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ORIGINAL ARTICLE

EXPOSURE ASSESSMENT OF INFANTS AND YOUNG CHILDREN ON SELECTED *FUSARIUM* TOXINS

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ABSTRACT

Background. Mycotoxins belong to substances harmful to human health. They are found mainly in cereal products and their preparations. In particular, infants and young children who consume cereal products, including porridge and gruel, are exposed to these substances.

Objective. The aim of the study is to assess the exposure of infants and young children in Poland to micotoxins (ochratoxin A. deoxynivalenol, nivalenol, fumonisins B1 and B1, T-2 and HT-2 toxins) derived from cereal products intended for infants and children.

Material and methods. Samples of products (302) were taken from all over the country in the following three years (2011, 2012 and 2013). HPLC-MS / MS method was used to determine the test compounds.

Results. Using the HPLC-MS / MS method, the assessment of population exposure in Poland to mikototoxins (ochratoxin A, deoxynivalenol, nivalenol, fumonisins B1 and B1, T-2 and HT-2 toxins) derived from cereal products (porridge, gruel) intended for infants and small children. Samples (302) were taken from across the country over the next three years. The exposure values obtained in the average exposure scenario range from 0.2 to 3% compared to the reference toxicological parameters. Considering that in the case of infants and young children, the tested products constitute a quantitatively significant part of the balanced diet of these consumers, and the remaining groups of foodstuffs, including vegetable products. fruit and meat and dairy products do not contribute significant amounts of mycotoxins to the diet can be accepted. that the level of contamination of cereal products does not pose a significant risk to the health of consumers. In the case of high exposure, it did not exceed 10% of the reference values for deoxynivalenol and the sum of fumonisins B1 and B2. These values were assessed as not relevant for the exposure of infants and young children. In contrast, in the case of zearalenone, the high level of exposure corresponded to 36% of the value of tolerable daily intake (TDI), and for the sum of T-2 and HT-2 toxins, the value of 48% of tolerable daily intake. In both cases, the contribution of pollutants to the diet was significant, but still remained 2-3 times less than the tolerable daily intake. Given, that cereal products are the main source of these contaminants, it can be estimated that exceeding the TDI value in relation to the total diet of infants and young children is unlikely.

Conclusions. The exposure values obtained in the average exposure scenario range from 0.2 to 3% compared to the reference toxicological parameters. In the case of zearalenone, the high level of exposure corresponded to 36% of the TDI value. and for the sum of T-2 and HT-2 toxins, 48% TDI. The contribution of pollutants to the diet in both cases was significant. however, it still remained 2-3 times less than the tolerable daily intake. Considering, that cereal products are the main source of these pollutants can be assessed. that exceeding the TDI value for the total diet of infants and young children is unlikely.

Key words: *exposure assessment, mycotoxins, cereal products, products for infants and young children, TDI, tolerable daily intake,*

STRESZCZENIE

Wprowadzenie. Mikotoksyny należą do substancji szkodliwych dla zdrowia ludzi. Wykazują właściwości mutagenne, kancerogenne, teratogenne i estrogenne. Stwierdzane są głównie w produktach zbożowych i ich przetworach. Na działanie tych substancji narażone są zwłaszcza niemowlęta i małe dzieci, które spożywają produkty zbożowe, w tym kaszki i kleiki.

Cel. Celem pracy jest ocena narażenia niemowląt i małych dzieci w Polsce na mikotoksyny (ochratoksyna A, deoksyniwalenlol, niwalenol, fumonizyny B1 i B1, toksyny T-2 i HT-2) pochodzące z produktów zbożowych przeznaczonych dla niemowląt i dzieci.

Materiał i metody. Próbki produktów (302) były pobrane z terenu całego kraju w ciągu kolejnych trzech lat (2011, 2012 i 2013). Do oznaczania badanych związków stosowano metodę HPLC-MS/MS.

Wyniki. Stosując metodę HPLC-MS/MS oceny narażenia populacji w Polsce na mikokotoksyny (ochratoksyna A, deoksyniwalen-

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lol, niwalenol, fumonizyny B1 i B1, toksyny T-2 i HT-2) pochodzące z produktów zbożowych (kaszki, kleiki) przeznaczonych dla niemowląt i małych dzieci. Próbki (302) były pobrane z terenu całego kraju w ciągu kolejnych trzech lat. Uzyskane wartości narażenia w przypadku scenariusza przeciętnego narażenia wynoszą od 0.2 do 3% w porównaniu z referencyjnymi parametrami toksykometrycznymi. Biorąc pod uwagę, że w przypadku niemowląt i małych dzieci badane produkty stanowi istotną ilościowo część zrównoważonej diety tych konsumentów, a pozostałe grupy środków spożywczych w tym produkty warzywne. owocowe i mięsne oraz nabiał nie wnoszą istotnych udziałów mikotoksyn do diety można przyjąć. że poziom zanieczyszczenia produktów zbożowych nie stanowi istotnego ryzyka dla zdrowia konsumentów. W przypadku wysokiego narażenia nie przekraczało ono poziomu 10% wartości referencyjnych dla deoksyniwalenolu oraz sumy fumonizyn B1 i B2. Wartości te oceniono jako nie mające istotnego znaczenia dla narażenia niemowląt i małych dzieci. Natomiast w przypadku zearalenonu wysoki poziom narażenia odpowiadał 36% wartości tolerowanego dziennego pobrania (TDI), a w przypadku sumy toksyn T-2 i HT-2 wartości 48% tolerowanego dziennego pobrania. W obu przypadkach udział wnoszonego zanieczyszczenia do diety był znaczący, jednak nadal pozostawał 2-3 krotnie mniejszy niż tolerowane dzienne pobranie. Biorąc pod uwagę. że produkty zbożowe są głównym źródłem tych zanieczyszczeń można ocenić, że przekroczenie wartości TDI w odniesieniu do całkowitej diety niemowląt i małych dzieci jest mało prawdopodobne.

Wnioski. Uzyskane wartości narażenia w przypadku scenariusza przeciętnego narażenia wynoszą od 0.2 do 3% w porównaniu z referencyjnymi parametrami toksykometrycznymi. W przypadku zearalenonu wysoki poziom narażenia odpowiadał 36% wartości TDI, a w przypadku sumy toksyn T-2 i HT-2 wartości 48% TDI. Udział wnoszonego zanieczyszczenia do diety w obu przypadkach był znaczący, jednak nadal pozostawał 2-3 krotnie mniejszy niż tolerowane dzienne pobranie. Biorąc pod uwagę, że produkty zbożowe są głównym źródłem tych zanieczyszczeń można ocenić, że przekroczenie wartości TDI w odniesieniu do całkowitej diety niemowląt i małych dzieci jest mało prawdopodobne.

Słowa kluczowe: *ocena narażenia, mikotoksyny, produkty zbożowe, produkty dla niemowląt i małych dzieci, TDI, tolerowane dzienne pobranie*

INTRODUCTION

Mycotoxins - secondary mould metabolites with undesirable effects for humans are common in the environment. Their presence is not related to the development of industry or transport. which in the common understanding are considered to be the main factors responsible for the occurrence of food contamination. These toxins are mainly produced by fungi of the genera *Penicilium*, *Aspergillus* and *Fusarium*. Their occurrence is associated with the inevitable presence of mould in the environment. Mycotoxins are present in agricultural products of basic importance for humans - in cereals (aflatoxins, ochratoxin A (OTA), *Fusarium* toxins and oilseeds (aflatoxins) and their preparations. They are also often detected in significant quantities in other foods – nuts, spices, dried fruit, coffee, wine, fruit preparations (aflatoxins, ochratoxin A, patulin). They may be present in raw materials of animal origin as a result of feeding with mycotoxin-contaminated feed. These substances are stable in most processes used in food processing, including at elevated temperatures. In many cases, they are characterized by significant acute toxicity, and they have carcinogenic, immunotoxic. nephro- and hepatotoxic effects.

In recent years, significant progress in food toxicology and the development of analytical techniques has caused attention to toxins produced by fungi of the genus *Fusarium*, widespread in the temperate climate zone, including in European agricultural areas. They are detected mainly in cereals and products obtained from them. The formation of Fusarium toxins occurs as a result of infection of plants (cereals) in the field, already during flowering. For this reason, their occurrence is dependent on climatic conditions during the growing season, rainfall and high humidity during the flowering of cereals are the cause of so-called "*Fusarium* years". There is then a particularly large toxin contamination. They constitute a large group of compounds with diversified chemical structure and a broad spectrum of toxic effects. To date, toxicological evaluation of the following *Fusarium* toxins: deoxynivalenol (DON), nivalenol (NIV), toxins T-2 and HT-2, zearalenone (ZAE) and fumonisins (FB) has been performed.

The increasing number of scientific publications on the contamination of foodstuffs with T-2 and HT-2 toxins, as well as the increasingly frequent detection of them in crops and food is noteworthy. In addition to the development of analytical methods, some authors explain this phenomenon in the recent decades of the effects of global warming. It is predicted that the temperature in the region of Central Europe may increase by 3 to 5 ºC. This may cause the migration of some mold species to new regions, as well as create conditions for the production of mycotoxins by species previously considered non-toxic.

T-2 and HT-2 toxins are substances with hematotoxic. neurotoxic and immunotoxic effects. The European Commission indicates that there is a risk of exceeding the tolerable daily intake (TDI) for these toxins by a particularly vulnerable population group - infants and young children. Due to not fully developed detox mechanisms young children are particularly sensitive to xenobiotics. It is underlined that the estimation of sampling can be an error due to the use of analytical methods with too high detection limits (LOD).

The project uses the method enabling the simultaneous determination of many mycotoxins. For the tests. in addition to the T-2 and HT-2 toxin. Deoxynivalenol, nivalenol and zearalenone were selected. DON and NIV were selected for evaluation due to the occurrence of reports of their occurrence independently of each other, whereas until now DON was considered to be an indicator mycotoxin. informing simultaneously about the presence of NIV. Zearalenone was indicated because of estrogenic activity, which is atypical for mycotoxins, especially harmful to small children's organisms, especially during the period of formation of reproductive organs.

Taking into account the above facts, it was considered advisable to estimate the exposure of infants and young children to T-2 and HT-2 toxins and to other indicated *Fusarium* toxins – dexinvalenol, nivalenol and zearalenone.

MATERIAL AND METHODS

Samples

Samples of grain products weighing not less than 1 kg were collected in accordance with the rules set out in the Commission Regulation (EC) No. 401/2006 [9] by inspectors of Sanitary and Epidemiological Stations. Samples were collected from retail sales throughout the country in 2011-2013 and sent to the Laboratory of the Food Safety Department of the National Institute of Public Health - National Institute of Hygiene. A total of 302 samples of products for infants and young children were delivered to the Laboratory.

Methodology of determination

A modified method of high performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS). developed by *Sulyok* et al. [11] was used to determine the level. Analytical grade reagents from Merck were used, with the exception of acetonitrile and ammonium acetate for mass spectrometry (Baker) and toxin standards, including those labelled with isotope (Biopure). The water was purified by reverse osmosis and demineralization.

A 4.0 g sample was weighed into a falcon tube with an accuracy of 0.01 g. 12 ml extraction solution (80 parts acetonitrile, 1 part acetic acid, 19 parts water) was added and homogenized in an UltraTurax homogenizer for 3 min. The homogenate was centrifuged for 20 min at 4000 x *g*. The l ml extract was transferred to autosampler vials and an internal standard solution (U-[13C22] HT-2 toxin or verrucol) was added. For chromatographic analysis, a Water Alliance 2695 liquid chromatograph was used with a C18-AtlantisTM dC18 3µm 100 Å. 2.1 x 100 mm column Waters.

Chromatograph operating conditions:

HPLC analysis

The mobile phase flow 0.2 ml / min

The composition of the mobile phase:

Phase A: 10% (V/V) aqueous solution of 5 mM ammonium acetate and 1% acetic acid with 90% methanol

Phase B: 90% (V/V) aqueous solution of 5 mM ammonium acetate and 1% acetic acid with 10% methanol.

Gradient profile:

Time (min)	Phase A (%)	Phase B (%)
0-5	0	100
5-30	100	0
30-60	100	0
60-65	0	100
65-70	0	100

The dispensed volume - 20 µl

Column temperature - 40ºC

The temperature of the sampler 10ºC

Calibration

6 point calibration, points 1-5 prepared by diluting the working solution for point 6. Concentrations according to the table below. Solvent mixture of chromatographic phases A and B 1 + 1 (V/V). Working solution 6 prepared from stock solutions.

Phase A: 5 mM AcoNH 4. 1% (V/V) AcOH in a mixture of 100 ml water + 900 ml methanol.

Phase B: 5 mM AcoNH 4. 1% (V/V) AcOH in a mixture of 900 ml of water + 100 ml of methanol.

Table 1. Concentration of standard solutions

Mycotoxin	Point 1 (ng/ml)	Point 2 (ng/ml)	Point 3 (ng/ml)	Point 4 (ng/ml)	Point 5 (ng/ml)	Point 6 (ng/ml)
DON	100	200	400	800	2 000	4 000
NIV	100	200	400	800	2 000	4 000
ZEA	10	20	40	80	200	400
OTA	0.25	0.5	1	2	5	10
HT-2	7.5	15	30	60	150	300
T-2	7.5	15	30	60	150	300
FB2	100	200	400	800	2 000	4 000
FB1	100	200	400	800	2 000	4 000
^{13}C-HT-2	15	15	15	15	15	15

Internal standard 13C-HT-2

The table shows the results obtained for the internal standard signal. The 13C labeled standard was added directly to the sample dispensed to the LC / MS measuring system. Samples are understood as separate calibration solutions or further extracts. In samples containing the RSD matrix is greater (probable impact of the matrix), but between successive dosing from the same sample, the stretch marks do not differ significantly for the standards and real samples (indicates the precision of the instrumental measurement).

Internal standard VER (verularol)

250 ng/ml based on the final solution dosed for measurement were used. For the assumed standard recovery of 100% (IS: VER/IS: 13C-HT-2). RRF 0.00397341 was obtained.

Preparation of validation samples. validation levels

Up to 25 g of wheat flour. an fortifying solution (6 calibration point) and 100 ml extraction solvent were added. The sample was shaken vigorously for 90 minutes. Next, 2 ml of extract (BLANK and LEVEL 1 samples) and 4 ml (LEVEL 3 and LEVEL 2 samples) were taken. which was evaporated in a gentle stream of nitrogen at 45˚C, then the sample was dissolved in 400 µl of phase A and B mixtures (1+1 V/V) was filtered through a syringe filter and 20 µl of 13C-HT-2 internal standard solution was added. The prepared samples were dosed into the LC/MS measuring system. For the savings of standards one extract was used for each validation level. Enrichment levels are listed in Table 2.

Table 2. Working conditions of MS / MS mass spectrometer (Waters Quattro Micro API MRM mode. ES + ionization

Toxin /IS	Parent ion (m/z)	Dauther ion potomny (m/z)	Cone voltage	Collisieon energy
DON	297.10	249.10	22	12
NIV	313.10	125.00	24	12
ZEA	319.20	301.00	21	10
OTA	404.00	239.00	26	24
HT-2	442.2	263.20	18	13
HT-2 C13 (IS)	464.3	278.1	20	14
T-2	484.3	305.1	24	14
FB1	722.30	334.3	46	40
FB2	706.4	336.2	48	40
Source parametrs				
Capillary	4.00 kV			
Cone	20.00 V			
Extractor	2.00 V			
Temperature	100 ºC			
Evaporation temperature	350 ºC			
Gas flow on the cone (N2)	50 l/h			
Evaporative gas flow (N2)	500 l/h			

Exposure assessment for infants and young children

The exposure assessment was performed taking into account the results obtained and various probable scenarios of consumption of cereal products intended for reconstitution for infants and young children. The obtained exposure values were compared with the reference values toxicological parameters - set by the European Food Safety Authority.

Evaluation of consumption of cereal products tested by infants and young children

In the case of consumption scenarios, i.e. the amount of consumption of cereal-based products for infants and young children, and intended for reconstitution with milk or water, multiple exposure scenarios can be considered.

Taking into account the guidelines for the diet of infants and young children, as well as suggestions of food producers associated with the consumption of the discussed products, two consumption variants were estimated. The first one refers to infants 12 months of age who have a body weight of 10 kg, equivalent to around the 50th percentile of body weight for both girls and boys. The average consumption of foodstuffs tested is about 20 g / day, however, due to the diversity of products (ie. their purpose for reconstitution with water or milk), the content of flour and cereal ranges in the range of 50-100%. The collection determined on this basis ranges from 1.0 to 2.0 g/kg bw/day. The second variant is related to consumption by 3-year-old children, whose body weight assume similar assumptions was estimated at 15 kg, and consumption

at the level of 60 g/day. Considering the adopted assumptions, the intake ranges from 1.5 to 4.0 g/kg bw./day. On the basis of this approach, the consumption of less than 1.0 g/kg bw./day and high consumption at 4.0 g/kg b.w./day were estimated.

Another important source of data on the consumption of the products in question by infants and young children is the database provided by EFSA. The results for the Polish population of young children (n = 53) indicate consumption at the median level of 0.24 g /kg bw/ day and at the 95th percentile level of 4.18 g/kg b.w./day. However, these studies took into account the consumption of all milling grain products, not only for feeding infants and young children.

Interesting insights are provided by German research and carried out in the United Kingdom. In the case of German studies carried out in the group of infants consuming foodstuffs produced with the participation of cereals, intended for reconstitution with water or milk (n = 140), the median value is 4.80 g/kg bw / day and the value 95./ percentile 27.97 g/kg mc/day. In the case of young children (n = 76) fed with similar preparations, the median value was 1.50 g/kg bw / day while the 95th percentile value was 22.53 g/kg bw / day. On the other hand, studies conducted in the United Kingdom on more numerous groups of consumers provide the following estimates of consumption: for infants (n = 1070) median 1.11 g/kg bw / day. 95th percentile 4.55 g/kg bw /day and for young children (n = 647) median 0.54 g/kg bw / day and 95th percentile 3.46 g/kg bw / day. German research indicates a clearly higher consumption of the product group in question. Considering consumption clusters designated under the GEMS/WHO program, they may be more adequate for Poland than British studies. However, the estimation obtained in British studies is closer to the previously designated consumption based on nutritional guidelines given to consumers in Poland.

When analysing GEMS/WHO data on intake, Spanish studies should also be considered, due to the same nutritional cluster as Poland and Germany. In this case, the value of food intake for infants and young children produced on the basis of cereals was at the median level of 3.3741 g/kg bw/day, while at the 95th percentile 17.7303 g/kg bw/day. The data on consumption compared are summarized in Table 3.

Table 3. Levels of fortification

Level (ng/g)	Blank	Level 1	Level 3	Level 2
DON, NIV, FB1, FB-2	0	200	400	100
ZEA	0	20	40	10
HT-2, T-2	0	15	30	7.5
OTA	0	0.5	1	0.25

The data on consumption discussed are very diverse. on their basis it is difficult to determine the intake value for the average and high consumption scenario, these values were proposed arbitrarily. In the case of average consumption, a value of 1.5 g/kg bw / day was assumed, which corresponds to lower, but realistically estimated values, while for high consumption, a value close to extreme consumption, i.e. 20 g/kg bw / day, was assumed to reflect the specific situations of consumer exposure.

RESULTS

The results of nd (not detected) were rejected in the performed statistical parameters. For comparison, results were also obtained using an internal standard added to the sample before preparation (VER verularol), the use of this standard may allow elimination of extraction errors. No verularol was introduced into the blank sample, the average recovery value for verularol obtained for the LEVEL 1 sample was 107.375%. The results are shown in Table 4.

Table 4. Performance of analytical method

Parameters	DON	NIV	ZEA	OTA	HT-2	T-2	FB2	FB1
Fortification level (µg/kg)	100	100	10	0.25	7.5	7.5	100	100
Recovery (%)	81.2	70.5	49.1	84.5	96.1	97.7	66.0	87.3
SD	17.7	5.0	0.71	0.1099	1.20	0.47	5.4	6.0
RSD (%)	5.1	7.1	14.4	65.1	14.3	6.5	6.0	5.7
Fortification level (µg/kg)	200	200	20	0.5	15	15	200	200
Recovery (%)	89.2	60.3	72.2	83.0	99.2	96.6	84.8	92.4
SD	28.5	4.5	1.80	0.0897	1.08	1.18	7.7	9.5
RSD (%)	6.4	3.8	12.5	14.5	6.8	8.2	4.0	4.7
Fortification level (µg/kg)	400	400	40	1	30	30	400	400
Recovery (%)	89.2	65.2	48.0	46.3	103.7	98.0	79.6	94.8
SD	32.3	13.5	1.49	0.0849	1.31	1.77	18.9	19.8
RSD (%)	5.2	5.2	7.8	12.7	4.0%	6.0	5.5	5.0

The BLANK sample contains DON and fumonisins B1 and B2 at a low level. The presence of OTA and HT-2 is at the noise level. The recovery results obtained in recalcurrol are generally lower by 3.9-6.3%. however, this is not a significant difference. RSD values are slightly higher (on average by 1.2%) while the maximum ranges between samples in a few cases significantly increased (NIV, HT-2. FB2). a clearly better result was obtained for DON.

The use of verularol as a reference internal standard does not bring about a significant improvement in recoveries. As it also does not bring about a significant improvement in recoveries, in this case the results are on the verge of acceptability, (repeating).

Estimation of LOD and LOQ values. The values estimated are analysed by the signal-to-noise ratio LOD (s / n) = 3 - LOQ (s / n) = 9; values are given in Table 5.

Table 5. Method performance parameters; detection limits (LOD) and quantification limits (LOQ)

Mikotoxine	LOD (3 x S/N)		LOQ (10 x S/N)	
	ng/ml	ng/g	ng/ml	ng/g
DON	5.0	2.0	16.3	6.5
NIV	46.5	18.6	154.8	61.9
ZEA	15.3	6.1	51.3	20.5
OTA	0.18	0.07	0.60	0.24
HT-2	2.8	1.1	9.3	3.7
T-2	0.3	0.1	0.8	0.3
FB2	3.5	1.4	1.0	0.4
FB1	1.3	0.5	3.8	1.5

The developed method of determination of Fusarium toxins in cereal products using HPLC-MS/MS meets the requirements for methods used in official control; the obtained efficacy parameters are given in the Table 6.

Table 6**.** Results of *Fusarium* toxin determination in products for infants and young children

Micotoxine	Samples			Values		Average				Mediane	P75	P90	P95	Max
	n	>LOQ	>LOD & <LOQ	LOD	LOQ	LB	MB	UB	>LOD					
DON	302	19	17	2	4	0.560	2.321	4.127	4.697	0	0	2.919	4.474	9.609
NIV	302	3	3	2	4	0.136	2.096	4.068	6.851	0	0	0	0	14.247
ZEA	302	14	14	1.8	3.6	0.435	2.069	3.758	4.697	0	0	0	3.589	19.660
OTA	302	7	4	0.07	0.14	0.007	0.075	0.143	0.196	0	0	0	0	0.386
FB1	302	36 3	0	0.5	1	0.560	1.000	1.441	4.697	0	0	2.919	4.474	9.609
FB2	302	1	1	1.4	2.8	0.033	1.414	2.800	2.455	0	0	0	0	2.807
HT-2	302	0	3	1.1	2.2	0.013	1.102	2.200	1.279	0	0	0	0	1.549
T-2	302	20 2	4	0.1	0.2	0.021	0.112	0.204	0.244	0	0	0	0.214	0.484

Obtained results of determination of *Fusarium* toxins in products for children and infants are given in the Table 7.

Table 7. The number of tested samples in which mycotoxins were found

Number of mycotoxins found	Number of samples (percentage of samples)
0	247 (81.8%)
1	14 (4.6%)
2	9 (3.0%)
3	18 (6.0%)
4	8 (2.6%)
5	4 (1.3%)
6	2 (0.7%)
7	0
8	0
Total number of positive results	150 (6.2%)
Total number of tested samples	302
Total number of samples in which at least 1 mycotoxin was found	55 (18.2%)

Obtained results of determination of toxins, corrected for recovery, are presented in the Table 6. It should be noted that in any sample no exceedance of the maximum allowable contamination established in the Commission Regulation (EC) No. 1881/2006, as amended. The number of samples in which at least one mycotoxin was found was 55 (18.2%). Detailed information on the distribution of individual mycotoxins is given in the table Table 8.

Exposure to the examined mycotoxins in no case exceeds the TDI value, also the maximum values do not exceed this parameter. It should be emphasized that for one sample. the level of contamination with zearalenone indicated about 50% of the value of tolerable daily intake. Detailed results are included in Table 9.

Table 8. Sample data on the consumption of food produced with cereals for infants and young children

	Own estimation		EFSA database (data for Poland)	EFSA database (data for Germany)		EFSA database (British data)		GEMS/ WHO (data for Spain)
			n=53	n=140	n=76	n=1070	n=647	n=8
Consumer group	infants	young children	young children	infants	young children	infants	young children	young children
Level of consumption (g/kg bw/day)								
Average	1	1.5	0.24	4.80	1.5	1.11	0.54	3.3741
High	2	4	4.18	27.97	22.53	4.55	3.46	17.7303

Table 9. Estimation of the exposure value of infants and young children to mycotoxins from food cereals products intended for infants and young children. Comparison of the exposure in relation to the toxicological reference values

Mycotoxin	Exposure scenario	Level of contamination	Intake	Exposure	Reference value	Exposure value in relation to the reference value
		µg/kg	*g/kg bw.*	*ng/kg bw*		
Deoksyniwlaenol	average	2.321	1.5	3.5	1 µg/kg b.w. (TDI)	0.4%
	high	4.474	20	89.5		9%
Nivalenol (NIV)	average	2.096	1.5	3.1	0.7 µg/kg b.w. (PTDI)	0.4%
Zearalenone (ZAE)	average	2.069	1.5	3.1	0.2 µg/kg b w. (TDI)	1.6%
	high	3.589	20	71.8		36%
Ochratoxin A	average	0.075	1.5	0.11 (0.77/week)	120 ng/kg b.w. (TWI)	0.6%
Sum of FB1+FB2	average	2.414	1.5	3.6	2 µg/kg b.w. (PTDI)	0.2%
	high	4.474	20	89.5		4.5%
Sum of T-2 + HT-2	average	1.214	1.5	1.82	0.1 µg/kg b.w. (TDI)	1.8%
	high	2.404	20	48.1		48.1%

DISCUSSION

Mycotoxins in the tested products

Analysis of the obtained results of the content of individual mycotoxins in foodstuffs allows the adoption of two levels of contamination for risk assessment purposes - the level of average and high contamination In the first case, a value was looked for reflecting the average level of contamination of products. Due to the significant number of samples below the limit of quantification, the mean value determined as medium bound was taken as the average exposure value, ie. the average calculated assuming that for values below the limit of quantification the half-valued limit value is taken to determine the average, which in this work is tantamount to accepting the limit of detection. At the same time, it should be noted that there are significant differences between mean values calculated as lower bound, i.e. assuming that if a result is below the limit of quantification, the averaged values are calculated and calculated as upper bound, i.e. assuming a value equal to the limit of quantification. A large discrepancy between the estimated values indicates a significant uncertainty of estimation at the level of average pollution. In the second approach. the 95th percentile of the occurrence of mycotoxins in the tested products was used to determine the high level of pollution. In the case of T-2 and HT-2 toxins, the mean *upper bound* value was assumed.

Obtained results do not allow for exposure assessment in the case of high levels of contamination with nivalenol and ochratoxin A - the 95th percentile is zero, which indicates incidental high levels of these mycotoxins. Due to the similar toxic effects and the determination of group toxicological parameters by EFSA. the exposure assessment was carried out for the sum of the occurrence of some mycotoxins. Taking into account the above assumptions. the exposure assessment was performed for both levels of contamination in the case of deoxynivalenol, zearalenone, sum of fumines B1 and B2 and sum of T-2 and HT-2 toxins, while for nivalenol and ochratoxin A the exposure assessment was limited to the average.

Evaluation of consumption of cereal products by infants and young children

In the case of consumption scenarios, i.e. the amount of consumption of cereal-based products for infants and young children, and intended for reconstitution with milk or water, multiple exposure scenarios can be considered.

Taking into account the guidelines for the diet of infants and young children, as well as suggestions of food producers associated with the consumption of the discussed products, two consumption variants were estimated. The first one refers to infants 12 months of age who have a body weight of 10 kg, equivalent to around the 50th percentile of body weight for both girls and boys. The average consumption of foodstuffs tested is about 20 g / day, however, due to the diversity of products (ie. their purpose for reconstitution with water or milk),

the content of flour and cereal ranges in the range of 50-100%. The collection determined on this basis ranges from 1.0 to 2.0 g/kg bw/day. The second variant is related to consumption by 3-year-old children, whose body weight assume similar assumptions was estimated at 15 kg, and consumption at the level of 60 g/day. Considering the adopted assumptions, the intake ranges from 1.5 to 4.0 g/kg bw/day. On the basis of this approach, the consumption of less than 1.0 g/kg bw./day and high consumption at 4.0 g/kg bw/day were estimated.

Another important source of data on the consumption of the products in question by infants and young children is the database provided by EFSA. The results for the Polish population of young children (n = 53) indicate consumption at the median level of 0.24 g/kg bw/day and at the 95th percentile level of 4.18 g/kg bw/day. However, these studies took into account the consumption of all milling grain products, not only for feeding infants and young children.

Interesting insights are provided by German research and carried out in the United Kingdom. In the case of German studies carried out in the group of infants consuming foodstuffs produced with the participation of cereals, intended for reconstitution with water or milk (n = 140), the median value is 4.80 g/kg bw / day and the value 95th percentile 27.97 g/kg bw / day. In the case of young children (n = 76) fed with similar preparations, the median value was 1.50 g/kg bw/ day while the 95th percentile value was 22.53 g/kg bw/ day. On the other hand, studies conducted in the United Kingdom on more numerous groups of consumers provide the following estimates of consumption: for infants (n = 1070) median 1.11 g/kg bw / day. 95th percentile 4.55 g/kg bw / day and for young children (n = 647) median 0.54 g/kg bw / day and 95th percentile 3.46 g/kg bw / day. German research indicates a clearly higher consumption of the product group in question. Considering consumption clusters designated under the GEMS/WHO program, they may be more adequate for Poland than British studies. However, the estimation obtained in British studies is closer to the previously designated consumption based on nutritional guidelines given to consumers in Poland.

When analysing GEMS/WHO data on intake, Spanish studies should also be considered, due to the same nutritional cluster as Poland and Germany. In this case, the value of food intake for infants and young children produced on the basis of cereals was at the median level of 3.3741 g/kg bw/day. while at the 95th percentile 17.7303 g/kg bw/day. The data on consumption compared are summarized in Table 8.

