place, and at home. Studies by *Jurakic et al.* [22], *Katz et al.* [23], and *Knuth et al.* [24] indicate that the physical activity levels in all these areas of modern life can be diverse.

## CONCLUSIONS

1. The physical activity levels of studied men were higher than in women, especially in high-intensity exercises.
2. The level of physical activity undertaken by the Katowice men can be considered satisfactory as 50% of them met the health-enhancing physical activity recommendations developed by the American College of Sports Medicine.
3. The highest percentage of respondents meeting the health-related physical activity recommendations was noted in the age group of 30-39 years, among inhabitants with a primary and basic vocational education.
4. Propagation of physical activity among adults and the elderly, and among women in particular, remains a continuing challenge facing health promotion professionals.

### Conflict of interest

*The authors declare no conflict of interest.*

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## ASSESSMENT OF NUTRITIONAL STATUS ON THE BASIS OF THE MINI NUTRITIONAL ASSESSMENT QUESTIONNAIRE IN PATIENTS ON THE INTERNAL MEDICINE WARD

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### ABSTRACT

**Background.** The inadequate nutritional status of hospitalized patients leading to the malnutrition is one of the crucial clinical problems.

**Objective.** The aim of the study was to assess nutritional status of patients on the basis of the mini nutritional assessment (MNA) questionnaire.

**Material and Methods.** The study group included 120 patients staying on the internal medicine ward. The average age for female and male was 65±9.7 and 63±8.4 years, respectively. To assess malnutrition the anthropometric measurements and the MNA questionnaire was conducted.

**Results.** The average MIS (Malnutrition Indicator Score) value in female and male was 25.0±3.0. Among 29% of women and 18.2% of men the risk of malnutrition occurrence was noted. The incorrect values of BMI (body mass index) were observed more frequently in male compared to female (84.0% vs. 67.0%). Incorrect values of the MAC (mid arm circumference) and CC (calf circumference) were found respectively in 6.6% and 10.5% of female and in 2.3% of male. 55.3% of women and 75% of men declared taking more than 3 prescription drugs a day. In women were observed a significantly higher deficiency of dairy products, meat, fish, or poultry as well as meals containing legume plants or eggs per week compared to men (21.0% vs. 4.5%). An insufficient daily intake of vegetables was declared by 20.4% of men and 15.8% of women.

**Conclusions.** The occurrence of malnutrition or its risk was observed in about a quarter of studied hospitalized patients. The malnutrition was worsened by a reported loss of appetite, a significant number of drugs being taken, and inadequate nutrition among the patients.

**Key words:** nutritional status, MNA questionnaire, malnutrition, obesity

### STRESZCZENIE

**Wprowadzenie.** Nieprawidłowy stan odżywienia, w populacji pacjentów hospitalizowanych, prowadzący do rozwoju niedożywienia, należy do istotnych problemów klinicznych.

**Cel badań.** Celem badania była ocena stanu odżywienia pacjentów na podstawie ankiety MNA (Mini Nutritional Assessment).

**Materiały i Metody.** Badaniem objęto 120 pacjentów hospitalizowanych na oddziale chorób wewnętrznych. Średnia wieku dla kobiet i mężczyzn wynosiła odpowiednio 65 ± 9,7 i 63 ± 8,4 lat. Aby ocenić stan odżywienia przeprowadzono badania antropometryczne i dokonano oceny stanu odżywienia na podstawie kwestionariusza MNA.

**Wyniki.** Średnia wartość wskaźnika MIS (ang. Malnutrition indicator score) u kobiet i mężczyzn wynosiła 25,0 ± 3,0. Spośród 29% kobiet i 18,2% mężczyzn odnotowano ryzyko wystąpienia niedożywienia. Nieprawidłowe wartości BMI (body mass index) obserwowano częściej u mężczyzn niż u kobiet (84,0% w porównaniu z 67,0%). Nieprawidłowe wartości MAC (ang. Mid arm circumference) i CC (ang. Calf circumference) stwierdzono odpowiednio u 6,6% i 10,5% kobiet oraz u 2,3% mężczyzn. 55,3% kobiet i 75% mężczyzn deklarowało przyjmowanie więcej niż 3 leków na receptę dziennie. U kobiet obserwowano znacznie większy niedobór produktów mlecznych, mięsa, ryb lub drobiu, a także posiłków zawierających rośliny strączkowe lub jaja w tygodniowej diecie, w porównaniu z mężczyznami (21,0% vs. 4,5%). Niewystarczające dzienne spożycie warzyw deklarowało 20,4% mężczyzn i 15,8% kobiet.

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**Wnioski.** Występowanie niedożywienia lub jego ryzyko obserwowano u około jednej czwartej pacjentów hospitalizowanych. Niedożywienie pogarszało się powodu zgłoszonej utraty apetytu, znacznej liczby przyjmowanych leków i nieprawidłowego odżywiania wśród pacjentów.

**Słowa kluczowe:** stan odżywienia, ankieta MNA, niedożywienie, otyłość

## INTRODUCTION

Inadequate nutrition, leading to the development of malnutrition, is one of the significant clinical problems of hospitalized patients on all hospital wards. According to the World Health Organization (WHO) in the years 2010-2012 the problem of malnutrition concerned about 12.5% of the world population [16]. The authors of the multi-center study, conducted in six European countries including Poland during the period 2006-2012, observed an occurrence of malnutrition in over 23% of the Polish seniors [18].

Adequate nutrition adjusted to metabolic and clinical conditions constitute a very significant yet still unappreciated element of treatment. The occurrence of malnutrition, as well as overweight and obesity, resulting in numerous quality deficiencies, is connected with inadequate nutrition in hospitals and at patients' homes. It can also result from the severity of disease and the method of treatment, thus adversely affecting a patient's prognosis [22].

The occurrence of malnutrition in hospitalized patients with underweight or obesity is significantly more costly for the public healthcare system than treating patients having a normal weight. It is estimated that the cost of treatment of a person who is overweight is 10% higher, while in an obese person it is 36% higher than in a person with normal body weight. Moreover, the occurrence of obesity significantly increases the risk for the development of complications and mortality [3].

Excess body weight is given as a reason accounting for 80% of type 2 diabetes, 55% of high blood pressure and 35% of ischemic heart disease [21]. Obesity does not exclude protein-energy malnutrition (PEM). In the elderly with overweight and obesity, seemingly well nourished, quality malnutrition is prevalent. Deficiencies of macro-nutrients as well as vitamins and minerals aggravate concomitant diseases [21, 29].

Standards of treatment on hospital wards should take into account the adequate nutritional status and the diet of patients with underweight as well as overweight and obesity. The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends the MNA questionnaire to assess nutritional status in patients after the age of 60 years [4]. By the MNA questionnaire, it is possible to predict the occurrence of malnutrition before deficiencies and concentrations of proteins appear in the blood serum. The occurrence of

malnutrition in hospitalized patients not only depends on current nutritional status but also the severity of the disease, pharmacotherapy, eating habits and the patient's mental state. The MNA questionnaire enables a broad evaluation of the nutritional status and the psycho-physical condition of the patient [8, 27].

The aim of the study was to assess nutritional status in hospitalized patients on the basis of the MNA questionnaire and selected anthropometric parameters.

The research received the approval of the Bioethics Commission at the Wrocław Medical University, Poland.

## MATERIAL AND METHODS

The study group included 120 patients (76 women; 44 men) hospitalized in the Internal Medicine Clinic of the 4th Military Hospital with Policlinic in Wrocław. The average age of female and male participants was  $65 \pm 9.7$  years (min- 50; max- 84) and  $63 \pm 8.4$  years (min- 50; max- 84), respectively. The characteristic of the study population is shown in Table 1.

In order to assess malnutrition the Mini Nutritional Assessment (MNA) questionnaire was conducted. On its basis the Malnutrition Indicator Score (MIS) was determined. The score of the MNA questionnaire is comprised by four parts: anthropometric measures (body mass index (BMI), mid arm circumference (MAC), calf circumference (CC), general assessment, nutrition and the patient's subjective self-evaluation (self-esteem). The final score qualifies the patients to one of the 3 groups: normal nutritional status, at risk of malnutrition or malnourished. Based on the MIS results, the research patients were divided accordingly: MIS < 17.5 points - patients malnourished

MIS: 17.5-23.5 points – patients at risk of malnutrition

MIS > 23.5 – patients with normal nutritional status

Waist measurement was taken at the narrowest place of the torso and as reference values the following were taken:  $\leq 80$  cm in women and  $\leq 94$  cm in men [34]. MAC was measured half-way between the coracoid process and the elbow in the non-dominant limb. The MAC reference value was assumed to be 23 cm (according to the values assumed in the MNA questionnaire).

On the basis of achieved anthropometric parameters the average value of BMI and the average value of waist to hip ratio (WHR) were calculated.

The average WHR was calculated for patients with confirmed overweight or obesity measured by the BMI [33, 34].

The measurement of blood pressure was taken twice, after a previous 5-minute-rest of the patient, in the sitting position with the blood pressure shoulder monitor Omron M6 Comfort (HEM-7000- E(V)). In order to determine irregular values of blood pressure the 2013 ESH/ESC guidelines were assumed [10].

Statistical analysis was performed using the "Statistica v 12.0 PL" program by StatSoft Inc. USA. The obtained results were summarized by average ( $X$ ), standard deviation (SD) and median (M). Assessment of the significance of differences between average values in the studied groups, in order to compare two independent samples, was conducted applying the U Mann-Whitney test. The statistically significant differences between the categorical variables was assessed by the  $Chi^2$  test. In case of fewer than 5 observations, Yates' correction, in the  $Chi^2$  test was applied. The level of statistical significance was assumed at  $p < 0.05$ .

## RESULTS

The percentage of malnourished female and male participants, at risk of malnutrition or with normal nutritional status according to the results of the MNA questionnaire was shown in Fig. 1.

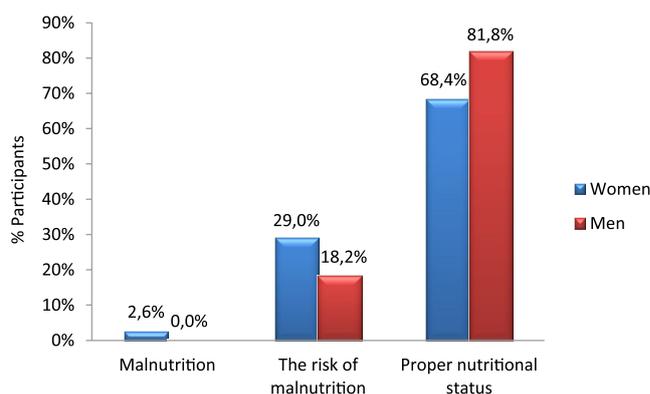


Figure 1. The prevalence of malnutrition, assessed on the basis of the MNA questionnaire, in the study population of women and men

The average value of the MIS index in the research population equaled  $25.0 \pm 3.0$ . Among 2.6% of female the malnutrition was observed. In 29% of women and 18.2% of men the risk of malnutrition was noted. The majority of participants, i. e. about 68% of women and about 82% of men were considered to have proper nutritional status.

Average values of body mass, height and waist circumference were significantly higher in the group of men, in comparison to the group of women. Incorrect average values of the WHR were observed in patients with overweight or obesity, indicating the occurrence of visceral obesity (Table 1.).

Table 1. Characteristics of the study population of women and men

Parameter	Women (W) n = 76		Men (M) n = 44		U M-W W vs M p
	X±SD	M	X±SD	M	
Age [yrs]	65±9.7	64.0	63±8.4	62	Ns
Body mass [kg]	72.7±16.7	72.0	86.8±17.0	86.5	0.00001
Height [cm]	158.5 ± 6.4	158.0	171.5 ± 7.6	172.0	0.00000
Waist circumference [cm]	92.4±15.7	94.0	104.5±11.9	104.8	0.00006
Hips circumference [cm]	104.6±16.5	103.5	105.2±8.5	105.5	Ns
WHR*	1.0±0.8	0.9	0.95±0.1	0.95	0.00
SBP [mmHg]	126.5±30.0	126.5	139.7±22.2	139.5	0.02279
DBP [mmHg]	77.7±17.3	78.8	86.7±13.0	86.8	0.00000

n- number of people studied; M- median; X±SD - average ± standard deviation; BMI- Body Mass Index; WHR\*- waist to hip ratio assessed for women (n=51) and men (n=37) with overweight and obesity; SBP- systolic blood pressure; DBP- diastolic blood pressure; U M-W - statistical test of U Mann-Whitney;  $p < 0.05$ - the value statistically important; Ns - non-statistically important value

It was evaluated that average values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in the group of male, in comparison to females (SBP:  $139.7 \pm 22.2$  mmHg vs.  $126.5 \pm 30.0$  mmHg,  $p = 0.02279$ ; DBP:  $86.7 \pm 13.0$  mmHg vs.  $77.7 \pm 17.3$  mmHg,  $p = 0.000$ ). Improper values of the blood pressure ( $\geq 140/90$  mmHg) were found in 47.7% of men and 34.2% of women.

The average values of anthropometric parameters and the incidence of occurrence of irregular values

of the researched parameters on the basis of the MNA questionnaire in the research population were presented in Table 2.

Average values of BMI indicated the occurrence of overweight both in women and men. Significantly more frequently the occurrence of abnormal values of BMI were observed in men compared to women (84.0% vs. 67.0%,  $p = 0.0000$ ). Incorrect values of MAC and CC were found respectively in 6.6% and 10.5% of women and in 2.3% of men.

Table 2. The average values and the prevalence of abnormal values of the analyzed parameters, based on a questionnaire MNA, in a study population of women and men

Mini Nutritional Assessment	Women (W) n = 76		Men (M) n = 44		W vs M* P
	X±SD	n (%)	X±SD	n (%)	
<b>Anthropometric parameters</b>					
BMI ≥ 25.0 kg/m <sup>2</sup>	29.1±6.3	51 (67.0)	29.8±4.3	37(84.0)	0.0000
MAC < 23 cm	29.5±4.7	5 (6.6)	30.5±3.4	1 (2.3)	Ns
CC < 31 cm	36.2±4.0	8 (10.5)	37.5±3.9	1 (2.3)	Ns
Weight loss during the last 3 months		<i>n (%)</i>		<i>n (%)</i>	
		16 (21.0)		8 (18.2)	Ns
<b>Overall assessment</b>		<i>n (%)</i>		<i>n (%)</i>	
Lives independently (not in nursing home or hospital)		63 (82.9)		33 (75.0)	Ns
Takes more than 3 prescription drugs per day		42 (55.3)		33 (75.0)	0.0314
Has suffered psychological stress or acute disease in the past 3 months		32 (42.1)		8 (18.2)	0.0074
Mobility- patient moves without any problems		76 (100.0)		44 (100.0)	Ns
No neuropsychological problems		73 (96.0)		43 (97.7)	Ns
Pressure sores or skin ulcers		1 (1.3)		2 (4.5)	Ns
<b>Nutrition</b>		<i>n (%)</i>		<i>n (%)</i>	
< 3 meals per day		5 (6.6)		7 (15.9)	Ns
In the daily diet no dairy products. meat. fish or poultry and food containing eggs or legumes per week		16 (21.0)		2 (4.5)	0.0147
Fruits and vegetables < 2 p/ day		12 (15.8)		9 (20.4)	Ns
Patients with decreased of appetite		19 (25.0)		10 (22.7)	Ns
Fluids < 5 cup's/ day		16 (21.0)		6 (13.6)	Ns
Patient unable to eat without assistance or self-fed with some difficulty		3 (3.9)		0 (0.0)	Ns
<b>Self - view of nutritional status</b>		<i>n (%)</i>		<i>n (%)</i>	
Patient views self as:					
- being malnourished		4 (5.3)		0 (0.0)	Ns
- is uncertain of nutritional state		11 (14.5)		0 (0.0)	0.0081
- having no nutritional problem		61 (80.3)		44 (100.0)	0.0016
In comparison with other people of the same age. the patient consider his / her health status is:					
- not as good		21 (27.6)		5 (11.4)	0.0371
- does not know		12 (15.8)		8 (18.2)	Ns
- as good		25 (32.9)		16 (36.4)	Ns
- better		18 (23.7)		15 (34.1)	Ns

n- number of people studied; X±SD- average ± standard deviation; BMI- Body Mass Index; MAC (Mid- arm Circumference); CC (Culff Circumference); p - portion; \*W vs M - Chi<sup>2</sup> statistical test; p<0.05 - the value statistically important; Ns- non- statistically important value

The loss of body mass within the last 3 months was observed in 21% of women and 18.2% of men. In the general assessment of the MNA questionnaire a significantly more frequent intake of more than three prescription drugs recommended by the doctor was found in men compared to women (75% vs. 55.3%, p=0.0314). The percentage of the patients who underwent severe stress or a serious illness within the previous 3 months was significantly higher in the group of women comparing to men (42.1% vs. 18.2%, p= 0.0074).