The data on consumption discussed are very diverse. on their basis it is difficult to determine the intake value for the average and high consumption scenario, these values were proposed arbitrarily. In the case of average consumption. a value of 1.5 g/kg bw / day was assumed, which corresponds to lower, but realistically estimated values, while for high consumption, a value close to extreme consumption, ie. 20 g/kg bw / day, was assumed to reflect the specific situations of consumer exposure.

Exposure estimation

Given the data on the occurrence of mycotoxins and the consumption of the product group in question, consumer exposure was assessed and comparisons were made with the toxicological reference values estimated by EFSA. Two exposure scenarios were selected: the first corresponding to the average medium bound and the average consumption, and the second corresponding to the high pollution (95th percentile) and high consumption. The results are summarized in table 9.

As reference parameters, TDI values were used, i.e. a tolerable daily intake, in some cases temporarily determined, designated as PTDI. For ochratoxin A, reference was made to the TWI value, i.e. the tolerable weekly intake - in this case, the daily intake was multiplied and the percentage was taken throughout the week. The exposure values obtained in the average exposure scenario range from 0.2 to 3% compared to the reference toxicological parameters. Considering that the product group in question in the case of infants and young children is a quantitatively part of the balanced diet of these consumers, and other food groups including vegetable, fruit and meat products and dairy products do not contribute significant mycotoxin content to the diet, it can be assumed that contamination of cereal products does not pose a significant risk to the health of consumers.

In the case of high exposure, it did not exceed 10% of the reference values for deoxynivalenol and the sum of fumonisins B1 and B2, these values were assessed as not relevant for the exposure of infants and young children. In contrast, in the case of zearalenone, the high level of exposure corresponded to 36% of the value of tolerable daily intake. and for the sum of T-2 and HT-2 toxins, the value of 48% of tolerable daily intake. In both cases, the contribution of pollutants to the diet was significant. but still remained 2-3 times less than the tolerable daily intake. Considering that cereal products are the main source of these contaminants, it can be estimated that exceeding the TDI value in relation to the total diet of infants and young children is unlikely.

The contamination levels obtained for the tested products are similar to those from other European countries. Obtained data indicate that the values of tolerable daily intake for tested toxins are not exceeded, however, it should be emphasized that the formation of Fusarium toxins depends on weather conditions and levels of contamination in years with unfavourable climatic conditions can be many times higher.

CONCLUSIONS

1. The developed method for the determination of mycotoxins in cereal products using HPLC-MS/MS meets the requirements of the methods used in official control in terms of the limit of quantification, precision, recovery and uncertainty of the result.
2. The obtained data indicate that the values of tolerable daily intake for the tested toxins are not exceeded, however, it should be emphasized that the formation of Fusarium toxins depends on weather conditions and levels of pollution in years with unfavourable climatic conditions can be many times higher.
3. It is advisable to continue monitoring studies in this area, expanding them with new product groups, especially for infants and young children.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

MINERAL CONSTITUENTS OF CONSERVED WHITE BUTTON MUSHROOMS: SIMILARITIES AND DIFFERENCES

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ABSTRACT

Background. Mushrooms are a food that is often considered as an important source of minerals and other nutrients for consumers. There is little data on the minerals in mushrooms processed culinary and on the impact of processing.
Objective. The research was aimed at understanding the similarities and differences in the mineral composition (Hg, Li, Mg, Al, Co, Ni, Cu, As, Se, Rb, Sr, Ag, Cd, Cs, Sb, Tl, Pb, U, Ba, Cr, Zn, Mn and V) of white button mushrooms (*Agaricus bisporus*) processed in industrial conditions.
Material and methods. Fungal materials came from six producers. The elements were determined by ICP-MS DRC and CV-AAS using validated methods and QA/QC protocol. The interdependencies of 18 elements and 10 batches of mushrooms examined were tested with the help of principal component analysis.
Results. Some significant differences were found in the content and composition of minerals in marinated white button mushrooms depending on producer. Conserved white button mushrooms are poorer in major essential elements but also in toxic Hg, As, Ag, Cd, Sb, Tl or Pb which has been reported for unprocessed mushrooms.
Conclusions. The relatively higher levels of Ag in some batches seem to be largely explained by the quality of the substrate used for mushrooms cultivation, while of Li, Rb, Cs, Cr, Al, U, V, As and Mn (in part also of Ba and Sr) largely by the quality of the marinade.

Key words: *white button mushroom*, *pickled mushrooms*, *mineral constituents*, *food*, *nutrition*

STRESZCZENIE

Wprowadzenie. Grzyby jadalne to surowiec spożywczy bogaty w niezbędne pierwiastki metaliczne ale też możliwie źródło narażenia konsumenta na toksyczne metale i metaloidy. Niewiele jest danych na ten temat odnośnie grzybów przetworzonych kulinarnie czy wpływu procesów przetwarzania.
Cel. Badania miały na celu poznanie podobieństw i różnic w składzie mineralnym (Hg, Li, Mg, Al, Co, Ni, Cu, As, Se, Rb, Sr, Ag, Cd, Cs, Sb, Tl, Pb, U, Ba, Cr, Zn, Mn and V) pieczarek (*Agaricus bisporus*) przetworzonych w warunkach przemysłowych.
Materiał i metody. Pieczarki pochodziły z sześciu przetwórni. W analizie zastosowano sprawdzone metodyki analityczne łącznie z bieżącą kontrolą i zapewnieniem jakości wyników analizy. Pomiar wykonano technikami ICP-MS-DRC i CV-AAS. Współzależności pomiędzy 18 pierwiastkami i 10 partiami grzybów badano metodą analizy głównych składowych.
Wyniki. Wykazano duże różnice w zawartości składników mineralnych w pieczarkach z różnych przetwórni. Konserwowe pieczarki są znacznie uboższe w główne pierwiastki niezbędne ale także w pierwiastki toksyczne takie jak Hg, As, Ag, Cd, Sb, Tl czy Pb w porównaniu z opublikowanymi danymi dla grzybów nieprzetworzonych.
Wnioski. Względnie większą zawartość Ag w określonych partiach badanych konserwowych pieczarek wydaje się tłumaczyć jakość podłoża zastosowanego w uprawie grzybów a w przypadku Li, Rb, Cs. Cr, Al, U, V, As i Mn (po części też Ba i Sr) główny wpływ wydaje się mieć jakość użytej zalewy (marynaty).

Słowa kluczowe: *pieczarka dwuzarodnikowa, grzyby marynowane, składniki mineralne, żywność, żywienie*

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INTRODUCTION

Mushrooms, either foraged or cultivated are foodstuffs relatively rich in many essential mineral constituents but in parallel they often accumulate toxic ions both of natural and anthropogenic origin [11, 12, 19, 24, 27]. For cultivated mushrooms, the grower may influence the content of minerals and contaminants accumulated in flesh by selection of a proper substrate and cultivation method [22, 34]. High quality information on the multi-mineral composition and nutritional characteristics of mushrooms is expanding in the recent years thanks to the modern methods of analysis [2, 20, 36-38]. A great biodiversity of mushrooms and their emergence at sites that differ in soil geochemistry or a degree of pollution makes study on the wild mushrooms perceived as organic food challenging. In addition, there is little data available on status of metals, metalloids and other elements in mushrooms processed culinary [6, 40, 42, 45]. The traditional methods of cooking and recipes for mushroom dishes as well as preservation techniques such as freezing and freeze drying are factors contributing variability in the chemical composition and nutritional value of cooked mushrooms [7, 8, 16, 17, 39].

White button mushroom *Agaricus bisporus* [MB#292246] is a popular foodstuff worldwide. This mushroom and other species collected from the wild or cultivated are considered as a good source of proteins, vitamins and mineral constituents (potassium, phosphorus, zinc and copper or selenium) but also of antioxidants and with antibacterial, anti-inflammatory, antitumor, and immunomodulatory properties - see reviews by several authors [4, 14, 32, 33, 37].

A. bisporus has a very short service life, only 1-3 days at ambient temperature [3] or from 5 to 7 days at 0 °C to 2 °C [20], due to its high water content (about 90%) and high enzymatic activity. A common drying or more advanced "traditional" and modern culinary technologies such as pickling, salting, frying, freezing and freeze-drying are used to preserve mushrooms. Apart from a simple household treatment procedures, e.g., salting, blanching/parboiling and pickling in glass [7, 8, 9], deep freezing of fresh mushrooms and canning and industrial pickling become more common. The nutritional properties of mushrooms may be changed by the processing condition [25, 30].

The objective of this study was to understand the similarities and differences in the mineral composition of conserved (pickled) white button mushrooms available in trade in Poland, and estimate their possible loss (Hg, Li, Mg, Al, Co, Ni, Cu, As, Se, Rb, Sr, Ag, Cd, Cs, Sb, Tl, Pb, U, Ba, Cr, Zn, Mn and V) during industrial processing as well as source in conserved product.

MATERIALS AND METHODS

Conserved white button mushrooms (*A. bisporus*) were produced by six manufacturers: the batches of the "marinated and lagoon natural" were by Notre Jardin, Ole, Rolnik, Urbanek and Smak companies, and the batches: "mushrooms marinated – a whole" and "mushrooms marinated – sliced/crushed" were by Bonduelle. In detail they were as: Notre Jardin Champignons de Paris, Pieds Et Morceaux "*Pieczarki marynowane krojone – pickled champignon sliced*"; Notre Jardin Champignons de Paris 1er Choix Entiers "*Pieczarki marynowane całe – pickled a whole champignons*"; Bonduelle "*Pieczarki łagodne marynowane – soft champignons pickled*"; Bonduelle "*Pieczarki marynowane – pickled champignons*"; Rolnik "*Pieczarki marynowane – pickled champignons*"; Urbanek „*Pieczarki marynowane – pickled champignons"*; Smak „*Pieczarki marynowane – pickled champignons* "; Smak „*Pieczarki w zalewie solnej – pickled champignons in a saline juice*"; Ole „*Pieczarki w zalewie naturalnej całe – a whole champignons in a natural marinate*" and Ole "*Pieczarki marynowane z marchewką i cebulą – marinated champignons with a carrot and onion*". In total 100 unit packages of the processed white button mushrooms were studied. 80 Unit packages (primary samples) of mushrooms were in a glass jars and 20 (Bonduelle) in cans. The original unit packages were sampled randomly from the shops in the city of Gdańsk in 2016.

Mushrooms if not sliced were young fruiting bodies (caps with a piece of a stipe). They were drained from a liquid, placed in a separate polyethylene bags, deep frozen (-30 °C) and next lyophilized to constant mass (model LYOVAC GT2; Steris, Germany), ground to a fine powder using a porcelain mortars and kept sealed in brand new polyethylene bags under clean and dry condition. Any laboratory vessel before use was submerged in solution of 10 % nitric acid for at least 24 hours and next washed with double deionized water and dried at room temperature.

The subsamples of dehydrated and powdered mushrooms subjected for analysis were around 300 to 400 mg. They were flooded with 5 ml solution of concentrated nitric acid (65% HNO_3 - Suprapur®, Merck, Darmstadt, Germany) and cold digested for 24 h in the open polytetrafluoroethylene (PTFE) vessels. The vessels were closed and pressure digested using the MARSXpress microwave oven (Microwave Accelerated Reaction System, CEM Corp., Matthews, NC, USA). The operating conditions for digestion were as follows: power - 1.2 kW; ramp 1-10 min; temp. 1-100 °C; hold 1-10 min, ramp 2-10 min; -800 psi pressure; temp. 2-100 °C; holding time 2-10 min; cooling down time 5 min. The digests were diluted up to 10 ml with double deionized water. The blank digests were carried out in the same way [8, 9].

The elements (except of mercury) were determined using inductively coupled plasma mass spectroscopy (Spectroscope type ELAN DRC II ICP-MS, Perkin Elmer SCIEX, Canada) with dynamic reaction chamber (Table I) [5, 7, 8]. Mercury was determined using a direct fungal matrices thermal decomposition and cold vapor atomic absorption spectroscopy (MA-2000, Nippon Instruments Corporation, Takatsuki, Japan) [11]. All procedures were validated, and the QA/QC protocol routinely followed every set of examined samples [5, 8, 11, 15, 23]. The Polish reference materials used were such as CS-M-2 (dried mushroom powder *Agaricus campestris*) and CS-M-3 (dried mushroom powder *Boletus edulis*) produced by the Institute of Nuclear Chemistry and Technology, Warsaw, Poland [8, 15, 23].

The computer software Statistica version 8.0 (Statsoft, Inc., computer software) was used for statistical analysis and graphical presentation of the results of two dimensional multiple scatter plot relationships between the variables [5, 44]. The interdependencies of 18 elements and 10 batches of mushrooms were tested with the help of PCA (*Principal Component Analysis*). Statistic data have been divided into comparable units with means of 0 and with standard deviation of 1. Correlation matrix reflects the analysis the Kaiser Criterion (factors with eigenvalues greater than 1) and scree test was used. In order to choose the right number of analyzed components, the values have been divided into groups: 0.75 - strong; between 0.75–0.5 - moderate and between 0.5–0.3 - weak. All of them were based on their absolute values. The strongest values of 0.75 have been taken for analyses and interpretation. Varimax normalized rotation was used in order to maximize the variances of normalized factor loadings across variables for each factor.

RESULTS AND DISCUSSION

As, Hg, Cd, Ag, Pb and Se

The definitely toxic elements such as As, Hg, Cd, Ag and Pb occurred in pickled mushrooms respectively (median values) in the range 0.006 to 0.19, 0.028 to 0.065, 0.02 to 0.08, 0.002 to 0.090 and 0.006 to 0.18 mg kg^{-1} dry matter (dm) (Table 2). The overall median value was 0.03 for As, 0.043 for Hg, 0.040 for Cd, 0.020 for Ag and 0.072 mg kg^{-1} dm for Pb. Those elements are common trace contaminants of mushrooms collected from the wild and cultivated, while sometimes at elevated levels [14]. Apart from the cultivated white button mushrooms, the oyster mushroom *Pleurotus ostreatus* can accumulate heavy metals at elevated amount if grew in polluted substrates [1, 13, 36]. The allowed content of Cd and Pb in cultivated *A. bisporus* offered at the European Union markets should not exceed respectively 0.20 mg kg^{-1} and 0.30 mg kg^{-1} (2.0 and 3.0 mg kg^{-1} dm; assuming 90% moisture content) [10].

Exposure to negligible doses of Ag, Hg, Cd and Pb taken with foods is generally considered safe and tolerated by human body due to adaptation mechanisms, e.g., antagonism between certain elements, lack or poor absorption of a particular chemical forms, complexation and excretion. Arsenic is perceived as toxic in every dose because chronic exposure to inorganic As may cause various types of cancers [19, 28].

The toxic mineral constituents and those that are nutritionally essential can substantially leak out of fruiting bodies during the household treatment, e.g. blanching [8, 9] or industrial conserving [42]. Marinated *A. bisporus* in this study was less contaminated with Hg than fresh mushrooms collected by us in 2016, which had 0.069, 0.080 and 0.11 mg kg^{-1} dm (n = 3; pooled samples, each of 1 kg).

In the past, *A. bisporus* collected randomly in Poland in 1984-1985 had Hg at level 0.0059 ± 0.0090 mg kg^{-1} fresh biomass (0.059 ± 0.090 mg kg^{-1} dm; n = 214, pooled samples) on average, and for three other pooled samples mercury results were 0.26, 0.27 and 0.54 mg kg^{-1} fresh biomass (data not included in the overall mean) [31]. In another study *A. bisporus* had 0.039 mg kg^{-1} fresh biomass (0.39 mg kg^{-1} dm; assuming humidity content at 90%) [21].

Regarding As, fresh *A. bisporus* (white strain) available in Poland contained 0.63 ± 0.37 mg kg^{-1} dm (total range 0.15 to 1.4 mg kg^{-1} dm) [37], and what is an order of magnitude higher than in pickled mushrooms in this study. Arsenic was < 0.05 mg kg^{-1} dm in sliced and whole pickled *A. bisporus*, while in the range 0.49 to 3.7 mg kg^{-1} dm (means) in fresh Hungarian mushrooms [42, 43]. Clearly, conserving of mushrooms by blanching or industrial pasteurizing/canning highly decrease content of As. Such tendency has been confirmed for experimentally blanched and pickled caps of *Amanita fulva* (decrease by 89%) [8]. A substantial drop of As from fruiting bodies during boiling can be explained by fact that it occurs in molecular forms such as arsenous acid, arsenic acid, monomethylarsonic acid, dimethylarsinic acid, tetramethylarsonium ion, arsenocholine and arsenobetaine, which are water soluble, dominant and species-specific (different proportion) in total As of mushrooms [19].

Fresh *A. bisporus* from Poland showed Cd, Ag and Pb at levels higher an order of magnitude than has been determined in pickled mushrooms in this study, i.e., 0.36 ± 0.47 mg kg^{-1} dm (total range 0.08 to 1.2) for Cd, 0.19 ± 0.05 mg kg^{-1} dm (0.15 to 0.23) for Ag and 0.54 ± 0.54 mg kg^{-1} dm (range 0.15 to 27) for Pb [37]. Fresh *A. bisporus* produced in Hungary had on the average from 0.12 to 0.22 mg kg^{-1} dm of Cd, and conserved mushrooms imported from Asia had 0.22 ± 0.03 mg kg^{-1} dm (sliced) and 0.22 ± 0.05 mg kg^{-1} dm (whole) [42, 43].

Table 1. Optimized experimental parameters used for elements determination by ICP-DRC-MS

Instrument	PE Sciex ELAN 6100 DRC II
Nebulizer gas flow	(0.88-0.92) L min^{-1}
Auxiliary gas flow	1.2 L min^{-1}
Plasma gas flow	16 L min^{-1}
RF power	1150 W
Lens voltage	(0.75-0.90) V
Lens setting	Autolens calibrated
Dwell time	50
Detector mode	Dual (pulse counting and analogue mode)
Scan mode	Peak hopping
Sweeps/Reading/Replicate	10/ 1/ 3
Measured mass	^{26}Mg, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{121}Sb, ^{133}Cs, ^{138}Ba, ^{205}Tl, ^{208}Pb, ^{238}U
DRC gas flow rate	NH_3 0.135 L min^{-1} (^{51}V) NH_3 0.45 L min^{-1} (^{27}Al, ^{52}Cr) NH_3 0.65 L min^{-1} (^{55}Mn, ^{66}Zn) O_2 0.45 L min^{-1} (^{75}As)
Internal standard	^{45}Sc, ^{74}Ge, ^{103}Rh, ^{159}Tb

Due to low concentrations of toxic As, Hg, Cd, Ag and Pb, it can be stated that consuming the conserved white button mushrooms available at Polish market does not show any threat to the consumer.

Similarly to As, Hg, Cd, Ag and Pb, also the Se in the environment is a seeker of sulfur, while is antagonistic to Hg [35]. Selenium at very low doses (ca. 3 µg per kg body mass daily) is essential to human [20, 26]. The medians of Se in pickled mushrooms were in the range 0.19 to 1.6 mg kg^{-1} dm, and the overall median was 0.60 mg kg^{-1} dm (Table 2). In a study, the element Se exceeded 38 times (molar basis) amount of Hg. Nevertheless, the pickled *A. bisporus* clearly can be considered as a weak source of Se. If taken at amount of 100 g can provide about 6 µg of bioavailable Se on average.

Selenium was undetected (< 0.05 mg kg^{-1} dm) in conserved in brine *A. bisporus* sold in Hungary, while in fresh mushrooms was in the range from 1.9 to 3.7 mg kg^{-1} dm (means) [42, 43].

Li, Cs and Rb

Amongst the alkali elements, the Li and Cs with medians respectively in the range 0.01 to 0.40 and 0.004 to 0.02 mg kg^{-1} dm (the overall medians 0.045 and 0.15 mg kg^{-1} dm) were highly minor minerals, and for Rb were in the range 1.1 to 4.1 mg kg^{-1} dm (the overall median was 1.8 mg kg^{-1} dm). In one study lithium was in caps of fresh *A. bisporus* in the range 0.15 ± 0.14 to 0.20 ± 0.13 mg kg^{-1} dm [43], and in a whole fresh individuals (white strain) in range 0.53 to 4.7 mg kg^{-1} dm (mean 2.6 ± 3.0 mg kg^{-1} dm) [36].

Ba and Sr

The medians of Ba were in the range 0.40 to 2.9 mg kg^{-1} dm (the overall median 0.65 mg kg^{-1} dm), while Sr was greater and accounted from 1.6 to 11 mg kg^{-1} dm (the overall median 2.7 mg kg^{-1} dm) (Table 2). Both Ba and Sr are among the lithophile elements. Nothing is known on their compounds in mushrooms. The sulphate of Ba and Sr are hardly or insoluble in water. Blanching and pickling slightly decreased Ba in *A. fulva*, while Sr even multiplied after blanching [8].

In a study in Poland, the mean of Ba in *A. bisporus* (white strain) was 0.76 ± 0.92 mg kg^{-1} dm (0.08 - 2.9 mg kg^{-1} dm), while surprisingly for Sr was 0.36 ± 0.35 mg kg^{-1} dm (range 0.04 to 6.0 mg kg^{-1} dm). As it has been pointed ahead, the Sr overwhelmed Ba in pickled mushrooms (Table 2). Also Vetter *et al*. (42, 43) noted Sr at higher level than Ba in *A. bisporus*: in fresh Sr was in the range 6.7 to 7.5 mg kg^{-1} dm (means), and conserved showed 16 ± 1 mg kg^{-1} dm in sliced and 3 ± 0 mg kg^{-1} dm in whole fruiting bodies. In turn, the Ba in fresh mushrooms was in the range 2.1 to 2.4 mg kg^{-1} dm (mean values), and in conserved from 6.9 ± 0.4 mg kg^{-1} dm (sliced) to 7.8± 0.1 mg kg^{-1} dm (whole) [42, 43].

Mg, Mn, Cu and Zn

The medians for Mg, Mn, Cu and Zn were in the range: 220 to 560 mg kg^{-1} dm (the overall median 330 mg kg^{-1} dm for Mg), 2.4 to 4.8 mg kg^{-1} dm (3.0 mg kg^{-1} dm for Mn), 8.3 to 19 mg kg^{-1} dm (10 mg kg^{-1} dm for Cu) and 13 to 71 mg kg^{-1} dm (26 mg kg^{-1} dm for Zn) (Table 2).

Magnesium occurs in fresh *A. bisporus* at relatively high level: one study reports 1250 ± 219 mg kg^{-1} dm (786 to 1650 mg kg^{-1} dm) [37], and other from 1100 to 1400 mg kg^{-1} dm [42, 43]. Magnesium is essential for mushrooms and its uptake and sequestration is regulated by them. Conserved *A. bisporus* clearly had much less of Mg than uncooked. In a study by Vetter [42] sliced had 390 ± 20 mg kg^{-1} dm, and marinated as a whole had 470± 22 mg kg^{-1} dm [42]. *A. bisporus* marinated or conserved with brine is by 70% depleted from Mg.

Table 2. Macro and trance element content of pickled white bottom mushrooms available from the Polish market (mg kg^{-1} dry mass, mean ± SD, range and median, respectively)

	1*	2	3	4	5	6	7	8	9	10
Li	0.43±0.03	0.16±0.02	0.10±0.02	0.04±0.01	0.07±0.02	0.02±0.01	0.04±0.03	0.03±0.02	0.05±0.03	0.02±0.02
	0.39 - 0.48	0.13 — 0.2	0.07 — 0.16	0.02-0.07	0.05-0.11	0.003-0.04	0.002-0.08	0.003-0.06	0.01-0.12	0.005-0.06
	0.40	0.16	0.10	0.04	0.07	0.01	0.04	0.03	0.05	0.01
Mg	540 ± 41	WD	WD	WD	WD	330±13	230±15	360±32	560±28	310±24
	470-620					310-350	200-250	330-420	510-620	280-360
	540					330	220	340	560	310
Co	0.013 ± 0.015	0.02±0.01	0.03±0.03	0.04±0.05	0.01±0.00	0.02±0.00	0.01±0.00	0.005±0.002	0.007±0.001	0.02±0.00
	0.004-0.04	0.01-0.03	0.01-0.13	0.01-0.22	0.008-0.01	0.01-0.02	0.009-0.01	0,002-0.007	0.004-0.009	0.01-0.02
	0.005	0.02	0.02	0.02	0.01	0.02	0.01	0.004	0.007	0.02
Cu	10 ± 1	20±3	9.5±0.6	11±1	9.9±1.1	13±0.9	11±1	15±1	15±1	8.1±1.6
	8.0-12	17-26	8.0-11	9.0-12	8.7-13	12-15	9.2-12	13-17	13-16	5.6-10
	10	19	9.6	10	9.6	13	10	14	15	8.3
Se	0.38 ± 0.10	0.19±0.05	0.49±0.07	0.66±0.10	0.58±0.11	0.67±0.09	0.60±0.10	1.0±0.2	1.6±0.3	0.51±0.13
	0.20 – 0.54	0.06-0.32	0.32-0.67	0.46-0.97	0.39-0.79	0.49-0.85	0.33-0.79	0.82-1.6	1.2-2.1	0.33-0.75
	0.39	0.19	0.49	0.65	0.59	0.67	0.62	1.1	1.6	0.50
Zn	39±4	54±5	17±9	22±10	10±4	24±2	13±2	29±4	75±14	26±4
	35-49	44-64	1.8-26	4.2-30	2.8-13	21-27	10-15	22-36	62-100	20-35
	38	53	22	27	13	23	13	28	71	25
Mn	2.8±0.8	3.6±0.2	2.5±0.6	4.7±0.5	2.7±0.2	3.9±0.3	2.8±0.6	2.9±0.4	4.7±0.6	3.3±0.3
	1.8-4.2	3.0-4.0	1.5-3.8	3.9-5.6	2.4-3.3	3.5-4.5	2.1-4.0	2.3-3.6	4.1-5.7	2.7-3.9
	2.7	3.6	2.4	4.8	2.7	3.8	2.7	2.8	4.5	3.3
Cs	0.029±0.002	0.019±0.001	0.009±0.001	0.007±0.001	0.004±0.001	0.002±0.001	0.004±0.001	0.001±0.001	0.007±0.001	0.001±0.001
	0.026-0.036	0.017-0.021	0.004-0.011	0.006-0.010	0.003-0.005	< 0.001-0.004	0.001-0.005	< 0.001-0.003	0.004-0.009	< 0.001-0.003
	0.029	0.019	0.009	0.007	0.004	0.002	0.004	0.001	0.006	0.001
Rb	4.1±0.5	3.2±0.4	1.5±0.3	3.4±0.4	1.6±0.1	2.0±0.1	1.1±0.2	1.7±0.2	2.7±0.2	1.2±0.1
	3.6-5.4	2.2-3.8	1.1-2.0	2.7-4.3	1.4-1.8	1.8-2.1	0.8-1.4	1.4-2.0	2.6-3.0	0.9-1.4
	4.1	3.1	1.4	3.4	1.5	2.0	1.1	1.6	2.7	1.3
Al	2.6±0.9	30±11	11±7	8.8±11	4.1±4.9	2.2±0.3	5.5±2.8	3.2±0.5	3.2±1.6	3.0±1.1
	1.4-4.3	12-50	3.6-24	1.5-29	0.8-14	1.7-2.8	2.7-11	2.3-4.2	1.4-6.2	1.3-5.4
	2.6	24	7.7	2.5	1.4	2.2	4.7	3.1	3.0	2.7
Ni	1.1±0.1	1.0±0.1	1.1±0.1	0.20±0.03	0.20±0.03	WD	WD	WD	WD	WD
	1.0-1.4	0.9-1.2	0.9-1.4	0.10-0.30	0.10-0.20					
	1.1	1.0	1.1	0.20	0.10					
Cr	0.08±0.02	0.31±0.03	1.9±3.1	0.76±1.20	0.39±0.59	0.13±0.02	0.20±0.07	0.22±0.01	0.90±0.07	0.32±0.05
	0.05-0.13	0.27-0.39	0.18-12	0.08-5.0	0.02-1.8	0.10-0.17	0.13-0.34	0.20-0.24	0.80-1.0	0.23-0.40
	0.07	0.30	0.32	0.12	0.08	0.13	0.18	0.22	0.88	0.31
V	0.007±0.002	0.063±0.017	0.025±0.008	0.020±0.013	0.026±0.006	0.025±0.002	0.021±0.007	0.015±0.004	0.014±0.003	0.030±0.004

	1*	2	3	4	5	6	7	8	9	10
	0.004-0.011	0.033-0.090	0.011-0.041	0.011-0.054	0.018-0.046	0.021-0.029	0.015-0.036	0.009-0.023	0.008-0.019	0.025-0.040
	0.007	0.059	0.024	0.015	0.023	0.025	0.018	0.014	0.013	0.029
Ba	3.0±0.5	2.0±0.1	0.70±0.01	1.3±0.1	0.70±0.10	0.50±0.20	1.2±0.7	0.60±0.20	1.2±0.1	0.60±0.20
	2.3-4.3	1.8-2.4	0.60-0.90	1.0-1.5	0.60-1.0	0.20-1.0	0.60-2.6	0.30-1.1	0.9-1.4	0.30-0.80
	2.9	2.0	0.70	1.3	0.70	0.40	0.80	0.60	1.2	0.50
Sr	11±1	8.0±1.1	3.1±0.6	3.8±0.6	2.4±0.2	2.1±0.3	1.7±0.3	2.4±0.3	4.0±0.5	2.1±0.4
	9.0-13	6.1-10	2.0-4.3	2.7-4.9	2.1-2.9	1.6-2.6	1.1-2.4	1.7-3.1	3.0-4.7	1.4-2.9
	11	8.0	3.0	3.9	2.3	2.1	1.6	2.4	3.9	2.1
As	0.16±0.02	0.19±0.02	0.03±0.01	0.04±0.01	0.03±0.01	0.01±0.01	0.020±0.019	0.04±0.01	0.03±0.02	0.008±0.008
	0.13-0.19	0.15-0.24	0.02-0.04	0.03-0.06	0.02-0.05	< 0.01-0.03	0.004-0.030	0.02-0.07	< 0.01-0.05	< 0.01-0.02
	0.15	0.19	0.03	0.04	0.03	0.01	0.020	0.040	0.03	0.006
Ag	0.090±0.008	0.070±0.007	0.002±0.001	0.010±0.006	0.030±0.007	0.020±0.004	0.020±0.006	0.020±0.003	0.040±0.020	0.020±0.002
	0.080-0.11	0.050-0.080	< 0.002-0.004	0.003-0.020	0.020-0.050	0.020-0.030	0.009-0.03	0.020-0.030	0.020-0.070	0.010-0.020
	0.090	0.070	0.002	0.010	0.020	0.020	0.010	0.020	0.040	0.020
Cd	0.04±0.01	0.04±0.01	0.02±0.03	0.05±0.01	0.08±0.01	0.03±0.00	0.08±0.02	0.04±0.01	0.04±0.01	0.03±0.00
	0.02-0.05	0.03-0.05	0.01-0.03	0.03-0.06	0.065-0.10	0.02-0.03	0.05-0.11	0.02-0.05	0.02-0.06	0.03-0.04
	0.04	0.04	0.02	0.04	0.08	0.02	0.08	0.03	0.04	0.03
Hg	0.041±0.001	0.047±0.004	0.051±0.003	0.034±0.003	0.061±0.012	0.059±0.007	0.057±0.010	0.028±0.006	0.036±0.005	0.036±0.005
	0.039-0.043	0.042-0.059	0.044-0.056	0.030-0.040	0.042-0.074	0.050-0.073	0.042-0.075	0.020-0.039	0.027-0.041	0.027-0.041
	0.041	0.045	0.052	0.032	0.065	0.058	0.056	0.028	0.038	0.038
Sb	0.008±0.003		0.005±0.002				0.002±0.001	0.003±0.001		0.009±0.003
	0.002-0.012	WD	0.002-0.010	WD	WD	WD	< 0.001-0.004	< 0.001-0.006	WD	0.004-0.012
	0.008		0.005				0.002	0.003		0.009
Tl	0.001±0.000					0.001±0.000	0.001±0.001	0.001±0.001	0.002±0.001	0.001±0.001
	< 0.001-0.002	WD	WD	WD	WD	0.001-0.002	0.001-0.002	< 0.001-0.001	0.001-0.002	< 0.001-0.002
	0.001					0.001	0.001	0.001	0.002	0.001
Pb	0.17±0.01	0.17±0.03	0.14±0.02	0.010±0.020	0.006±0.003			0.020±0.007		
	0.15-0.20	0.13-0.22	0.11-0.18	< 0.001-0.050	0.001-0.020	WD	WD	0.008-0.024	WD	WD
	0.18	0.17	0.13	0.007	0.006			0.014		
U	0.0004±0.0002	0.029±0.004	0.001±0.00	0.002±0.000	0.002±0.001	0.003±0.001	0.001±0.000	0.001±0.000	0.001±0.001	0.001±0.000
	0.0001-0.0006	0.020-0.034	0.001-0.002	0.001-0.003	0.001-0.003	0.002-0.004	0.001-0.002	0.001-0.002	0.001-0.003	0.001-0.002
	0.0004	0.030	0.001	0.002	0.002	0.003	0.001	0.001	0.001	0.001

Notes: *(sample ID) 1. Notre Jardin Champignons de Paris Pieds et morceaux "pieczarki marynowane krojone – pickled champignon sliced"; 2. Notre Jardin Champignons de Paris 1er Choix entiers "pieczarki marynowane całe – pickled a whole champignons"; 3. Bonduelle "Pieczarki łagodne marynowane – soft champignons pickled"; 4. Bonduelle "Pieczarki marynowane – pickled champignons"; 5. Rolnik "Pieczarki marynowane – pickled champignons"; 6. „Pieczarki marynowane – pickled champignons"; 7. Smak „Pieczarki marynowane – pickled champignons "; 8. Smak „Pieczarki w zalewie solnej – pickled champignons in a saline juice"; 9. Ole „Pieczarki w zalewie naturalnej całe – a whole champignons in a natural marinate"; 10. Ole "Pieczarki marynowane z marchewką i cebulą – marinated champignons with a carrot and onion"; WD, without data.