16% of men and 7% of women declared having fewer than 3 meals a day in their daily diet. Among diet of

women, the insufficient intake of dairy products, meat, and also meals containing legumes or eggs weekly was observed significantly more frequently compared to men (21% vs. 4.5%, p=0.0147). Insufficient intake of vegetables in the diet (<2 portions/day) was declared by 20.4% of men and 15.8% of women. There was observed a significant percentage of the studied patients in whom the loss of appetite was caused by an illness (25% of women, 22.7% of men). An incorrect fluid intake (< 5 cups/a day) was observed in 21.0% of womendiet and 13.6% of men. It was noted that only about 4% of women were incapable of eating without assistance or had meals with difficulties.

In self-perception of health and nutrition the 14.5% of women declared a worse nutritional status than men. About 20% of studied women declared problems with nutritional status. Additionally, 27.6% of the women assessed their nutritional status as worse in comparison to their peers. The percentage of men

evaluating their health condition in such a way was significantly lower and equaled 11.4%.

Table 3 and Table 4 present a comparison of the average values of selected anthropometric parameters and the blood pressure in the group of female and male participants, depending on the score of the MNA questionnaire.

Table 3. Comparison of mean values of selected parameters (anthropometry, body composition analysis, blood pressure) between the examined women with malnutrition or risk of malnutrition and women with proper nutritional status, according to MNA questionnaire

Parameter	Incorrect value	Women G1 MNA ≤ 23.5 points (n=24)			Women G2 MNA > 23.5 points (n=52)			U M-W GI vs GII	Chi <sup>2</sup> GI vs GII
								<i>p</i>	<i>p</i>
		<i>X</i> ± SD	M	<i>n</i> (%)	<i>X</i> ± SD	M	<i>n</i> (%)		
Body mass [kg]	-	67.2±15.9	64.4	-	75.2±16.6	74.5	-	Ns	-
WC	≥ 80 cm	87.5±14.8	84.5	15(62.5)	94.7±15.6	95.0	38 (73.1)	Ns	Ns
CC	< 31.0 cm	35.3±3.8	35.7	4 (16.7)	36.7±4.1	37.0	4 (7.7)	Ns	Ns
MAC	< 23.0 cm	28.1±5.0	28.5	4 (16.7)	30.1±4.4	29.5	1 (1.9)	Ns	0.0160
BMI	≥ 25.0 kg/m <sup>2</sup>	26.8±6.0	25.7	13(54.2)	30.1±6.1	30.7	38 (73.1)	0.0288	0.0421
WHR*	>0.85	0.85±0.1	0.84	13(100)	1.4±1.2	0.90	34 (89.5)	Ns	Ns
SBP	≥ 140 mmHg	136.2±23.7	127.5	7 (29.2)	123.9±31.4	126.5	13 (25.0)	Ns	Ns
DBP	≥ 90 mmHg	78.4±11.6	78.3	3 (12.5)	77.3±20.0	80.7	6 (11.5)	Ns	Ns

*n*- number of people studied; *M*- median; *X*±SD- average ± standard deviation; WC - waist circumference; MAC - Mid- arm Circumference; CC - Culf Circumference; BMI - Body Mass Index; WHR\*- waist to hip ratio assessed for women (n=51) and men (n=37) with overweight and obesity; SBP- systolic blood pressure; DBP- diastolic blood pressure; U M-W- statistical test of U *Mann-Whitney*; *p*<0.05 - the value statistically important; *Ns*- non-statistically important value

Table 4. Comparison of mean values of selected parameters (anthropometry, body composition analysis, blood pressure) between the examined men with malnutrition or risk of malnutrition and men with proper nutritional status, according to MNA questionnaire

Parameter	Incorrect value	Men G1 ≤ 23.5 points (n= 8)			Men G2 > 23.5 points (n= 36)			U M-W GI vs GII	Chi <sup>2</sup> GI vs GII
								<i>p</i>	<i>p</i>
		<i>X</i> ± SD	M	<i>n</i> (%)	<i>X</i> ± SD	M	<i>n</i> (%)		
Body mass [kg]	-	74.8 ± 24.0	72.8	-	89.5 ± 14.2	89.5	-	0.0319	-
Waist circumference	≥ 94 cm	100.4 ± 12.6	95.5	6 (75.0)	105.4 ± 11.7	105.0	30 (83.3)	Ns	Ns
CC	< 31.0 cm	36.2 ± 5.0	36.0	0 (0.0)	37.8 ± 3.6	38.0	1 (2.8)	Ns	Ns
MAC	< 23.0 cm	28.7 ± 5.0	29.0	1 (12.5)	30.8 ± 2.8	30.0	0 (0.0)	Ns	0.0319
BMI	≥ 25.0 kg/m <sup>2</sup>	26.7 ± 5.1	24.4	4 (50.0)	30.53.9	30.1	33 (91.7)	0.0308	0.0036
WHR*	>0.90	1.04±0.1	1.06	3 (75.0)	1.00±0.1	1.00	15 (45.4)	Ns	Ns
SBP	≥ 140 mmHg	126.0 ± 24.1	135.0	3 (37.5)	142.8 ± 23.2	141.0	18 (50.0)	Ns	Ns
DBP	≥ 90 mmHg	81.7 ± 14.5	80.0	1 (12.5)	88.1 ± 12.8	89.0	16 (44.4)	Ns	Ns

*n*- number of people studied; *M*- median; *X*±SD - average ± standard deviation; MAC- Mid- arm Circumference; CC - Culf Circumference; BMI- Body Mass Index; WHR\*- waist to hip ratio assessed for women (n=51) and men (n=37) with overweight and obesity; SBP- systolic blood pressure; DBP- diastolic blood pressure; U M-W - statistical test of U *Mann-Whitney*; *p*<0.05- the value statistically important; *Ns*- non-statistically important value

The occurrence of improper values of the MAC (< 23 cm) was observed significantly more frequently in the group of malnourished women in the study in comparison with the women in whom malnutrition was not found (16.7% vs. 1.9%; *p*=0.0160). A similar relationship was found in groups of men (G1 vs. G2; *p*=0.0319). Moreover, in the group of malnourished men having significantly lower average values of body mass were noted in comparison to the participants with normal nutritional status (74.8 ± 24.0 kg vs. 89.5 ± 14.2 kg; *p*=0.0319).

Average values of BMI were incorrect both in the groups of female and male and were significantly higher in the group of female and male participants who were not found to be malnourished in comparison with the malnourished participants. The percentage of participants with abnormal values of the BMI was significantly higher in women and men with proper nutritional status, in comparison to the malnourished women and men (73.1% vs 54.2%; *p*=0.0421 and 91.7% vs 50.0%; *p*=0.0036).

## DISCUSSION

Both excess and shortage of body mass negatively influence the patients' health. They lead to numerous disturbances in the body functioning, metabolism of drugs and regulation of food intake. Malnourished patients are at risk of qualitative and quantitative deficiencies in the diet. Disturbances in the nutritional status in hospitalized patients additionally deepen complex pharmacotherapy and periods of posts related to the diagnosis, affecting the metabolism of nutrients [14, 30].

In the study population malnutrition connected with underweight was not found. In the majority of the research participants the occurrence of overweight or obesity was noted. In the study conducted by *Skop-Lewandowska et al.* [26] in a group of patients with cardio-vascular diseases at the age over 39 years, the incorrect body weight was recognized in over 70%. *Koltuniuk and Rosińczuk et al.* [17] recognized overweight and obesity in a majority of patients from surgical wards in the elderly. In that study, the occurrence of excessive body mass was confirmed in 38% of participants and obesity in 30% of them.

The occurrence of abdominal obesity is connected with a higher share of metabolically active visceral fat tissue. Numerous clinical studies indicate a positive correlation of the occurrence of abdominal obesity and the higher risk of metabolic disease [2, 6]. In the research population both in the group of women and men, average values of waist circumference and WHR index indicated existence of abdominal obesity. In the study conducted by *Gacek et al.* [11] among the patients suffering from diabetes type 2, at the average age of 55 years, the occurrence of abdominal obesity was indicated in 72% of women and 74% of men. Average values of waist circumference and WHR indicated the occurrence of abdominal obesity, as it was observed in our study.

In the study conducted by *Bator et al.* [1], among Wrocław patients with recognized the metabolic syndrome the occurrence of abdominal obesity was indicated in 100% of women and 70% of men. Over 90% of all the participants had incorrect body waist. *Lakowska et al.* [20], conducted research in patients with hypertension. Abdominal obesity was found in 50% of women and 39.2% of men. Additionally, in the study conducted by *Goluch-Koniuszy and Fabianczyk* [12], in the group of persons aged 49-60 years, the percentage of persons with excess body mass and abdominal obesity was significantly higher (76% of women and 89% of men), in comparison with own research. In half of the participants, additional diseases were noted such as: hypertension, diabetes, rheumatoid arthritis, or hormonal disorders.

It can be anticipated that the increased incidence of obesity and the increased share of metabolically active abdominal fat tissue, worsens the course and treatment of the disease, resulting in hospitalization,

and also affects the outcome of the final evaluation of the nutritional status of the patient. The incidence of overweight and obesity does not exclude deficiencies of nutrients and protein-caloric malnutrition, particularly frequently observed in the elderly [31].

The incidence of malnutrition in hospitalized patients depends not only on the current nutritional status, but also on the stage of the disease, the pharmacotherapy, dietary habits as well as of the mental condition [19]. In the research population, malnutrition was found in about 24% of the patients. The incidence of malnutrition was smaller in the group of women and it was not indicated in the group of men. The Swedish research study conducted by *Persson et al.* [25] indicated that assessment of the nutritional status with the MNA questionnaire significantly correlated with mortality. In malnourished patients, where the average MNA equaled < 17.5 points, the level of mortality after a year was 40% and after 3 years 80%. As regards the patients with normal nutritional status, where the MNA was on average > 23.5 points, after a year mortality equaled 20%, and after 3 years 50%. Moreover, in the study conducted by *Diekmann et al.* [9] it was indicated that the results of the MNA questionnaire correlated with the albumin concentration and predicted a longer hospitalization of patients, as opposed to the Subjective Global Assessment (SGA) and the Nutrition Risk Score (NRS) scale. The MNA questionnaire, out of the three, best specified the normal nutritional status and the patients' survive-ability.

In the same area as in the own study *Wyka et al.* [35], in research among elderly, obtained similar results of assessment of the nutritional status with the MNA questionnaire as in our study. Malnutrition was not found in the participants who were found at risk of malnutrition constituted about 16% of the population. In the groups of patients with normal nutritional status, higher results of anthropometric parameters were noted: BMI, WHR, and waist in comparison to the groups with recognized risk of malnutrition. *Strugala et al.* [27], assessing the nutritional status in patients of the geriatric ward at the ages from 65 to 89 years, identified malnutrition in 7% of patients and a risk of malnutrition in 60%. *De Luis et al.* [7], in research among Spanish patients at ages over 70 years from internal medicine wards, indicated that 24% of the population constituted malnourished patients and 50% persons with a risk of malnutrition. In Brazil the risk of malnutrition was assessed in the elderly living in communal hostels in Rio de Janeiro with the MNA questionnaire. According to the MNA, 8.3% of the participants were malnourished, in 56% a risk of malnutrition was indicated and 36.1% of the participants had normal nutritional status. A significant correlation was found between the results of the MNA questionnaire and BMI ( $r=.391$ ,  $p=0.000$ ) [24].

Among the patients from our research the majority of the population took more than 3 drugs a day. *Saliburska*

and *Bogdanski* [28], in research conducted among the sick suffering from diabetes type 2, indicated that 60% of the patients take over 4 drugs a day and 9% over 3 drugs a day. Additionally, it was recognized that the eating habits of patients can affect correct metabolism and medication efficacy. It is believed that taking 2 drugs simultaneously by a patient can lead to the occurrence of adverse interactions in about 5,6% of people, 5 drugs at the same time in about 50%, and the intake of 8 pharmacological products increase the risk of the occurrence of adverse interactions in 100% patients [32].

Over 60% of the patients with a recognized risk of malnutrition indicated a significant or moderate loss of appetite. Loss of appetite is the result of many factors, such as: the disease stage, oral health, disturbances of taste and smell resulting from the disease and/or senility, disorders of the digestive system and the mental state of the patient. *Miwa et al.* [23] noted significant changes in the intake of food products, resulting from disturbances in smell and taste in the elderly. In this research, 36% of respondents pointed out that smell disturbances resulted in a more seldom choice of fresh food. Among 49% of the elderly, disturbances of smell determined the preparation of meals. In 75% they resulted in scorching meals and reducing the amount of food eaten. A chronic disease, limitations in physical activity, and the solitude of hospitalized elderly can cause depression which affects about 15% of persons after 65 years old. It is documented that depression can decrease the appetite of patients [15].

In our study, a smaller general number of MNA points was connected with lower self-evaluation of one's health condition. A higher percentage of patients evaluating their health as worse was noted in the group with a confirmed risk of malnutrition in comparison with persons with normal nutritional status. A similar regularity was noted by *Jachimowicz et al.* [13] in the research of students of the third age university, at the age over 65 years. Worse nutritional status was connected with a more frequent occurrence of emotional disturbances, reduction of life energy, increased pain perception and more frequent movement limitations.

The assessment of nutritional status on the basis of anthropometric parameters and the MNA questionnaire is a simple, non-invasive and effective screening tool administered to recognize malnutrition in the elderly, and is also recommended to identify the risk of malnutrition in this group of the population earlier. The results of the MNA questionnaire are reliable and can be easily interpreted by doctors and health-care professionals, in hospitals, nursing homes, or for early detection of threats connected with malnutrition [5]. Early recognition of malnutrition and the appropriate intervention in hospital patients may prevent later complications in treatment and convalescence.