Table 3. Factor loadings (Varimax normalized)

Element	PC1	PC2	PC3	PC4
Li	**0.94**	-0.14	-0.09	-0.25
Al	0.25	0.01	**0.94**	-0.03
Co	-0.30	-0.58	0.32	0.59
Cu	0.19	0.58	0.69	0.11
As	**0.85**	-0.01	0.51	-0.02
Se	-0.30	**0.84**	-0.42	0.12
Rb	**0.85**	0.10	0.01	0.47
Sr	**0.98**	0.00	0.15	0.00
Ag	**0.92**	0.14	0.20	-0.07
Cd	-0.02	-0.07	-0.13	-0.01
Cs	**0.96**	-0.09	0.16	-0.08
U	0.27	0.03	**0.95**	0.12
Ba	**0.98**	0.02	0.11	0.06
Cr	-0.16	**0.83**	0.11	0.09
Zn	0.41	**0.77**	0.28	0.26
Mn	-0.04	0.34	0.05	**0.92**
V	-0.05	-0.15	**0.97**	0.03
Hg	-0.21	-0.35	0.17	-0.28
Eigenvalue	7.491714	3.472054	3.245906	1.610754
Total Variance (%)	41.62063	19.28919	18.03281	8.94864
Cumulative (%)	41.62063	60.90982	78.94263	87.89127

Manganese is also essential for mushrooms. In fresh *A. bisporus* is in the range from 5.7 to 8.3 mg kg^{-1} dm (means) and 6.1 ± 2.0 mg kg^{-1} dm [37, 42, 43]. Conserved has it in the range 6.0± 0.1 (a whole) to 12 ± 0 (sliced) mg kg^{-1} dm [43]. It can be suggested that quality of water used for preparation of marinade or brine can have an impact on content of Mn in processed mushrooms. If marinade is free of Mn, pickling can decrease its content in *A. bisporus* by around 50%. On the other side, a lack of a difference between the fresh mushrooms and those conserved can be because of elevated content of Mn in brine. This is known that sodium can be absorbed by and highly elevated in fruiting bodies conserved with a brine [43].

A. bisporus conserved in brine (two batches) had Cu in the range 15 ± 0 to 15 ± 1 mg kg^{-1} dm [43]. Hence, mushrooms from our study and conserved with brine were > 50% poorer in Cu when relate to fresh mushrooms, which showed 36 ± 14 mg kg^{-1} dm (total range 13 to 75 mg kg^{-1} dm) [37], or from 39 to 65 mg kg^{-1} dm (means) [42, 43]. Marinated mushrooms in this study (Table 2) also had > 50% less of Zn than has been reported for fresh *A. bisporus*: 66 ± 14 mg kg^{-1} dm (range 41 to 95 mg kg^{-1} dm) [37], and 60 to 65 mg kg^{-1} dm (means) [42, 43].

Co and Ni

Both Co and Ni were minor trace elements in marinated *A. bisporus*, while content widely varied between the batches: for Co medians were in the range 0.004 to 0.02 mg kg^{-1} dm (the overall median 0.015 mg kg^{-1} dm), and for Ni in the range 0.10 to 1.1 mg kg^{-1} dm (1.0 mg kg^{-1} dm) (Table 2).

Curiously, Vetter et al. noted cobalt in imported conserved mushrooms at higher level of 0.86 ± 0.07 mg kg^{-1} dm (sliced) and 0.37 ± 0.04 mg kg^{-1} dm (a whole), than in fresh (< 0.002 to 0.09 mg kg^{-1} dm; means) [42, 43]. Nickel in other studies has been found in fresh and conserved mushrooms in roughly similar concentrations, *i.e.* in fresh 1.1 ± 1.4 mg kg^{-1} dm (0.08 to 6.2 mg kg^{-1} dm) [37] and from 0.35 to 2.2 mg kg^{-1} dm (means), and in conserved 1.6 ± 0.2 mg kg^{-1} dm (sliced) and 1.3 ± 0.1 mg kg^{-1} dm (whole) [42, 43].

Al, Cr, V and U

The elements Al, Cr or U are without known role in mushrooms, while V is essential to certain *Amanita* mushrooms and specifically at high level is in some *Amanita* mushrooms, e.g. *Amanita muscaria*, *Amanita regalis* and *Amanita velatipes*. In *A. muscaria* V content was 22 ± 11 to 130 ± 45 mg kg^{-1} dm in caps and 120 ± 29 mg kg^{-1} dm in stems [18, 29].

Processed *A. bisporus* in this study showed Al, Cr, V and U in relatively a wide range of concentrations both for the same element within the product batches and between the elements. They contained respectively Al, Cr, V and U in the range (medians): 1.4 to 24 mg kg^{-1} dm (the overall median 2.8 mg kg^{-1} dm for Al), 0.071 to 0.88 mg kg^{-1} dm (0.20 mg kg^{-1} dm for Cr), 0.007 to 0.059 mg kg^{-1} dm (0.020 mg kg^{-1} dm for V) and 0.0004 to 0.003 mg kg^{-1} dm (0.001 mg kg^{-1} dm for U) (Table 2).

Aluminum in this study was in roughly similar concentration as has been reported in fresh *A. bisporus* in Poland, i.e., 3.0 ± 1.2 mg kg^{-1} dm range (1.7 to 6.5 mg kg^{-1} dm) [37], and what may suggest on a lack of leaching out from mushrooms during industrial marinating. Vetter et al. [42, 43] reports aluminum in fresh *A. bisporus* in the range 19 to 46 mg kg^{-1} dm (means), and for conserved in brine 74 ± 9 mg kg^{-1} dm (sliced) and 54± 3 mg kg^{-1} dm (whole).

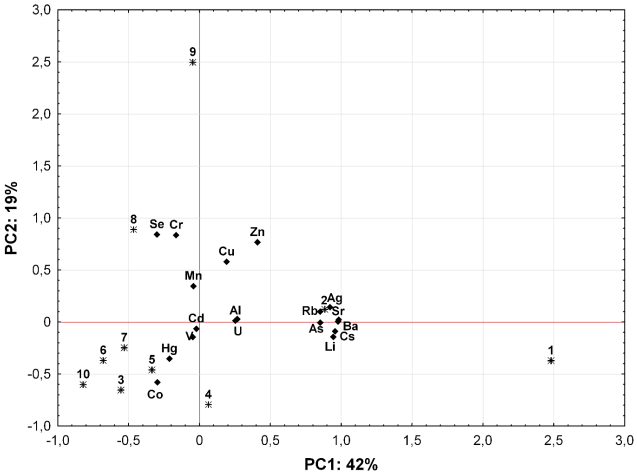

Figure 1. Projection of the elements concentrations in conserved white bottom mushrooms consignments set on the first and second factor-plane

Chromium with the overall median value of 0.20 mg kg^{-1} dm in this study is close to 0.21 ± 0.06 mg kg^{-1} dm (range 0.08 to 0.30 mg kg^{-1} dm) for fresh mushrooms by Rzymski et al. [37], and what may suggest on a lack of decrease during industrial processing. Vetter et al. [42, 43] reported on a somehow higher level of chromium in fresh mushrooms (means in the range 0.73 to 0.83 mg kg^{-1} dm) and mushrooms conserved in brine (1.9 ± 0.4 mg kg^{-1} dm in sliced and 1.6 ± 0.5 mg kg^{-1} dm in a whole).

Vanadium is weakly accumulated by *A. bisporus*. This element in fresh fruiting bodies occurred at level 0.02 ± 0.01 mg kg^{-1} dm (0.01 to 0.04 mg kg^{-1} dm) [37], and from 0.02 to < 0.05 mg kg^{-1} dm [42, 43]. Those values are close to the overall median level of 0.020 mg kg^{-1} dm in marinated mushrooms in this study, and what may imply on lack of a substantial leaking during culinary processing. The white button mushrooms conserved with brine showed vanadium at level higher an order of magnitude than was in mushrooms in this study, *i.e.*, 0.35 ± 0.03 mg kg^{-1} dm (sliced) and 0.25 ± 0.09 mg kg^{-1} dm (whole) [42].

A single result available for uranium in fresh *A. bisprus* (white strain) showed on level < 0.01 mg kg^{-1} dm, while for brown strain it was 0.29 ± 0.14 mg kg^{-1} dm (total range 0.53 - 0.08 mg kg^{-1} dm) [37]. The overall median value of uranium in marinated mushrooms in this study was 0.001 mg kg^{-1} dm.

Principal Component Analysis (PCA)

Major differences in the mineral composition of white button mushrooms between the producers have been observed. Five statistically significant ($p < 0.05$) principal components (PC) were identified, which accounted for 93% of total variability.

The PC1, which accounted for 42% of total variability, was determined by positively correlated Li, As, Rb, Sr, Ag, Cs and Ba (Table 3, Figure 1). Statistically significantly higher content of these elements in mushrooms from the batch marked as ID 1 (Notre Jardin Champignons de Paris Pieds et morceaux "Pieczarki marynowane krojone – pickled champignon sliced" it distinguishes it from the others (Table 2, Fig. 1).

The PC2, which accounted for 19 % of total variability was determined by positively correlated Se, Cr and Zn (Table 3, Fig. 1), which elements at statistically higher level were in a batch of white button mushrooms ID 9 (Ole: „Pieczarki w zalewie naturalnej całe – a whole champignons in a natural marinate") (Table 1, Figure 1). Mushrooms from that batch had also more Cu while less Co (correlations moderately significant) (Tables 2 and 3).

The PC3, which accounted for 18% of total variability was determined by positively correlated Al, U and V (Table 3, Figure 2), which elements at statistically higher level were in a batch of white button mushrooms with ID 2 (Notre Jardin Champignons de Paris 1er Choix entiers "pieczarki marynowane całe – pickled a whole champignons") (Table 2). Also As and Cu were moderately at higher levels in those mushrooms (Table 2, Table 3, Figure 2).

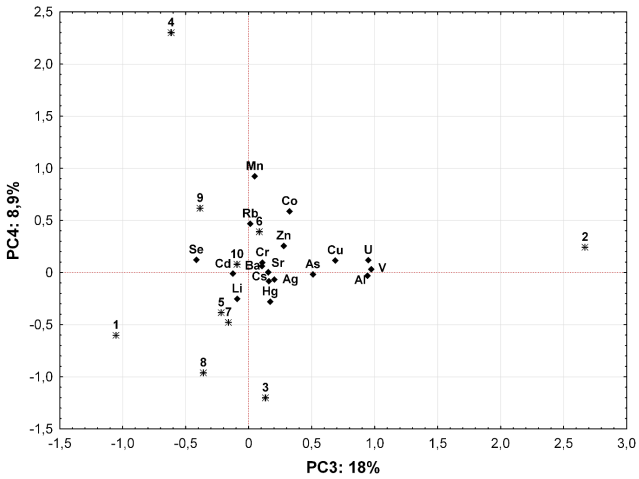

Figure 2. Projection of the elements concentrations in conserved white bottom mushrooms consignments set on the third and fourth factor-plane

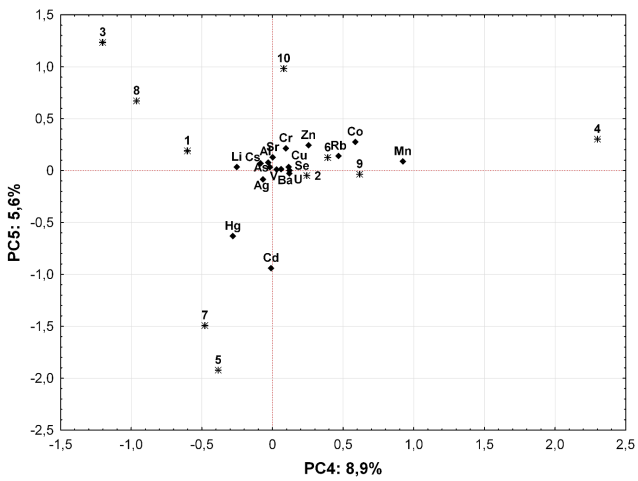

Figure 3. Projection of the elements concentrations in conserved white bottom mushrooms consignments set on the fourth and fifth factor-plane.

The PC4, which accounted for 8.9 % of total variability was determined by Mn, while moderately by Co (Table 3, Figure 2 and Figure 3), and what differentiated mushrooms from the batch with ID 4 (Bonduelle "Pieczarki marynowane – pickled champignons") from others. Manganese at similar level was in mushrooms from the consignments ID 4 and ID 9 (Table 2).

With PC5 were moderately associated the elements such as Cd and Hg (mushrooms from the batches ID 5 and ID 7) (Tables 2 and 3, Fig. 3).

The PC analysis based on total concentration of each chemical element, which could leak out from mushrooms into the marinade at different rate from one side, while some could be absorbed (added in the vinegar or table salt as well as leaked from the vegetables if present) on the other. Hence, it can provide only some piece of information about general tendencies and to feature major differences in technology or quality of marinade and/or containers used by producers. The elements Li, Rb, Sr, Cs and Ba are lithophile and in addition Ba and Sr are seekers for Ca. The elements As and Ag (PC1) and as mentioned already they are seekers for sulphur (Ag also correlated with Cd under PC4) (Figs. 1-3). A difference in a bulk content of those elements in mushrooms depending on the producer was clear for a batch no. 1 (and no. 2) and 9 (Fig. 1; Table 2). White button mushrooms are able to accumulate efficiently Ag [13]. Also As is well accumulated by some *Agaricus* mushrooms [19]. Silver seems to be very hard to remove from mushrooms during blanching and pickling [8]. Arsenic can occur in mushrooms in form of a several compounds while those which usually dominate basically are well water soluble and leak out during blanching and pickling [13, 19]. An external source of contamination (water, table salt, vinegar) cannot be excluded in those cases (samples ID 1 and 2; Table 2).

The components forming PC1, PC2, PC3, PC4 had positive charge values while the PC5 element had a negative one. The differences and similarities in the content of the given elements in the fruiting bodies of the mushrooms from different manufacturers were presented in linear maps. Major differences in the mineral composition of mushrooms from different producers were found. It has been shown that fruit mushrooms from different producers differ in the content of the determinants of PC1, PC2, PC3, PC4 and PC5.

CONCLUSIONS

Some significant differences were found in the content and composition of minerals in marinated white button mushrooms for producers. The relatively higher levels of Ag in some batches seem to be largely explained by the quality of the substrate used for mushrooms cultivation, while of As, Li, Rb, Cs, Cr, Al, U, V and Mn (in part also of Ba and Sr) largely by the quality of the marinade. Conserved white button mushrooms are poorer in major essential elements but also in toxic Hg, As, Ag, Cd, Sb, Tl or Pb than has been reported for unprocessed mushrooms. Due to low concentrations of toxic As, Cd, Hg and Pb, it can be stated that consuming the conserved white button mushrooms available at Polish market does not show any threat to the consumer.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

EFFECT OF CONJUGATED LINOLEIC ACID AND DIFFERENT TYPE OF DIETARY FAT ON SERUM LIPID PROFILE, LIVER ENZYMES ACTIVITY AND OXIDATIVE STRESS MARKERS IN WISTAR RATS

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ABSTRACT

Background. Nutritional recommendations emphasize the need to limit consumption of saturated fatty acids and to increase the intake of polyunsaturated fatty acids in the prevention of non-communicable chronic diseases, particularly cardiovascular diseases. Among the fatty acids with health-related effects on the body, conjugated fatty acids are mentioned (i.e. CLA).

Objective. The current study was designed to determine the effects of conjugated linoleic acid (CLA) on serum lipid profile, glucose, liver enzymes activity (AST and ALT), malonic dialdehyde (MDA) as well as lipid hydroperoxide (LPO) concentrations in rats fed diet differing in type of dietary fat.

Material and methods. Male *Wistar* rats were divided into six groups and fed the following diets: control AIN-93G diet contained soybean oil (O) and diets with modification of fat source: butter (B) and margarine (M). The experimental diets were supplemented with 1% of conjugated linoleic acid (O+CLA, B+CLA, M+CLA). After 21 days the blood was collected and lipid profile, glucose, liver enzymes, MDA as well as LPO were analyzed.

Results. The dietary treatments had no significant effect on the body weight and liver weight of the animals. The concentrations of total cholesterol (TC) and LDL+VLDL cholesterol were unchanged. Both experimental factors (fat source and CLA) had a significant influence on the TAG and HDL levels. Margarine (M) significantly increased the TAG concentration, whereas CLA had a significant impact on the TAG reduction (M+CLA). Glucose level was significantly decreased in all groups fed diets supplemented with CLA. Serum ALT significantly increased in all CLA groups. Fat source had statistically significant influence on the MDA concentration. The LPO level was significantly elevated in all CLA groups. There was statistically significant interaction of experimental factors (fat source and CLA supplementation) on LPO level.

Conclusions. Margarine had an adverse effect on the rat's lipid profile. However, in the group fed with margarine, the addition of CLA decreased the concentration of TAG. Regardless of the type of the dietary fat, CLA supplementation increased the level of LPO in the blood serum of animals.

Key words: *conjugated linoleic acid, dietary fat, lipid profile, liver enzymes activity, oxidative stress markers, rats*

STRESZCZENIE

Wprowadzenie. Zalecenia żywieniowe podkreślają potrzebę ograniczania spożycia nasyconych kwasów tłuszczowych i zwiększania spożycia wielonienasyconych kwasów tłuszczowych w prewencji przewlekłych chorób niezakaźnych, szczególnie chorób układu krążenia. Spośród kwasów tłuszczowych o prozdrowotnym oddziaływaniu na organizm wymienia się sprzężone kwasy tłuszczowe (tu: CLA).

Cel. Badanie miało na celu określenie wpływu sprzężonego kwasu linolowego (CLA) na profil lipidowy, aktywność enzymów wątrobowych (AST i ALT), dialdehyd malonowy (MDA) oraz stężenie wodoronadtlenku lipidów (LPO) w surowicy krwi szczurów żywionych dietami zawierającymi różne źródła tłuszczu.

Materiały i metody. Samce szczurów *Wistar* żywiono dietami AIN-93G o różnym źródle tłuszczu: olej sojowy (O), masło (B) i margaryna (M). Diety eksperymentalne uzupełniono 1% dodatkiem CLA (O+CLA, B+CLA, M+CLA). Zwierzęta otrzymywały dietę oraz wodę *ad libitum*. Po 21 dniach doświadczenia w pobranej krwi wykonano analizy profilu lipidowego, glukozy, aktywności enzymów wątrobowych (AST i ALT), MDA oraz LPO.

Wyniki. Skład diet eksperymentalnych nie miał istotnego wpływu na masę ciała i masę wątroby zwierząt. Stężenia cholesterolu całkowitego (TC) i cholesterolu frakcji LDL + VLDL nie uległy zmianie. Zarówno źródło tłuszczu jak i CLA miały znaczący wpływ na poziomy TAG i frakcji HDL cholesterolu. Margaryna (M) istotnie zwiększała stężenie TAG, podczas gdy CLA obniżył

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istotnie stężenie TAG w grupie M+CLA. Poziom glukozy we wszystkich grupach, którym podawano diety z dodatkiem CLA, istotnie się zmniejszył. Aktywność ALT w surowicy istotnie wzrosła we wszystkich grupach z dodatkiem CLA. Źródło tłuszczu miało statystycznie istotny wpływ na stężenie MDA. Poziom LPO był istotnie podwyższony we wszystkich grupach CLA. Wykazano istotną statystycznie interakcję czynników eksperymentalnych (źródło tłuszczu i CLA) na poziom LPO.
Wnioski. Żywienie zwierząt dietą z dodatkiem margaryny miało niekorzystny wpływ na profil lipidowy szczurów. Natomiast w grupie żywionej margaryną dodatek CLA obniżył stężenie TAG. Bez względu na rodzaj spożywanego tłuszczu suplementacja CLA podniosła poziom LPO w surowicy krwi zwierząt.

Słowa kluczowe: *sprzężony kwas linolowy, tłuszcz w diecie, profil lipidowy, aktywność enzymów wątrobowych, markery stresu oksydacyjnego, szczury*

INTRODUCTION

Over the years there have been studied to examine the various components of dietary fat and their effect on serum lipids. Several studies reported serum lipid responses to butter, margarine or vegetable oils added to control diets. Moreover, the *trans* fatty acids resulting from the partial hydrogenation of vegetable oils and their replacement of the saturated fatty acids in many processed food have been reported to produce undesirable serum lipopotein profiles [19]. Additionally, it was shown that polyunsaturated fatty acids are effective in lowering serum cholesterol.

Conjugated linoleic acid (CLA) is a term that refers to a collection of positional and geometric isomers of linoleic acid (LA)- 18:2 *n*-6 with conjugated double bonds. This fatty acids are the natural food component occurring in the lipid fraction of meat, milk and other dairy products [13]. It has been largely demonstrated that CLA has positive effects in cancer [2, 3, 7], cardiovascular disease [1, 22, 28, 30], diabetes [20, 23, 25] and obesity [6, 27, 31].

According to knowledge about oil, butter and margarine consumption on human health, the aim of this study was to determine the effect of diets with different fat sources and CLA on serum lipid profile, glucose, liver enzymes activity as well as oxidative stress markers in rats.

MATERIAL AND METHODS

Animal and diets

Male *Wistar* (n=36) rats, weighing about 100 g were housed during the experimental period of 21 days in an isolated room under conditions of controlled temperature, humidity and a 12 h light-dark cycle. After the adaptation period (5 days), the *Wistar* rats were divided into six groups (n=6) and fed purified base diet AIN-93G [24]. Modifications of diet consist in different fat source and supplementation with CLA as follows: O- soybean oil, B- butter, M- soft margarine, O+CLA- soybean oil and CLA, B + CLA – butter and CLA, M + CLA – margarine and CLA. The CLA oil (Luta-CLA® 60), obtained from BASF (Ludwigshafen, Germany) contained 600 g CLA/kg, with equal representation of two major CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12). The final composition of the diets is shown in Table 1. After 21 days of feeding rats were anesthetized with an *ip* injection of thiopental: 120-150 mg/kg (Biochemie; Vienna, Austria). All procedures involving animals were conducted according to the Guidelines for Animal Care and Treatment of the European Union and were approved by the Local Animal Ethics Commission.

Table 1. The composition of the experimental diets

DIETS * INGREDIENTS	O	B	M	O + CLA	B + CLA	M + CLA
	g/kg					
Cornstarch	532.486	532.486	532.486	532.486	532.486	532.486
Casein	200	200	200	200	200	200
Sucrose	100	100	100	100	100	100
Soybean oil	70	-	-	53.33	-	-
Butter	-	70	-	-	53.33	-
Soft Margarine	-	-	70	-	-	53.33
Fibre	50	50	50	50	50	50
Mineral mix	35	35	35	35	35	35
Vitamin mix	10	10	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Tert-butylhydroquinone	0.014	0.014	0.014	0.014	0.014	0.014
CLA**	-	-	-	16.67	16.67	16.67

* O- oil diet: AIN-93G; B- butter diet: AIN-93G + butter; M- margarine diet: AIN-93G + margarine; O+CLA- oil diet supplemented in CLA: AIN-93G + 1% CLA; B+CLA- butter diet supplemented in CLA: AIN-93G + 1% CLA; M+CLA- margarine diet supplemented in CLA: AIN-93G + 1% CLA.

** CLA - the CLA oil (Luta-CLA® 60), obtained from BASF (Ludwigshafen, Germany) contained 600 g CLA/kg, with equal representation of two major CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12).

Blood analysis

Blood samples were taken from the left ventricle of the heart, collected into test tubes and centrifuged (4 000 g, 10 min) to obtain serum samples. Serum samples were analyzed using commercially available kits for total cholesterol (TC; Liquick Cor-Chol 60 no 2-204; Cormay, Lublin, Poland), triacylglycerols (TAG; Liquick Cor-TG 30 no 2-262; Cormay, Lublin, Poland) and HDL cholesterol (HDL; Liquick Cor-HDL no 2-181). LDL+VLDL level was calculated. Blood glucose concentrations were measured using an Accu-Chek Active glucosemeter (Roche DiagnosticsGmbH, Mannheim, Germany). Malonic dialdehyde (MDA; OXI-TEK TBARS Assay kit; Alexis Biochemicals, USA) and Lipid Hydroperoxide (LPO Assay kit No 705002, Cayman Chemical, Michigan, USA) were analyzed in rat's serum. Also theactivity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rat's serum were analyzed using commercially available kits (No A6624-050, A6661-050 AlphaDiagnostics, Warszawa, Poland).

Statistical analysis

Results are expressed as means. The data were subjected to two-way analysis of variance (ANOVA) calculated by STATISTICA 13 package (StatSoft Inc., USA), followed by *post-hoc* Duncan's multiple range test. Statistical significance was considered to be $P \leq 0.05$.

RESULTS

Effects of CLA and different fat source on body and liver weight

The effect of dietary treatments on body and liver weight is shown in Table 2. There was no significant effect on body weight and liver weight of animals fed different diets. The lowest liver weight was observed in control group (O), whereas the highest liver weight were in B+CLA and M+CLA groups.

Table 2. Effect of dietary treatments (fat source and/or CLA supplementation) on body weight and liver weight of *Wistar* rats.

Body and liver weights	O	B	M	O+CLA	B+CLA	M+CLA	SEM	Effect of:		
								Fat	CLA	Interaction
Final body weight (BW) [g]	331.55	347.81	344.92	324.63	323.78	315.53	6.303	0.882	0.131	0.768
Body weight gain [g]	192.15	211.64	209.75	187.96	187.28	179.53	6.528	0.852	0.156	0.718
Liver weight/ 100g BW [g]	3.76	3.97	3.96	3.95	4.16	4.16	0.052	0.998	0.173	0.064

Effects of CLA and different fat source on lipid profile

The effect of dietary treatments on lipid profile is shown in Table 3. In our experiment there was no statistically significant influence of experimental factors (fat source and CLA supplementation) on the TC and LDL+VLDL levels. However, both experimental factors (fat source and CLA) had a significant influence on the TAG and HDL levels. The highest level of HDL was recorded in the group fed butter (B). Addition of CLA to butter as well as margarine groups significantly decreased the HDL cholesterol concentration. Margarine diet significantly increased triacylglycerol level in rats compared to other groups. At the same time, CLA supplementation decreased triacylglycerol level in M+CLA rats compared to margarine group.

Table 3. Effect of dietary treatments (fat source and/or CLA supplementation) on serum lipid profile in *Wistar* rats

Serum lipid profile	O	B	M	O+CLA	B+CLA	M+CLA	SEM	Effect of:		
								Fat	CLA	Interaction
TC [mmol/L]	1.61	1.89	1.80	1.46	1.58	1.72	0.051	0.130	0.070	0.595
TAG [mmol/L]	2.07	1.96	2.73	1.55	1.33	1.81	0.110	0.017	0.000	0.634
HDL [mmol/L]	1.03	1.43	1.28	0.91	0.98	0.95	0.045	0.019	0.000	0.113
LDL+VLDL [mmol/L]	0.65	0.46	0.55	0.54	0.60	0.77	0.040	0.425	0.294	0.238

Effects of CLA and different fat source on glucose concentration

Dietary treatments affected the glucose concentration (Table 4). Glucose level was significantly decreased in all groups fed diets supplemented with CLA compared to those without supplementation. The significant difference was observed in rats administered O+CLA treatment compared to those received control (O) diet .