## CONCLUSIONS

1. The assessment of nutritional status on the basis of the MNA questionnaire indicated the occurrence of malnutrition or the risk of malnutrition in about a quarter of the study population.
2. The average values of anthropometric indices indicated the occurrence of overweight and obesity in women and men. It was pointed out that the diets of the patients contained an incorrect amount of products containing plant and animal protein, fruit and vegetables as well as fluids.
3. Hospitalized patients from the study require a multi-aspect assessment of nutritional status because a seemingly normal nutritional status resulting from excessive body mass may lead to incorrect assessment of the patient's health condition.

### Conflict of interest

*The authors declare no conflict of interest.*

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## ASSESSMENT OF NUTRITIONAL BEHAVIOURS OF CHILDREN AGED 3-7 FROM SELECTED KINDERGARTENS IN BIALA PODLASKA COUNTY<sup>1</sup>

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### ABSTRACT

**Background.** Children constitute a group that is particularly susceptible to the effects of improper nutrition. The knowledge of parents and guardians about proper nutrition affects their children's nutritional habits and behaviours. Children develop their patterns of nutritional behaviours on the basis of their observations of nutritional habits of adults, nutrition trends and nutritional education.

**Objective.** The aim of the study was to assess nutritional behaviours of children aged 3-7 from selected kindergartens in Biała Podlaska county.

**Material and methods.** The research included 549 children, i.e. 258 girls (47%) and 291 boys (53%) aged 3-7 years. The study was conducted in 2015 within the county health prevention and promotion programme titled "Together for heart" in several selected kindergartens in Biała Podlaska county. A questionnaire prepared by the authors and distributed among parents of the examined children was used in the study. The questions regarded the frequency and number of meals, types of products consumed between the meals and the frequency of consuming fruit and vegetables, sweets, fizzy drinks, fast foods and milk or dairy products (natural yoghurt, kefir, cottage cheese). Differences between the responses to the questions in groups divided according to sex were analysed with the use of  $\chi^2$  test (G function).

**Results.** It was revealed that 219 girls (84.9%) and 273 boys (93.8%) had at least four meals a day while 23.7% of the children left home without breakfast. The main meal, i.e. dinner, was eaten by 227 girls and 256 boys every day, which constituted 88% of each gender group. In turn, 20 girls and 27 boys (8% and 9% of the study participants, respectively) regularly skipped supper. The most common products consumed between the main meals were fruit and vegetables (eaten by 44% of the girls and 39% of the boys). It was declared that dairy products (milk, natural yoghurt and/or kefir) were consumed once a week or less frequently by 43% of the girls and 45% of the boys. Cottage cheese was equally rarely eaten, i.e. 48% of the girls and 44% of the boys reported having it once a week or less frequently. The study also revealed insufficient consumption of fish. It was eaten once a week or less frequently by 60% of the girls and 50% of the boys.

**Conclusions.** The assessment of nutritional behaviours revealed mistakes which may negatively affect proper development if they are repeated. It seems necessary to implement nutritional education among children and their parents in order to shape proper nutritional behaviours and correct mistakes.

**Key words:** *eating behaviours, preschool children, assessment of consumption, children nutrition*

### STRESZCZENIE

**Wprowadzenie.** Dzieci w wieku przedszkolnym to grupa szczególnie podatna na skutki niewłaściwego żywienia. Na kształtowanie postaw i zachowań żywieniowych w dużym stopniu ma wpływ wiedza rodziców i opiekunów na temat prawidłowego odżywiania się. Dzieci budują swoje wzorce na podstawie zauważonych nawyków żywieniowych osób dorosłych, poprzez modę żywieniową oraz poziom edukacji żywieniowej.

**Cel pracy.** Celem pracy była ocena zachowań żywieniowych dzieci w wieku 3-7 lat uczęszczających do kilku wybranych placówek przedszkolnych z terenu powiatu białskiego.

**Material i metody.** Badaniem objęto 549 dzieci, w tym 258 dziewcząt (47%) i 291 chłopców (53%) w wieku 3-7 lat. Badania zrealizowano w 2015 roku w ramach powiatowego programu profilaktyki i promocji zdrowia pn. „Razem dla serca” w kilku przedszkolach powiatu białskiego. Wykorzystano autorski kwestionariusz ankiety rozdawany rodzicom badanych dzieci. Pytania w ankiecie dotyczyły częstotliwości i liczby spożywanych posiłków, rodzaju produktów spożywanych między posiłkami, a także częstotliwości spożycia

<sup>1</sup> The study was conducted within the county health prevention and promotion programme aimed at reducing cardiovascular diseases morbidity and mortality among the inhabitants of Biała Podlaska county titled "Together for heart" and financed from the resources of the Norwegian Financial Mechanism 2009-2014 and the national budget.

warzyw i owoców, słodczy, napojów gazowanych, produktów typu fast-food oraz mleka i produktów mlecznych (jogurtu naturalnego, kefiru, serów twarogowych). Analizę różnicowania odpowiedzi udzielonych na pytania zawarte w ankiecie w grupach wyodrębnionych na podstawie płci przeprowadzono za pomocą testu  $\chi^2$  w postaci funkcji G.

**Wyniki.** Wykazano, że 219 dziewcząt (84,9%) i 273 chłopców (93,8%) spożywało co najmniej cztery posiłki w ciągu dnia oraz, że 23,7% dzieci wychodziło do przedszkola bez spożycia I śniadania. Główny posiłek – obiad – spożywało codziennie 227 dziewcząt i 256 chłopców, co stanowiło 88% w każdej z grup uwzględniając płeć badanych. Natomiast regularnie kolacji nie spożywało 20 dziewcząt i 27 chłopców, co stanowiło odpowiednio 8 i

9% badanych. Najczęściej spożywanymi produktami pomiędzy głównymi posiłkami były owoce i warzywa; spożywało je 44% dziewcząt i 39% chłopców. Spożycie produktów mlecznych (mleka, jogurtu naturalnego i/lub kefiru) raz w tygodniu lub rzadziej deklarowało

43% dziewcząt i 45% chłopców. Równie rzadko spożywane były sery twarogowe, ich spożycie raz w tygodniu lub rzadziej dotyczyło zaledwie 48% dziewcząt i 44% chłopców. W badaniach wykazano również niedostateczne spożycie ryb. Raz w tygodniu lub rzadziej zjadało je odpowiednio 60% dziewcząt oraz 50% chłopców.

**Wnioski.** Ocena zachowań żywieniowych wykazała błędy popełniane przez dziewczęta i chłopców, których utrwalenie może mieć negatywny wpływ na ich prawidłowy rozwój. Koniecznym wydaje się prowadzenie edukacji żywieniowej wśród dzieci oraz ich rodziców w celu kształtowania odpowiednich zachowań żywieniowych i korygowania popełnianych w tym zakresie błędów.

**Słowa kluczowe:** zachowania żywieniowe, dzieci w wieku przedszkolnym, ocena konsumpcji, żywienie dzieci

## INTRODUCTION

Proper nutrition of pre-school children means providing them with regular, diverse meals including all the necessary nutrients in the amounts meeting the needs of a young body. The nutrients should ensure an optimal supply of energy enabling the body to function as well as facilitating proper physical, mental, emotional and motor development [3, 11]. Children should consume a proper amount of all the necessary products, particularly fruit and vegetables as well as protein. Insufficient consumption of the above-mentioned products and eating large amounts of sweets between meals may lead to the development of nutrition-related diseases and cause improper nutritional habits in adult life [7, 19, 21].

## MATERIAL AND METHODS

The research included 549 children, i.e. 258 girls (47%) and 291 boys (53%) aged 3-7 from selected kindergartens in Biala Podlaska county, which is situated in the northern part of Lubelskie province. The study was conducted in 2015 within the county health prevention and promotion programme titled "Together for heart" aimed at reducing cardiovascular diseases morbidity and mortality rate. The programme was financed from the resources of the Norwegian Financial Mechanism 2009-2014 and the national budget. The research was conducted in state kindergartens selected by Biala Podlaska County authorities on the basis of their responses to the offer of joining the programme. Out of 16 kindergartens, 11 responded positively, which constituted 68.8% of all the state kindergartens in the county. Only children whose parents gave a consent to complete the questionnaire were included in the study. The questionnaire applied in the study was prepared by the authors and included questions

regarding the frequency and number of meals eaten at home and in a kindergarten, types of products consumed between the meals and the frequency of consuming fruit and vegetables, sweets, fizzy drinks, fast foods and milk or dairy products (natural yoghurt, kefir, cottage cheese). Differences between the responses to the questions in groups divided according to sex were analysed with the use of  $\chi^2$  test (G-test).

## RESULTS

Table 1 includes data regarding the type and number of meals consumed by children every day. A vast majority of them, i.e. 53% of the girls and 58% of the boys had 5 meals a day. A maximum of 3 meals a day were eaten by 39 girls (15%) and 18 boys (6%). Differences between the girls and boys regarding the number of consumed meals proved statistically significant ( $G=12.43$ ;  $p\leq 0.05$ ). Moreover, 191 girls (74%) and 228 boys (78%) had breakfast before going to the kindergarten, while 88% of the study participants from each group had dinner every day and the numbers (227 boys and 256 girls) were significantly higher ( $G=6.03$ ;  $p\leq 0.05$ ) than the remaining 31 girls and 35 boys (12% of the children from both groups) who had dinner less than 7 times a week. Supper was eaten regularly by 91-92% of the girls and boys.

Table 2 presents data regarding products eaten for breakfast by children before going to the kindergarten. Both girls and boys started their day from cereals or groats with milk most often (42% and 45%, respectively). Slightly lower numbers of girls and boys had bread or sweet rolls for breakfast (34% and 38%, respectively). Dairy products as the first meal were eaten by 19 girls (7%) and 11 boys (4%). Differences regarding types of products consumed by children for breakfast proved statistically significant ( $G=6.25$ ;  $p\leq 0.05$ ).

Table 1. Type and number of meals consumed by girls and boys

Question	Research category	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How many meals does a child have a day?	More than 5 meals	19	7	27	9	12.43*
	5 meals	136	53	169	58	
	4 meals	64	25	77	26	
	3 meals	30	12	15	5	
	Fewer than 3 meals	9	3	3	1	
Does a child have breakfast before going to the kindergarten?	yes	191	74	228	78	1.42
	no	67	26	63	22	
How often does a child have dinner?	Every day	227	88	256	88	6.03*
	5-6 times a week	28	11	23	8	
	2-4 times a week	3	1	12	4	
Does a child have supper every day?	yes	238	92	264	91	3.27
	no	20	8	27	9	

Note: \*p<0.05 – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 2. Types of products consumed by children for breakfast

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
What does a child's breakfast include?	sandwiches, sweet rolls	89	34	111	38	6,25*
	dairy products	19	7	11	4	
	scrambled eggs	4	2	1	1	
	cereals/groats with milk	109	42	131	45	
	other inne	11	4	12	4	

Note: \*p<0.05 – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 3 illustrates data on the consumption of wholemeal bread. Statistically significant differences between girls and boys (G=10.96; p<0.05) regarding the frequency of consuming these products were revealed. Wholemeal bread was eaten daily by 19% and 20% of the examined children, while 93 girls

and 76 boys (36% and 27%, respectively) had it once a week or less frequently. White bread and rolls were consumed daily by 108 girls (42%) and 111 boys (38%), while 8% of the girls and 5% of the boys had them once a week or less frequently.

Table 3. Frequency of consuming white and brown bread and rolls

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How often does a child eat brown bread or rolls?	every day	52	20	56	19	10.96*
	4-6 times a week	33	13	65	22	
	2-3 times a week	60	23	60	21	
	once a week or less	93	36	79	27	
How often does a child eat white bread or rolls?	every day	108	42	111	38	4.64
	4-6 times a week	74	29	105	36	
	2-3 times a week	45	17	52	18	
	once a week or less	21	8	15	5	

Note: \*p<0.05 – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 4 demonstrates data concerning the consumption of products between the main meals. It was revealed that a similar percentage of girls and boys (44% and 39%, respectively) most often had fruit and vegetables between the main meals. Also, cakes and sweets were popular as 27% of the girls

and 22% of the boys consumed them. A considerably lower percentage of girls (7%) and boys (14%) chose crisps or breadsticks. The difference between types of products selected by girls and boys between the main meals was statistically significant ( $G=8.08$ ;  $p \leq 0.05$ ).

Table 4. Types of products eaten between the main meals

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
What types of products does a child eat between meals?	fruit and vegetables	114	44	115	39	8.08*
	sweets or cakes	70	27	66	22	
	crisps, breadsticks	19	7	42	14	

Note: \* $p \leq 0.05$  – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 5 includes data on the frequency of consuming fruit and vegetables in one week. Everyday vegetable consumption was declared by 20% of the girls' parents and 18% of the boys' parents, while 19% of the girls and 21% of the boys had vegetables no more than once a week. Children had fruit considerably more

often. According to the parents' declarations, 33% of the girls and 30% of the boys had fruit every day. No significant differences between the girls and boys were found as far as the consumption of fruit ( $G=2.19$ ) and vegetables ( $G=1.43$ ) is concerned.

Table 5. Weekly frequency of consuming fruit and vegetables

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How many times a week does a child eat vegetables?	every day	51	20	53	18	2.19
	4-6 times a week	75	29	73	25	
	2-3 times a week	71	28	87	30	
	once a week or less	49	19	61	21	
How many times a week does a child eat fruit?	every day	86	33	87	30	1.43
	4-6 times a week	101	39	117	40	
	2-3 times a week	52	20	60	21	
	once a week or less	14	5	18	6	

Note: \* $p \leq 0.05$  – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 6 shows data on the consumption of highly processed food. Differences regarding weekly consumption of sweets were statistically significant ( $G=70.13$ ;  $p \leq 0.05$ ). According to the parents' declarations, 14% of the girls and 18% of the boys had sweets every day, while 16% of the girls and 36% of the boys ate them no more than once a week. Also, differences concerning the frequency of fast food consumption proved statistically significant ( $G=4.94$ ;  $p \leq 0.05$ ). These products were eaten every day by 4% of the girls and 7% of the boys, while 64% of the girls and 58% of the boys consumed them once a week or less frequently. Parents also responded to the question concerning the frequency of consumption of sweet drinks. This type of beverages was consumed once a week or less frequently by 26% of the girls and 22% of

the boys. When it came to daily consumption, a reverse trend was noted, i.e. 22% of the girls and 26% of the boys drank them every day.

Data on the frequency of dairy products consumption are illustrated in table 7. Differences between the declared frequency of eating fermented dairy products by girls and boys were statistically significant ( $G=15.37$ ;  $p \leq 0.05$ ). 10% of the girls and 18% of the boys consumed natural yoghurt and kefir every day, while 43% of the girls and 45% of the boys consumed these products no more than once a week. The consumption of cottage cheese was at a similar level. Half of the children (44-48%) consumed this product once a week or less frequently, while 2% of the girls and 1% of the boys had it every day. Differences between the declared frequency of cottage cheese consumption by girls and boys were statistically significant ( $G=3.87$ ;  $p \leq 0.05$ ).