Effects of CLA and different fat source on liver enzymes (AST and ALT) activity

The composition of experimental diets did not affect the serum AST activity (Table 4). However, the serum ALT significantly increased in all experimental groups after CLA supplementation.

Table 4. Effect of dietary treatments (fat source and/or CLA supplementation) on serum glucose, AST and ALT activity, MDA and LPO concentrations in *Wistar* rats

Serum	O	B	M	O+CLA	B+CLA	M+CLA	SEM	Effect of:		
								Fat	CLA	Interaction
Glucose [mg/dl]	139.00	141.17	134.33	126.80	132.00	131.67	1.632	0.541	0.014	0.446
AST [U/I]	69.84	50.49	55.14	55.87	72.75	54.71	4.246	0.725	0.764	0.241
ALT [U/I]	19.06	25.17	26.48	24.88	35.07	31.43	1.586	0.058	0.023	0.757
MDA [nmol/ml]	14.20	14.82	17.88	14.37	16.63	23.27	1.056	0.036	0.222	0.548
LPO [nmol/ml]	26.06	27.04	42.48	67.32	85.95	58.38	4.245	0.237	0.000	0.003

Effects of CLA and different fat source on oxidative stress markers (MDA and LPO) concentrations

CLA supplementation had no statistically significant effect on malonic dialdehyde concentration in rat's serum (Table 4). However, fat source had statistically significant influence on the MDA concentration. The lipid hydroperoxide level was significantly elevated in all CLA groups. The highest level of LPO was in the B+CLA group. In our experiment there was statistically significant interaction of experimental factors (fat source and CLA supplementation) on LPO level.

DISCUSSION

Various components of dietary fat and their effect on serum lipids are investigated. Dietary fat has an essential role in modulating immune and inflammatory responses. Both quantity and quality of fats have shown to affect these processes [13]. The type and degree of fatty acid unsaturation has also shown to affect immune and inflammatory responses [3]. Estimates for the daily consumption of margarine and butter are: in the United States - 11 and 6 g/person, respectively, in the Netherlands: 22 and 3 g/person, and in the Great Britain: 12 and 7 g/person [12]. Recommendations to reduce the risk of cardiovascular disease usually stress the importance of reducing intake of SFAs. In practical terms, this advice frequently implies restricting the intake of products rich in SFAs and cholesterol, such as butter, and replacing them in part with equivalent products lower in cholesterol and SFAs, but higher in unsaturated fatty acids, such as margarines. Over the past years, evidence has shown that, in addition to SFAs, *trans* unsaturated fatty acids (TFAs) also raise plasma total cholesterol and LDL cholesterol and may lower plasma HDL cholesterol concentrations. Some, but not all, studies indicated that high intakes of TFAs increase the risk of cardiovascular disease, which agrees with the observed effects of TFAs on blood lipids.

Therefore, the issue arises as to whether there are benefits to replacing a product rich in SFAs, such as butter, with a product lower in SFAs but higher in *cis*- and TFAs, such as margarine. It is now feasible to manufacture margarines that have no TFAs and/or have high amounts of unsaturated fatty acids without major increases in SFAs. In nutrition recommendations there are advices telling us that our goal is to limit intake of SFAs and to avoid TFAs altogether.

There are many studies showing positive effects of CLA as a bioactive ingredient. Up to now, there was no study examined the effects of CLA supplementation in diets containing different fat sources. Therefore, the aim of the present study was to determine effects of CLA and common sources of dietary fat (soybean oil, butter and margarine) on serum lipid profile, glucose and liver enzymes activity and oxidative stress markers in rats.

In our study there was no statistically significant influence of fat source on the TC and LDL+VLDL levels. However, it had a significant influence on the TAG and HDL levels. The highest level of HDL was recorded in the group fed butter (B). No effect on plasma lipoproteins was shown in growing pigs fed diets containing unsaturated plant fatty acids compared to pigs fed diet with saturated animal fat [18]. In contrast to the above findings, *Wood* et al. (1993) showed that total serum cholesterol levels were significantly higher in butter received group but significantly lower in soft margarine patients relative to baseline diet. There were no changes in TG content among dietary groups [32]. In contrast to our findings, *Dorfman* at al. (2005) showed, that in hamster fed butter, the level of TG increased more than in margarine group compared to soybean oil. However, the aoric lesion surface in margarine group was the highest (0.84%) and it was double then soybean and butter group (0.4 vs 0.36%) [5]. In *Milewska* et al. (2007) study it was shown that cholesterol enriched diets differing in dietary fat type (butter, margarine with stanols, margarine with rapeseed oil and sunflower oil) excluding diet containing margarine with stanols, had hypercholesterolemic effects on *Wistar* rats [21].

In the last years, attempts have been made to enrich animal-derived foods in CLA isomers through animal nutrition strategies (CLA-enriched milk, butter, cheese). In addition to natural foodstuff, dietary CLA supplements can also contribute to CLA intake in humans. In our study addition of CLA to butter

as well as margarine groups significantly decreased the HDL cholesterol concentration. However, CLA supplementation decreased triacylglycerol level in M+CLA rats compared to margarine group.

In human study, dietary treatment of CLA enriched butter resulted in a significant reduction in TC level compared to diet without CLA, while there were no differences in TG level between experimental groups [4]. Similar results have been presented by *Lock* et al. (2005) where hamsters fed butter naturally enriched in CLA had significant lower TC and TG concentration compared to animals fed no CLA diet [17]. Additionally, *Franczyk-Żarów* et al. (2008) shown, that CLA-enriched eggs exerted an anti-inflammatory effect more effectively than CLA-supplemented eggs in apoE/LDLR$^{-/-}$ mice [8]. However, effect of CLA supplementations produced equivocal results. Conjugated linoleic acid decreased serum triacyloglycerol and changed fatty acid composition in rat's liver [16]. *Kostogrys* et al (2012) do not support the notion that CLA isomer supplementations to the oil or margarine possess anti-atherosclerotic effect [14, 15]. Moreover, *Franczyk-Żarów* et al. (2015) shown, that margarine supplemented with CLA significantly increased liver weight and induced steatosis in apoE/LDLR$^{-/-}$ mice, but CLnA supplementation alleviated the liver steatosis and affected the expression of lipid metabolism genes [9].

In our experiment CLA decreased glucose concentration. The discussion concerning antidiabetic effects of CLA is controversial. An experiment using Zucker diabetic fatty rats showed a normalization of impaired glucose tolerance and improved hyperinsulinemia induced by CLA [11]. A recent study showed inverse effects. C57BL/6J mice fed a CLA-enriched diet developed a state resembling diabetes, with a marked insulin resistance (hyperinsulinemia) [29]. Also, in studies of *Halade* et al. (2010) the fasting serum glucose concentration was significantly increased in CLA-fed mice compared to the Control groups [10].

In our study serum ALT activity significantly increased in all experimental groups after CLA supplementation. The same effect observed *Liu* et al. (2012) in hamsters which presented elevated ($p < 0.05$) ALT levels after feeding 2% and 3% t10,c12 CLA isomers. They concluded that a diet enriched with more than 2% t10, c12 led to liver malfunction and poses unfavorable changes on plasma lipid profiles [25]. Also in study of *Kim* et al. (2010) there was a prominent increase in ALT levels in high-fat-diet-induced obese C57BL/6J mice that received esterified and free forms of CLA [21]. Moreover, dietary t10, c12 CLA induces a severe hepatic steatosis in mice with a more muted response in other species [41]. In contrast, dietary CLA did not affect serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) activities after a partial hepatectomy (PH) in Sprague-Dawley rats [14]. Additionally, conjugated linoleic acid supplement or foods enriched with CLA was associated with a significantly increased circulating AST without any significant effect on alkaline phosphatase (ALP) and ALT levels in human studies [31].

In our experiment fat source had statistically significant influence on the MDA concentration. *Santos-Zago* et al. (2007) showed that CLA reduced MDA and thus reduces oxidation for the group that received CLA as compared to the control group [26]. The reactive aldehydes like malondialdehyde (MDA) - decomposition products of lipid peroxides, are used as reliable indicators of lipid peroxidation. Lipid peroxidation is a well-characterized consequence of oxidative stress and leads to further cell and tissue damage. In several studies it was shown that CLA regulates lipid metabolism in various tissues by modulating lipid oxidation, lipolysis and *de novo* lipogenesis [6].

In our study the lipid hydroperoxide (LPO) level was significantly elevated in all CLA groups. In paper by *Kilian* et al. [19] in order to evaluate the impact of dietary LA and CLA on liver metastasis and lipidperoxidation (LPO) Syrian hamsters were BOP-induced pancreatic cancer. SOD activity and lipid peroxidation were increased in liver metastases [19]. Furthermore both diets decreased the activity of glutathion peroxidase and increased the level of lipid peroxidation in pancreatic intratumoral tissue [18]. *Diniz* et al. [6] concluded that CLA may be disadvantageous because it can lead to oxidative stress and dyslipidemic profile. Moreover, CLA accelerates the decomposition of storage lipids in WAT and the clearance of serum non-esterified fatty acid levels, resulting in lipid peroxidation and a morphological change in the liver [44]. In the contrary, the antioxidative effect of CLA was examined by *Kim* et al. (2005) by determining lipid peroxidation and antioxidative enzyme activities. Male *Sprague-Dawley* rats were fed diets: normal diet, vitamin E-deficient control diet, 0.5% CLA vitamin E-deficient diet, or 1.5% CLA vitamin E-deficient diet for 5 wk. Dietary CLA reduced lipid peroxidation by increasing oxidative stability in rats [20].

CONCLUSIONS

1. Margarine had an adverse effect on the rat's lipid profile.
2. The addition of CLA decreased the concentration of TAG in the group fed with margarine.
3. Regardless of the type of the dietary fat, CLA supplementation increased the level of LPO in the blood serum of animals.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE INFLUENCE OF NUTRITIONAL INFORMATION UPON CUSTOMER ATTITUDE AND BEHAVIOUR IN EATING OUT ESTABLISHMENTS

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ABSTRACT

Background. Providing nutritional information in catering establishments in Poland, it is not mandatory, at the same time this type of information may affect the attitudes and behavior of consumers.
Objective. The purpose of this research was to define the influence of nutritional information upon customer attitude and behaviour in eating out establishments.
Material and methods: An online consumer survey was conducted in 2016. The quantitative research was undertaken in the form of a questionnaire among a group of 403 people selected in terms of age (18-35 years old), residence (big cities) and frequency of eating out or away from the home.
Results. The results of this research show that the respondents' perception of information was positive and influenced both their perception of the eating out establishment, as well as their purchasing decisions. Only gender was statistically important for the differentiation of the consumers' behaviour within the scope analyzed.
Conclusions. The results obtained lead to the conclusion that providing nutritional information may increase the competitiveness of eating out establishments. It may also lead to a more rational marketplace, where choices in terms of health may impact social health, taking into consideration the growing popularity of eating out or away from home.

Key words: *nutritional information, eating out establishments, consumer behaviour*

STRESZCZENIE

Wprowadzenie: Dostarczanie informacji o wartościach odżywczych w placówkach gastronomicznych w Polsce nie jest obowiązkowe, a tego typu informacje mogą wpływać na postawy i zachowania konsumentów.
Cel badań: Celem tego badania było określenie wpływu informacji o wartości odżywczej na postawy i zachowania klientów w lokalach gastronomicznych.
Materiał i metody: Badanie konsumenckie zostało przeprowadzono przez Internet w 2016 r. Badania ilościowe przeprowadzono w formie ankiety wśród 403 osób wybranych pod względem wieku (18-35 lat), miejsca zamieszkania (duże miasta) i częstotliwości spożywania posiłków poza domem.
Wyniki: Wyniki badań pokazują, że postrzeganie informacji przez respondentów było pozytywne i wpłynęło zarówno na ich postrzeganie placówki gastronomicznej, jak i na decyzje zakupowe. Tylko płeć była statystycznie istotna dla zróżnicowania zachowań konsumentów w analizowanym zakresie.
Wnioski: Uzyskane wyniki prowadzą do wniosku, że dostarczanie informacji żywieniowych może zwiększyć konkurencyjność lokali gastronomicznych. Może to również prowadzić do bardziej racjonalnego rozwoju rynku, gdzie wybory w zakresie zdrowia mogą wpływać na zdrowie społeczne, biorąc pod uwagę rosnącą popularność jedzenia poza domem.

Słowa kluczowe: *informacje o wartościach odżywczych, lokale gastronomiczne, zachowania konsumentów*

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INTRODUCTION

In recent years, in EU countries and in the USA offer by eating out establishments has become more diversified [7]. Also the demand for gastronomic services has grown, and this has been boosted by economic changes (increase of income), demographic changes (increase of households of 1 or 2 persons), social and professional changes (increase of women's professional activity) and cultural (changes of lifestyle) [38].

As a result the meals eaten in eating out establishments have a considerable influence upon the consumers' diet [4, 20, 21, 35, 41]. Moreover, the gastronomy sector has begun to play an important role in the realisation of nutrition policy [24] by shaping consumers' nutritional habits. This in turn is what determines the necessity to pay particular attention to the nutritive aspect of meals served by eating out establishments [14].

Gastronomy may play an important role in the popularisation of healthy nutrition. One of the activities undertaken in this field is the education of consumers by giving the nutritional information of meals served [18]. The information may be placed on trays, in folders, menu cards or menu boards [1]. Providing nutritional information of meals served in gastronomy establishments is a more frequent practice [14, 16, 40, 41].

Opinions about the influence of nutritional information on consumer behaviour in gastronomy are not unambiguous.

According to *Burton* et al. [6], the availability of nutritional information in eating out establishments may limit the consumption of unhealthy food. In the opinion of *Bollinger* et al. [5], information on the caloric value of dishes may positively influence the decisions of clients without negative impact on the foodservice sector because consumers are ready to pay higher prices for healthier products [17, 31, 33]. On the other hand, *Harnack and French* [15], *Krukowski* et al. [23] have found that information on the caloric value of meals in eating out establishments does not influence consumer behaviour a lot. But in opinion of *VanEpss* et al. [36] restaurant menu labeling should not be expected to reduce energy consumption by enough to address obesity on its own, but it should be viewed as a reasonable place to start.

Mayfield [25], *Mills and Thomas* [27] have noticed that it is particularly important for clients of eating out establishments to have information on the amount of fats, saturated fats and trans fats. *Josiam and Foster* [19] have obtained similar results. They have in addition stated that female consumers aged between 35-65, people who were 'well-situated' and educated and people who care about healthy nutrition were particularly interested in nutritional information. Also *Radwan* et al. [30] noticed that young females are seen to favor menu labeling restaurants.

Providing nutritional information in gastronomy establishments is relatively rare in Poland where nutritional information is mostly, only given by foreign restaurant chains. In some domestic eating out establishments only iconographic identifiers are used (products for vegetarians, light products, etc.). Taking into account the fact that Poles are eating out more and more in the gastronomy sector [22], at the same time more and more are suffering from obesity and associated diseases [41]. It seems, as a result, therefore, to be important to analyse the influence of the nutritional information provided in eating out establishments on the Polish consumers' attitude and behaviour. This was the aim of the research undertaken.

The following research questions have been formed:

1. What are the consumers' opinions about the nutritional information placed in eating out establishments?
2. Does nutritional information provided at eating out establishments influence the customers' purchasing decisions?
3. Does nutritional information influence the customers' perception of eating out establishments?

MATERIAL AND METHODS

To find the answers to the research questions formulated, a quantitative research (carried out with use an original questionnaire, based on literature review) was conducted (in June 2016). The sample consisted of 403 respondents selected purposefully. Criteria of the selection were: age (between 18 and 35), living in big cities and eating out of the home in the last 3 months. The research was conducted using the method of an online survey.

Men and women were of almost equal number in the group surveyed. Over 50% of respondents visited bars and restaurants at least once a week. The average age of the respondent was 28. Almost half declared they had a higher education (Table 1).

Table 1. Description of population surveyed

Specification	%
Gender	
Women	50.87
Men	49.13
Frequency of attendance at eating out establishments	
Several times a week	25.80
Once a week	26.55
2-3 times a month	27.30
Once a month	13.65
Once a quarter	6.70
Age	
18 -29	53.85
30 -35	46.15
Education	
Primary, vocational, secondary	51.12
Higher	48.88

In the main part of the survey a 5-point Likert scale was used (where: 1-strongly disagree, 2-disagree, 3-undecided, 4-agree, and 5-strongly agree). From the twelve statements used we formed three indicators: indicator of perception of nutritional information (PNII), indicator perception of eating out establishment (PEOEI) and indicator of declared purchasing behaviour (DPBI).

Perception of nutritional information indicator

Perception of nutritional information indicator (PNII) described the respondents' attitude towards nutritional information. It was composed of four statements:

- nutritional information is given understandably (PNI1),
- nutritional information is useful and important from the nutritional point of view (PNI2),
- nutritional information influences more deliberate and reasonable choices (PNI 3),
- nutritional information should be available in eating out establishment in a visible place (PNI4).

Perception of eating out establishment indicator

Perception of eating out establishment indicator (PEOEI) was supposed to specify the respondents' attitude towards eating out places providing nutritional information, with the consideration of four statements:

(1) eating out establishments which provide nutritional information influence my confidence (PEOE1),
(2) providing nutritional information in eating out establishments means that they are innovative (PEOE2),
(3) providing nutritional information in eating out establishments means that they have high-quality services or dishes on offer (PEOE3),
(4) an eating out establishment, where nutritional information is given, has a healthier offer than others that do not provide such information (PEOE4).

Declared purchasing behaviour indicator

Declared purchasing behaviour indicator (DPBI) allowed us to recognise the respondents' intentions as regards to eating away from the home, considering the provision of nutritional information. It was composed of four statements:

- while choosing food, I rely on nutritional information (DPB1),
- the knowledge of a meal's nutritional value would influence my choice of a meal (DPB2),
- I'd rather eat in a place where nutritional and caloric information about the product is available (DPB3),
- caloric value placed in a visible place, nearby the product, would make it easier for me to choose a dish (DPB4),

Research model

The research also assumed that there is a correlation between the consumers' perception of nutrition information (PNII) and the perception of an eating out establishment (PEOEI) and declared purchasing behaviour (DPBI).

Statistical analysis

The results obtained were subject to statistical analysis using the Statistica 10 program.

The values of the indicators analysed were calculated as an arithmetical average with conformity of the assessment of responses to the statements, which consisted of particular indicators. The only feature of a consumer, which made a difference between the results obtained, was that of gender and this was assumed as the grouping variable in the analysis results. To compare the differences in compliance with particular statements (measured in the 5-degree *Likert* scale) the U *Mann-Whitney* test was used because of the gender issue. To compare the differences of the average indicators PNII, PEOEI, PBI depending on gender, the t-*Student* test was applied. *Pearson* correlation analysis was also made in order to check the dependencies assumed in the research model. In all analyses, the level of statistical importance has been assumed as $p \leq 0.05$.

RESULTS AND DISCUSSION

As far as perception of nutritional information is concerned, the respondents declared a high compliance with most statements, except for the opinion, that "nutritional information is given understandably" (PNI1) (Table 2). One of the reasons for this phenomenon should be considered as the result of the low scale of education concerning nutrition within the scope of nutritional information in Poland [28]. The research completed so far shows that the level of knowledge regarding nutrition has a considerable influence on perception, evaluation and use of nutritional information [2, 23]. Another reason for the poor understanding of information about nutrition may be that too much information is given, which also limits the possibility of using it [1, 30, 32].

Most respondents, highly valued the usefulness of nutritional information, the need for its provision in eating out establishments and also where the information is shown i.e. in an easily visible place (PNI4) (Table 2). Analogical opinions were noted in effect in other research concerning this issue [3, 9, 32].

The analysis of respondents' opinions on the statements accounting for the perception of an eating out establishment (PEOEI) showed, that providing nutritional information influences the increase of trust towards an eating out establishment (PEOE1) as well as the perception of quality of the meals offered (PEOE3) (grades 3.96 and 3.91) (Table 2). Also in the opinion of *Thomas and Mills* [34] eating out establishments that

provide nutritional information have the consumers' confidence and offer, in the clients opinion, better quality meals. Moreover, *Din* et al. [9] suggest that consumers will more often choose eating out establishments where nutritional information is given, and as a result may force the establishment to offer meals with a healthier, and higher nutritional value [37, 39].

Lower grades of compliance were noted for the statements about innovativeness, and the healthier food on offer by the eating out establishments, which provided nutritional information (3.78 and 3.63) (Table 2). A reason for this may be that nutritional information is most often provided by network enterprises (overseas food chains for example), which offer fast food and are accused of a low nutritive value of their products [2].

Accounting for the elements regarding declared purchasing behaviour indicator (DPBI), it was stated that the highest compatibility (3.88) was for the statement concerning consumer preference of eating out establishments, which provide nutritional information (DPB3). Lower grades (3.66-3.7) have been noted for statements about the influence of nutritional information upon the choice of products offered in eating out establishments (DPB1, DPB2, DPB4) (Table 2). Also, in the opinion of *Auchincloss* et al. (2013), for the consumer the very fact of the provision of nutritional information is important, even if it is not used/referred to. Other research also suggests that nutritional information is not perceived as the key factor for choosing a meal in a restaurant [3, 11, 1, 29].

The correlation analysis between the level of compliance with the statements and features of respondents showed that statistically important differences appear only in the case of gender. Women have expressed greater compatibility with all the statements of the PNI indicator. As far as the PEOEI indicator is concerned, the evaluation given by women were higher for statements PEOE1, PEOE3, PEOE4, whereas regarding the DPB indicator women declared a higher compliance with statements DPB2 and DB3 (Table 2). This suggests that women are more aware of nutritional information, which has also been stated in other research [8, 9, 10, 26, 31]. Women are also more determined when it comes to the introduction of changes in the nutrition model and this further embraces the use of nutritional information while choosing dishes at an eating out establishment [13, 42].

After averaging out the results obtained, the highest rating was obtained for the indicator statement (forming individual indicators) where it was stated that in the general, the best evaluation/score was obtained for indicators PEOEI (3.82) and PNII (3.73). Mark 3.72 was noted in terms of the DPBI indicator.

Table 2. Respondents' compliance with statements that make up indicators of perception of nutritional information, perception of an eating out establishment and declared purchasing behaviour accounting for gender

Statements	Total		Women		Men		p-value (UWM test)
	Mean	SD	Mean	SD	Mean	SD	
Perception of nutritional information (PNI)							
PNI1 Nutritional information is easy to understand (PNI1),	2.881	1.142	3.000	1.167	2.758	1.105	0.042
PNI2 Nutritional information is useful and important, from the point of view of nutrition (can delete)	4.072	0.905	4.239	0.802	3.899	0.972	0.001
PNI3 Nutritional information influences more deliberate and reasonable choices	3.943	0.962	4.073	0.944	3.808	0.963	0.005
PNI4 Nutritional information should be available in eating out establishments in a visible place	4.007	0.882	4.146	0.833	3.864	0.911	0.003
Perception of an eating out establishment (PEOE)							
PEOE1 Eating out establishments which provide nutritional information influence my confidence	3.963	0.887	4.112	0.887	3.808	0.863	0.000
PEOE2 Providing nutritional information in eating out establishments means that they are innovative	3.779	0.964	3.912	0.919	3.641	0.991	0.006
PEOE3 Providing nutritional information in eating out establishments means that they have higher-quality on offer (services or dishes)	3.906	0.965	3.976	0.977	3.833	0.949	0.108
PEOE4 An eating out establishment where nutritional information is given has a healthier offer than one which does not provide it	3.625	1.116	3.761	1.083	3.485	1.134	0.013
Declared purchasing behavior (DPB)							
DPB1 While choosing a meal, I rely on nutritional information	3.660	1.118	3.771	1.048	3.545	1.177	0.080
DPB2 Knowledge of nutritional value would influence my choice of a meal	3.695	1.094	3.820	1.049	3.566	1.128	0.027
DPB3 I'd rather eat in a place where nutritional and caloric information about the product is available	3.876	1.015	4.039	0.944	3.707	1.059	0.002
DPB4 Caloric value placed in a visible place nearby the product would make it easier for me to choose a dish	3.682	1.085	3.780	1.087	3.581	1.076	0.054

The result of the verification of the dependencies between the indicators (accounted for in the research in accordance with the rules assumed in the model prepared) found that the perception of nutritional information quite strongly correlates with declared purchasing behaviour and the evaluation of the eating out establishment (Figure 1).

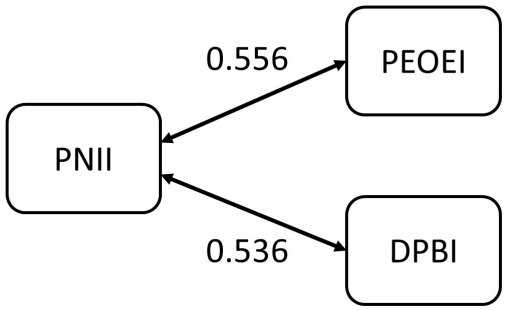

Figure 1 Dependencies between the perception of nutritional information (PNII) and declared purchasing behaviour (DPBI) and evaluation of eating out establishment (PEOEI).

CONCLUSIONS

The results of the research show that a high level of consumer acceptance of nutrition information, at eating out establishments, was found, even though it was poorly understood. The influence of perception of nutrition information on consumer opinion of eating out establishments and their purchasing decisions was also noted.

The results lead to the conclusion that providing nutritional information may be an activity, which increases the competitiveness of eating out establishments. It may also lead to more rational marketplace choices in terms of health. Moreover, when taking into consideration the growing popularity of eating out/away from the home, this may even have an impact upon social health.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

NUTRITION KNOWLEDGE OF PEOPLE WITH EATING DISORDERS

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ABSTRACT

Background: Eating disorders are an increasingly common health problem that is a major therapeutic challenge. For many years, the basic form of therapy used to be psychiatric and psychotherapeutic treatment, but now it is postulated that the dietetician should also be part of the therapeutic teams.

Objective: The main purpose of the study is to assess nutrition knowledge of people with eating disorders with consideration to their age, place of living, education, BMI, type of disease, participation in dietary consultations and in therapy.

Material and methods: Nutrition knowledge of the respondents was assessed by means of an author's survey questionnaire. The questionnaire was published in one of the social portals in the "Eating disorders – tackling" group gathering people with different types of eating disorders. The survey questionnaire consisted in 33 questions. Arithmetic mean and standard deviation for the number of correct answers provided by the respondents by the selected criteria.

Results: In terms of age, the least nutrition knowledge was attributable to the persons below 20 years of age (25.24 points in average). When considering the place of living, the least nutrition knowledge was revealed among the subjects living in medium cities (between 20 and 100 thousand of population) i.e. 25.31 points. In terms of education, the least nutrition knowledge was recorded in people with vocational education (24.83 points). When classifying the respondents by BMI, the highest average score was gained by the respondents with normal body mass index (BMI) (26.42 points).

Conclusions: The study on the level of nutrition knowledge among the people with eating disorders demonstrated that this knowledge was selective and insufficient to provide rational nutrition. It aimed at teaching the rules of healthy lifestyle and nutrition and thorough discussing of all nutrients, their functions and effect on the body.

Key words: *nutrition knowledge, eating disorders, nutrition, lifestyle*

STRESZCZENIE

Wprowadzenie. Zaburzenia odżywiania są coraz powszechniejszym problemem zdrowotnym stanowiącym duże wyzwanie terapeutyczne. Przez wiele lat podstawową formą terapii było leczenie psychiatryczne i psychoterapeutyczne, obecnie postuluje się, aby w skład zespołów terapeutycznych wchodził również dietetyk.

Cel badań. Głównym celem badania jest ocena wiedzy żywieniowej osób z zaburzeniami odżywiania się z uwzględnieniem ich wieku, miejsca zamieszkania, wykształcenia, BMI, rodzaju choroby, udziału w konsultacjach dietetycznych i terapii.

Materiał i metody. Wiedzę żywieniową respondentów oceniano za pomocą autorskiego kwestionariusza ankiety. Kwestionariusz został opublikowany w jednym z portali społecznościowych „Zaburzenia odżywiania - walka", gromadzącej osoby z różnymi rodzajami zaburzeń. Kwestionariusz ankiety składał się z 33 pytań. Obliczono średnią arytmetyczną i odchylenie standardowe dla liczby poprawnych odpowiedzi udzielonych przez respondentów według przyjętych kryteriów.

Wyniki. Pod względem wieku najmniejszą wiedzą żywieniową można przypisać osobom poniżej 20 roku życia (średnio 25,24 pkt). Biorąc pod uwagę miejsce zamieszkania, najmniejszą wiedzę żywieniową stwierdzono ród osób zamieszkujących średnie miasta (od 20 do 100 tysięcy ludności), tj. 25,31 punktów. Pod względem wykształcenia najmniejszą wiedzę żywieniową odnotowano u osób z wykształceniem zawodowym (24,83 pkt). Przy klasyfikacji respondentów według BMI najwyższy średni wynik uzyskali respondenci z prawidłowym wskaźnikiem masy ciała (BMI) (26,42 pkt).

Wnioski. Badanie dotyczące poziomu wiedzy żywieniowej wśród osób z zaburzeniami odżywiania wykazało, że wiedza ta była selektywna i niewystarczająca do zapewnienia racjonalnego żywienia. Istnieje konieczność tworzenia zespołów terapeutycznych, w których powinien znajdować się dietetyk. Jego zadaniem powinno być nauczenie zasad zdrowego stylu życia i odżywiania oraz dokładne omówienie wszystkich składników odżywczych, ich funkcji i wpływu na organizm.