Table 6. Frequency of consuming processed food products

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How often does a child eat sweets?	every day	36	14	52	18	70.13*
	4-6 times a week	83	32	101	35	
	2-3 times a week	91	35	28	10	
	once a week or less	40	16	106	36	
How often does a child eat fast food?	every day	10	4	21	7	4.94*
	4-6 times a week	23	9	35	12	
	2-3 times a week	36	14	41	14	
	once a week or less	165	64	168	58	
How often does a child have sweet drinks?	every day	58	22	77	26	2.21
	4-6 times a week	55	21	70	24	
	2-3 times a week	68	26	70	24	
	once a week or less	66	26	65	22	

Note: \* $p \leq 0.05$  – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 7. Frequency of consuming natural yoghurt, kefir and cottage cheese

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How often does a child have natural yoghurt and kefir?	every day	25	10	51	18	15.37*
	4-6 times a week	39	15	35	12	
	2-3 times a week	72	28	52	18	
	once a week or less	110	43	131	45	
How often does a child eat cottage cheese?	every day	4	2	4	1	3.87*
	4-6 times a week	44	17	59	20	
	2-3 times a week	75	29	76	26	
	once a week or less	123	48	129	44	

Note: \* $p \leq 0.05$  – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 8 includes information regarding the frequency of consuming fat products added to meals. It was revealed that 12% of the girls and 14% of the boys had margarine every day, while 41% and 34% of the girls and boys, respectively, ate it no more than once a week. Everyday consumption of vegetable oil was declared by 7% of the girls' parents and 12% of the boys' parents. In turn, according to the declarations, this product was consumed once a week or less frequently by 34% of the girls and 21% of the boys. Differences regarding the frequency of consuming vegetable oil by girls and boys were statistically significant ( $G=14.12$ ;  $p \leq 0.05$ ). Daily consumption of butter was declared by one third of all the parents. In turn, 8% of the girls and 10% of the boys ate it no more than once a week. Differences concerning a weekly intake of butter by girls and boys were not statistically significant ( $G=1.11$ ).

Table 9 demonstrates the frequency of consumption of red meat, poultry and fish. Everyday consumption of red meat was declared by 7% of the girls' parents and 5% of the boys' parents, while as many as 45% and 47% of the parents, respectively, declared that their children ate this product 2-3 times a week. Differences regarding a weekly intake of red meat by girls and boys were statistically significant ( $G=6.26$ ;  $p \leq 0.05$ ). According to the parents' declarations, poultry was eaten most frequently, i.e. 2-3 times a week by more than a half of the children (51-53%), and less than once a week by 13% of the girls and 15% of the boys. Differences concerning weekly consumption of fish by girls and boys were also statistically significant ( $G=9.88$ ;  $p \leq 0.05$ ). Fish was eaten every day by 3-5% of the girls and boys, while its consumption no more than once a week was declared by 60% of the girls' parents and 50% of the boys' parents.

Table 8. Frequency of consuming fat products added to meals

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How often does a child have margarine?	every day	30	12	40	14	5.16
	4-6 times a week	36	14	58	20	
	2-3 times a week	48	19	50	17	
	once a week or less	105	41	99	34	
How often does a child have vegetable oils?	every day	18	7	35	12	14.12*
	4-6 times a week	46	18	69	24	
	2-3 times a week	86	33	97	33	
	once a week or less	88	34	62	21	
How often does a child have butter?	every day	84	33	92	32	1.11
	4-6 times a week	90	35	100	34	
	2-3 times a week	54	21	58	20	
	once a week or less	20	8	30	10	

Note: \* $p \leq 0.05$  – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

Table 9. Frequency of consuming meat and fish

Question	Research categories	Girls n <sup>#</sup> =258		Boys n <sup>#</sup> =291		G function
		n <sup>##</sup>	%	n <sup>##</sup>	%	
How often does a child eat red meat?	every day	19	7	15	5	6.26*
	4-6 times a week	77	30	103	35	
	2-3 times a week	116	45	138	47	
	once a week or less	42	16	30	10	
How often does a child eat poultry?	every day	21	8	13	4	3.72
	4-6 times a week	64	25	77	26	
	2-3 times a week	137	53	149	51	
	once a week or less	33	13	44	15	
How often does a child eat fish?	every day	9	3	14	5	9.88*
	4-6 times a week	20	8	44	15	
	2-3 times a week	68	26	81	28	
	once a week or less	155	60	145	50	

Note: \* $p \leq 0.05$  – statistically significant differences between the number of responses provided by the parents of girls and boys ( $\chi^2$  test – G-test); n<sup>#</sup> number of participants; n<sup>##</sup> number of responses

## DISCUSSION

The studies conducted to date have indicated that nutritional habits of children developed in early childhood include not only behaviours observed in their parents but also in their kindergarten teachers and they determine their nutritional choices in adult life [4]. Kindergartens are obliged to provide children with 70% of daily energy requirements with an appropriate supply of particular nutrients [3, 4, 14, 23]. According to the recommendations of the Food and Nutrition Institute and Mother and Child Institute, pre-school children should eat 4-5 meals a day regularly every 3-4 hours [8, 9, 15].

A tendency to having an improper number of meals, i.e. three or even less, was noted among the observed children, mainly among the girls (15% of the girls and 6% of the boys). It may result in an insufficient daily

calorie intake, deficiency in nutrients that leads to being underweight as well as developmental disorders. Among the examined children, 88% of the girls and boys had dinner every day; however, as many as 47 children did not have supper every evening (Table 1). Similar behaviours in pre-school children were noted by *Kostecka* [11], who concluded that children attending kindergartens very often ate too few meals which were additionally badly composed. On the other hand, the data included in table 1 also showed that 7% of the girls and 9% of the boys had more than 5 meals, which may lead to excessive energy intake as well as obesity and diseases related to it, as it was pointed out by *Harton* [7]. The results presented in table 1 also indicated that main meals are eaten irregularly, which is not a unique phenomenon, as similar tendencies were noted by *Sadowska* and *Krzymska* [8, 16].

The data demonstrated in table 3 show that a small number of children, i.e. only 19-20% of all the participants followed the recommendations presented in the Healthy Diet and Physical Activity Pyramid for Children and Youth and had sandwiches with wholemeal bread every day, paying particular attention to ingredients. In the case of children having their first meal at home, the type of carbohydrates included in this meal is important. In our research, the daily intake of the recommended wholemeal products was not presented but it was revealed that less than half of both girls and boys (47% and 49%, respectively) had carbohydrates in their first meal in the form of cereals or groats (Table 2). Skipping breakfast at home, which was noted in 26% of the girls and 22% of the boys (Table 1), or an improper composition of the meal may reduce psychophysical capacities and cause morning hypoglycemia [7, 8, 16].

The study also revealed a habit of eating between meals, which is widespread among children (Table 4). It was noted in 34% of the girls and 36% of the boys who additionally had sweets, cakes, crisps or breadsticks during the day and consumed unnecessary calories coming mainly from simple carbohydrates. Similar observations were also made by Kolarzyk et al. and Kostecka [10, 11]. Tooth decay poses a serious threat to children, with all the consequences in the future. It is caused, inter alia, by increased fermentation of carbohydrates by bacteria found at dental plaque. Children with milk teeth or not fully mature permanent teeth are particularly prone to tooth decay, which was confirmed by Tkaczuk [19]. Therefore, pre-school children should be encouraged to eat nutritious meals taking into account their aversion to things they do not know or like [8, 15, 21].

Everyday diet of children should include fruit, vegetables, low-fat dairy products, wholemeal products, fish and lean meat. The assessment of the level of consumption of these products by children revealed that vegetables were eaten every day only by 20% of the girls and 18% of the boys (Table 5), which was also confirmed in the research by Kostecka, who pointed to low consumption of vegetables on a daily basis [11]. A child's diet should include fruit and vegetables [6], and, according to the WHO, children should consume approximately 400 g of fruit and vegetables every day [20].

The period of intensive growth in children is also characterised by a high demand for complete protein which can be found, inter alia, in milk and its products which simultaneously exert an influence on the intake of calcium necessary for bone mass development. The research revealed that the guidelines on providing catering for children included in the Ordinance of the Minister of Health [24], which determines the frequency of consuming particular groups of products,

e.g. two portions of milk or dairy products daily, are not followed. The examined children (both girls and boys) consumed an insufficient amount of these products and the difference between them was only slight, which was also noted by Gacek [5]. Other researchers also confirm such a low intake of milk products and at the same time they highlight the fact that calcium intake should be kept at a high level, particularly until peak bone mass is achieved [1, 15].

The Ordinance of the Minister of Health [24] also determines the consumption of fish by pre-school children. It is stressed that it should be eaten at least once a week. The study revealed that fish was eaten more than once a week by more boys than girls (48% vs. 37%). The remaining children had fish once a week or less frequently, which was also confirmed by the findings of Gacek [5, 24].

One of the determinants of excessive body mass in children and the development of diet-related diseases is the consumption of highly-processed food. It was revealed that 46% of the girls and 53% of the boys had sweets four times a week or more often (Table 6). In their guidelines concerning children, the Food and Nutrition Institute recommends limiting or excluding sweets and sweet drinks from the diet and indicates that they cause obesity and lead to the development of diet-related diseases [8]. Sochacka-Tatara and Gruszka [18] claimed that overweight and obesity are caused by excessive consumption of sweet products and, as a result, by long-lasting positive energy balance. The study also revealed considerable consumption of fast food by children (Table 6); 13-19% of the children had these products more frequently than 4 times a week. It is also confirmed by other researchers who indicated that excessive consumption of fast food resulted in the formation of improper food habits and led to the development of excessive body mass [2].

Our research also showed that 22% of the girls and 26% of the boys had sweet drinks every day (Table 6). A negative influence of excessive consumption of sweet drinks was noted by numerous authors who pointed out that products which contain simple carbohydrates in a liquid form, just like other processed products containing large amounts of sugar, change daily energy balance of a child and should be excluded or limited as quickly as possible [5, 11, 22].

In their research, *Merkiel* and *Chalcarz* [13] concluded that consuming excessive amounts of saturated fats and limiting the consumption of polyunsaturated fats in childhood increase the risk of obesity and may lead to improper brain development and learning difficulties. In our study, it was revealed that 32-33% of the children had butter every day, while vegetable oils, which are the source of unsaturated fats, were consumed only by 7-12% of the children. Fat intake was at a similar level in girls and boys.

Excessive intake of animal fats, which is mainly associated with red meat consumption (noted in 39% of the children), may lead to obesity, which was also described by *Kostecka* [11] as well as *Merkel* and *Chalcarz* [12, 13]. Moreover, our research revealed that the study participants rarely had fish which is a significant source of unsaturated fats (Table 9). As many as 60% of the examined girls and 50% of the boys had fish once a week or less frequently, which was also confirmed by other researchers. They also indicated that this may be the cause of an insufficient intake of polyunsaturated fats by children [12, 17].

1. Nutritional mistakes of the children under investigation were related to irregularity of meals, insufficient consumption of dairy products, fruit and vegetables, wholemeal products, unsaturated fats and fish as well as excessive consumption of sweets, highly processed food and sweet drinks.
2. The prevention of diet-related diseases in the nutrition of children requires limiting the consumption of simple carbohydrates and saturated fats.
3. The qualitative assessment of children's nutrition indicated the need for nutritional education of parents and children aimed at developing proper nutritional habits.

#### Conflict of interest

*The authors declare no conflict of interest.*

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## IMPROVING THE EFFECTIVENESS OF OBESITY TREATMENT BY COMBINING A DIET AND MOTIVATIONAL TECHNIQUES

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### ABSTRACT

**Background.** Obesity is considered a disease. The treatment of obesity should include the modification of health behaviors in order to achieve long-term weight loss.

**Objectives.** The comparison between traditional weight loss treatment with and without a motivational therapy.

**Materials and Methods.** The study included 104 overweight women or women with obesity with a median age of 34. They were under the supervision of a dietician. The patients were divided into two groups: the standard group which was on an individualized and low-calorie diet developed by a dietitian and received substantial support, and the study group which additionally used motivational techniques.

**Results.** Mean weight loss, mean reduction in waist measurement and body fat after a 5-month treatment were calculated. The mean body weight loss in control group was 10.56 kg ( $\pm 8.65$ ), the waist measurement was 11.84 cm ( $\pm 10.62$ ), and fat loss was 5.11 ( $\pm 3.17$ ) kg on average - it corresponds to 8.02% ( $\pm 6.55$ ). The study in which additional motivational techniques were used, the mean weight loss was 17.31 kg ( $\pm 9.29$ ), the waist measurement was 15.25 cm ( $\pm 8.28$ ), and fat loss was 8.34 kg - it corresponds to 12.19% ( $\pm 7.32$ ) of adipose tissue. All the differences between treatment results in both groups were statistically significant ( $p < 0.05$ ).

**Conclusions.** The dietary treatment process employing motivational techniques has greater therapeutic efficacy than a traditional approach.

**Keywords:** *motivation, obesity, nutrition, overweight, weight reduction*

### STRESZCZENIE

**Wprowadzenie.** Otyłość uważana jest za chorobę. Leczenie otyłości powinno obejmować modyfikację zachowań zdrowotnych w celu osiągnięcia długoterminowej utraty masy ciała.

**Cele.** Porównanie efektywności tradycyjnego postępowania dietetycznego z postępowaniem wykorzystującym w terapii techniki motywacyjne.

**Materiał i metody.** Badaniem objęto 104 osoby z nadwagą lub otyłością, z medianą wieku 34 lat. Wszystkie pacjentki uczestniczyły w procesie dietoterapii pod kontrolą dietetyka. Pacjentki podzielono na dwie grupy: standardowa grupa stosowała zindywidualizowaną i niskokaloryczną dietę opracowaną wraz z wsparciem merytorycznym. Grupa badana dodatkowo stosowała techniki motywacyjne i była poddana modyfikacji zachowań zdrowotnych podczas stosowania dietoterapii.

**Wyniki.** Obliczono średnią utratę masy ciała, średnie zmniejszenie pomiaru talii i tkanki tłuszczowej po 5-miesięcznym leczeniu. Średnia utrata masy ciała w grupie kontrolnej wynosiła 10,56 kg ( $\pm 8,65$ ), pomiar talii 11,84 cm ( $\pm 10,62$ ), a utrata tkanki tłuszczowej średnio 5,11 ( $\pm 3,17$ ) kg - odpowiada 8,02% ( $\pm 6,55$ ). W badaniu, w którym zastosowano dodatkowe techniki motywacyjne, średnia utrata masy ciała wynosiła 17,3 kg ( $\pm 9,29$ ), pomiar talii 15,25 cm ( $\pm 8,28$ ), a utrata tłuszczu 8,34 kg - odpowiada 12,19% ( $\pm 7,32$ ) tkanki tłuszczowej. Wszystkie różnice między wynikami leczenia w obu grupach były statystycznie istotne ( $p < 0,05$ ).

**Wnioski.** Proces leczenia dietetycznego wykorzystujący techniki motywacyjne ma większą skuteczność terapeutyczną niż tradycyjne podejście.

**Słowa kluczowe:** *motywacja, otyłość, żywienie, nadwaga, redukcja masy ciała*

## INTRODUCTION

Obesity is a pathological state endangering human life [25]. People with obesity are predisposed to develop type 2 diabetes, hypertension and cardiovascular diseases, liver and biliary tract diseases, and the dysfunction of the skeletal system [20]. Obesity contributes to a high psychological burden and significantly increases the expenses of national health care [14]. The genesis of obesity is multifactorial. Health and nutrition behaviors constitute the main role in the prevalence of obesity. Irregular eating habits, eating ready-made meals, the increased consumption of highly processed food, including Fast Food and lack of physical activity are the most common reasons for weight gain in patients. In the dietetic practice, BIA (bioelectrical impedance analysis) method is used for the diagnosis of obesity. The analysis also automatically reports the patient's current body weight, BMI (body mass index), obesity grade and weight. Additionally, anthropometric measurements are used, primarily the waist measurement [7, 15]. The aim of treating obesity is to normalize body weight and the improvement of patients' well-being. It includes the modification of nutritional behaviors and introducing a proper, low-calorie diet under the supervision of a dietitian [3].

During the weight reduction process, the patient's individual approach and psychological support are very important. Motivational techniques are based on behavioral modification (cognitive behavioral therapy). Abnormal eating habits have been continued by obese patients over the years, so it requires a great deal of time and commitment to change them [10]. Motivational techniques and behavior modification also allow the patient to understand the causes of obesity and all its consequences. These include working on negative beliefs about weight reduction and self-observation. They also teach them how to control the quantity and quality of food they eat [8]. The strongest incentive to change is the internal motivation which comes directly from the person involved in the process. In this case, a patient is on a diet to improve his appearance, health or well-being. Giving nutritional lectures and creating support groups for people with obesity is an eminently effective method [17].

The aim of the study was to compare the effectiveness of traditional dietary treatment with or without the motivational techniques in the group of overweight women or women with obesity and the comparison of psychological aspects of the treatment using the author's questionnaire.

## MATERIALS AND METHODS

### *Study subjects*

One hundred and four overweight women or women with obesity were included in the study. The patients were under the supervision of a dietitian from January 2015 to June 2016 in the Diet Clinic in Jaworzno, Poland. The participants were on a low-calorie diet that was developed individually for each patient. The age-group selection was determined by several factors: the emotional maturity of the women, the low likelihood of disease occurrence, the premenopausal age, the same nutritional standards and the reference values recommended by the manufacturer of the body composition analyzer. A questionnaire in the course of treatment was conducted among the subjects. The survey has been validated. The study was anonymous and the patients responded on their individual forms. Overweight women or women with obesity, identified on the basis of calculated BMI > 25, were included in the study. The criteria of inclusion and exclusion was shown in Table 1.

The effectiveness of both slimming methods was examined by appropriate measurements.

### *Nutritional plan*

All patients used a diet developed for them individually by a dietitian in accordance with the methodology of planning a low-energy diet. The slimming treatment was carried out based on the standards developed by the Polish Society of Dietetics (PTD). The low-calorie diet used by the participants of the study aimed at weight reduction, the improvement of well-being in patients and minimizing their hunger. The energy value of the diet was reduced from 500 to 1000 kcal compared with the total energy requirements of the patient. The nutritional plan was balanced in terms of nutrient content. The energy level of the menu was developed individually. It considered a patient's primary metabolic value (PPM), calculated by means of the *Mifflin-St. Jeora's* equation, according to the standards of the low-calorie diet. In the diet we limit the supply of fats, whose energy content should not exceed 25% of the diet. The recommended amount of fat intake is in the range of up to 40 g of fat per day, of which 25-30 g should be invisible fat being a natural component of food products. Protein supply is in the physiological standard (1g per 1 kg of body weight). Carbohydrates in the diet complement the total energy demand and should include mainly products with a low glycemic index (IG) containing a low percentage of simple sugars and a high content of dietary fiber. The menu in a low-energy diet should be balanced for five meals a day. It is necessary to appoint with the patient hours of meals consumed, depending on the lifestyle.

### *Measurements*

To accomplish the purpose of the study, the women were divided into two subgroups (Table 3).

The patients were assigned to groups according to their preferences. The control group were 52 women who followed the previously discussed low-energy diet. The visits with the dietician were held once a month. During those visits, weight loss was measured using a Tanita's body composition analyzer. The waist was measured using a SECA meter ruler. The task of the dietitian in this group was primarily substantial support. At each visit, the researcher touched on the aspects of the use of low-energy diet and checked the diet schedule. The study group of 52 women was on the same diet. The dietary inspection visits took place weekly. The motivational techniques and psychological support were used to improve the effectiveness of the slimming treatment. The effectiveness of the methods was measured in the same way as in the first group. The qualification for a particular group was based on the conscious decision of the patient about the treatment.

### *Motivational techniques*

Motivation training in the study group was developed for the study and composed so that each follow-up visit contained its elements. Expectations, among other things, were analyzed: the first visit examined the patient's individual needs, the dietician's expectations and treatment ideas. An analysis of past weight loss tests was also performed, each patient could present, from their point of view, the reasons why previous weight loss was not successful. Then the past weight loss tests were compared with the current ones to avoid repeating the same mistakes. The contracts were concluded with each participant and the terms of cooperation were discussed. The contract included the responsibilities of both the patient and the dietician.

The dietician presented the patients an individual weight reduction goal and the amount of time necessary to achieve it. The attention was also paid to the other benefits of the weight reduction process. Only positive goals were set. At every visit, the dietician did not use ready-made solutions and tried to be patient by showing the right way to accomplish the goal. The members kept diet diaries during the weight reduction process. At one of the first follow-up visits, the patients were asked to list their strengths and weaknesses in the table, which could affect the weight loss process. At follow-up visits, the dietician tried to visualize the patients' final weight loss goals. At subsequent visits, the dietician tried to keep the patients' motivation high. The important element of obesity treatment is identifying bad habits and proposing a change in thinking to a patient. Each

patient was supposed to prepare a vision of the "new self" on the piece of paper with the help of photo collages from women's magazines. Once a month, the patients participated in support group meetings. During the joint talks they shared ideas and experiences related to slimming. Once every two months, a series of lectures were organized to allow patients to learn the importance of sensible nutrition. In order to maintain constant contact with the patients, the Internet was used to solve arising problems. In order to monitor daily physical activity, the patients in the study group used devices measuring the steps taken. All the above methods aimed at increasing the efficiency of the treatment [4, 5, 11, 12, 24].

### *Effectiveness of dietary treatment*

To evaluate the effectiveness of dietary treatments in both groups, the following parameters were calculated for the first twenty weeks of weight loss (5 months):

- average weight loss in kilograms;
- average waist circumference reduction in centimeters, measured midway between the highest point of the hip plate and the lowest rib curve;
- fat loss measured by bioimpedance expressed in percentages and kilograms;
- authorized survey in the course of treatment.

Each patient was weighed on the SECA 711 medical scale. This is a 100 g medical column. This scale also includes the SECA 220 height gauge. The Tanita BC 420 was used to analyze the composition of the body every week. Anthropometric measurements in each participant were measured every three weeks.

### *Ethical standards*

All participants gave oral informed consent and written informed consent on the questionnaire. The project was approved by the behavioral and ethical review committee of the Medical University of Silesia (KNW/0022/KB/89/I/16).

### *Statistical analysis*

The quality of the slimming methods was also compared with the questionnaire. STATISTICA 12 and Microsoft Excel 2013 were used to perform statistical analyzes. In the analysis of quantitative variables, the *Shapiro-Wilk* test was used. In a statistical test for two independent variables, *U-Man Whitney* was used. The statistical analysis of dependence of non-measurable features was performed on the basis of descriptive statistics and chi-square test, V-Cramer correlation coefficient and Fi factor ( $\phi$ ). All statistical analyzes were carried out at significance level  $\alpha = 0.05$ .

## RESULTS

The median age of the whole group was 34 (30-39). Twelve of the whole group of patients (11.5%) had vocational education, 36 (34.5%) secondary and 56 (54%) higher education. Seventy five percent of the study population was professionally active. Thirty-six (34.5%) women were childless, with the largest proportion 38% of women having one child. The average weight at the beginning of the reduction in the study population was 80.50 kg ( $\pm 15.70$ ). The average weight of the study group was 80.00 kg ( $\pm 14.61$ ) kg, with the lowest body weight being 64 kg and the highest 130 kg. The average BMI for the entire population was 31.5 ( $\pm 5.08$ ). Forty-three (41%) women included in the study were overweight, 38 (36.5 %) of the whole population had obesity of 1 degree, 13 (12.5 %) suffered from obesity of 2 degree, 10 (10 %) had obesity of 3 degree, and there was no case of obesity of 4 degree. The distribution of BMI in both group shown in Table 2. There were no significant differences in the analysis in the examined parameters before treatment in the studied groups ( $p > 0.01$ ).

Table 1. Study qualification criteria

Inclusion criteria	Exclusion criteria
Age 30-39	No patient's consent
Overweight or obesity (BMI > 25)	Psychiatric care or eating disorders
Consent to attending follow-up visits in Diet Clinic	Pregnancy or breastfeeding
Use of a diet that is designed individually for each person	Diseases in which a low-calorie diet is contraindicated

BMI – body mass index

The average waist measurement in the study population was 99.00 cm before the treatment. In the control group and in the study group it was 100.00 and 97.50 cm respectively. In the total studied population, the mean weight loss was 13.90 kg ( $\pm 9.55$ ), the waist measurement decreased by 13.54 cm ( $\pm 9.63$ ). Fat loss on average is 10.10 kg ( $\pm 7.22$ ), which corresponds to 6.37% ( $\pm 4.28$ ) of adipose tissue. The comparison of weight loss effectiveness in both groups has shown that the mean weight loss in the study group expressed in kg is statistically significantly higher by 6,75 kg ( $p < 0.001$ ) in the study group using additionally motivational techniques than in the control group. The mean reduction in waist measurement is also higher by 3.41 cm in the study group and the difference is statistically significant ( $p = 0.002$ ). Fat reduction in % and kg in the same way as the previous assessed values is statistically significant ( $p < 0.001$ ) higher in the study group by 3.23 and 4.17, respectively. In the study group using motivational techniques in weight reduction, the women exhibited internal motivation, which prompted them to lose weight. In this group, the women declared that they had tried treatment to take care of their appearance (38%) and because of the desire to improve their well-being (30%). In contrast, the women in the control group were characterized by weaker motivation, which came from their environment. In this group, the patients most frequently attempted to reduce weight due to the perceived social stigma associated with their appearance (19%).

To assess the effectiveness of nutrition education during the treatment, the patients were asked whether they had changed their eating habits through weight loss. In the study group, 86.5% ( $n=45$ ) women answered that they had changed their eating habits. It was statistically lower in the control group, because only 36.5% ( $n=19$ ) ( $p < 0.0001$ ), it was high correlation.

The patients who completed a weight loss diet statistically more frequently ( $p < 0.0001$ ) reduced their body weight to a normal BMI ( $< 25$  kg/m<sup>2</sup>) in the

Table 2. BMI value before beginning dietary treatment in both groups

Body Mass Index	Control group (n = 52)		Study group (n = 52)	
	No of women n (%)	Average BMI [kg / m <sup>2</sup> ] (OS)	No of women n (%)	Average BMI [kg / m <sup>2</sup> ] (OS)
Overweight	20 (38.5)	27.65 ( $\pm 0.88$ )	23 (44)	27.42 ( $\pm 1.08$ )
Obesity I degree	18 (34.5)	32.44 ( $\pm 1.28$ )	20 (38.5)	32.47 ( $\pm 1.13$ )
Obesity II degree	7 (13.5)	37.36 ( $\pm 1.24$ )	6 (11.5)	37.50 ( $\pm 1.32$ )
Obesity III degree	7 (13.5)	43.21 ( $\pm 2.01$ )	3 (6)	43.36 ( $\pm 1.31$ )
Obesity IV degree	0 (0)	-	0 (0)	-

n – numer

study group using motivational techniques. There is therefore a link between the BMI value obtained after the weight loss treatment and the type of group that the patient belonged to. To determine the strength and direction of this relation, correlation was 0.48, which means that the average force is correlated.

Another important aspect during the treatment is a well-adjusted diet plan. In the current study, the authors also investigated these issues, according to them, during the weight reduction process, there may be a fixation on the diet used, which manifests itself with burdensome thinking about food, energy metabolism and mental discomfort. Such statistically significant findings ( $p < 0.0001$ ) occurred during a weight loss regimen in the control group, in which 29 women (55.8%) declared such feelings, it was a high correlation power. In the motivational group, only 11 women (21.2%) declared their occurrence.

Table 3. The comparison of methods of the therapy in the control group and the study group

Control group	Study group
<b>FIRST VISIT</b>	
preliminary interview, medical and nutritional history body composition analysis, anthropometric measurements	
-	examining patient's needs
-	analysis of the previous slimming attempts
-	contract determining the rules of cooperation between a dietician and a patient
<b>FOLLOW UP VISITS</b>	
monthly	weekly
medical history, discussing possible dietary problems, checking the meal schedule from the previous visit	
body composition analysis by bio-impedance, anthropometric measurements	
Substantial discussion of the next nutrition plan	
-	use of motivational techniques
-	support groups, nutritional talks

In the motivational group, 78.9% (n=41) declared that during the course there had been no doubts or desire to discontinue treatment, compared to only 9.6% in the control group. In contrast, only 22 subjects in the control group (42.3%) responded the same. The study showed that the people in the study group were statistically more determined in the weight loss process compared with the control group ( $p < 0.0001$ ).

The coefficient was used to determine the strength and direction of this relation, which showed that the correlation between the variables was strong (0.77).

During the current study on weight loss, the women in both groups were advised to exercise regularly. In the control group, the patients used pedometers to improve their exercise motivation. Comparing the two groups statistically significantly ( $p = 0.0003$ ), 40 women in the study group were motivated (76.9%) and only 19 (36.5%) in the control group. The strength of the coefficient  $F_i$  is 0.41, which means average correlation. In the control group, the women were most likely to experience physical activity alone, since statistically more frequently ( $p < 0.0001$ ) they felt shame (43.3%) in front of other people in comparison with the women in the second group. The correlation was 0.32, which means average correlation. In the study group, the women preferred physical activity in the group as it increased their overall motivation.

## DISCUSSION

Currently, there are many methods of treating excessive body mass, including unconventional methods. Patients with overweight or obesity often seek easy and quick ways to reduce body fat and become slim. Obesity is a chronic disease and its treatment is long-lasting. It requires a professional approach from the doctor and a dietitian, and great involvement of the patient in the therapeutic process. Studies indicate that obesity is incidental to many destructive attitudes that impair the process of weight loss, including: negative beliefs, impaired self-image, inadequate expectations, low self-esteem of the patient [4]. The lack of significant treatment and prevention progress highlights the need for a more expanded strategy because psychological and emotional distress is a fundamental link between socioeconomic disadvantage and weight gain [12]. According to *Hamilton et al.*, the holistic approach to treatment is the most important aspect of the treatment process [11]. Good dietitians and supportive life partners or family are key to weight loss treatment. In the current study, the patients were asked to evaluate the nutritionist's work. In the study group, all the women declared that the dietician had spent enough time being properly involved in the weight loss process. *Ryden et al.* have shown that in difficult and stressful situations, people with obesity cannot actively solve problems [24]. They use less effective strategies and approach the problems too emotionally, which makes the weight reduction process easier to discourage. People with obesity often have a feeling of the powerlessness caused by the low efficiency, which Bandura introduced as the first [5]. *Bak-Sosnowska* [4] has shown that overweight patients have lower self-esteem compared to those with normal

body weight. In difficult situations, people with obesity overexerted themselves and could not actively solve the problem. In the present study, the patients were asked about the problematic moments and the desire to doubt while being on the low-calorie diet. Studies indicate that multicomponent behavioral weight management programs, incorporating diet, exercise and behavioral counselling, can lead to significant weight loss [9]. According to the study, people with obesity should boost their confidence. Studies show that obese individuals are highly stigmatized and face multiple forms of prejudice and discrimination because of their weight [22]. Studies indicate that the best way to reduce overweight is the implementation of proper, low-calorie diet and the increase of physical activity. Patients with obesity should be on a diet for a long period of time. This is the only way to reduce body fat [4].