Słowa kluczowe: *wiedza żywieniowa, zaburzenia odżywiania, odżywianie, styl życia*

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INTRODUCTION

Eating disorders pose a great challenge to the contemporary medicine. According to data provided by the National Eating Disorder Association, nearly 70 million of people throughout the world suffer from different eating disorders [18]. This group of diseases is the cause of the greatest number of deaths from among all mental diseases and therefore developing the effective treatment methods is crucial [14]. Eating disorders should not be perceived as a reflection of the prevailing pursuit of weight loss. The underlying cause of these diseases is multifaceted and complex. The fact that people living in the era of wealth and prosperity choose a path of "hunger at the times of repletion" is extremely difficult to explain [5], so as the reason behind attributing the emotions to eating. Equating this essential physiological need of the body with consolation, a sign of bonds, love or entertainment deprives the food of its physiological functions and makes it a way to satisfy the higher needs [21]. Numerous scientific publications bring up the need of taking up studies on nutrition knowledge of people with eating disorders. This need stems from the reports clearly demonstrating that psychotherapy combined with diet therapy has better therapeutic indices than psychotherapy alone [8]. The studies by *Waisberg* and *Woods* [23] demonstrated that education in the area of nutrition significantly increased the nutrition knowledge, which was reflected in changed attitudes and behaviours of the subjects. According to the body of literature, the level of nutrition knowledge and health views determine the health behaviours [5]. Thus, studying the behaviours and knowledge is a must for understanding the activities underlying the change of nutrition habits and practices [5].

Patient education in the scope of nutrition knowledge plays a significant role in the recovery process. The key position of dietician in treatment of eating disorders has been emphasized in numerous studies [7, 8, 9, 13, 21]. The essential aim of nutrition education is to teach the patient how to compose the meals well and to extend knowledge on the rules of proper nutrition. In addition, while on diet therapy, the subject needs to understand that the food satisfies the physiological rather than mental needs.

The main purpose of the study is to assess nutrition knowledge of people with eating disorders with consideration to their age, place of living, education, BMI, type of disease, participation in dietary consultations and in therapy.

MATERIAL AND METHODS

100 respondents (94 women and 6 men) with eating disorders, regardless of their type, were enrolled in the study. The exclusion criteria were targeted on healthy individuals or individuals with diseases failing to meet the eating disorder criterion. The study involved people whose eating disorders were diagnosed by a general practitioner, psychiatrist or other specialist. During the study, no medical certificate was required and the respondents were informed that only people diagnosed by a specialist could take part in it. The study included patients with an active form of the disease.

Participation in the study was voluntary. The respondents were informed about the purpose of the study and its process. The respondents were assured that the surveys were anonymous and the results of the study would be used only for scientific purposes.

The study was conducted electronically. The questionnaire was published in one of the social portals in the "Eating disorders – tackling" group gathering people with different types of eating disorders and aiming at mutual support and encouraging treatment.

51% of the respondents were below 20 years of age. The second largest group included the respondents aged 20-25, whereas the smallest groups were the respondents aged 26-30 and above 30 years of age.

30% of the respondents lived in large cities with population of 100 thousand and above. Similar number of the respondents (29%) lived in medium cities (between 20 and 100 thousand of population). A significant number of the respondents inhabited the rural areas, whereas the smallest group originated from small cities of population below 20 thousand.

Substantially all the respondents were the persons with anorexia (55%) and bulimia (31%) and only few percent's with night eating syndrome. The other eating disorders (7%) included: bigorexia, lack of the sensation of satiety after eating (acoria), lack of appetite and selective eating disorders.

Vast majority of the respondents had upper secondary or higher education. Nutrition knowledge of the respondents was assessed by means of an author's survey questionnaire. The questionnaire contained 20 questions related to nutritional knowledge and 13 questions about metric data. 7 test questions were of multiple choice ones. The respondents were asked about current body mass and height in order to calculate the body mass index (BMI) [kg/m²], interpreted according to the updated standards [10]. The survey developed for the purposes of this study included the questions about the essential nutrients: proteins, fats, carbohydrates (including dietary fiber) and vitamins as well as about caloric value of these components, their functions, sources and percentage share of these components in a balanced diet. The other questions referred to the proper number of meals a day, caloric intake, BMI value and an example of a well composed dinner.

The responses provided by the respondents were assessed by their correctness. The maximum score

was 36. 20 questions referring to nutrition knowledge were assessed. 1 point was scored for a correct answer in a single choice question and 4 points for a correct answer in a multiple choice question (scores were given for checking of a specific number of correct answers and for non-checking of the incorrect ones). The number of points scored by the respondents was interpreted according to the following scale: 0-21 points – insufficient knowledge, 22-27 points – sufficient knowledge, 28-33 points – good knowledge, 34-36 points – excellent knowledge.

Knowledge of the respondents was analysed in terms of: age, place of living, education, BMI, type of disease, participation in dietary consultations and in therapy.

The gamma coefficient was calculated to demonstrate correlations between the actual knowledge of the respondents (assessed on the basis of the provided answers) and self-assessment of the respondents. The adopted significance level was ≤ 0.05. Analysis of correlations between the features referred to above was performed with the use of scale according to Table 1.

RESULTS

Considerable majority of the respondents (68%) used no assistance of the psychotherapy specialist. Only 32% of them decided to participate in such form of treatment. Also the substantial majority (as many as 72%) used no assistance of the human nutrition specialist (Figure 1).

Table 1. The strength of dependence resulting from the dependence ratio

The value of the dependency coefficient	The strength of dependence
0	No dependency
(0; 0.2>	Weak
(0.2; 0.4>	Low
(0.4; 0.7>	Moderate
(0.7; 0.9>	High
(0.9; 1.0)	Very high
1	Functional dependence

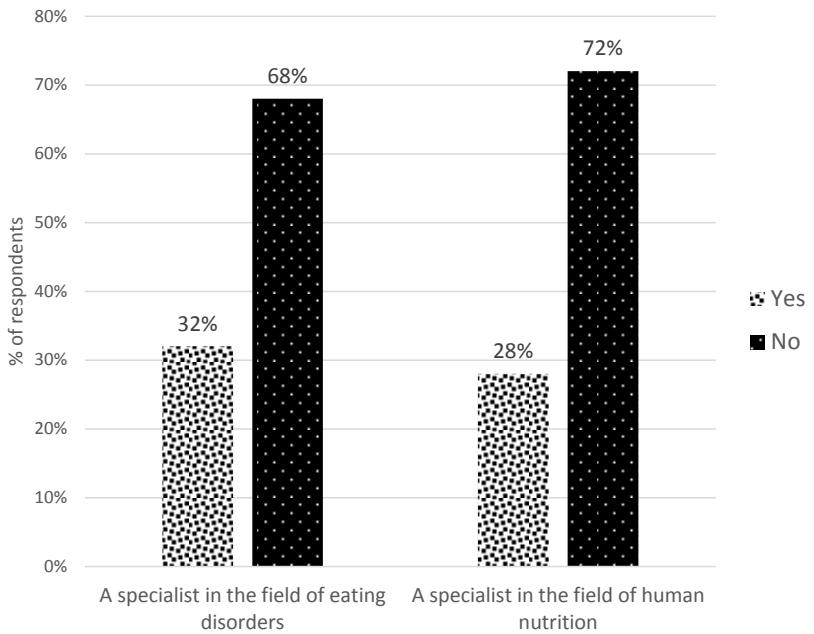

Figure 1. Participation of the respondents in psychotherapy and dietary consultations

Substantially all respondents displayed nutrition knowledge at sufficient level, while only 5 of them had excellent knowledge on human nutrition. Almost 1/5 of the respondents demonstrated insufficient knowledge (Figure 2).

The respondents were asked to rate their nutritional knowledge on a scale of 1 to 5, where 1 meant the lowest (insufficient) and 5 - the highest (very good) grade. Considerable majority of the respondents assessed their nutrition knowledge as good and sufficient and only 1 respondent considered its level as insufficient (Figure 3).

Arithmetic mean and standard deviation for the number of correct answers provided by the respondents by the selected criteria, including: age, place of living, education, BMI, type of disease, participation in dietary consultations and in therapy, was calculated. In terms of age, the least nutrition knowledge was attributable to the persons below 20 years of age (25.24 points in average) (Table 2). When considering the place of living, the least nutrition knowledge was revealed among the subjects living in medium cities (between 20 and 100 thousand of population) i.e.

25.31 points. The respondents inhabiting rural areas gained the highest average score in the questionnaire (26.32 points). In terms of education, the least nutrition knowledge was recorded in people with vocational education (24.83 points). When classifying the respondents by BMI, the highest average score was gained by the respondents with normal body mass index (BMI) (26.42 points). While adopting the type of disease as a differentiating criterion, the individuals with compulsive eating obtained the lowest average score (24.26 points). The respondents not consulted by a human nutrition specialist as well as these not participating in therapy had lower nutrition knowledge comparing to the respondents that took part in dietary consultations and therapy. The above results were not statistically significant.

On the basis of the performed Gamma correlation coefficient test, correlation between actual knowledge (knowledge assessment) and self-assessment of the respondents, people with eating disorders, for $\alpha = 0.05$, $p = 0.007$, was determined.

Calculated value of the Gamma correlation coefficient amounts to 0.27, which means, that the magnitude of correlation between the actual knowledge (knowledge assessment) and self-assessment of the respondents in the studied group is low (Figure 4).

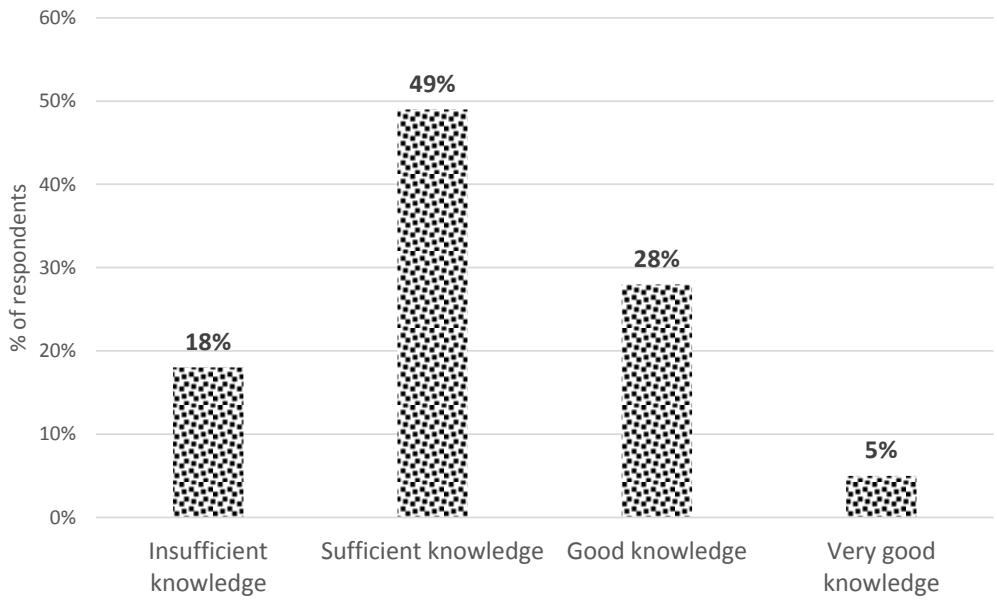

Figure 2. Assessment of nutritional knowledge of the respondents

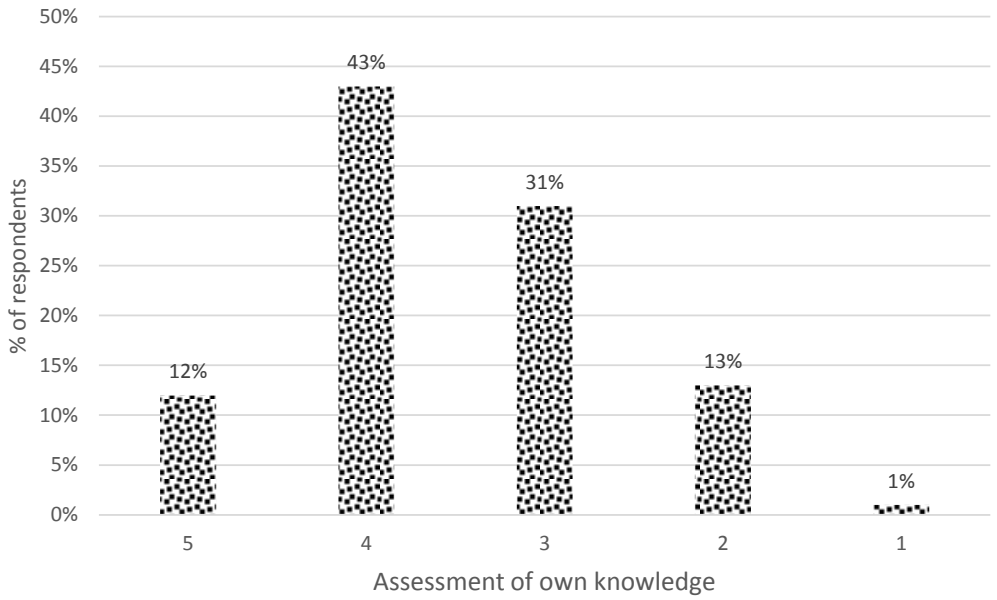

Figure 3. Opinion of the respondents on their own knowledge in the field of human nutrition

Since >0 and as the result of the Gamma correlation coefficient test is statistically significant, the values of analysed random variables for a certain part of subjects in the sample and in the studied general population are ordered in the same direction.

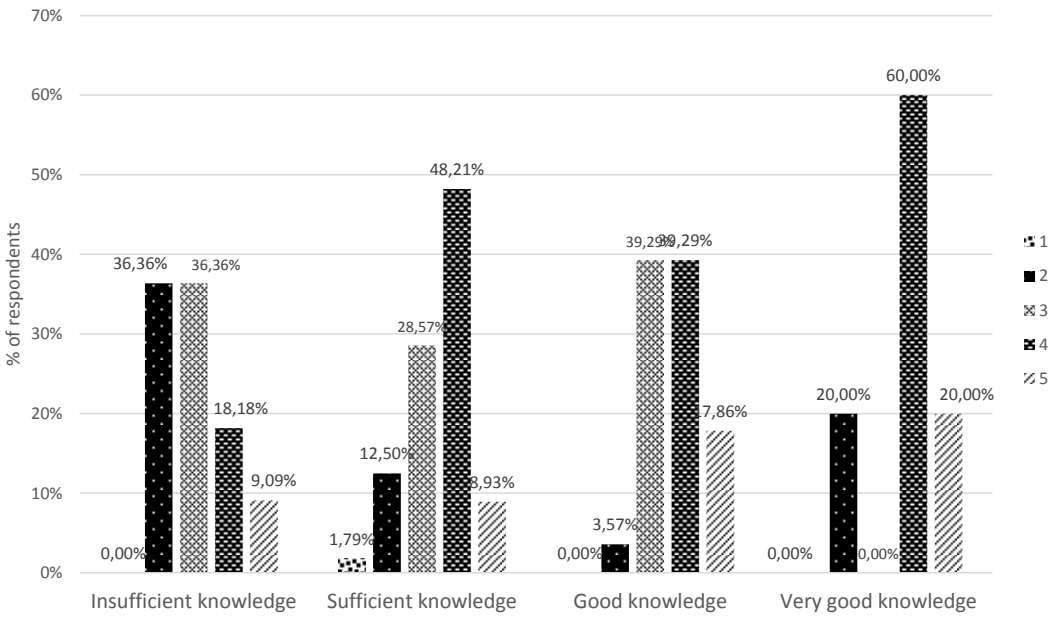

Figure 4. Knowledge assessment obtained on the basis of the questionnaire and self-assessment of the respondents

Table 2. Analysis of the average number of points obtained from the survey depending on the selected criterion

Criterion	Classification (n)	Arithmetic mean of the number of points obtained (±SD)
Age [years]	< 20 (51)	25.24 (±3.81)
	20 – 25 (37)	26.43 (±4.67)
	26 – 30 (6)	26.67 (±6.31)
	> 30 (6)	25.33 (±4.76)
Place of residence	Village (25)	26.32 (±4.64)
	Small city (16)	26.00 (±3.85)
	Medium city (29)	25.31 (±4.21)
	Big city (30)	25.63 (±4.58)
Education	Basic (24)	25.00 (±4.04)
	Vocational (12)	24.83 (±2.76)
	Secondary (46)	26.24 (±4.58)
	Higher (18)	26.22 (±4.95)
BMI	Underweight (57)	25.56 (±4.00)
	Normal weight (38)	26.42 (±4.91)
	Overweight or obesity (5)	23.20 (±2.17)
Type of disease	Anorexia nervosa (55)	25.78 (±4.19)
	Bulimia nervosa (31)	25.90 (±3.81)
	Orthorexia nervosa (14)	28.10 (±3.53)
	Compulsive overeating (25)	24.26 (±3.19)
	Night eating (10)	25.30 (±3.19)
	Other (7)	24.33 (±5.85)
Consultation with a dietitian	Yes (28)	27.62 (±4.72)
	No (72)	25.04 (±3.98)
Participation in therapy	Yes (32)	27.84 (±4.82)
	No (68)	24.79 (±3.74)

DISCUSSION

Many scientists emphasize the important role played by a multi-disciplinary team in the recovery process. This includes primarily the role of dieticians focused for the most on nutrition education, correcting improper nutrition behaviours and control over the nutrition treatment [2, 8]. Nevertheless, the fact that such form of assistance was used by only 32% of the respondents might be disturbing. This imposes the need for improving the treatment conditions in this area and for establishment of the specialist centers offering simultaneous therapy provided by a multidisciplinary team. Analysis of the results of studies performed on the subjects with bulimia demonstrated that nutrition education combined with psychotherapy brought a significant reduction of eating attacks or their remission [15]. *Hsu* et al. [10] compared the effectiveness of cognitive therapy and nutrition therapy as well as combination of these two methods. It was demonstrated that these methods resulted in significant reduction in the number of eating episodes and the use of compensation methods, while simultaneous nutrition and cognitive therapies contributed to better self-control and higher reduction of improper behaviours. Therefore, proper nutrition education and increased nutrition knowledge can influence discontinuation of bulimic behaviours. This thesis was confirmed in the study by *O'Connor* et al. [19]. Similar studies performed on the subjects with anorexia provide no unambiguous results. *Sarfety* et al. [22] demonstrated that better nutrition knowledge in the subjects with anorexia does not affect the recovery process, while *Waisberg* and *Woods* [23] proved that nutrition education resulted in a significant change of nutrition behaviours in this group of subjects.

While assessing the nutrition knowledge, it was found that this knowledge differed depending on the group. In context of age, the highest average score was obtained by the respondents in the 26-30 age group (26.67 points, ±6.31). The least nutrition knowledge was recorded in the persons below 20 years of age (25.24 points, ±3.81).

Analysis of the average score demonstrated that the persons inhabiting rural areas have the most extensive nutrition knowledge (26.32 points, ±4.64). These results may surprise since according to numerous scientific studies the persons living in urban areas display greater nutrition awareness comparing to these living in rural areas [4]. Considering the size of the city, it can be noted that the respondents living in a medium or large city have lesser nutrition knowledge comparing to the respondents living in a small city.

In terms of education of the respondents, it can be found that the most extensive nutrition knowledge is recorded among the persons with upper secondary education (26.24 points, ±4.58), while the subjects with higher education obtained a similar average score (26.22 points, ±4.95). This study proves also that people with vocational education have the least nutrition knowledge. The study of *Ostachowska-Gąsior* et al. [20] obtained distinct results. In this study, the best knowledge was recorded in the respondents with higher education. It is surprising that in the quoted study the persons with vocational education have better nutrition knowledge than these with upper secondary education.

In terms of BMI classification, the most extensive nutrition knowledge was demonstrated by the respondents with normal body mass index (26.42 points, ±4.91), whereas the least knowledge in this area was displayed by overweight or obese people (23.20 points, ±2.17). Also the underweight persons demonstrated lesser knowledge comparing to these with normal BMI. This may prove the theory that each deviation from normal body mass and presence of eating disorders are correlated with improper nutrition knowledge. However, the *Weker* et al [24] study, which included children aged 13-15, shows that the level of knowledge about proper nutrition was higher in obese adolescents than in children with right body weight.

While analysing the average score obtained by the respondents in terms of type of their disorder, it can be found that the least nutrition knowledge is specific for the persons with compulsive eating disorder (24.26 point, ±3.19), whereas the best knowledge is attributable to the persons with orthorexia (28.10 points, ±3.53). It can be suspected that these results are closely correlated with the type of disease. Compulsive eating consists in consuming large volumes of food in a short time period, with loss of control over the types of the consumed products and their quantity [1, 3, 6]. During the attack, people with compulsive eating disorder frequently consume the products that are commonly considered unhealthy, of high caloric value and with high content of fat and carbohydrates. There is no division into "good" and "bad" products. People with compulsive eating disorder believe that all that is eatable is food and do not care about the caloric intake and nutrients provided by a given product [3, 6], while the subjects with orthorexia impose numerous dietary restrictions on themselves, exclude processed products and chemical additives in a form of chemicals [12, 17]. Restrictions in consumption of specific types of products are far more extensive than the ones recommended by the specialists, therefore it can be concluded that people with orthorexia have broad knowledge on human nutrition and advanced nutrition knowledge.

This study demonstrated no significant differences between the level of nutrition knowledge of the

subjects with anorexia and bulimia. The persons with diagnosed anorexia nervosa obtained 25.78 points (±4.19) in average, while these with bulimia nervosa obtained 25.90 (±3.81) in average in the questionnaire. Similar results were provided also in two other independent studies. One of the first studies in the world that assessed the level of nutrition knowledge in people with eating disorders was performed in Germany in 1988 [16]. It was found that the results assessing the level of nutrition knowledge of the patients with anorexia and bulimia were equivalent. The other study [5], carried out in Poland in 2007, also displayed no differences in the level of nutrition knowledge for these two diseases.

The study showed however certain differences in the level of nutrition knowledge in the respondents who were consulted by a dietician and the persons without such consultation. Average score obtained by the first of the discussed groups was 27.64, whereas the persons without consultation gained only 25.04 points. Therefore, it can be stated that an appointed visit to a human nutrition specialist contributed to better nutrition knowledge which is reflected in the obtained score.

The survey performed for the purposes of this study assessed also the level of nutrition knowledge depending on participation in the therapy. The methods commonly applied in treatment of eating disorders include psychotherapy and pharmacotherapy. Psychotherapy consists in behavioural therapy, interpersonal therapy, family therapy and familiarises with the methods of handling high-stress situations [6]. The most commonly applied therapeutic methods in eating disorders include cognitive and behavioural therapy aiming at identifying the core cause of disease and changing the previous behaviours [3]. According to the obtained results, the knowledge of people participating in these forms of treatment is by 3 points higher comparing to the persons avoiding therapy. These results can confirm the effectiveness of therapies and argue for the need of introducing the clinical dieticians to the group of specialists treating the nutrition disorders.

CONCLUSIONS

1. The study showed that the lowest level of nutritional knowledge was characteristic of respondents under the age of 20, living in a small town, with vocational education, and not using a dietician's or therapist's help.
2. The study on the level of nutrition knowledge among the people with eating disorders demonstrated that this knowledge was selective and insufficient to provide rational nutrition. It aimed at teaching the rules of healthy lifestyle and nutrition and thorough discussing of all nutrients, their functions and effect on the body.
3. The persons with eating disorders hardly use the assistance of dietary specialists. The task of such specialists is to offer nutrition education, correct improper nutrition behaviours and control over nutrition therapy.

Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

CHARACTERISTICS OF A DIET AND SUPPLEMENTATION OF AMERICAN FOOTBALL TEAM PLAYERS - FOLLOWING A FASHIONABLE TREND OR A BALANCED DIET?

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ABSTRACT

Background. American football is based on speed and strength efforts players forming both defensive and offensive units in the team. Players' diet's may differ with regard to the variety of physical efforts performed and their different nutritional needs.
Objective. The aim of the study was to evaluate a diet and the supplementation, including the comparison of both the defensive and offensive player's formations, which may constitute a background of sportsmen' balanced menus permitting them to achieve better sports results.
Material and methods. The study included 44 American football players (24 defensive, 20 offensive players). The study group completed a three-day-dietary recall containing the time of consumption as well as a questionnaire about a type and amount of supplements used, including questions of one or multiple choice.
Results. The mean age of defensive players was 25.1±5.8 years, while of offensive players was 23.4±3.7. The mean body mass equaled 101±15.6 kg vs. 88.7±22.2 kg. The mean height of defensive players was 183.8±6 cm vs. 182.4±7.3 cm of offensive players. Offensive players consumed on average 2471.9±838.6 kcal/24 hs, whilst defensive players 3086.1±908.9 kcal/24 hs. The mean level of cholesterol level consumption equaled 667.81±300 mg in defensive players, while 546.2±285 mg in offensive players. Of energizing preparations, protein powder supplement and coffee were most frequently chosen by players.
Conclusions. Defensive unit players were characterized by higher consumption of certain nutritional components and more frequent diet supplementation. A too low caloric intakes well as water, vitamin D, carbohydrates and dietary fiber intake was observed in both units. A high intake of high level cholesterol products requires reduced consumption of saturated fatty acids and increased consumption polyunsaturated fatty acids.

Key words: *diet, American football, team sport, nutrients*

STRESZCZENIE

Wprowadzenie. Futbol amerykański będący sportem zespołowym, bazuje na wysiłkach szybkościowych i siłowych, zaś w skład drużyny wchodzą zawodnicy formacji defensywnej oraz ofensywnej. Sposób żywienia zawodników, może różnić się ze względu na różnorodność wykonywanych wysiłków fizycznych i odmienne potrzeby żywieniowe.
Cel. Celem pracy była ocena sposobu żywienia i suplementacji z uwzględnieniem porównania obu formacji zawodników: defensywnej oraz ofensywnej, która stanowić może podstawę do zbilansowania jadłospisów sportowców, umożliwiając im uzyskiwanie lepszych wyników sportowych.
Materiał i metody. W badaniu udział wzięło 44 zawodników futbolu amerykańskiego Lowlanders Białystok (24 formacji defensywnej oraz 20 ofensywnej). Grupa badana uzupełniła 3-dniowy dzienniczek żywieniowy z uwzględnieniem godzin spożycia, gramatury poszczególnych produktów/ posiłków oraz kwestionariusz ankiety dotyczący rodzaju oraz ilości stosowanych suplementów.
Wyniki. Średni wiek badanych zawodników defensywy wyniósł 25.1±5.8 lat, zaś formacji ofensywnej 23.4±3.7. Średnia masa ciała była równa 101±15.6 kg vs. 88.7±22.2 kg. Średni wzrost zawodników defensywy wynosił 183.8±6 cm vs. 182.4±7.3 cm u zawodników ofensywy. Gracze ofensywy przyjmowali średnio 2471.9±838.6 kcal/dobę, zaś defensywy 3086.1±908.9 kcal/dobę. Średni poziom spożycia cholesterolu u zawodników defensywy wynosił 667.81±300 mg zaś ofensywy 546.2±285 mg. Najczęściej wybieranymi przez zawodników były odżywki białkowe, a z preparatów energetyzujących - kawa.
Wnioski. Zawodnicy formacji defensywnej, charakteryzowali się wyższym spożyciem poszczególnych składników odżywczych oraz częstszym przyjmowaniem suplementów diety. W obu formacjach zaobserwowano zbyt niską energetyczność diety, podaż wody, witaminy D, węglowodanów i błonnika pokarmowego. Wysoka podaż produktów bogatych w cholesterol, wskazuje na konieczność redukcji spożycia nasyconych kwasów tłuszczowych i zwiększenie spożycia kwasów tłuszczowych wielonienasyconych.

Słowa kluczowe: *dieta, futbol amerykański, sporty zespołowe, składniki odżywcze*

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INTRODUCTION

Team sport is the most popular sport discipline of the 20 century predominating in the world. Due to the characteristics of American football, its team contains both defensive and offensive unit players. Sportsmen involvement is enormous during a game or training, doing aerobics and speed efforts. An appropriate model of nutrition taking into consideration an increased intake of some nutrients, such as proteins, carbohydrates, minerals and vitamins is essential in this discipline. Properly personalized supplementation as well as biological regeneration are equally important. They contribute to sportsmen's achievements and improve the quality of regeneration [6]. Individual diets may differ regarding various physical efforts, different nutritional and supplemental needs. According to American Dietetic Association, Canada Dieticians and American College of Sports Medicine, a proper diet influences significantly the physical efficiency of sportsmen's organisms. A diet should be rationalized and adapted and customized to the type of physical activity [20].

The aim of the study was to evaluate American football players' diet and supplementation. The analysis of the study results will enable to obtain the information regarding dietary mistakes made by the American football players. This may lead to balancing sportsmen's proper menus allowing them to achieve better sports results.

MATERIAL AND METHODS

The study includes 44 players of American football team of Lowlanders Bialystok, training actively this sports discipline. A defensive unit consists of 24 players (54%), while an offensive (attack) unit - 20 players (46%).

The approval of the Bioethical Committee, Medical University of Bialystok, number R-I-002/496/2014 was obtained for the test. All participants have given their informed consent for participation in the research study. Every precaution was taken to protect the privacy of patients.

The study group completed a three-day-dietary recall designed on the grounds of a 24-hour review questionnaire, which provided anthropometric data as well as the information about frequency, quality and quantity of meals consumed during two working days and a day off. The consumption time, grammage of individual products, the amount of liquids were taken into consideration. A portion size was verified based on 'Album of photographs of products and dishes' published by Institute of Food and Nutrition in Warsaw [22]. Analyzing a questionnaire, the caloric values of individual meals and the content of essential nutrients and liquids were taken into consideration. Computer program Diet 5 developed by the Institute of Food and Nutrition was used for qualitative analysis. The norms of daily calorie intake were established for healthy men in the age interval 19-30, at the PAL-physical activity level equaled 1.75 [12].

Due to lack of randomized studies determining precisely the daily intake level of basic nutrients for American football players, the studies referring to the team sport because of the similar characteristics of physical efforts were used in the discussion. The intake level of basic nutrients was compared to the norm for proteins [11], lipids [12], carbohydrates [18], minerals [12], vitamins [12], and liquids [15].

The survey questionnaire referring to the supplementation applied contained one-and multiple-choice questions. They concerned preparations used, their type, frequency, quantity and the way of administration.

The results were analyzed in the program STATISTICA 12.0 of StatSoft firm. Descriptive statistics was developed and an arithmetic mean, standard deviation, ranges of maximum and minimum values were calculated by means of this program. *Chi-square Pearson* and *Mann-Whitney* tests were used to calculate the results.

RESULTS

The mean age of the examined equaled 24.3±4.9 years (16-40 years). In the defensive unit, the mean age was 25.1±5.8 years, while 23.4±3.7 years in the offensive unit. However, these values were not statistically significant.

In defensive players, the mean body mass was 101±15.6 kg, and 88.7±22.2 kg in offensive players. The mean height of defensive players equaled 183.8±6 cm, while of offensive players, 182.4±7.3 cm. A statistically significant difference, p=0.02, was determined only for the body mass of respective unit players.