Body mass reduction in people with obesity can be performed in a variety of ways. Today's methods of pharmacotherapy, bariatric surgery, physical activity, and a proper diet are available. The *Nurses' Health Study* found that women who had low-energy diets did not gain as much weight as women who did not follow any diets [13]. In *Ostrowska et al.* study obesity treatment with a low-energy diet was conducted. The women with obesity were on a diet, but were not routinely monitored at a dietitian's and no motivational techniques were introduced. As a result, the weight loss in this study was significantly smaller than in our study. The treatment of obesity is time-consuming and requires good planning of the entire course of the diet. Therapy must lead to a permanent modification of the behavior, because only a momentary adherence to dietary recommendations, and then return to the old eating habits, cause a rapid increase in body weight [21]. The TOHP II study also reported patients who had no weight loss in spite of following a low-calorie diet. It is necessary to find an expert dietitian during the treatment process and to implement appropriate treatment, based on the principles of rational nutrition. It is important to reduce your body weight at the right pace. It is possible that follow-up visits and regular measurements in the current study make it more effective than the implementation of individual spontaneous self-monitoring [2].

An important indicator in the diagnosis of obesity in patients is the measurement of waist circumference. In our study, the effectiveness of the weight reduction process in both groups of women was also compared using the measurement [7]. The waist circumference as well as the waist-hip ratio anthropometric index is often used in practice. This is a simple way to determine the amount of fat tissue, central obesity and excessive fat accumulation in the abdominal region [27]. Long-term MORGEN study shows that a waist circumference of >88 cm leads

to increase of cardiovascular risk factors [20]. According to *Romanowska-Tolloczko et al.* study, comprehensive actions during the treatment constitute the main role in an overweight therapy. There are the three most important components in this process: a diet, physical activity and a psychological influence. Dietetics have to support the change of eating habits [23]. *Adachi's* study has shown that psychological support in weight loss treatment maximize the patient's own ability to maintain new healthy eating habits after a slimming cure. Motivational techniques used in the process of weight reduction can be a permanent element of the treatment carried out through individual consultations of the patient with a psycho-dietitian or multi-person consultations, such as support groups, lectures, and independent exercises prepared by a dietitian to perform by the patient at home alone [1]. Creating effective motivation in patients' attitude is a very important factor for their weight loss. The most fundamental element in the process of weight loss during the obesity treatment is a correct behavior therapy [26]. It may increase the patients' capacity for self-control and boost their confidence [16]. Motivational techniques teach patients how to improve their eating habits, be healthier and maintain this healthy lifestyle for the rest of their lives [6, 18].

## CONCLUSIONS

The combination of motivational techniques and diet therapy for overweight women or women with obesity who are slimming is more effective in reducing body weight, adipose tissue and waist circumference than diet therapy only.

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### Conflict of interest

*Nothing to declare.*

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## HYPOMETHYLATION OF THE *C-MYC* PROMOTER REGION INDUCED BY PHENOBARBITAL IN RAT LIVER

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### ABSTRACT

**Background.** The changes in DNA methylation are considered as one of the early events in hepatocarcinogenesis.

**Objective.** We evaluated the ability of phenobarbital (PB) – the most widely used anticonvulsant worldwide and classical rodent liver carcinogen – to cause the promoter region of the *c-myc* protooncogene hypomethylation as well as changes of mRNA level of this gene. Moreover, the expression of Dnmt1 protein in rat treated with this compound was analyzed.

**Material and Methods.** Male *Wistar* rats received PB in daily oral doses of 92.8 mg kg<sup>-1</sup> b.w. day<sup>-1</sup> (at 24-h intervals; for one, three and fourteen days). Methylation of the *c-myc* promoter region was measured by PCR-based methylation-sensitive restriction enzyme analysis (MSRA). Levels of mRNA for *c-myc* and protein Dnmt1 were assayed using Real-Time PCR and Western Blot, respectively.

**Results.** The study showed that phenobarbital stimulated persistent changes in DNA methylation, i.e. loss of methylation in the promoter region of the *c-myc* gene and up-regulated its mRNA level. In addition, a significant increase in protein level of Dnmt1 in the *c-myc* over-expressing liver cells was observed.

**Conclusion.** The opposite relationship between Dnmt1 activity and methylation status of *c-myc* gene was demonstrated. The *c-myc* over-expression by demethylation might represent an important, early events in the mechanism of action (MOA) of phenobarbital.

**Key words:** *c-myc*, DNA methylation, Dnmt1, genes expression, liver, rats

### STRESZCZENIE

**Wprowadzenie.** Zmiany metylacji DNA są rozważane jako jeden z wczesnych mechanizmów hepatokancerogenezy.

**Cel pracy.** Celem badań była ocena wpływu fenobarbitalu (PB) - leku przeciwpadaczkowego, modelowego promotora raka wątroby - na poziom metylacji regionu promotorowego i ekspresji na poziomie mRNA protoonkogenu *c-myc*. Ponadto dokonano analizy poziomu ekspresji białka Dnmt1.

**Material i metody.** Samce szczurów szczepu *Wistar* otrzymywały PB w dawce 92,8 mg/kg m.c. x dzień<sup>-1</sup> jednorazowo, 3-krotnie i 14-krotnie. Ocena poziomu zmian metylacji genu *c-myc* dokonano metodą MSRA (ang. Methylation-Sensitive Restriction Enzyme Analysis). Analizę względnego poziomu transkryptów genu *c-myc* i białka Dnmt1 przeprowadzono odpowiednio metodą PCR w czasie rzeczywistymi i techniką Western Blot.

**Wyniki.** W wyniku oddziaływania fenobarbitalu wykazano trwałe zmiany metylacji DNA - obniżenie metylacji w rejonie promotorowym genu *c-myc* i nadekspresję badanego genu na poziomie mRNA. Jednocześnie obserwowano statystycznie istotny wzrost poziomu białka Dnmt1.

**Wnioski.** Wykazano odwrotną zależność między aktywnością Dnmt1 a poziomem metylacji genu *c-myc*. Nadekspresja *c-myc* w wyniku demetylacji może stanowić istotne, wczesne zdarzenie w mechanizmie działania (ang. Mechanism of Action (MOA)) fenobarbitalu.

**Słowa kluczowe:** *c-myc*, metylacja DNA, Dnmt1, ekspresja genów, wątroba, szczury

### INTRODUCTION

The introduction of short-term tests for the detection of genetic damage has partly solved the problem of an initial and early evaluation of carcinogenic potential

of chemical substances. There is, however, a need to supplement them with tests aimed at identification of carcinogens which act through non-genotoxic, i.e. epigenetic mechanisms. According to current knowledge, epigenetic mechanisms play an important

role in carcinogenesis, including hepatocarcinogenesis [15, 40, 46, 49]. In spite of intensive research in this field, the selection of markers, which would serve as tests for the identification and evaluation of the interactions of non-genotoxic potential carcinogens, still remains an open issue.

Literature from the last years [22, 35, 37] indicates that using short-term changes in gene expression in target organ as effect of epigenetic alteration, may be one of the approaches to address the problem. It has been hypothesized that epigenetic alternations may be used as early biomarker in the evaluation of the carcinogenic potential of the chemicals [10, 22, 26, 27]. Furthermore, abnormal changes in DNA methylation and mRNA expression of *c-myc* are believed as an important stimulatory factors in the development of liver cancer [9, 30].

The *c-myc* protooncogene encodes a transcription factor which has a well described role in cell growth and proliferation [1, 5]. Moreover, direct role of *c-myc* in the control of DNA synthesis is suggested [6, 41]. The ability to induce DNA hypomethylation and/or *c-myc* protooncogene in mouse liver tumors has been reported for many non-genotoxic carcinogens, including peroxisome proliferators (PPs) and phenobarbital [2, 34, 38, 39]. However it should be pointed out that the most of long-term studies, which were already performed, did not detect early changes in the genome [16, 45].

Phenobarbital (PB) is classical rodent liver carcinogen whose carcinogenic potency may be linked with its ability to cause epigenetic effects. *In vitro* and *in vivo* tests demonstrated that the carcinogenic activity of PB is not a result of its genotoxic activity. IARC has classified PB to group 2B, as non-genotoxic agent, carcinogenic in animals and possibly carcinogenic in humans [17].

The purpose of this study was to determine the methylation status and expression of the *c-myc* protooncogene, which is one of the so-called immediate early genes associated with increased cell proliferation, in the liver of *Wistar* rats treated of PB up to 14 days. The ability of PB to affect Dnmt1 expression was also analyzed.

## MATERIAL AND METHODS

### Chemicals

Phenobarbital (95%) was obtained from "Galenus", Poland. Genomic DNA Purification Kit, the HpaII restriction enzyme and phage  $\lambda$  DNA were purchased from Fermentas, USA; FastStart Taq Polymerase was obtained from Roche, Germany. RNeasy® Mini Kit – Qiagen, Germany; RT-PCR – Advantage® RT-for-PCR Kit – Clontech, USA; Kapa™ SYBR® Fast qPCR Kit PCR – Kapa Biosystem, USA. Other chemicals were

obtained from Sigma Chemical Company, USA, and were of the highest quality and purity.

### Animals and outline of experiment

The studies were performed on male *Wistar* rats, *Crl:WI(Han)* weighing  $200 \pm 10$  g. All procedures involving animals were performed according to national animal welfare regulations after authorization by the Local Ethics Committee for the conducting of research studies in live vertebrates (permissions No. 6A/01 and 23/2010).

All animals were housed in groups of five in cages at  $22 - 24^\circ\text{C}$ , relative air humidity  $50 \pm 10\%$  and a 12-h light/dark cycle. Prior to the use, the rats were acclimated for 2 weeks before the start of the experiment. During the adaptation and experimental phases standard feed LSM and filtered tap water *ad libitum* was administered to the animals. The animals were observed daily and their body weight as well as food and water consumption were monitored.

PB was dissolved in olive oil (vehicle) immediately before use and was administered orally *via* gavage at doses of  $92.8 \text{ mg kg}^{-1} \text{ b.w. day}^{-1}$  (dose corresponding to 1/10 of the  $\text{LD}_{50}$  value) for 1, 3 and 14 days (at 24-h intervals), respectively. The dose of PB was identical to this used in previous study [20].

Animals were anesthetized by inhalation isoflurane/ $\text{O}_2$ , then were euthanized by decapitation. Livers were removed, rinsed of surface blood, blotted to dryness, weighed, frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . Representative samples of liver tissue from the right lobe were taken for analysis.

### Analysis of DNA methylation

After DNA isolation using the Genomic DNA Purification Kit (Fermentas), methylation status of selected fragment of *c-myc* gene was determined based on the restrictive analysis sensitive to methylation (MSRA), as described previously [21].

### RNA extraction and Real-time PCR for *c-myc*

Total RNA was extracted from frozen livers using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA synthesis was done in a 20  $\mu\text{l}$  reaction mix starting with 1  $\mu\text{g}$  of total RNA using the reverse transcription system of Clontech (Advantage RT-for-PCR kit) following the manufacturer's recommendations. Real-time PCR was performed on an MiniOpticon system (Bio-Rad) using Kapa Sybr®Fast qPCR Kit (Kapa Biosystems, USA). *c-myc* (GeneBank No NM\_12603) cDNA was amplified using primers: 5'-CTGCTGTCCTCCGAGTCCTC-3'(F), 5'-GGGGTTGCCTCTTTCCAC-3'(R). *b-actin* (GeneBank No V01217) was used as the endogenous control and amplified using the following primers: 5'-GTGGGTATGGGTCAGAAGG-3'(F)

and 5'-CAATGCCGTGTTCAATGGGG-3'(R). Cycling conditions of Real-Time PCR were defined: 95°C for 2 min, followed by 40 cycles of denaturing at 95°C for 30 s, 55°C for 20 s, 72°C for 30 s, 80°C for 5 s. This number of cycles had been previously estimated to be the optimal one for detecting the signal in the linear range. PCR products were verified by melting curves and were also run on a 6% polyacrylamide gel to confirm the appropriate size. All real-time PCRs were done in triplicates. Amplification and threshold calculations were generated using the CFX Manager Software v1.6 (Bio-Rad). Relative gene expression was calculated using Pfaffl model, which provides for correction of data as regards differences in the efficacy of amplification of individual transcripts [32]. In the model the gene mRNA level in test samples is estimated relative to the level of the same gene in control samples and normalized to the mRNA level of a selected reference gene ( $\beta$ -actin).

#### Western blot analysis

Proteins from the animal's liver were isolated in T-Per Mammalian Tissue Reagent (ThermoFisher Scientific, USA) containing protease inhibitor (Complete Protease Inhibitor Cocktail, from Roche, Switzerland). Equal amounts of the protein (25 mg) were mixed with Laemmli buffer and boiled 5 min. Protein samples were then separated on 10% acrylamide gel in the presence of sodium dodecyl sulphate (SDS) and  $\beta$ -mercaptoethanol (SDS-PAGE) and transferred onto Immobilon-P membranes (Millipore, USA) using semi-dry electroblotting. The membranes were blocked in TBST (137 mM NaCl; 20 mM Tris-Cl; 0,1% Tween-20; pH 7,6) containing 5% non-fat milk and subsequently incubated overnight at 4°C with monoclonal mouse b-actin and polyclonal goat Dnmt1 antibodies (Santa Cruz), diluted respectively 1:5000

and 1:2000 in 5% milk in TBST. The membranes were washed four times for 10 min in TBST and then incubated with horseradish peroxidase (HRP) conjugate goat anti-mouse IgM antibodies and donkey anti-goat IgM antibodies (1:5000 dilution, Santa Cruz, USA) for 1 hour. The membranes were again washed four times for 10 min in TBST and then analyzed via an enhanced chemiluminescence method (Immobilon™ Western Chemiluminescent HRP Substrate, Millipore) and ultra-sensitive photographic X-ray Kodak film according to the manufacturer's instructions. The films were scanned and used for densitometry analysis by Image Quant v5.2. Dnmt1 proteins expression level was normalized relative to  $\beta$ -actin expression level.

#### Statistical analysis

The *c-myc* promoter methylation frequency was calculated with Fisher's test ( $p < 0.05$ ). REST-384 software tool was used to estimate the relative mRNA levels of particular genes. The software allows to compare mRNA levels in two groups (the control group and the test group) and to assess the statistical significance of demonstrated differences in mRNA levels using randomization tests (*Pair Wise Fixed Reallocation Randomization Test*) [31]. Proteins level was expressed as the mean  $\pm$  SEM for five animals. The two-tailed Student's *t* test was employed to calculate the statistical significance between control and treated groups ( $p < 0.05$ ).

## RESULTS

#### DNA methylation status of *c-myc* gene

The methylation-sensitive restriction endonuclease HpaII was used to assess the methylation status in the promoter regions of *c-myc* gene. A scheme of the *c-myc* gene promoter region is illustrated in Figure 1.

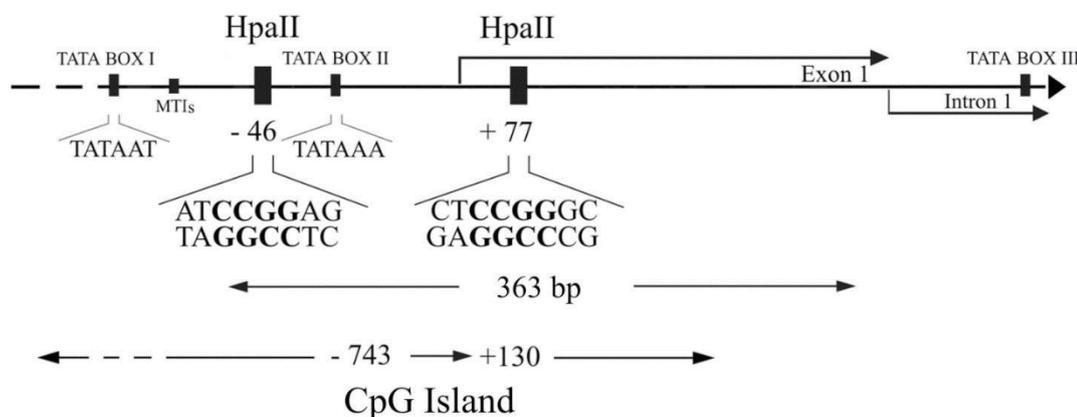


Figure 1. Scheme of the *c-myc* promoter region

The *c-myc* promoter region contains CpG Island at position -743 bp+130 bp, which was defined according to Gardiner-Garden and Frommer [11]. Within this region are located two CpG sites recognized by the methylation-dependent enzyme HpaII at position -46 bp and +77 bp. Two of the three independent non-overlapping promoters (TATA BOX I and II) occur in a region rich in CpG dinucleotides.

The ability of PB to induce *c-myc* hypomethylation in male rat liver is presented in Table 1 and Figure 2.

The *c-myc* promoter region was unmethylated at a frequency of 50% (in 8 out of 16 samples analyzed) after single dose of PB (Table 1). Three doses of the PB, administered every 24h, induced a further increase demethylation of *c-myc*. The unmethylated cytosines (in 10 out of 16 DNA samples) were indicated in HpaII-digested DNA from the liver of male rats exposed to PB at dose of 92.8 mg kg<sup>-1</sup> b.w., which exceeded control by 63% - a frequency 13% higher than this obtained after a single dose of compound ( $p < 0.05$ ). After 14 days of exposure, the *c-myc* promoter region was also unmethylated at a frequency of 63% (in 10 out of 16 samples) ( $p < 0.05$ ). Hypomethylation of *c-myc* gene did not occur in the control rats.

Table 1. Summary of methylation-sensitive digestion with HpaII of DNA isolated from the liver of rats treatment with PB for 1, 3 or 14 days<sup>1</sup>

Group of rats	1 day	3 days	14 days
	(M/U)	(M/U)	(M/U)
PB	8/8*	6/10*	6/10*
Control	8/0	8/0	8/0

<sup>1</sup>DNA material from the livers of four PB-treated rats was digested with HpaII in two independent repeats. Each restricted DNA sample was used for two independent PCR runs. Total the 16 DNA samples were analyzed. The results for each DNA sample from the control rats are presented for one PCR. M - DNA samples in which investigated CpG sites were methylated; U - DNA samples in which investigated CpG sites were unmethylated; (\*) Significance calculated using Fisher's test.

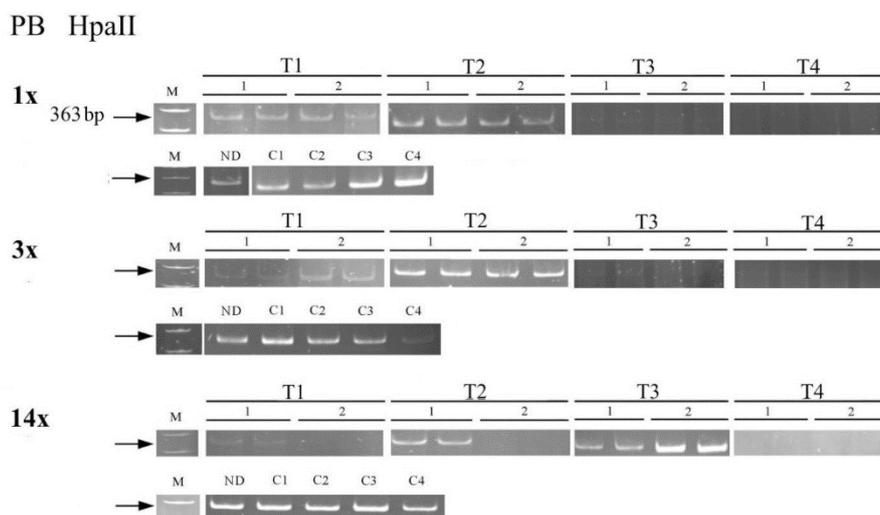


Figure 2. Effect of PB on the methylation status of the *c-myc* gene in the liver of *Wistar* rats

Methylation status of the *c-myc* investigated CpG sites in rat liver determined by methylation sensitive restriction enzyme analysis (MSRA). The rats were administered PB for 1, 3 or 14 days in doses 92,8 mg kg<sup>-1</sup> b.w. day<sup>-1</sup>. The absence of a visible PCR product (363 bp) indicates the presence of unmethylated investigated CpG sites. T1-T4 - DNA isolated from rat livers administered with PB; C1-C4, DNA isolated from the livers of control rats. Number 1 and 2 - represents two of DNA digestion with HpaII. ND (not digested) - positive control of PCR. The arrows in the left margin indicate the size of the bands (363 bp) for *c-myc*.

Representative results of methylation sensitive restriction enzyme analysis (MSRA) showing effect of PB on the methylation status of the *c-myc* promoter region in the rat liver are illustrated in Figure 2.

In the analyzes of 4 independent samples of DNA isolated from the livers of rats exposed to a single dose of PB, HpaII endonuclease digestion yielded no PCR product of the size of 363 bp in samples T3 and T4. This shows that no cytosine methylation occurs in the examined CpG sequences. Further reduction of cytosine methylation levels at the examined positions -46 bp and +77 bp was observed after 3 and 14 days of exposure to PB. No PCR product was obtained in samples T3 and T4 as well as in one run of the restriction analysis conducted on sample T1 after 3 doses of PB. No DNA methylation was observed

following prolonged exposure (14 days) to PB in samples T1, T2 (one run) and T4, as evidenced by the lack of 363 bp product. In four control samples (C1-C4), restriction analysis afforded the appropriate PCR product.

#### *mRNA expression of c-myc gene*

DNA methylation has an essential role in the transcriptional regulation of gene expression and has been recognized as an important factor in the activation of protooncogenes such as *c-myc* [9]. Therefore, we assessed the effect of exposure to PB on the level of the *c-myc* mRNAs in rat liver. Changes of *c-myc* mRNA levels induced by studied compound are presented in Table 2.

Table 2. Effect of PB on the mRNA expression of the *c-myc* gene in the liver of *Wistar* rats<sup>2</sup>

<i>c-myc</i>	Expression ratio		
	1	3	14
PB	1,719*	1,934*	1,666*

<sup>2</sup>Expression levels of *c-myc* were analyzed by real-time RT-PCR. The data were normalized to the housekeeping gene *β-actin* and expressed as the relative to control value. (\*) Significance calculated using randomisation test.

As shown in the Table 2, PB caused a sustained increase the transcript levels of *c-myc* as compared with the control group. The expression of mRNA of the *c-myc* gene was increased by 70 and 90% after a single dose and 3 doses of the compound, respectively.

This tendency was also observed after 14 days of exposure to PB.

## DISCUSSION

Literature from the last years indicates that investigations of the response of the cellular genome to the action of chemical agents give the possibility of both in-depth understanding of the mechanisms of their action as well as form the basis for earlier evaluation of their carcinogenic potential [35, 46].

The results of studies assessing the effect of non-genotoxic carcinogens (NGCs) on the methylation levels of key genes involved in regulation of the cellular cycle and apoptosis may be particularly important due to the role of DNA methylation in regulation of gene expression [28, 29, 30] and the NGCs' capability to interfere with processes of cell proliferation and apoptosis at the early stages of the tumorigenic process [23, 33].

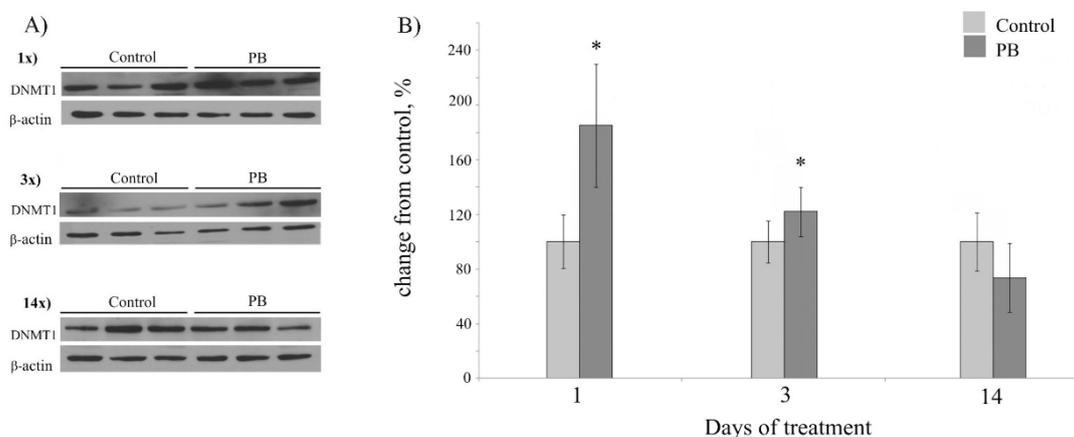


Figure 3. Western blot analysis of Dnmt1 protein in the liver of control rats and rats treated with PB

The signal intensity from the chemiluminescent detection was analyzed by ImageQuant software. *β-actin* was used as a loading control. A) The representing images of Dnmt1 and *β-actin* protein expression. B) Quantitative analysis of Dnmt1 protein level. Control values at each time point were considered as 100%. (\*) Significantly different from the control at the same time point,  $p < 0,05$ ; These results are representative of three independent experiments.

### Expression of Dnmt1 protein

The DNA methylation process is catalyzed by three independently coded DNA methyltransferases (DNMTs): Dnmt1, Dnmt3a and Dnmt3b. The main enzyme responsible for reproduction of methylation patterns upon DNA replication in somatic mammalian cells is Dnmt1. Disruption in its activity and/or expression may lead to alteration in DNA methylation [9, 19, 28, 42].

The Western blot analysis of Dnmt1 levels revealed statistically significant differences in the levels of the tested protein in the livers of rats exposed to the examined compound (Figure 3).

Over-expression of Dnmt1 was observed in protein extracts isolated from the livers of rats exposed to the single dose of PB. Dnmt1 expression level was 60% higher than that in the control group. Upon continued exposure of animals to PB, statistically significant increase in Dnmt1 levels (by 22%) was observed only after 3 days of exposure to studied compound.

The results reported in this work indicate that phenobarbital (PB) stimulated hypomethylation of the tested sequences of the *c-myc* promoter region, which is one of the pathways for regulating gene expression at a transcription level. Indeed, we found that expression of *c-myc* was up-regulated after short (1 and 3 doses at 24-h intervals) and prolonged exposure (14 doses at 24-h intervals) of the animals to PB. The relationship of the methylation status of promoters and gene transcription level was also reported with respect to other non-genotoxic liver carcinogens. Hypomethylation of *c-myc* gene has been found in the liver of rodent exposed to peroxisome proliferators (PPs), such as a model PP, Wy-14,643 [12] and dibutyl phthalate [21]. The prolonged (7-8 weeks) treatment with dibromoacetic acid (DBA) induced hypomethylation of promoter regions of the *c-myc* and *Igf2* (insulin-like growth factor 2) protooncogenes in mouse liver. DBA also increased the mRNA

expression of the two genes that was associated with their hypomethylation in mouse liver [43]. In other study, it has been shown [2], that phenobarbital causes hypomethylation of *Ha-ras* (encodes a protein that is involved primarily in regulating cell division) and increased expression of the protooncogene in 50% of B6C3F1 mice. Moreover, Lempiäinen et al. [24] and Luisier et al. [25] found *Cyp2b10*, the cytochrome P450 member, to be concomitantly hypomethylated and transcriptionally activated in the liver of phenobarbital-treated mice.

Furthermore, we have demonstrated that PB induces a transient increase of Dnmt1 expression. According to current knowledge, Dnmt1 is considered the main enzyme responsible for the maintenance of the normal methylation pattern in repeated replication cycles that precede cell division [2]. Dnmt1 plays an essential role in the control of cellular cycle. Synthesis of Dnmt1 is induced of the moment when cells enter S-phase of the cellular cycle [18, 19]. This process is associated with the requirement of methylation of the newly synthesized DNA strand in hemimethylated sequences. This indicates that Dnmt1 activity/expression inhibition can cause loss of methylation during DNA synthesis. However, we found the methylation status of *c-myc* gene was not associated with decreased of Dnmt1 expression. It is suggested that Dnmt1 protein level was not a critical determinant of promoter demethylation of *c-myc* gene. On the other hand, a well-established link between expression of Dnmt1 and DNA replication, as mentioned previously, was also confirmed by results from this work and our previous studies [20, 47] conducted under identical experimental conditions (the same dose level and dosing regimen). Treatment of rats with PB for 1 and 3 days produced an increase in nuclear Dnmts activity and DNA synthesis (previous study) [20] and Dnmt1 protein level (this study). Taking into account Dnmt1 activity/expression and DNA synthesis, our results are consistent with a study by Ge et al. [13] who not only reported increased cell proliferation and Dnmts activity, but also DNA hypomethylation of the *c-myc* gene after single administration of Wy-14,643 to mice. However, a long-term (22 weeks) exposure to Wy-14,643 [39, 38] had no effect on *c-myc* methylation, although up-regulation of *c-myc* proteins was detected. It is worth noting, that Wy-14,643-induced global hypomethylation of DNA was associated with a decrease in Dnmt1 protein levels and increased cell proliferation. Thus, coexistence of hypomethylation of *c-myc* gene and transient over-expression of Dnmt1, reported in this paper, indicates that there must be a more complex mechanisms responsible for these findings.

One of the hypotheses regarding the carcinogenic potential of PB [8, 44] takes into account potential

induction of oxidative stress as a result of PB-stimulated metabolism, particularly of CYP2B induction, leading to generation of reactive oxygen species (ROS) and DNA damage. Moreover, the integrated transcriptomic and metabolomic analyses of livers and plasma of the *Fisher F344* rats exposed to PB for up to 14 days [48], also suggest that oxidative stress is involved in the development of early lesions that are caused by this compound. On the other hand, there is a growing conviction that oxidative stress has significant consequences for epigenetic processes, including DNA methylation [7, 29, 50].