The comparison of daily calorie intake and basic nutrients among players of an offensive and defensive unit was presented in Table 1. Offensive players consumed on average 2471.9±838.6 kcal/24h, which squared Estimated Average Requirement (EAR) only in 69% , whereas defensive players, 3086.1±908.9 kcal/24h (80% of EAR). These differences were statistically significant at the level of significance p=0.02.

The mean consumption of protein was higher in players of a defensive unit, reaching 156.3±42.7g (on average 1.6 g/kg of body mass /24h) than in players of an offensive unit, 127.3±37.7g (on average 1.4 g/kg of body mass /24h). The mean intake of vegetable protein was also higher in a defensive unit and equaled 39±15.5g. These results were statistically significant (Table 1.)

Table 1. Comparison of the daily level of energy supply and basic nutrients among players of defensive and offensive formation

Parameter	Defensive (n=24)			Offensive (n=20)			p
	Average±SD	Min.	Max.	Average±SD	Min.	Max.	
Energy value of the diet (kcal)	3086.1±908.9	1644	4517.7	2471.9±838.6	1558.1	4602.7	0.02
EAR (%)	80.1±26.7	45.7	131.6	68.9±23.9	43.3	127.8	0.05
Protein (g)	156.3±42.7	84.9	268.9	127.34±37.7	78.1	218.0	0.02
Plant protein (g)	39±15.5	10.7	76.2	30.1±12.1	16.1	57.9	0.03
Animal protein (g)	113.3±38.7	51.5	230.5	93.9±32.7	55.4	182.3	0.07
Fat's (g)	114±46.7	44.2	220.4	85.0±41.5	27.3	193.6	0.03
Saturated fat's (g)	41.9±18.9	14.8	93.3	29.7±12.6	7.5	59.8	0.02
Mono-unsaturated fats (g)	46.9±21.3	14	91.1	33.2±15.7	10.1	61.6	0.03
Poly-unsaturated fat's (g)	16.3±7.0	4.2	29.2	14.3±19.5	4.1	93.6	0.01
Food cholesterol (mg)	667.81±300	241.5	1176.2	546.2±285	209.4	1158	0.13
Carbohydrates (g)	373.8±117.2	209.8	604.4	317.8±123.3	136.9	695.2	0.10
Simple carbohydrates (g)	347.0±111.1	192.7	575.2	295.9±119.6	123.9	665.7	0.09
Fiber (g)	25.5±7.9	10.3	44.6	21.4±6.9	11.6	34.6	0.10

Next the mean total lipids consumption and respective groups of fatty acids and food cholesterol were compared. The mean total lipid intake with a diet equaled 114±46.7 g (average 1.1 g/ kg of the body mass per 24 hours) in a defensive unit, while 85±41.5 g (on average 0.95 g/kg of the body mass per 24 hours) in a offensive unit, respectively. These differences were statistically significant (p=0.03). Defensive players also consumed more saturated acids (41.9±18.9 g), while offensive players consumed on average 29.7±12.6 g. These differences were statistically significant (p=0.02). Defensive players consumed also more monounsaturated, of which mean level was 46.9±21.3 g in contrast to offensive players' mean consumption reaching the level of 33.2±15.7 g (p=0.03). The mean consumption of polyunsaturated lipids was similar in both study groups and equaled: 16.3±7.0 g in defensive players and 14.3±19.5 g (p=0.01) in offensive players. In both groups, a very high level of food cholesterol was reported: on average 667.81±300 mg in defensive players and 546.2±285 mg in offensive players. Maximum levels of this component intake were also high and equaled 1176.2 mg in a defensive unit and 1158.4 mg in a offensive unit.

Daily intake of carbohydrates and food fiber consumed with a diet was analyzed. The mean total consumption of carbohydrates was higher among defensive players than in offensive players, equaling on average 373.82±117.25 g. Defensive players also consumed more assimilable carbohydrates and food fiber. The differences presented above were statistically insignificant.

Mineral components and vitamin D intake in a diet were analyzed statistically (Table 2). The higher consumption of all minerals and vitamin D was observed in defensive players with statistically significant differences regarding sodium and iron.

Table 2. Comparison of the average daily intake of selected minerals and vitamin D among the players of defensive and offensive formation

Parameter	Defensive (n=24)			Offensive (n=20)			P
	Average±SD	Min.	Max.	Average±SD	Min.	Max.	
Sodium (mg)	5195.3±1650.1	2624.4	8674.5	4061.2±1306.9	2285.8	6057.9	0.01
alcium (mg)	1043.1±769.9	98.2	3031.2	847.5±547.3	280.7	1911.7	0.34
Magnesium (mg)	491.6±158.2	169.3	759.3	429.1±170.1	224.8	706.5	0.13
Iron (mg)	19.1±10.5	9.0	51.4	15.2±9.0	8.6	41	0.05
Vitamin D (ug)	4.6±2.5	1.9	11.5	3.8±2.3	0.9	8.8	0.19

The mean consumption of liquids was another parameter assayed in the study. The mean daily intake was higher among defensive players (1925 ml) than offensive players (1750 ml). The differences were statistically insignificant (p=0.44).

The survey questionnaire aimed at evaluation of the kinds and frequency of diet supplementation among the sportsmen with 58% of defensive players and 65% of offensive players who declared consumption of vitamin and mineral supplements. More than 77% of players of both units continued supplementation for the whole year.

Sportsmen drank also energy drinks, enriched with vitamins or minerals. A total of 96% of defensive players and 90% of offensive players declared their consumption in the survey. These were not products

chosen every day, but drank during training or competitions, several times in a month in the amount not exceeding 2 liters.

In the defensive and offensive unit, 83% and 65% of players consumed functional food, respectively. Some players used more than one type of functional food. Types of preparation with the division into units were presented in Table 3.

Of defensive players, 67% and 40% of offensive players took protein powder (differences close to statistical significance (p=0.07)). Protein-carbohydrates powder supplements (in which the ratio of carbohydrates to proteins is 1:1) and the type of Gainer (the ratio of carbohydrates to proteins is 4:1) were rarely chosen by players of units. Functional food of the Carbo type (carbohydrates) was slightly more frequently chosen by defensive players. Then sportsmen were asked about substances improving concentration. Defensive and offensive units were compared revealing statistically significant differences (p=0.02) in the regularity of their consumption among the players, which were presented in Table 4.

Table 3. Comparison of the frequency intake of sports nutrition supplements

Type of supplement	Defensive (n=24)		Offensive(n=20)		p
	n	%	n	%	
Protein supplements	16	67	8	40	0.07
Protein and carbohydrate suppl.	1	4	2	10	0.44
Gainer type supplements	2	8	2	10	0.84
Carbo type supplements	8	35	7	33	0.90

Table 4. Comparison of the frequency of intake of supplements to improve concentration

Frequency of intake	Defensive (n=24)		Offensive (n=20)		P
	n	%	n	%	
Regularly	5	21	4	20	0.02
Irregularly	7	29	2	10	0.02
Rarely	11	46	6	30	0.02
I do not use	1	4	8	40	0.02

It was demonstrated that 46% of defensive players rarely took these preparations, while only 21% did this regularly. Most offensive players (40%) did not use these preparations, while only 20% took them regularly. The players were also asked about the type of preparations consumed to improve concentration. The results obtained were shown in Table 5.

Table 5. Comparison of the type of intake of supplements to improve concentration

Type of supplement	Defensive (n=24)		Offensive (n=20)		P
	n	%	n	%	
Coffee	14	58	9	45	0.37
Guarana	0	0	1	5	0.26
Energy drinks	13	54	5	25	0.05
Other	4	17	1	5	0.22

Interestingly, when comparing the types of substances improving concentration, the differences revealed referred only to energy drinks. As many as 54% of defensive players and only 25% of offensive players drank these drinks (statistically significant differences p=0.05). Defensive players more often chose coffee, though these differences were statistically insignificant.

DISCUSSION

A properly balanced diet and supplements applied in justified cases influence beneficially the results achieved by American football players. Additionally, since a diet affects the players' body composition, it should be personalized and adjusted to the type of training and competition. This plays a significant role in forming players' cardiovascular and respiratory capacity [1].

The food players of both offensive and defensive units consumed did not provide the necessary amount of calories at the level required according to the norm of Estimated Average Requirement [12]. The offensive unit players providing the energy value at the level of 2471.9±838.9 kcal with a diet covered energy requirements only in 69% of EAR norm, while the defensive unit players in 80% (3086.1±908.9 kcal/24hs). Taking into consideration intensive trainings, a daily energy intake should reach about 3850 kcal in a defensive unit and 3600 kcal in an offensive

unit. A similar conclusion was drawn in the study of *Borrows* et al. in 2016 year, assessing the consumption of individual nutritional components among 25 rugby players [3]. In this study, a mean energy value of a diet equaled 2450 kcal, which was similar to the value obtained in our study. Since rugby players were preparing to a season, a coefficient of physical activity was established at the Physical Activity Level (PAL)= 1.4 (compared to 1.75 in our study). The conclusion can be drawn that a similar calorie value of players' diet resulted from a similar amount and intensity of trainings. Players' diet should be personalized with regard to requirements depending on their position in the playing field and physical efforts undertaken as well as the kind of a training season [7].

A total protein, vegetable and animal protein consumption is essential in sportsmen' diet, mainly, when trainings are based on strengths exercises. The proper amount of this component influences the regeneration of the muscle fibers damaged during intensive training or competition. In our study, players more frequently chose food products providing animal than vegetable protein. The mean total protein consumption was higher in a defensive unit (156.3±42.7 g/24hs) than in a offensive unit (127.34±37.7 g/24 hs). The studies carried out by *Berning* in 2015 year showed the mean protein consumption of 131-139 g in the group of American football players [2], which was a slightly lower than the values obtained in our study. American football players should consume on average 1.6-1.7g/kg of the body mass. Our results indicate that a protein intake in grams per kilogram of the body mass was 1.6 g in defensive players, whereas only 1.4 g in offensive players. Defensive players' requirement of total protein over their body mass and the type of physical efforts performed should equal about 172 g/day, while offensive players required about 151 g/day [11]. Thus, as our study showed the norm for this component requirement was not covered. The players' menus should be varied complying the food products being the source of vegetable protein simultaneously adjusting animal protein intake with regard to players' individual requirement.

Lipids were another nutrient analyzed in the study. The consumption of total lipids, saturated, monounsaturated fatty acids and cholesterol was higher in a defensive unit than in an offensive unit. Total lipid consumption per a kilogram of the body mass equaled on average 1.1 g in defensive players, while 0.95 g in offensive players. Similar values were reported in the study of *Potgieter* et al. from 2014 year carried out in the group of rugby players [19]. This study showed that the mean consumption of lipids was 97g/ 24 hs, which constituted about 1g/kg of the players' body mass. When comparing our and *Potgieter's* et al. study results, it was found that the data obtained were very close and consumption of lipids was at the similar level oscillating within a proper range in these players. However, the level of cholesterol consumption is disturbing. In our study, its mean level was 667.81±300 mg in defensive players and 546.2±285 mg in offensive players. Its maximum consumption per day was 1176.2 mg and 1158 mg in both groups, respectively. In the study from 2014 year mentioned above, the mean daily consumption of food cholesterol was 766.3±371.8 mg, which was very close to the results presented in our study. In both studies, this component consumption was very high, significantly exceeding the norm of 300 mg/24 hs [12]. Planning players 'diet, the intake of products with cholesterol and saturated fatty acids should be personalized. A healthy 19-30 year-old man, physically active at the coefficient of PAL= 1.75 should consume on average 120 g of lipids in total per 24 hours (the body mass about 90 kg). At the same time, each player should have the intake of mono and polyunsaturated acids personalized, which may protect against cardiovascular diseases [12].

Carbohydrates should be the main source of energy in a rational personalized diet of healthy people. This component is a basis of a sportsman's properly balanced diet, especially, when he does endurance sport, where a level of glycogen in the body is very important.

In our study, defensive players consumed significantly higher amount of carbohydrates (373.8±117.2 g) compared to offensive players (317.8±123.3 g), though still too low in comparison to the norm. In our study, consumption of carbohydrates was about 3.7 g/kg/bw/24 hs in defensive players and about 3.6 g/kg/bw/24 hs in offensive players. In *Tooley's* et al. study [23], diets of ten professional rugby players were analyzed, defining the mean consumption of total carbohydrates as 476,77 g (about 4.9 g/kg of a player's body mass). These levels were higher than in our study. According to American College of Sports Medicine, carbohydrates intake should be about 6-10 g over a kilogram of the body mass in 24 hours in a diet of people active physically, especially, taking into account such a discipline like American football. The ratio of carbohydrates intake to proteins intake should be 4:1 or 3:1.The results of our study showed that consumption of carbohydrates was insufficient, which might have caused a worse response to training or faster fatigue [18].

In many sports, food fiber consumption can be improper due to intestinal disorders caused by its excess. Players being afraid of diarrhea give up the products with fiber. In our study, the mean daily consumption of this component was 25.5±7.9 g in defensive players and 21.4±6.9 g in offensive players. In the study referring to fiber consumption among rugby players,

the daily mean consumption of food fiber equaled 36.4±8.1 g [14]. These were obviously higher levels than those obtained in our study. Based on this data, it can be concluded that fiber consumption should be higher, especially, in the offensive unit. Players should be educated about the products providing fiber and its proper personalized amount adjusted to training seasons and competition.

An adequate level of calcium, magnesium and iron consumed is necessary for a sportsman's organism to function properly. Calcium affects beneficially the bone mass formation and the calcium-phosphorous product in the body. Magnesium with potassium prevents from cumbersome and frequent cramps (especially in lower extremities) occurring after trainings. Iron, a main component, responsible for a proper structure and functions of erythrocytes, prevents from microcytic anemia.

In our study, the daily mean consumption of minerals covered the norm of EAR. The mean consumption of calcium was 1043.1±769.9 mg among defensive players and 847.5±547.3 mg among offensive players. The norm of EAR for this component equals 800 mg for healthy and physically active men aged 19-30 years [12]. The daily mean consumption of magnesium was 491.6±158.2 mg in defensive players and 429±170 mg in offensive players. These were levels covering completely the requirement compared to the norm of EAR for this product, equaling 330 mg [12]. In our study, players exceeded significantly the norm of EAR for iron consumption, 6 mg [12], with the mean consumption of 19±10.5 mg in the defensive unit and 15.25±9 mg in the offensive unit. Taking into consideration these results, the conclusion can be drawn that the requirement for minerals listed above was covered in sportsmen's menus. The increased level of magnesium and iron consumption exceeding the norm may result from consuming the fortified and functional food.

However, sportsmen's menus are not always balanced with regard to the content of mineral components. In the study carried out among rugby players, *Imamura* et al. showed different results; the mean consumption of calcium was 668±268 mg, magnesium - 311±81 mg and iron - 8.7±2.9 mg [10]. Obviously, these were lower levels than those obtained in our study and not covering the recommended amount. This could result from an improperly balanced diet, poor in the sources of these components or lacking the fortified or functional food usually consumed by sportsmen.

American football players' intensive physical efforts may cause deficiency of D vitamin in the body. These sportsmen not usually consuming D- vitamin-rich products, training and competing indoors, which makes D vitamin production in the skin impossible, are, especially, prone to this condition [13]. In our study, the mean consumption of vitamin D in a daily diet was 4.6±2.5 µg in defensive players, while 3.8±2.3 ug in offensive players. Our study showed that players 'menus should be varied, containing D vitamin rich products, due to significantly lower its consumption compared to the norm of EAR equaling 10 µg/24 hs [12]. *Garrido* obtained similar results in the study assessing vitamin D intake in a diet of soccer players [9]. The consumption of this component was compared in the usual diet and the diet prepared and balanced for the sports team. The results revealed that the mean vitamin D consumption was 3.2±1.6 µg in the imbalanced diet and 5.9±2.0 µg in the diet customized to sportsmen. Apart from the balanced diet, an additional advantage is exposure to the sun during sports competitions held outdoors. The attention should be turned to rehydrating sportsmen in this period, because this sports activity may lead to dehydration [9].

It is commonly known that proper sportsmen's rehydration is a factor affecting their sports results, enabling the lower loss of mineral components and preventing from a very dangerous phenomenon, dehydration. Defensive players consumed higher amounts of liquids during a day (1925 ml) than offensive players (1750 ml). According to the literature, the mean total amount of liquids consumed (water and drinks for sportsmen), only during the game, that is, a training unit lasting 3 hours, should be about 2200-2600 ml [15]. As it was observed in our study, the results are surprisingly low compared to the recommendations for American football players or soccer players. The differences in the liquids drunk may to some extent depend on the season of the year when the studies were performed. Our study took place in winter, while in the studies compared to ours, trainings were assessed at temperature of about 25°C [15].

The amount of sodium consumed in a diet was also essential together with an adequate level of rehydration. Intensive trainings and games cause a huge loss water and sodium with sweat. However, the amount of sodium should not exceed the recommended level, because this may contribute to developing hypertension. In the study from 2014 year, *Żyła* et al. reported that rugby players consumed on average 3369.5±1392.8 mg of sodium [26]. The level was lower than our study (a defensive unit, 5195.3±1650.2 mg and an offensive unit, 4061.2±1307 mg), which may suggest excessive consumption of sodium justified only during intensive trainings and games but regarded too high in a usual diet. When discussing a level of sodium consumption, the kind of drink consumed during the physical activity is important as well as highly mineralized water and sports drinks, which usually contain a big quantity of sodium.

Numerous sportsmen use dietary supplementation. Preparations are often chosen without the adequate knowledge and adjustment to the type of physical activity and players' life style. The discussion about probable supplementation should be based on the evaluation of a usual diet and quantity and the type of physical effort the players undertake. American football players were asked about vitamin and mineral preparations they took and the frequency of their consumption. Offensive players took them more willingly (65% of the study group) and most of them declared their consumption during the whole year. In 2013 year, *Casiero* et al. concluded that a balanced rich diet did not require additional supplementation with these preparations [4]. If a given component cannot be provided at the adequate level with a diet, preparations supplementing deficiency should be introduced. However, this should be consulted with a physician or a dietician. Dietary supplements should not be chosen, when there are no clear indications [8].

During intensive physical efforts, a huge loss of water and electrolytes occurs, mainly, when trainings and competitions are held at high temperatures. Dehydration is a very dangerous phenomenon that must be prevented from, because this condition affects negatively a player's efficacy in the game field and in critical situations may lead to death. The players of the study group were asked about drinking drinks intended for sportsmen. There is variety of products chosen more frequently than water. In the study group, 96% of defensive and 90% of offensive players declared using drinks for sportsmen (isotonic drinks, rehydrating enriched with vitamin and mineral complexes). However, these products were chosen only few times in a week, during longer and more intensive activities. As few as 2% of the study players reported drinking more than 2 liters of these products daily. In his study, *Shirreffs* indicated that drinking rehydrating preparations, including isotonic drinks is recommended in long strenuous efforts, when loss of electrolytes, mainly sodium, occurs [21]. This may prevent players from dehydration, mainly, during efforts at high temperatures. It should be underlined that players must be educated about the type of drinks chosen and necessity of drinking water, avoiding sweetened products which contain food coloring. Drinks containing glucose can be consumed during very intensive and long-term efforts.

Lack of time, intensive trainings and desire to build up muscles quickly predispose players to taking sports supplements. These available in the market contain various amounts of nutrients from protein, protein and carbohydrate of the Gainer type to the Carbo type supplements. Sportsmen often ignorantly take the amounts of some nutrients, inadequately to their needs, which may burden excessively their organisms. Protein supplements were chosen most willingly by both players of a defensive (67%) and offensive (40%) unit compared to other supplements for sportsmen. *Van Loon's* study (2013), proved that consumption of protein supplements combined with a rational personalized diet may increase muscles and improve the results among players of strength and endurance sport [25]. This affects beneficially training effectiveness and better results in the sports field. However, a supplement should never replace a balanced diet and be personalized for a sportsperson and their needs as well as completely safe.

Protein and carbohydrate supplements were chosen more rarely, only by 9% of the examined (n=3). This type of the supplement is characterized by the ratio, on average 50:50 of protein to carbohydrates. They are supplemented with complexes of vitamins and minerals. In the study group, a similar number of players (n=4) took the Gainer type andprotein and carbohydrate supplements. In the study, *Nacleiro et al.* examined the effect of taking protein and carbohydrate supplements on football players' sports achievements [16]. Each study participant took the preparation consisting of 53 g of carbohydrates and 14.5 g of proteins, enriched with 1.5g of L-carnitine and 5 g of L-glutamine. The rise in the fat-free body mass (FFM) of the players and their speed and effort tolerance were assessed in the study. It was established that taking these preparations may contribute to a rise in the fat-free body mass. However, no beneficial effect was proved in the relief of fatigue after intensive trainings, improved speed or fitness during training. Supplements and dietary supplements should not replace a balanced diet. Though, the supplementation of a player's menu with them should be taken into consideration when a caloric value of the food consumed is high and it should be fortified with some nutrients.

The Carbo type supplementation was the last preparation the players were surveyed about in the study. This preparation is used to supplement losses after an intensive physical effort and to rebuild glycogen reserve In our study, 33% of defensive players and 35% of offensive players used the type Carbo supplements (n=15). In the study of *Nicholas*, it was determined that using carbohydrate preparations may influence beneficially sportsmen's results via an increase in effort fitness, maintenance of proper glycemia level and conserving glycogen during an effort and restoring it in the muscles after the activity [18]. The necessity of the preparation chosen individually for each player during dietary consultation was underlined.

Energizing and concentration – improving products were assessed among the study participants. Coffee was the most frequently chosen energizing product (66% of the surveyed), while guarana, the most rarely chosen (only one player). Energy drinks

were chosen by 52% of the surveyed. *Tunnicliffe* et al. examined 270 players of various sports and assessed consumption of energizing preparations [24]. Caffeine the players took may contribute to the increased fitness endurance and their improved metabolism. The average caffeine consumption was 0.85±13 mg/kg in the players. The increased consumption of this substance was not revealed in any sport discipline. Coffee was drunk as frequently as caffeine consumed in the form of a preparation. The highest level of caffeine drunk with coffee was 193-895 mg/day. In the study, it was underlined that excessive consumption of coffee and its preparations may cause gastrointestinal problems among players, sleep disorders and go into interactions with other supplements.

Energizing drinks often contain caffeine or guarana and are enriched with vitamin and mineral complexes. In the study of 2013 year, *Del Coso* et al. showed consumption of these preparations among rugby players [5]. Sportsmen were given energizing drinks containing caffeine and without it. It was concluded that players taking energizing drinks with caffeine were faster in the field and ran longer distances compared to players from the placebo group. The emphasis was put on the moderate consumption of these preparations as a diet supplementation in the clear conditions as well as monitoring of their influence on the players organisms.

CONCLUSIONS

1. The defensive formation players were characterized by a higher intake of individual nutrients and more frequent intake of dietary supplements than offensive players.
2. The players' diet should be more balanced. Insufficient supply of energy, water, vitamin D, carbohydrates and dietary fiber has been observed. The consumption of these ingredients should be increased in both formations.
3. The study showed the adequate intake of magnesium, calcium and iron and a significantly higher level of sodium exceeding the norm, which needs to be corrected.
4. A high intake of cholesterol–rich products in both units indicates that players'diet must be modified via reduced consumption of saturated fatty acids and increased consumption of polyunsaturated fatty acids.
5. Players' menus should be more varied including products that are the source of vegetable protein, simultaneously adjusting the intake of animal protein depending on players' individual needs.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSESSING THE EFFECT OF SUGAR TYPE AND FORM OF ITS INTAKE ON SELECTED PARAMETERS OF CARBOHYDRATE-LIPID METABOLISM AND PLASMA ATHEROGENIC INDICES IN RATS

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ABSTRACT

Background. Over the past 50 years, the average consumption of sugar worldwide has tripled, also the type of consumed sugar has changed. Due to high price of sucrose and its technological disadvantages, high fructose corn syrup (HFCS) has become one of the most commonly used substitutes.

Objective. The aim of the study was to compare, on the animal model, the effect of sugar type (sucrose vs high fructose corn syrup 55% of fructose) and the sugar form (solid vs fluid and solid) on the chosen parameters of carbohydrate-lipid metabolism.

Material and methods. The experiment was carried out on 40 *Wistar* male rats aged 5 months, fed four isocaloric diets, containing: group I (SUC 15%) fodder with 15% sucrose, group II (HFCS 15%) fodder with 15% HFCS-55%, group III (SUC 7.5%+7.5%) – 7.5% sucrose in solid fodder and 7.5% sucrose water solution, group IV (HFCS 7.5%+7.5%) – 7.5% HFCS-55% in solid fodder and 7.5% HFCS water solution.

Results. The effect of HFCS-55 on the parameters of carbohydrate and lipid metabolism was not equivalent of the effect of sucrose. Dietary use of HFCS-55 instead of sucrose causes adverse changes in blood parameters of carbohydrate and lipid metabolism, particularly when provided in beverages, as at comparable weight gains to that of sucrose. More intense changes, manifesting in increased blood levels of glucose, triglycerides and uric acid, as well as increased liver fat content, were observed at simultaneous intake of sweeteners in solid foods and fluids, even with less sugar consumption, compared to solid food only.

Conclusions. Dietary use of HFCS-55 causes adverse changes in blood parameters of carbohydrate and lipid metabolism, as at comparable weight gains to that of sucrose. But liquid form of sugar intake is more important insulin resistance and cardiovascular disease risk factor than the sugar type.

Key words: *carbohydrate-lipid metabolism, HFCS, sucrose, plasma atherogenic indices*

STRESZCZENIE

Wprowadzenie. Konsumpcja cukrów przypadająca na statystycznego mieszkańca Ziemi na przestrzeni ostatnich pięćdziesięciu lat zwiększyła się trzykrotnie, zmienił się także rodzaj spożywanych cukrów. Ze względu na wysoką cenę sacharozy i jej wady technologiczne, wysokofruktozowy syrop kukurydziany (HFCS) stał się jednym z najczęściej używanych jej substytutów.

Cel badań. Celem pracy było porównanie, na modelu zwierzęcym, wpływu rodzaju cukru (sacharoza vs syrop fruktozowo-glukozowy 55%) oraz sposobu ich podania (pasza/płyn) na wielkość spożycia paszy, przyrosty masy ciała, odkładanie tkanki tłuszczowej oraz stężenia wybranych składników przemian węglowodanowo-lipidowych we krwi.

Materiał i metody. Doświadczenie przeprowadzono na 40 samcach szczura rasy Wistar w wieku 5 miesięcy, żywionych izokalorycznymi paszami: grupa I (SUC 15%) – paszą z 15% dodatkiem sacharozy, grupa II (HFCS 15%) – paszą zawierającą 15% syropu fruktozowo-glukozowego 55%, grupa III – (SUC 7.5%+7.5%) – paszą zawierającą 7.5% sacharozy i 7.5% roztworu sacharozy podane w płynie, grupa IV (HFCS 7.5%+7.5%) – paszą zawierającą 7.5% HFCS-55% i 7.5% roztworu HFCS podane w płynie.

Wyniki. Wpływ syropu fruktozowo-glukozowego 55% na badane parametry gospodarki węglowodanowo-lipidowej nie był równoważny z wpływem sacharozy. Zastosowanie w diecie syropu fruktozowo-glukozowego 55% zamiast sacharozy, powoduje niekorzystne zmiany parametrów węglowodanowo-lipidowych krwi, zwłaszcza jeśli podawany jest on w formie napoju, przy porównywalnym do sacharozy wpływie na przyrosty masy ciała. Bardziej intensywne zmiany, manifestujące się wyższym stężeniem glukozy, triglicerydów i kwasu moczowego we krwi, a także zwiększoną zawartością tłuszczu

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w wątrobie, obserwowano przy jednoczesnym przyjmowaniu cukrów w pokarmach stałych i płynach, nawet przy mniejszym spożyciu cukru, w porównaniu do podawania ich tylko z pokarmem stałym.
Wnioski. Zastosowanie w diecie HFCS-55 powoduje niekorzystne zmiany w parametrach węglowodanowo-lipidowych krwi, przy porównywalnych przyrostach masy ciała jak przy podaniu sacharozy. Jednak płynna forma podania cukrów jest większym czynnikiem ryzyka insulinooporności i chorób sercowo-naczyniowych niż rodzaj cukru.

Słowa kluczowe: *metabolizm węglowodanowo-lipidowy, HFCS, sacharoza, wskaźniki aterogenności osocza*

INTRODUCTION

Over the past 50 years, the average consumption of sugar worldwide has tripled, also the type of consumed sugar has changed [25, 44]. Due to high price of sucrose and its technological disadvantages, high fructose corn syrup (HFCS) has become one of the most commonly used substitutes. Recently, the use of HFCS in food production in the Europe has increased by almost 100% [7].

Taking into account the time coincidence between the common introduction of HFCS in food production and observed increasing prevalence of obesity, the hypothesis was made that HFCS is one of the culprits for obesity [11, 38]. Moreover, such suggestions were made as some researchers equate the effect of HFCS with the one of pure fructose or high-fructose diet, of which negative effect on the lipid metabolism has been confirmed in numerous studies [21]. However, considering the composition of HFCS-42 (42% fructose) and HFCS-55 (55% fructose), the commonly used name 'high-fructose syrup' is misleading as they contain the amount of fructose similar to sucrose.

Yet still, causative links between the common use of HFCS and increasing prevalence of obesity are tested. So far, a correlation between the body weight and HFCS consumption in certain cases has not been assessed, because individual consumption of HFCS has not been monitored. Moreover, the experiments carried out did not result in unequivocal answers. Some of them show that the metabolic effects differ depending on the form of dietary intake, i.e. solid versus fluid [32]. Therefore, it was decided to test on an animal model how the intake of HFCS would affect the metabolism of carbohydrates and lipids and compare it to the effect of sucrose with regard to the form of sugar intake.

Studies on the effect of sucrose and HFCS on carbohydrate-lipid metabolism were carried out by *Sadowska & Bruszkowska* [34], using a 10% addition of the above mentioned sugars in solid form in the diet. They found a varied effect of the sugars used on the parameters tested. In our research, we decided to use the 15% amount of sugars and compare the intake of solid sugars in diet to sugars in solid and fluid form. The amount and form of sugars used reflects the average human intake. The greater amount of sugars can mask the differences in their impact caused by their different composition.

The aim of the study was to compare, on an animal model, the effect of sugar type (sucrose vs HFCS-55) and the form of its administration (solid vs fluid and solid) on the amount of consumed feed, body weight gain, fatty tissue depots, blood concentration of certain products of carbohydrate and lipid metabolism and plasma atherogenic indices.

MATERIAL AND METHODS

The experiment, after approval of the Ethics Commission (Approval no. 5/2013), was carried out on 40 Wistar male rats aged 5 months, of initial body weight 349 ±15.1 g.