It is widely known that oxidative damage can lead to formation of a variety of modified bases in DNA [4] as well as can be involved in the 5-hydroxymethylcytosine (5hmC) formation in a demethylation pathway [14]. Considering above assumption we cannot exclude conversion of promoter 5mC to cytosine (without Dnmt1) through a 5hmC intermediate for PB-induced demethylation of *c-myc* gene.

## CONCLUSION

In conclusion, the results of our study showed early changes in the genome of the liver of *Wistar* rats exposed to a tumor-promoter such as phenobarbital. Decreased methylation in the promoter region of the *c-myc* gene and increased level of its mRNA in the presence of a transient increase in Dnmt1 expression were found. Given the ability of *c-myc* gene to collaborate in the regulation of key cancer-related processes, i.e. cell proliferation and apoptosis, its deregulated expression (overexpression) by epigenetic events, can play crucial role for PB toxicity, including its carcinogenic activity.

Further study will be needed to verify these results by use more sensitive method to quantify methylation levels. Moreover, the additional experiments are required to establish the details of the mechanisms that are responsible for PB-stimulated demethylation of *c-myc* gene as well as to unravel a link between change of methylation status *c-myc* and induction of oxidative stress by PB.

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## Conflicts of interest

*The authors declare no conflicts of interest.*

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## DISTRIBUTION OF POLYETHYLENIMINE IN ZEBRAFISH EMBRYOS

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### ABSTRACT

**Background.** Polyethylenimine (PEI) plays important roles in the pharmaceutical design of non-viral gene delivery systems. Due to a set of unique physicochemical properties this cationic polymer has a great potential in modern gene therapies.

**Objective.** The aim of the present study was to determine the distribution of branched PEI (0.8 kDa) in zebrafish embryos (*Danio rerio*).

**Material and methods.** Zebrafish embryos at 3 hours post-fertilization (hpf) were incubated with PEI (10 µg/ml) for 24 and 48 hours and studied using the confocal laser microscopy.

**Results.** The obtained results show that PEI effectively distributed into the layers of the chorion and yolk sac in developing embryos due to first 24 hours of exposure. In contrast, PEI was found in the yolk, head, trunk and tail of the embryos due to prolonged treatments (48 hours).

**Conclusion.** The study evidences a high distribution of the branched PEI (0.8 kDa) polymer in the zebrafish embryo tissues.

**Key words:** polyethyleneimine (PEI), distribution, zebrafish embryos.

### STRESZCZENIE

**Wprowadzenie.** Polietylenoimina (PEI) odgrywa ważną rolę w projektowaniu niewirusowych transporterów genów. Ze względu na unikalne właściwości fizykochemiczne, ten kationowy polimer posiada duży potencjał aplikacyjny w nowoczesnych terapiach genowych.

**Cel badań.** Celem niniejszego badania było określenie dystrybucji rozgałęzionego PEI (0,8 kDa) w zarodkach *Danio pręgowanego* (*Danio rerio*).

**Materiały i metodyka.** Trzy godziny po zapłodnieniu zarodki *Danio pręgowanego* inkubowano z PEI (10 µg/ml) przez 24 godziny i 48 godzin po czym badano przy użyciu konfokalnej mikroskopii laserowej.

**Wyniki.** Wykazano, że PEI ulega dystrybucji w warstwach chorionu oraz żółtku rozwijającego się zarodka w pierwszych 24 godzinach ekspozycji. W wyniku przedłużonej ekspozycji (48 godzin), wykazano obecność PEI w żółtku, głowie, tułowiu i ogonie zarodków.

**Wnioski.** Badania wskazują na znaczącą dystrybucję rozgałęzionego polimeru PEI (0,8 kDa) w tkankach zarodka *Danio pręgowanego*.

**Słowa kluczowe:** polietylenoimina, PEI, dystrybucja, zarodki *Danio pręgowanego*

### INTRODUCTION

Polyethyleneimine (PEI) is a synthetic polymer, which has been recently used in novel non-biological gene delivery systems [16]. Studies evidence that PEI forms stable complexes with DNA, and the binding is

achieved mainly due to direct interaction between the protonated imino groups on PEI and the electronegative oxygens on the DNA backbone [4]. The high density of secondary and tertiary amino groups in PEIs confers significant buffering capacity to the polymers over a wide pH range [15]. This property known as

“proton sponge effect” is mainly recognized as one of the crucial factors for the high transfection efficiency due to PEI associated cargos [2].

Recent studies elucidate that PEI mediated toxicity depends on its molecular weight and type of the PEI structure [9]. The low molecular weight linear or branched PEI has low cytotoxicity compared to its high molecular weight counterparts [6]. Moreover, the linear PEIs are more tolerable than the branched ones [5]. Generally, it is assumed that the backbone linkages (carbon-carbon or carbon-amine bonds) in branched PEIs are non-degradable at physiological pH and are resistant to systemic clearance and accumulate in cells leading to further toxicities [12]. Because using branched PEIs as non-viral vectors for different nucleic acid cargos is very challenging, a more thorough understanding of the whole-body fate of these cationic polymers is really needed.

In the present study, we evaluated the distribution of branched polyethylenimine in zebrafish embryos (*Danio rerio*). Both early and late stages of embryonal developments were studied due to PEI exposures.

## MATERIAL AND METHODS

### Reagents

Branched polyethylenimine (PEI; Mw (by LS): 0.8 kDa), fluorescein isothiocyanate (FITC), dimethyl sulfoxide (DMSO) and tricaine were purchased from Sigma-Aldrich (Germany). All the reagents were used as received without purification. Dialysis against water was carried out using 0.5 k MCWO Snake-Skins Dialysis Tubes (Thermo-Fisher Scientific, MA USA). Lyophilization was performed using a FreeZone 1 liter Laboratory Lyophilizer (Labconco, MO USA). All manipulations were made in the dark with a red bulb.

### Synthesis of PEI-FITC labels

Branched PEI-FITC derivatives were synthesized using the methods described elsewhere with slight modifications [10, 13]. To a stirred solution of PEI (0.8 kDa; 140 mg) in distilled water (10 ml) a solution of FITC (11.2 mg; 28.7  $\mu$ mol) in DMSO (5 mL) was added. The reaction mixture was stirred at room temperature for 24 hours and then dialyzed (0.5k MWCO membrane for PEI 0.8 kDa) against distilled water for 48 h. Finally, after lyophilization for 24 h *ca.* 70 mg of the final product (PEI-FITC) was obtained respectively.

### Animals

Zebrafish embryos (*Danio rerio*) of the AB strain were obtained from the International Institute of Molecular and Cellular Biology (Zebrafish Core Facility) in Warsaw (Poland). In brief, adults zebrafish

(male and female) were kept at the standard laboratory condition of 28.5°C on a 14 hours light/10 hours dark photoperiod in the so-called zebrafish water. After photo-induced spawning of ten pairs (male and female; ratio 1:1), the embryos were collected and staged as described by Kimmel et al. [11]. Well-developed zebrafish embryos at the 8-32 cellular stage were selected under a dissecting microscope and used for the distribution studies.

### Zebrafish embryo exposures to PEI

The zebrafish embryos at 3 hpf were incubated with a water solution of PEI labeled with FITC at 10  $\mu$ g/ml for 24 and 48 hours, respectively. The embryos treated with ultrapure water were served as controls. To date, the exposures were performed using 96-well plates with one embryo in each well. The embryos were held continuously under a 14:10 light/dark photoperiod at 28.5  $\pm$  0.5°C.

### Confocal microscopy studies

Randomly selected zebrafish embryos from 24 and 48 hours exposures were transferred on a petri dish and rinsed with zebrafish medium [1]. In the case of the embryos at 24 hours exposure, the study was performed with embryos possessing with and without the chorions. The embryos were then transferred in a drop of the zebrafish medium into 96-well plate (one embryo per one well) and anesthetized with a drop of tricaine. Each anesthetized embryo was covered with melting agarose (42°C) and immediately positioned on its side for imaging from the lateral side. The embedded embryos were imaged with an Olympus X1000 confocal microscope. Images were taken with 10X objective and excitation wavelength 488 nm. Fluorescence signal was acquired together with DIC imaging.

## RESULTS AND DISCUSSION

The zebrafish species are the most frequently used animal models in modern toxicological studies [3]. It owes its great popularity due to the availability of a large number of individuals at the embryonic development, low cost, transparency, susceptibility to genetic manipulation and usefulness in high-throughput screening research [14]. In the present study, the distribution of non-modified branched polyethylenimine (0.8 kDa) was examined using the embryonic zebrafish model (*in vivo*). To date, PEI was conjugated with FITC and used in the study in zebrafish embryos. The results evidence the substantial accumulation of the PEI material in the layers of the chorion resulting in the inhibition of the hatching process in the early embryos (Figure 1).

Interestingly, PEI also distributes into the tissues of the developing embryos because the increase of fluorescence intensities was noted in the yolk sac due to dissection of the chorion (Figure 2).

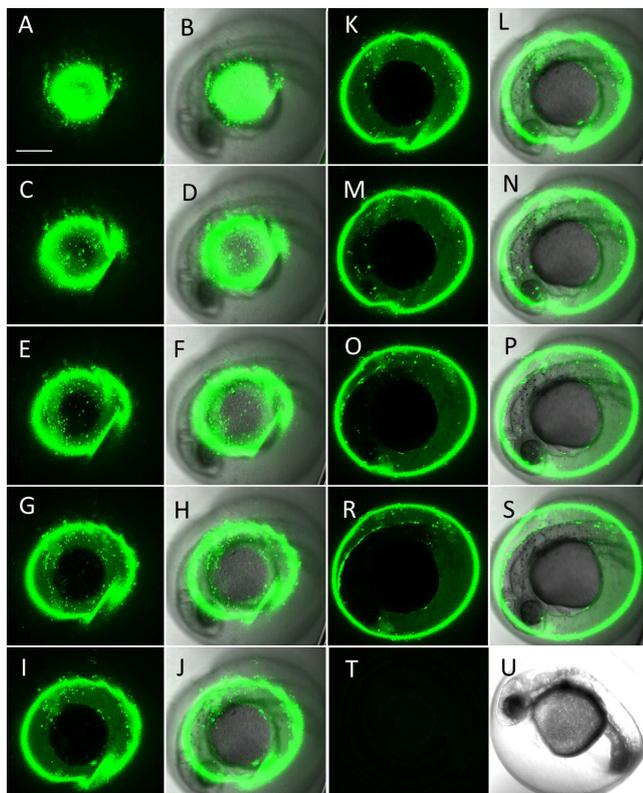


Figure 1. Distribution of PEI 0.8 kDa within the chorion of the *Danio rerio* embryo after 24 hours of exposure (10 µg). Serial optical sections (z-stacks, 24 µm/slice) of the confocal images of exposed (A-S) and non-exposed (T-U) embryos. Fluorescence measurements (green, A-U) indicate localization of the PEI polymer. Fluorescent signal is also merged with DIC (differential interference contrast) (grey, B,D,F,H,J,L,N,P,S,U). Scale bar 300 µm.

In recent studies, polyethylenimine was shown to have a high affinity for parenchymal cells, especially the lung, liver, spleen, kidney, heart and brain of laboratory mice [8]. After intraventricular injection of the PEI-DNA complex into the cerebrospinal fluid of mice, a good cerebral distribution of this complex was also observed [7]. In other studies, the PEI-DNA complex were mainly deposited in zebrafish embryos due to short-term exposures [17]. In the present study, the distribution of PEI was found within the yolk sac and head of the developing embryo due to first 24 hours of exposures (Figure 2). Interestingly, PEI was mainly determined in the yolk, trunk and head of embryos due to prolonged (48h) exposures (Figure 3).

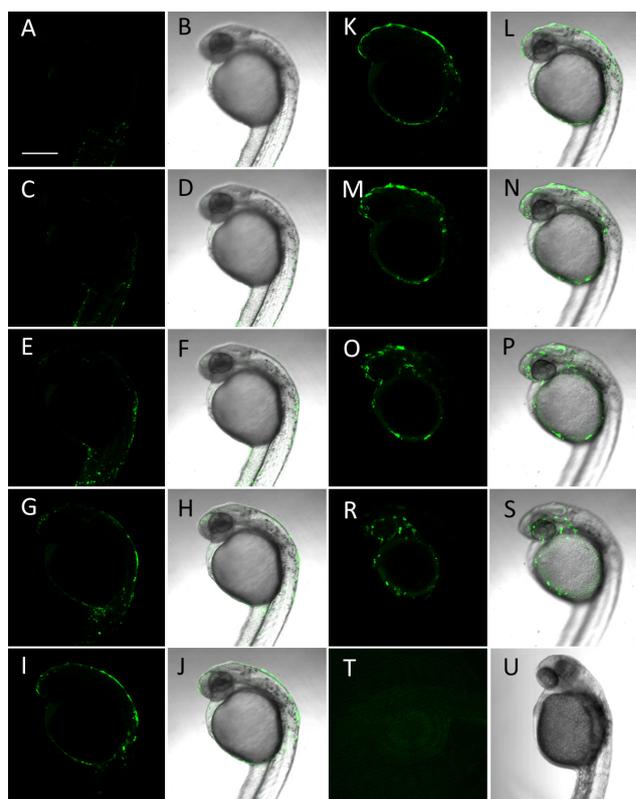


Figure 2. Distribution of PEI 0.8 kDa in the yolk sac and head in the dechorionated zebrafish embryo after 24 hours of exposure (10 µg). Serial optical sections (z-stacks, 33 µm/slice) of confocal images of treated (A-S) and non-exposed (T-U) embryos. Fluorescence (green, A-U) indicates localization of PEI and fluorescent signal merged with DIC imaging of embryo (grey, B,D,F,H,J,L,N,P,S,U). Scale bar 300 µm.

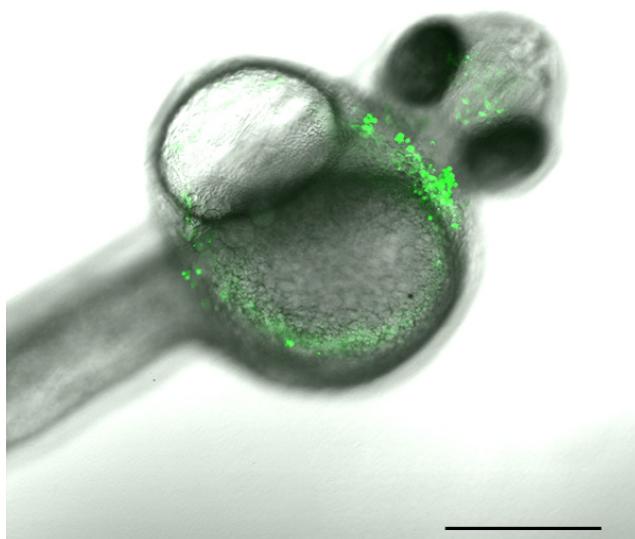


Figure 3. Distribution of PEI 0.8 kDa in the head and yolk sac in the dechorionated zebrafish embryo after 48 hours of exposure (10 µg). The yolk sac edema is found. Scale bar 300 µm.

## CONCLUSION

The study evidences that PEI easily distributes into all organs and tissues in zebrafish embryos. A large body of PEI is found at the chorion, yolk sac and head of the growing embryos due to 24- and 48-hours exposures.

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### Conflict of interest

The authors declare that there are no conflicts of interest.

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