Following a week long conditioning, the animals were sorted into four groups (n=10) of equal body weight (349.6 ±14.7 g), housed in individual cages, fed *ad libitum* on pelleted feeds.

Group I (SUC 15%) received fodder 1 containing 15% sucrose, group II (HFCS 15%) – fodder 2 containing 15% HFCS-55%, group III (SUC 7.5%+7.5%) – fodder 3 with 7.5% sucrose, group IV (HFCS 7.5%+7.5%) – fodder 4 containing 7.5% HFCS-55%. Selection of HFCS-55% resulted from the fact that it contains more fructose (than HFCS-42), which exerts adverse metabolic effects (than glucose) and is more popular in food technology. Fodders were designed to reflect the changes taking place today in the composition of diets, which contains simple sugars and refined carbohydrates. Detailed composition of fodders used in the experiment are presented in Table 1. The prepared fodders were subjected to chemical analysis [3] to determine the contents of total nitrogen (by *Kjeldahl's* method, on *Kjeltec* 2100 apparatus), converted to quantity of protein, crude fat (by *Soxhlet's* method, on Soxtec 1046 apparatus), dry matter, ash (by a gravimetric method) and fiber (in an ANKOM 220 apparatus). The content of digested carbohydrates was calculated from the difference between dry matter and the remaining solid components – Table 1. The metabolic energy was calculated using commonly applied energy equivalents: protein – 4.0 kcal/g (16.76 kJ/g), fat – 9.0 kcal/g (37.71 kJ/g), digested carbohydrates – 4.0 kcal/g (16.76 kJ/g).

Table 1. Component and chemical composition of fodders used in the experiment

Component	Fodder 1	Fodder 2	Fodder 3	Fodder 4
Wheat (g/100 g)	6	6	6	6
Corn grain (g/100 g)	10	10	10	10
Wheat bran (g/100 g)	20	20	20	20
Dry whey (g/100 g)	3	3	3	3
Fodder salt[1] (g/100 g)	0.3	0.3	0.3	0.3
Soya-bean grain (g/100 g)	17	17	17	17
Fodder chalk[2] (g/100 g)	1.5	1.5	1.5	1.5
Phosphate 2-CA[3] (g/100 g)	0.8	0.8	0.8	0.8
Premix LRM[4] (g/100 g)	1	1	1	1
Wheat flour (g/100 g)	25.4	25.4	32.9	32.9
Sucrose (g/100 g)	15	-	7.5	-
High fructose corn syrup-55 (in powder) (g/100 g)	-	15	-	7.5
Total protein (%)	15.3±0.54	15.3±0.62	16.1±0.71	16.2±0.48
% of diet energy	18.6	18.4	19.6	19.7
Crude fat (%)	2.91±0.11	2.99±0.09	3.15±0.22	3.16±0.12
% of diet energy	8.0	8.10	8.64	8.64
Carbohydrates (%)				
total	65.5±0.54	66.2±0.48	63.8±0.51	64.0±0.62
fiber	5.36±0.13	5.21±0.15	5.07±0.09	5.08±0.10
digested	60.1±0.51	61.0±0.45	58.7±0.54	59.0±0.65
% of diet energy	73.3	73.5	71.6	71.7
Dry matter (%)	89.1±0.20	89.9±0.12	88.7±0.17	89.0±0.11
Total ash (%)	5.38±0.14	5.38±0.10	5.67±0.08	5.59±0.09
Metabolizable energy	3.28±0.03	3.32±0.04	3.28±0.07	3.29±0.02
(kcal/g) (kJ/g)	13.7±0.20	13.9±0.19	13.7±0.22	13.8±0.17
Part of energy from added sugars in fodder (%)	18.3	18.1	9.1	9.1
Part of energy from added sugars in diet* (%)	18.3	18.1	18.3	18.2
including fructose (%)	9.15	9.95	9.15	10.0

[1]- Mainly NaCl, [2]- Mainly $CaCO_3$, [3]- $CaHPO_4$, [4]- Vitamin-mineral composition used in animals feeds content per kg: IU: A 1500000, vit. D3, 100000; mg: vit. E 8000; vit. K 300, vit. B1 1200, vit. B2 1200, vit. B6 1000, vit. B12 8, Se 100, Fe 16000, Mn 4500, Zn 6000, Cu 1300, I 100, Co 200; * - from fodder and fluid

For drinking animals from I and II group received settled tap water, animals in group III received 25 ml of 7.5% sucrose water solution, and the animals of group IV - 25 ml of 7.5% HFCS-55% water solution. After drinking sugars solutions animals received settled tap water. The application rate of solutions and the concentration of sugars in them calculated so that the consumption of added sugars in all groups of animals was similar and amounted to approx. 18% of the energy value of the diet. This amount was used, taking into account the composition of the modern diet of many people in the diet which the share of added sugars is up to 20%, provided both in the solid foods and beverages [41].

Analyses

The experiment lasted for six weeks, the amount of diets consumed by the animals were recorded daily, once a week the animals were weighted (Radwag PM 10.C32 Precision Scales). On the completion of the experiment, the animals were fasted overnight (12 h), and anaesthetised with an intramuscular injection (10 mg/kg b.m.) of Ketanest (Pfizer Ireland Pharmaceuticals). During fasting, the animals of all groups only had access to water. Blood was sampled from heart to tubes with anticoagulant and centrifuged, at 2000 g for 10 min. at 4ºC. Plasma samples were stored at 4ºC and assayed within 24 h.

Peri-cardial, intraperitoneal and retroperitoneal fat was dissected and weighted. Thigh muscles (*triceps femoris*) and livers were dissected and used to determine the percentage of crude fat, with the Soxhlet technique in Soxtec HT6 apparatus (Foss Tecator).

Blood plasma was assayed for the concentration of glucose (BioSystems ref. no 11503), triglycerides (BioSystems ref. no 11528), HDL-chol. (BioSystems ref. no 11557), LDL-chol. (BioSystems ref. no 11579), total cholesterol (BioSystems ref. no 11505), uric acid (BioSystems ref. no 11521) with colorimetric method on the Metertech spectrophotometer. Insulin (ref. no DE2048, Rat ELISA kit Demeditec Diagnostics, Kiel, Germany) was assayed using a monoclonal antibody against rat insulin. To quantify insulin resistance and beta-cell function homeostatic model assessment

(HOMA) was used, where: HOMA-IR = fasting glucose [mmol/L] x fasting insulin [mU/L] / 22.5.

The plasma atherogenic ratios were calculated as follows [4]:

Atherogenic Index of Plasma (AIP) = log {TG [mmol/L]/ HDL-C [mmol/L]}

Castelli's Risk Index I (CRI-I) = TC [mmol/L]/HDL-C [mmol/L]

Castelli's Risk Index II (CRI-II) = LDL-C [mmol/L]/ HDL-C [mmol/L]

Atherogenic Coefficient (AC) = {TC [mmol/L]– HDL-C [mmol/L]}/HDL-C [mmol/L].

Statistics

The resulting data, after checking normality of distribution (*Shapiro-Wilk* test) and homogeneity of variance (*Leven's* test), were analyzed by two-way ANOVA statistics for factorial designs, with regard to sugar type (ST) and sugar form (SF) of intake. If the interaction occurred, the post hoc *Tukey's* test was used. Analyzes were carried out in the Statistica 12.0® computer program.

RESULTS

Analysis of the obtained results has revealed, that the type of sugar had no statistically significant effect on the feed and energy intake (Table 2). The feed intake (g/100 g body weight/day) was affected by the sugar form and the interaction occurred between sugar type and sugar form. Administration of sugars in the liquid decreased feed intake, but statistically significant differences were observed only in animals fed on sucrose compared to the other groups.

Table 2. Effect of diet type on feed and energy intake in rats, $\bar{x}$ ± SD, n = 40

Trait	SUC 15%	HFCS 15%	SUC 7.5+7.5%	HFCS 7.5+7.5%	ST	SF	I STxSF
Feed intake (g/day)	29.3 ± 1.82	28.7 ± 1.61	26.3 ± 1.84	27.2 ± 1.55	p≤0.239	p≤0.000**	p≤0.171
Feed intake (g/100 g body weight/day)	6.31 ± 0.21[b]	6.14 ± 0.25[b]	5.57 ± 0.18[a]	5.83 ± 0.38[b]	p≤0.591	p≤0.000**	p≤0.018*
Energy value of feed consumed (kcal/day)	97.5 ± 6.14	98.3 ± 5.52	87.2 ± 6.21	92.1 ± 5.29	p≤0.120	p≤0.000**	p≤0.259
Energy value of liquid consumed (kcal/day)	0	0	6.55 ± 0.60	6.16 ± 0.94	p≤0.272		
Energy value of diet (kcal/day)	97.5 ± 6.14	98.3 ± 5.52	93.7 ± 5.92	98.3 ± 5.39	p≤0.143	p≤0.302	p≤0.300
Energy value of diet (kcal/100 g b.w./day)	21.4 ± 0.72	21.1 ± 0.86	20.3 ± 0.53	21.1 ± 1.27	p≤0.088	p≤0.364	p≤0.053
Added sugar intake in fodder (g/day)	4.39 ± 0.32[b]	4.3 ± 0.12[b]	1.97 ± 0.22[a]	2.04 ± 0.10[a]	p≤0.359	p≤0.000**	p≤0.355
Added sugar intake in fluid (g/day)	0	0	1.64 ± 0.18	1.54 ± 0.15	p≤0.469		
Total added sugar intake (g/day)	4.39 ± 0.32	4.3 ± 0.12	3.61 ± 0.28	3.58 ± 0.40	p≤0.259	p≤0.039*	p≤0.371
Total added sugar intake (g/100 g b.w. /day)	0.96 ± 0.058	0.92 ± 0.074	0.78 ± 0.051	0.77 ± 0.061	p≤0.339	p≤0.034*	p≤0.472

a, b – means denoted different letters in the same line are statistically different, p≤0.05.
*, ** - statistical difference, * p ≤ 0.05, ** p ≤ 0.01.
ST – sugar type, SF – sugar form, I STxSF – interaction sugar type x sugar form, SUC – sucrose groups, HFCS – high fructose corn syrup groups.

It was found that sugar type and the form of its intake had no statistically significant effect on the weight gains in tested animals, nor on the amount of periorgan fatty tissue and muscle fat (Table 3). But significant influence of the sugar form and the interaction between the sugar type and form of its administration on the hepatic fat content were found. The hepatic fat content was higher in animals received sugars in feed and fluid compared to those receiving them only in feed. The liver fat was the highest in the SUC 7.5% + 7.5% group and the lowest in the SUC 15% group.

Table 3. Effect of diet type on body weight gain and fat deposition in male rats, $\bar{x}$ ± SD, n = 40

Trait	SUC 15%	HFCS 15%	SUC 7.5+7.5%	HFCS 7.5+7.5%	ST	SF	I STxSF
Initial body weight (g)	349.3 ± 15.0	349.6 ± 13.7	351.1 ± 14.6	348.7 ± 15.7	p≤0.920	p≤0.822	p≤0.772
Final body weight (g)	456.3 ± 28.9	466.6 ± 25.9	461.1 ± 26.8	464.7 ± 14.8	p≤0.335	p≤0.835	p≤0.688
Body weight gain (g/6 weeks)	107 ± 21.1	117 ± 26.5	110 ± 14.7	116 ± 13.8	p≤0.175	p≤0.855	p≤0.781
Body weight gain (g/100 kcal)	2.60 ± 0.341	2.83 ± 0.544	2.79 ± 0.245	2.83 ± 0.353	p≤0.259	p≤0.435	p≤0.449
Peri-cardial fat (g)	0.678 ± 0.157	0.647 ± 0.093	0.732 ± 0.154	0.710 ± 0.111	p≤0.517	p≤0.157	p≤0.914
Peri-cardial fat (g/100 g body weight)	0.147 ± 0.03	0.140 ± 0.02	0.160 ± 0.04	0.153 ± 0.02	p≤0.344	p≤0.132	p≤0.918
Peri-cardial fat (mg/100 kcal)	16.4 ± 3.14	15.7 ± 1.99	18.7 ± 4.50	17.2 ± 2.21	p≤0.231	p≤0.059	p≤0.687
Peri-intestinal fat (g)	1.618 ± 0.268	1.740 ± 0.188	1.628 ± 0.291	1.823 ± 0.270	p≤0.054	p≤0.565	p≤0.653
Peri-intestinal fat (g/100g body weight)	0.354 ± 0.054	0.372 ± 0.030	0.352 ± 0.056	0.392 ± 0.058	p≤0.072	p≤0.582	p≤0.493
Peri-intestinal fat (mg/100 kcal)	39.4 ± 5.78	42.2 ± 4.19	41.1 ± 5.62	44.2 ± 6.61	p≤0.106	p≤0.298	p≤0.921
Muscle fat (%)	3.16 ± 0.321	3.37 ± 0.516	3.16 ± 0.250	3.36 ± 0.441	p≤0.117	p≤0.996	p≤0.942
Liver fat (%)	2.86 ± 0.111[a]	2.99 ± 0.095[b]	3.20 ± 0.120[c]	3.09 ± 0.105[bc]	p≤0.693	p≤0.000**	p≤0.001**

a, b, c – means denoted different letters in the same line are statistically different, p≤0.05.
** - statistical difference, p ≤ 0.01.
ST – sugar type, SF – sugar form, I STxSF – interaction sugar type x sugar form, SUC – sucrose groups, HFCS – high fructose corn syrup groups.

The sugar type and form affected statistically significantly plasm concentration of glucose and triglycerides – table 4. They were higher in animals on HFCS-55 diet comparing to those on sucrose diet, and in animals receiving sugars in fodders and fluids comparing to those on solid sugars only (Table 4). The form of sugar intake had a statistically significant effect on the insulin concentration and the HOMA-IR index value. They were higher in animals provided with solid and fluid sugars, even with less sugar consumption, compared to those fed on solid sugars only.

Table 4. Effect of diet type on glucose, lipids, the value of atherogenic indices and uric acid level in plasma of male rats, $\bar{x} \pm$ SD, n = 40

Trait	SUC 15%	HFCS 15%	SUC 7.5+7.5%	HFCS 7.5+7.5%	ST	SF	I STxSF
Glucose (mmol/L)	7.88 ± 0.74	8.52 ± 0.96	8.60± 0.57	8.93 ± 0.86	*p≤0.041	*p≤0.039	p≤0.472
Insulin (mU/L)	6.54 ± 1.41	5.82 ± 1.15	7.97 ± 1.0	8.23 ± 1.63	p≤0.523	*p≤0.012	p≤0.312
HOMA-IR	2.29 ± 0.51	2.20 ± 0.47	3.05 ± 0.61	3.27 ± 0.39	p≤0.689	*p≤0.026	p≤0.283
Triglycerides (mmol/L)	0.333 ± 0.052	0.426 ± 0.092	0.446 ± 0.075	0.522 ± 0.088	**p≤0.001	**p≤0.000	p≤0.743
Total cholesterol (mmol/L)	2.12 ± 0.31	2.0 ± 0.16	2.05 ± 0.30	2.14 ± 0.23	p≤0.889	p≤0.648	p≤0.223
HDL-chol. (mmol/L)	0.85 ± 0.20	0.87 ± 0.14	0.79 ± 0.08	0.79 ± 0.13	p≤0.888	p≤0.112	p≤0.764
LDL-chol. (mmol/L)	0.91 ± 0.09	0.88 ± 0.11	0.85 ± 0.09	0.94 ± 0.11	p≤0.302	p≤0.982	p≤0.084
AIP	-0.420 ± 0.044	-0.333 ± 0.049	-0.254 ± 0.084	-0.179 ± 0.120	**p≤0.001	**p≤0.000	p≤0.822
CR-I	2.44 ± 0.33	2.33 ± 0.25	2.59 ± 0.36	2.78 ± 0.53	p≤0.664	*p≤0.017	p≤0.226
CR-II	1.05 ± 0.14	1.02 ± 0.10	1.08 ± 0.13	1.23 ± 0.53	p≤0.231	*p≤0.045	p≤0.129
AC	1.44 ± 0.33	1.33 ± 0.25	1.59 ± 0.36	1.78 ± 0.53	p≤0.664	*p≤0.017	p≤0.226
Uric acid (mg/dl)	0.997 ± 0.339	0.937 ± 0.311	1.256 ± 0.343	1.370 ± 0.405	p≤0.948	**p≤0.002	p≤0.537

*, ** - statistical difference, * $p \leq 0.05$, ** $p \leq 0.01$.
ST – sugar type, SF – sugar form, I STxSF – interaction sugar type x sugar form, SUC – sucrose groups, HFCS – high fructose corn syrup groups. AIP - Atherogenic Index of Plasma, CRI-I - Castelli's Risk Index I, CRI-II - Castelli's Risk Index II, AC - Atherogenic Coefficient.

Neither sugar type nor the form of its intake had any statistically significant effect on the concentrations of total cholesterol and its fractions (HDL and LDL). The form of sugar intake had a significant effect on the concentration of uric acid. Its higher concentration was observed in animals on sugars derived from solid foods and fluids comparing to those on sugars derived from solid foods only.

The form of sugar had a statistically significant effect on all the atherogenic indices. They were more atherogenic in animals provided with solid and fluid sugars compared to those fed on solid sugars only. Only AIP was decreased both by dietary HFCS and if added sugars were provided in a liquid and solid form (Table 4).

DISCUSSION

A lower intake of feed was observed in animals provided with sugar-sweetened fluid, but in this respect only the effect of sucrose was statistically significant. Similar results were obtained by *Tsanzi* et al. [40] and *Light* et al. [23] in female rats provided with HFCS-55 or sucrose-sweetened water.

Lower feed intake by animals provided with sugar solution resulted in the comparable energy intake in all the treatment groups. Such compensational decrease in energy intake from solid foods resulting from energy added in drinks was reported by *Roy* et al. [33] for rats, and by *Gadah* et al. [15] for humans. Studies conducted by *Chen* et al. [8] and *O'Connor* et al. [31], however, demonstrated that energy provided with drinks is considered additional one, it is not compensated by lower feed intake, which results in increased energy value of diet. Therefore, meeting the energy requirements in animals seems to be predominant in the regulation of food intake. Emotional factors, increasing significantly food intake in humans, are non-existent in the studies based on animal models. The lack of differences in the energy intake observed in this study could have resulted from the fact that the sweetened fluids were not provided

ad libitum but only in the amount providing 7.5% of dietary sugar, which was adequate to the sugar intake by rats only on solid foods.

Weight gains of the tested animals were not affected by the sugar type and form. Opposite results were obtained by *Bocarsly* et al. [5] who reported higher weight gains in animals receiving HFCS compared to animals on sucrose drinks. However *Bocarsly* et al. [5] provided only fluid sugars. The form of sugar intake may modify the rate of absorbing monosaccharides, elevate the glycemic effect and stimulate insulin release. Additionally, sweetened drinks may cause lower thermogenesis which contributes to a positive energy balance [14]. The lack of differences in the weight gains in our experiment could have resulted from the high total sugar intake, regardless of sugar type. Sugars accounted for as much as 15% of diet, and weight gains were considerable in all the animals (ca. 110 g/6 weeks). In a similar study, but with different amount of sucrose (10%), *Goluch-Koniuszy & Sadowska* [17] observed weight gains in males at 42 g/6 weeks.

No differences between treatment groups were found in the amount of pericardial and per-intestinal fat. Also studies carried out by *Light* et al. [23] on female rats did not result in statistically significant differences in fatty depots around gonads or in the retroperitoneal space between the groups treated with 13% HFCS-55 or 13% sucrose. Sugar type had no statistically significant effect on the liver and muscle fat. Also *Bravo* et al. [6] stated that consumption of sucrose or HFCS does not increase liver fat or ectopic fat depots in muscles. But in this study the liquid sugar form increased liver fat content.

The sugar type and form of its intake affected statistically significantly the concentrations of glucose and triglycerides. They were higher in animals on the HFCS diet and in animals fed on sugars in solid foods and fluids. The crucial role in regulating fasting blood glucose concentration is played by the glucostatic function of liver in which the gluconeogenesis takes place. The process goes faster at lower insulin levels or with insulin resistance. Studies conducted by *Dirlewanger* et al. [10] demonstrated that fructose induces the hepatic insulin resistance and impaired inhibition of gluconeogenesis by insulin in liver. It has been also reported that fructose may reduce the synthesis of adiponectin which stimulates binding glucose to cells and inhibits gluconeogenesis [36].

The sugar type had no effect on insulin concentration and HOMA-IR index value. Similar results were also reported by *Monsivais* et al. [28] in rats, *Akhavan & Anderson* [2] in men, and *Melanson* et al. [27] in women and *Soenen & Westerterp-Plantenga* [37] in men and women.

The elevated concentration of blood glucose in male rats fed on the HFCS-55 could be also result of elevated concentration of triglycerides that decrease tissue sensitivity to insulin and stimulate glucose synthesis in liver. But on the other hand elevation of triglycerides may also be a consequence of insulin resistance. Considering higher insulin level and HOMA-IR index values in animals receiving sugars in feed and liquid, it can be assumed that higher triglycerides concentrations in these animals may be a consequence of insulin resistance.

Similar results were obtained by *Akar* et al. [1] and *Bocarsly* et al. [5]. They reported elevated triglyceride levels in the blood of male rats fed on HFCS-55 compared to those fed on sucrose. But no influence on the short term endocrine and metabolic effects of consuming HFCS-sweetened beverages stated *Heden* et al. [18].

The concentration of triglycerides reflects the amount of plasma VLDL. A number of studies demonstrate that fructose increased VLDL secretion [35]. Fructose also reduces the plasma elimination of VLDL [9]. Impaired hydrolysis of triglycerides from VLDL hinders their storage in fatty tissue and elevates their blood level. It explains why animals fed the HFCS-55 diet have changed lipid parameters in blood without significantly increased fatty tissue depots.

The difference between the amount of fructose derived from sucrose or HFCS in this study was not big and accounted for 9.3% (9.15% vs. 10.0%). However, the obtained results allow to state that due to multiplicity of mechanisms through which fructose may affect parameters of carbohydrate and lipid metabolism, adverse changes in these parameters may occur even at slight changes in dietary fructose content.

The elevated blood levels of triglycerides and glucose observed in animals receiving sugars in solid and fluid form, with slightly increased depots of periorgan fat may indicate that the animals were developing insulin resistance, which was confirmed by higher insulin concentration and a higher HOMA-IR index. *Ma* et al. [26] showed that regular sweet beverages intake is associated with an insulin resistance and a higher risk of developing prediabetes.

In this study no effect of the examined factors was observed on blood cholesterol levels and its fractions. Also *Akar* et al. [1], *Light* et al. [23] and *Lowndes* et al. [24] did not observe any HFCS effect of the levels of total cholesterol and HDL-cholesterol in male rats. The opposite results were obtained by *Figlewicz* et al. [13] and *Ferder* et al. [12].

In order to assess the CVD risk in this study, the following atherogenic markers were applied: CR-I, CR-II, AIP, and AC, which prove to be very useful

in estimating the CVD risk when LDL-C levels are similar [29, 19, 39]. All of the indices were work out for humans. There are no reference values for them in rats, but they can be useful in animals groups comparisons in terms of the risk of cardiovascular diseases. Values of these indicators were less favorable in rats receiving sugars in liquid and solid form. Sugar type has no effect. *Sadowska & Bruszkowska* [34] were found that using HFCS-55 instead of sucrose has an adverse effect on blood lipid parameters and plasma atherogenic indices. However, in their studies only solid sugars were used, and their amount was lower (10%).

The level of uric acid is often used as a marker for cardiovascular diseases. The acid is a key factor in the pathomechanism of forming and developing atherosclerotic plaque, and by causing endothelium dysfunctions it contributes to hypertension [20]. The study also demonstrated uric acid relation to the development of insulin resistance [43].

Similarly to this study, in the studies carried out by *Yu* et al. [42] and *Le* et al. [22] no difference in the effect of sucrose and HFCS was found on uric acid blood level. But in our study, the concentration of uric acid was influenced by the sugar form. It was higher when sugars were partly used in beverages. It was found in the studies conducted by *Nguyen* et al. [30] and *Gao* et al. [16] that excessive use of sugars derived from sweetened beverages is associated with elevated levels of plasma uric acid. Probably, the observed results were associated with a faster rate and other region of sugar absorption when the provided sugars were in fluid form.

The observed effect could also result from specific fructose metabolism. With excessive dietary intake of fructose, the use of adenosine triphosphate in phosphorylation of fructose increases. ATP breakdown results in elevated level of adenosine monophosphate which is the substrate for the uric acid synthesis, therefore its formation is enhanced.

In rats, uric acid is converted by urate oxidase to allantoin. In humans, uric acid is an end product of purine metabolism. Anthropoids and humans do not have urate oxidase activity, therefore purine metabolism ends with poorly soluble uric acid. Therefore, it may be assumed that elevated level of blood uric acid observed in the tested rats would be even higher in people.

CONCLUSIONS

The effect of HFCS-55 derived from diet on the parameters of carbohydrate and lipid metabolism was not equivalent of the effect of sucrose. Dietary use of HFCS-55 as a sweetener instead of sucrose causes adverse changes in blood parameters of carbohydrate and lipid metabolism, particularly when provided in beverages, as at comparable weight gains to that of sucrose.

Taking into account the value of HOMA-IR index value and atherogenic plasma indices, it was found that form of sugar intake is more important risk factor of insulin resistance and cardiovascular diseases than the sugar type. The HOMA-IR index value and atherogenic plasma indices were higher in animals provided with solid and fluid sugars, even with less sugar consumption, compared to those fed on solid sugars only.

Liquid form of sugar intake is more important insulin resistance and cardiovascular disease risk factor than the sugar type.

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Conflict of interest

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

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ORIGINAL ARTICLE

NUTRITION BEHAVIOURS AND THE OCCURRENCE OF DEPRESSIVE SYMPTOMS AMONG THE STUDENTS IN THE INSTITUTIONS OF HIGHER EDUCATION IN SILESIA (POLAND)

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ABSTRACT

Background. Depressive symptoms may appear at any age, yet they are most likely to be observed among young people aged 20-30. According to numerous scientific records, a properly balanced diet may prevent depression or enhance a therapeutical process.

Objective. The aim of the study was the assessment of nutrition behaviours and prevalence of depressive symptoms among the students, as well as investigation whereas there is a connection between these variables and the sexes of the examined group. Furthermore, the aim was to determine, whether there is a relation between proper nutrition behaviours and an intensification of depressive symptoms.

Material and methods. The research was conducted on a group of 959 students. In order to collect that data on nutrition behaviours an original anonymous questionnaire was used. In order to collect the data on a depressive symptoms occurrence Beck Depression Inventory (BDI-II) was used.

Results. Recommended number of meals was taken by 54.95% of students, including 59.20% of women and 48.56% of men. Only 6.25% of students, including 6.94% of women and 5.22% of men, ate a recommended number of 5 or more portions of fruit and vegetables. 11.16% of students, including 10.94% of women and 11.49% of men, drank at least 2 glasses of milk or dairy fermented products. 22.84% of students presented depressive symptoms. 11.57% of the examined people suffered from mild depression, 7.1% - of moderate depression and 4.17% – of a severe one.

Conclusions. It was found that there are relations between nutrition behaviours and sexes of the examined people. Women tend to present more beneficial behaviours. Almost every fourth student presented depressive symptoms – an intensification of the symptoms was more frequent among women. A growth in the rate of proper nutrition behaviours was connected with a drop in the depressive symptoms.

Key words: *nutrition, students, depression*

STRESZCZENIE

Wprowadzenie. Objawy depresyjne mogą pojawić się w każdym wieku, ale najczęściej młodych osób w wieku 20-30 lat. Z wielu doniesień naukowych wynika, że odpowiednio zbilansowana dieta może wykazywać działanie prewencyjne lub wspomagające proces terapeutyczny.

Cel badań. Ocena zachowań żywieniowych i częstości występowania objawów depresyjnych wśród studentów oraz sprawdzenie czy istnieją zależności pomiędzy tymi zmiennymi a płcią badanych osób. Ponadto celem było stwierdzenie, czy istnieje zależność pomiędzy prawidłowymi zachowaniami żywieniowymi a nasileniem objawów depresyjnych.

Materiał i metody. Badania przeprowadzono wśród 959 studentów. W celu zebrania danych dotyczących zachowań żywieniowych wykorzystano autorski anonimowy kwestionariusz ankiety, w celu zebrania danych dotyczących wystąpienia objawów depresyjnych wykorzystano skalę depresji Becka (BDI-II).

Wyniki. Zalecaną liczbę posiłków w ciągu dnia spożywało 54,95% studentów, w tym 59,20% kobiet i 48,56% mężczyzn. Jedynie 6,25% studentów, w tym 6,94% kobiet i 5,22% mężczyzn spożywało codziennie zalecane 5 lub więcej porcji warzyw i owoców. 11,16% studentów, w tym 10,94% kobiet i 11,49% mężczyzn wypijało co najmniej 2 szklanki mleka lub mlecznych produktów fermentowanych. Wykluczenie słodyczy zadeklarowało 2,09% studentów, w tym 1,39% kobiet i 3,13% mężczyzn,

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natomiast napojów słodzonych 11,37% studentów, w tym 15,63% kobiet i 4,96% mężczyzn. Objawy depresji stwierdzono u 22,84% studentów, depresja łagodna dotyczyła 11,57%, umiarkowana – 7,1%, ciężka – 4,17% badanych osób.
Wnioski. Stwierdzono, że istnieją zależności między zachowaniami żywieniowymi a płcią badanych. Kobiety mają tendencję do prezentowania korzystniejszych zachowań. Niemal co czwarty student prezentował objawy depresyjne - nasilenie objawów było częstsze u kobiet. Wzrost odsetka prawidłowych zachowań żywieniowych związany był ze spadkiem objawów depresyjnych.

Słowa kluczowe: *żywienie, studenci, kobiety, mężczyźni, depresja*

INTRODUCTION

In the previous decades, along with the growth of availability and diversity of nutrition, the level of knowledge about its influence on health changed. More and more importance is attached to the promotion of a healthy lifestyle, i.e. a combination of a well-balanced diet, a systematic physical activity, attention to a psychophysical balance and a lack of addictions [9]. Proper nutrition is one of basic factors which influence health as well as physical and psychological well-being. Proper nutrition is meant by regular meals and their optimal composition which covers organism's demand for energy and all the necessary nutrients. Therefore, daily nutrition habits are crucial, including food preferences, dietary patterns and the frequency of an intake of various food products [15].

According to numerous scientists, apart from social and environmental factors, it is a modern diet – poor in nutrients and mainly in vitamins and minerals, and rich in sugar, saturated fatty acids and *trans* type unsaturated fatty acids, that may have an impact on a higher rate of depression cases [2, 8, 20, 22, 25]. It was observed that such a diet often results in obesity which may lead to a higher risk of an occurrence of depression or its symptoms, especially among women [1, 2, 5]. Other authors claim that despite numerous gaps in the literature of this subject, there is strong evidence that a combination of healthy dietary patterns (including a higher intake of fruit, vegetables, legumes, whole-grain cereals, nuts and seeds, as well as a high consumption of food rich in polyunsaturated omega-3 fatty acids and a lower intake of processed food, fast food and sweets) may result in a lower risk of an occurrence of depression [13, 17, 23].

The aim of the research was to assess nutrition behaviours and estimate the frequency of an occurrence of depressive symptoms among the students in the institutions of higher education in Silesia, as well as the determination if there is a connection between these variables and the sexes of the examined groups. Furthermore, the aim was to determine whether there is a connection between proper nutrition behaviours and an intensification of depressive symptoms.

MATERIAL AND METHODS

The research was conducted on a group of 959 people including 576 (60.06%) women and 383 (39.94%) men, all of whom were students in the institutions of higher education in Silesia. 860 people (89.68%), including 487 (84.55%) women and 373 (97.39%) men, were the undergraduate students and 99 people (10.32%), including 89 (15.45%) women and 10 (2.61%) men were master-degree students or post-graduate student. 296 people including 213 (36.98%) women and 83 (21.67%) men studied Medicine or Medicine-related studies, and 663 (69.13%) people, including 363 (63.02%) women and 300 (78.33%) men attended studies not related to Medicine. 60 people (6.25%) were underweight, including 57 (9.90%) women and 3 (0.78%) men, 702 people (73.20%) maintained a healthy weight, including 460 (79.86%) women and 242 (63.19%) men. 154 (16.06%) students, including 49 (8.50%) women and 105 (27.42%) men were overweight. 41 (4.28%) people, including 10 (1.74%) women and 31 (8.09%) men were obese. 2 (0.21%) students did not reveal their body mass. Average BMI was 21.50 kg/m^2 (SD±3.00 kg/m^2) for women, and 24.49 kg/m^2 (SD±3.99 kg/m^2) for men.

The research was being conducted personally from May to June 2017. It was preceded by obtaining permission from the institution's authorities and the examined students. At the beginning of the research, participants were familiarized with an aim and a method of the research.

In order to collect that data on nutrition behaviours, an original anonymous questionnaire created and validated at the Department of Dietetics at Medical University of Silesia in Katowice, Poland was used. The questionnaire was composed of questions about nutrition habits, frequency of an intake of selected groups of food products, taken medicines, dietary supplements and physical activity, all of which with regard to 30 days preceding the examination. On the basis of a declared body mass (kg) and height (cm), Body Mass Index (BMI) was calculated for every examined student. It was interpreted in accordance with the criteria by the World Health Organization (WHO) [24]:

- < 18.5 kg/m^2 – underweight,
- 18.5-24.99 kg/m^2 – healthy body mass,
- 25.0-29.99 kg/m^2 – overweight,
- ≥ 30.0 kg/m^2 – obesity.

In order to collect the data on a depressive symptoms occurrence (also in the last 30 days before

the examination), Beck Depression Inventory (BDI-II) was used. The following criteria were taken into consideration:

- 0-13 points – no depressive symptoms,
- 14-19 points – an intensification of depressive symptoms at a mild level (mild depression),
- 20-28 points – an intensification of depressive symptoms at a moderate level (moderate depression),
- 29-63 points – an intensification of depressive symptoms at a severe level (severe depression) [19].

Results were processed in Microsoft Office Excel 2010. Responses were analysed in total for the entire respondent and by sexes. A statistical analysis was conducted with Statistica 12.0 (StatSoft, Inc.). In order to determine nutrition behaviours of the examined people, proper behaviours was granted 1 point (in the analysis of results, presented with grey). It was possible to be granted with a maximal number of 17 points. Next it was investigated whether quantitative variables comply with the assumptions of a regular arrangement with a W *Shapiro-Wilk* test and charts of regularity. In order to determine the connection between a sex and a frequency of an intake of the selected food groups and an intensification of depressive symptoms (i.e. a result on a BDI-II scale), $\chi 2$ test of compatibility was used. Differences between proper nutrition behaviours by women and men were assessed with U *Mann-Whitney's* test. A connection between proper nutrition behaviours and an intensification of depressive symptoms (i.e. the number of points obtained on a BDI-II scale) was measured with a nonparametric *Tau Kendall's* test. Value of $p<0.05$ was accepted as statistically important for all the analyses.

RESULTS

Nutrition behaviours

Students' selected nutrition behaviours are presented in Tables 1-4.

A recommended number of meals was eaten by 54.95% of students, including 59.20% of women and 48.56% of men. Meals were eaten every 3-4 hours by 43.59% of students, including 48.09% of women and 36.81% of men. Drinking a recommended amount of water was declared by 33.16% of students, including of 23.61% women and 47.52% of men. Thirst was most often quenched with water (68.40%) – such a variant was chosen by 65.63% of women and 72.58% of men. Most of the students (51.40%), including 57.81% of women and 41.78% of men did not add sugar to hot drinks (Table 1).

Table 1. Basic nutritional behaviours

Nutrition behaviours	Variants of the answer	Women		Men		Total	
		N=576	60.06%	N=383	39.94%	N=959	100%
Number of meals intake during the day	1-2 meals	28	4.86	46	12.01	74	7.72
	3 meals	175	30.38	133	34.73	308	32.12
	4-5 meals	341	59.20	186	48.56	527	54.95
	> 5 meals	32	5.56	18	4.70	50	5.21
Breaks between meals	3-4 h	277	48.09	141	36.81	418	43.59
	4-6 h	142	24.65	116	30.29	258	26.90
	> 6 h	9	1.56	26	6.79	35	3.65
	Irregularly	148	25.70	100	26.11	248	25.86
The amount of water during the day	< 0.5 l	32	5.56	4	1.04	36	3.75
	0.5-1.0 l	186	32.29	52	13.58	238	24.82
	1.0-1.5 l	222	38.54	145	37.86	367	38.27
	> 1.5 l	136	23.61	182	47.52	318	33.16
The most intake drink to satisfy your thirst	Water	378	65.63	278	72.58	656	68.40
	Tea / coffee	130	22.57	45	11.75	175	18.25
	Carbonated drinks	39	6.77	42	10.97	81	8.45
	Juice	29	5.03	18	4.70	47	4.90
Sweetening of hot drinks with sugar*	1 teaspoon	146	25.35	97	25.33	243	25.34
	2 teaspoons	85	14.76	111	28.98	196	20.44
	> 2 teaspoons	12	2.08	15	3.91	27	2.82
	No sugar	333	57.81	160	41.78	493	51.40

*adding sugar to a 250 ml glass

A relation between a sex of the examined people and a number of meals eaten a day was found ($p<0.01$). According to the results for this group, women ate a recommenced number of meals a day much more often than men (Table 1).

According to an analysis of frequency of an intake of plant origin products, 93.33% of students had bread, however, a type of bread most often chosen was white bread – such a response was given by 48.91% of students, including 41.85% of women and 59.53%

of men. Only 6.25% of students, including 6.94% of women and 5.22% of men had recommended 5 or more portions of fruit and vegetables a day. Maintaining a ratio of ¾ vegetables and ¼ fruit in an everyday diet was declared by 23.77% of students, including 22.05% of women and 26.37% of men. Oils were eaten on a daily basis by 6.78% of students, including 6.77% of women and 6.79% of men (Table 2).

A relation between sexes of the examined people and a consumed type of bread was found ($p<0.01$). According to the result, women chose brown bread much more often than men (Table 2).

Table 2. Intake of plant origin food products

Nutrition behaviours	Variants of the answer	Women		Men		Total	
		N=576	60.06%	N=383	39.94%	N=959	100%
The most intake type of bread	White bread	241	41.85	228	59.53	469	48.91
	Brown bread	269	46.70	111	28.98	380	39.62
	Other	26	4.51	20	5.22	46	4.80
	Never	40	6.94	24	6.27	64	6.67
Daily consumption of vegetables and fruits	1-2 portions	342	59.38	280	73.11	622	64.86
	3-4 portions	185	32.12	67	17.49	252	26.28
	5 or more portions	40	6.94	20	5.22	60	6.25
	Never**	9	1.56	16	4.18	25	2.61
Proportion of vegetables and fruits in the daily diet	½ vegetables & ½ fruit	256	44.45	151	39.43	407	42.44
	¾ vegetables & ¼ fruit	127	22.05	101	26.37	228	23.77
	¼ vegetables & ¾ fruit	170	29.51	111	28.98	281	29.30
	Only vegetables	11	1.91	11	2.87	22	2.29
	Only fruit	12	2.08	9	2.35	21	2.20
Frequency of intake of plant oils	Every day	39	6.77	26	6.79	65	6.78
	Several times a week	185	32.12	123	32.11	308	32.12
	Several times a month	268	46.53	170	44.39	438	45.67
	Never	84	14.58	64	16.71	148	15.43

**the answer relates to the daily intake of vegetables and fruit

According to an analysis of frequency of an intake of animal origin products, 11.16% of students, including 10.94% of women and 11.49% of men drank at least 2 glasses of milk or dairy fermented products such as natural yoghurt or kefir. Eating fish a several times per week was declared by 11.16% of students, including 10.59% of women and 12.01% of men. Having meat and cold meat a several times per week was confirmed by 42.55% of students, including 48.26% of women and 33.94% of men (Table 3).

Table 3. Intake of animal origin food products

Nutrition behaviours	Variants of the answer	Women		Men		Total	
		N=576	60.06%	N=383	39.94%	N=959	100%
Frequency of intake of milk or dairy fermented products	≥ 2 glasses a day	63	10.94	44	11.49	107	11.16
	<2 glasses a day	224	38.89	152	39.69	376	39.21
	Occasionally	265	46.00	176	45.95	441	45.98
	Never	24	4.17	11	2.87	35	3.65
Frequency of intake of fish	Every day	0	0.00	2	0.52	2	0.21
	Several times a week	61	10.59	46	12.01	107	11.16
	Several times a month	458	79.51	303	79.11	761	79.35
	Never	57	9.90	32	8.36	89	9.28
Frequency of intake of meat and cold meat	Every day	176	30.56	239	62.40	415	43.27
	Several times a week	278	48.26	130	33.94	408	42.55
	Several times a month	91	15.80	10	2.61	101	10.53
	Never	31	5.38	4	1.05	35	3.65

According to the analysis of an intake of products not recommended, exclusion of sweets was declared by 2.09% of students, including 1.39% of women and 3.13% of men and of sweet drinks – by 11.37% of students, including 15.63% of women and 4.96% of men. Elimination of fast-food products from a diet was declared by 17.31% of students, including 22.74% of women and 9.14% of men and of instant products – by 48.49% of students, including 53.82% of women and 40.47% of men (Table 4).

Table 4. Intake of non-recommended products

Nutrition behaviours	Variants of the answer	Women		Men		Total	
		N=576	60.06%	N=383	39.94%	N=959	100%
Frequency of intake of sweets	Every day	141	24.48	58	15.14	199	20.75
	Several times a week	243	42.19	143	37.34	386	40.25
	Several times a month	184	31.94	170	44.39	354	36.91
	Never	8	1.39	12	3.13	20	2.09
Frequency of intake of sweet drinks	Every day	39	6.77	48	12.53	87	9.07
	Several times a week	148	25.69	133	34.73	281	29.30
	Several times a month	299	51.91	183	47.78	482	50.26
	Never	90	15.63	19	4.96	109	11.37
Frequency of intake of fast-food products	Every day	6	1.04	5	1.31	11	1.15
	Several times a week	47	8.16	54	14.10	101	10.53
	Several times a month	392	68.06	289	75.45	681	71.01
	Never	131	22.74	35	9.14	166	17.31
Frequency of intake of instant products	Every day	4	0.69	9	2.35	13	1.35
	Several times a week	28	4.86	28	7.31	56	5.84
	Several times a month	234	40.63	191	49.87	425	44.32
	Never	310	53.82	155	40.47	465	48.49

A relation between sexes of the examined people and a frequency of an intake of sweet soft drinks was found ($p<0.01$). According to the results, women chose such products much less often than men (Table 4).

Based on the *Shapiro-Wilk* test and charts of regularity the rate of proper nutrition behaviours variable was not found to be with the assumptions of a regular arrangement ($p<0.05$). In the next analysis, differences between a rate of proper nutrition behaviours by women and men were observed ($p<0.01$) (Table 5).

Table 5. Rate of proper nutrition behaviours

Variable		Median	Lower-upper quartile	Name and test result
Sex	Women	29.41	23.53-41.18	U *Mann-Whitney's* test; $p<0.01$
	Men	29.41	17.65-35.29	
Total		29.41	17.65-41.18	

Taking medicines and dietary supplements and physical activity

Taking medicines and dietary supplements by students is presented in Table 6, and their physical activity – in Figure 1.

Taking medicines was declared by 20.23% of students, including 23.61% of women and 15.14% of men. Sedatives were taken by 9.07% of students, including 10.59% of women and 6.79% of men. Dietary supplements were used by 34.62% of students, including 35.94% of women and 32.64% of men. Supplementation of vitamin D was declared by 6.78% of people, including 9.38% of women and 2.88% of men (Table 6).

Table 6. Taking medicines and using dietary supplements

Nutrition behaviours	Variants of the answer	Women		Men		Total	
		N=576	60.06%	N=383	39.94%	N=959	100%
Taking medicines	Yes	136	23.61	58	15.14	194	20.23
	No	440	76.39	325	84.86	765	79.77
Taking sedatives	Yes, regularly	11	1.91	6	1.57	17	1.77
	Yes, occasionally	50	8.68	20	5.22	70	7.30
	No	515	89.41	357	93.21	872	90.93
Using dietary supplements	Yes	207	35.94	125	32.64	332	34.62
	No	369	64.06	258	67.36	627	65.38
Vitamin D supplementation	Yes	54	9.38	11	2.88	65	6.78
	No	522	90.62	372	97.12	894	93.22

The examined students undertook physical activity for at least 30-45 minutes, most often for a several times a week. Such a respond was given by 41.08% of people, including 40.08% of women and 41.52% of men. Physical activity was undertaken daily by 12.72% of students, including 8.16% of women and 19.58% of men (Figure 1). A relation between sexes of the examined people and undertaking physical activity was found (p<0.01). According to the results, women undertake physical activity much less often than men (Figure 1).

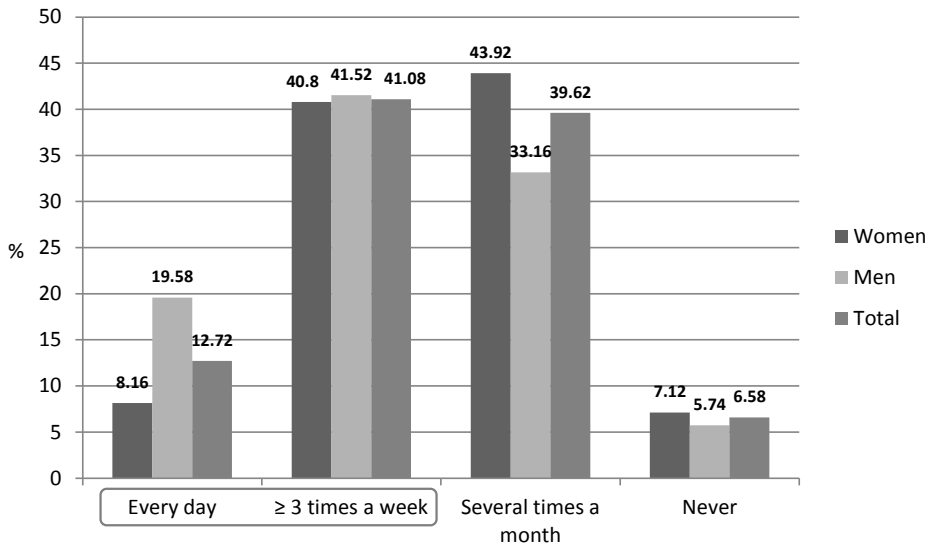

Figure 1. Physical activity taking 30-45 minutes

Role of food

The examined students claimed that apart from nutrition, eating is relaxing and gives them pleasure – such a respond was given by 47.76% of students, including 48.96% of women and 45.95% of men. For 21.48% of students food has mainly a pro-health role – students declared to pay special attention to health and nutrition values of food. 17.41% of students like eating with other people and for them eating has got a social role. The lowest number of students (13.95%) considered an emotional role of food (reduction of stress) (Figure 2).

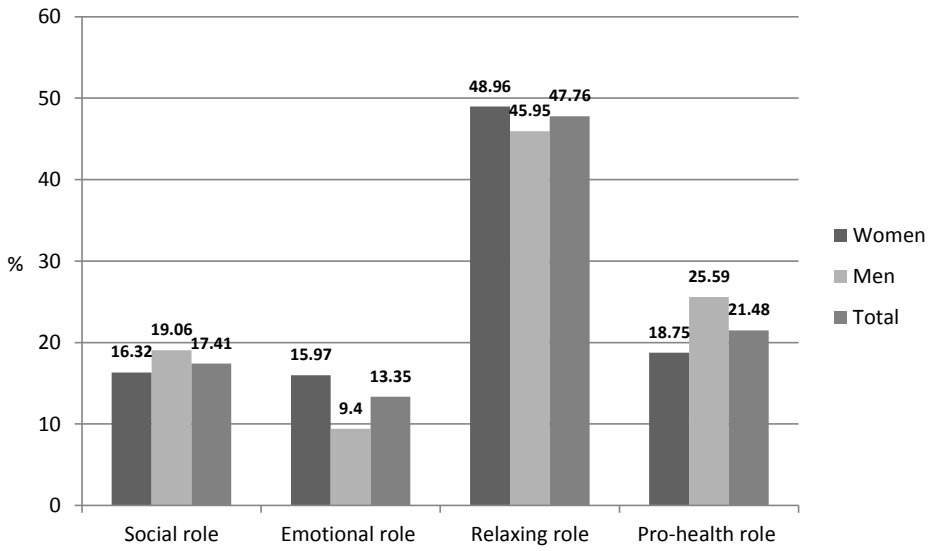

Figure 2. Role of food, beyond satisfying hunger

Intensification of depressive symptoms on a BDI-II scale

Mood of the examined people was assessed using a BDI-II scale. It oscillated between 0 and 53 points with a median of 7 points (bottom-top quartile: 3-13 points). For women, the results were between 0 and 50 points with a median of 5 (bottom-top quartile: 3-14 points) and for men, the results were between 0 and 53 points with a median of 5 (bottom-top quartile: 2-11 points).

Depressive symptoms were observed in 22.84% of students: symptoms which might suggest mild depression – in 11.57%, moderate

depression – in 7.1%, and severe depression – in 4.17% of the respondents (Figure 3).

A relation between sexes of the examined people and an intensification of depressive symptoms was found (a result on a BDI-II scale), ($p<0.01$). According to the results, depressive symptoms were observed much more often in women than men (Figure 3).

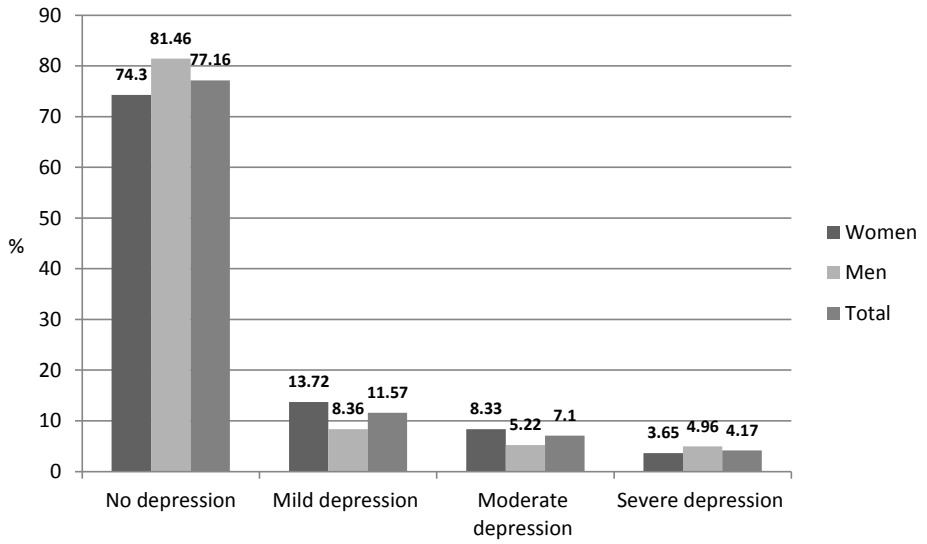

Figure 3. Severity of depression symptoms according to the BDI-II scale

It was also investigated, whether there is a relation between a percent of proper nutrition behaviours and an intensification of depressive symptoms (i.e. points on a BDI-II scale). According to the results in the examined group, an increase in proper nutrition behaviours generated a drop in depressive symptoms (Tau Kendall's = -0.09; $p <0.01$).

DISCUSSION

In the recent years, more and more young people have been diagnosed with a mood decline. However, in Poland this data is only fragmentary and results of the conducted research are difficult to compare due to methodology [4]. According to WHO, by 2020 depression will have been on a 2nd position in the global burden of diseases [6]. People at any age may be diagnosed with it, yet the first symptoms usually appear at the age of 20-30 and an incidence rate is higher in a group of women. This may be determined by neurobiological, hormonal and social and cultural differences between the sexes [4, 6]. According to the results of the personal research, women are diagnosed with a mood decline more often. Also in the research by *Jaworska* et al., which aim was to assess mood and humour of 153 students at the University School of Physical Education in Wroclaw, Poland, more cases of an occurrence and/or an intensification of depressive symptoms were observed in a group of women, which is an evidence that this sex may be a risk factor [4].

People diagnosed with depressive symptoms tend to have eating disorders. Most often, these are problems with appetite and changes in food preferences. Eating too much or too less and irregularity in meals may result in improper body mass, nutrition deficiencies and worse mood [5, 21]. People whose BMI indicated overweight or obesity frequently had problems of psychological origin. Low self-esteem and lack of self-confidence are just a few emotional states that may result in a withdrawal from an active life [5].

In a research by *Leszczyńska* et al., in which an impact of emotions on nutrition behaviours of 90 women aged 18-30 years was estimated, it was observed that in a group of women who tended to suffer from a mood decline, overweight was more common than in a group of women who did not. Furthermore, women classified to a group with mood decline tendencies ate sweets more often, which may be connected with a stress-eating phenomenon [10]. According to a personal research, everyday sweets intake was higher in a group of women than in a group of men (24.48% vs 15.14%). What is more, it was a group of men, which declared to undertake everyday physical activity more frequently. Such results may suggest that in case of men, physical activity is a preferred method for stress relieve and dealing with other difficult situations in everyday life.

A well balanced diet, both in terms of quantity and quality, is beneficial for the functioning of a nervous system. Long-term deficiencies of vitamins and minerals may account for the occurrence of psychological disorders [21]. According to the latest research on the relation between vitamin D_3 and an occurrence and/or an intensification of depressive symptoms, deficiency of this vitamin enhances the risk of depression. Supplementation of vitamin D_3

by people with depression disorders had an antidepressant effect and prevented its relapses [20]. In Poland, there are deficiencies of this vitamin in a population. According to personal research, only 6.78% of students supplements this vitamin. Such a respond was chosen more often by women than men (9.38% vs 2.88%).

In the recent years, perception of food and its impact on health has undergone changes. A role of a dietician and various nutrition patterns in the treatment of various diseases, including depression, are more often emphasized [17]. Nevertheless, relations between a diet quality and nutrition behaviours and depression are not fully comprehensible yet. According to a systematic review of scientific papers conducted in 2013 in order to estimate these relations, diet modifies key biological factors connected with the development of depression, yet there are no sufficient evidences for it. Various types of diet were investigated: a Mediterranean diet, a Norwegian diet, a Japanese diet, a "western" diet and a healthy diet in a broad sense [14].

In a review of scientific papers published in 2016, connections between eating food by children and the adolescent, and depression and depression-related psychological problems, lack of a homogenous methodology and negligence of important environmental and social and economic factors, which influence the research parameters, were criticized. Yet despite some inconsistent results, this research is an evidence for the relation between healthy eating habits and a high-quality diet, and a lower level of depression occurrence. They also present a connection between a low-quality diet and an occurrence of depression or a bad psychological condition [7]. According to the research on 1000 men in Finland, it was proven that a balanced diet determined lower risk of a depressive symptoms occurrence, whereas a "western" diet model correlated with a more frequent occurrence depressive symptoms. Compliance with a healthy diet model was also connected with a lower risk of depression requiring hospitalization [18].

According to some research, some nutrition patterns – especially a Mediterranean and a Norwegian one – may result in a lower risk of depression among adults. This seems to be consistent in various countries, cultures and populations. Patterns potentially connected with a lower risk of depression are the ones based on seafood, fruit and vegetables and nuts. However, current evidence is still limited and research in this subject should be continued [11]. In the research by *Nasir* et al. all the possible relations between main nutrition patterns and depression, stress and anxiety among adults were shown. According to the authors, better compliance with a healthy diet led to a decrease in an average depression result (p=0.03) and an anxiety rate (p=0.03) [12].

Based on the current scientific information, it seems important to complete an assessment of nutrition behaviours among young people, especially among those who are prone to depressive symptoms. Apart from the authors, such assessment was completed inter alia by *Ilow* et al., who analysed nutrition habits of students from Wroclaw Medical University. According to the authors, improper nutrition habits were observed among a significant part of students, especially men [3]. When interpreting the results, it is important to take into account the fact that the respondents in both personal research and the research by *Ilow* et al. were Medicine students. Based on the review of the research, depression is observed relatively frequently in this group – it is estimated to be ca. 27.2%. It might be caused by the specificity of these studies, which is connected with strong stress, anxiety and competition between students [3, 16].

Our research have shown that nutrition behaviours are varied in the examined group. Women tend to present more beneficial nutrition behaviours. However, in this group depressive symptoms are observed more frequently.

CONCLUSIONS

1. Students' nutrition behaviours varied. It was found that there are relations between nutrition behaviours and sexes of the examined people. Women tend to present more beneficial behaviours. Differences between a rate of proper behaviours by women and men were observed.
2. Almost every fourth student presented depressive symptoms. It was found that there is a relation between an intensification of depressive symptoms and sexes of the examined people – an intensification of the symptoms was more frequent among women.
3. There is a relation between proper nutrition habits and an intensification of depressive symptoms – a growth in the rate of proper nutrition behaviours was connected with a drop in the depressive symptoms.

Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

WHAT ARE THE DIETS OF PATIENTS BEFORE BARIATRIC SURGERY?

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ABSTRACT

Background. Obesity is regarded as the most common disease of affluence, gradually getting an epidemic status.
Objective. The aim of the study was to assess the frequency of consumption of selected food products among the patients qualified for bariatric surgery, together with the analysis of the potential effect of the diet on the development of obesity in examined group of people.
Material and Methods. The study involved 57 patients qualified for bariatric treatment of obesity. A standardized food frequency questionnaire (FFQ) was used and anthropometric measurements were performed.
Results. In examined group of people, along with increasing BMI, the frequency of cheeses consumption decreased, whereas the frequency of consumption of vegetable and fruit - vegetable juices increased. In addition to that, it was observed that with higher frequency of consumption of animal fat (lard, bacon) and beer, the value of WHR increased, whereas the frequency of consumption of dairy products was in direct proportion to body weight of examined people. It was also noted that people living in the country consumed fruits and potatoes significantly more often than people living in the city, and that people with higher education significantly more often ate coarse grits, high quality meats and fatty fish, as compared to people with vocational training.
Conclusions. Inappropriate selection of food products and numerous dietary mistakes made by the patients directly contributed to the development of extreme obesity. The patients prepared for bariatric surgery should receive a dietician support during the preparation for the procedure and afterwards, later in life, in order to maintain a reduced body weight after the surgery.

Key words: *bariatric surgery, feeding behavior, obesity, diet, body mass index, BMI*

STRESZCZENIE

Wprowadzenie. Otyłość jest uznawana za najczęściej występującą chorobę cywilizacyjną, stopniowo przybierającą postać epidemii.
Cel. Celem pracy była ocena częstości spożycia wybranych produktów spożywczych wśród pacjentów zakwalifikowanych do operacji bariatrycznej wraz z określeniem potencjalnego wpływu sposobu odżywiania na rozwój otyłości w badanej grupie osób.
Materiał i Metody. W badaniu wzięło udział 57 pacjentów zakwalifikowanych do chirurgicznego leczenia otyłości. Wykorzystano standaryzowany kwestionariusz częstości spożycia (FFQ) oraz wykonano pomiary antropometryczne.
Wyniki. W badanej grupie osób wraz ze wzrostem wskaźnika BMI spadało spożycie serów, a jednocześnie zwiększała się częstość spożywania soków warzywnych i warzywno-owocowych. Dodatkowo zaobserwowano, że wraz ze wzrostem częstotliwości spożycia tłuszczów zwierzęcych (smalcu, słoniny) oraz piwa zwiększeniu ulegała wartość wskaźnika WHR, natomiast częstość spożycia produktów nabiałowych była wprost proporcjonalna do masy ciała badanych osób. Odnotowano także, iż mieszkańcy wsi istotnie częściej niż mieszkańcy dużych miast spożywali owoce oraz ziemniaki, a także że osoby z wyższym wykształceniem istotnie częściej sięgali po kasze gruboziarniste, wędliny wysokogatunkowe oraz tłuste gatunki ryb w odniesieniu do osób z wykształceniem zawodowym.
Wnioski. Nieodpowiedni dobór produktów spożywczych oraz liczne błędy żywieniowe popełniane przez pacjentów, bezpośrednio przyczyniły się do rozwoju otyłości olbrzymiej. Pacjenci przygotowywani do operacji bariatrycznej powinni otrzymać wsparcie dietetyka podczas przygotowania do zabiegu oraz w celu utrzymania zredukowanej po operacji masy ciała, w kolejnych etapach życia.

Słowa kluczowe: *chirurgia bariatryczna, nawyki żywieniowe, otyłość, dieta, wskaźnik masy ciała, BMI*

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INTRODUCTION

Obesity is regarded as the most common disease of affluence, gradually getting an epidemic status [38]. According to the World Health Organization (WHO) the percentage of overweight and obese people increased three-fold compared to the year 1975. It means that 1.9 milliard people in the world are overweight, including more than 650 million suffering from obesity [39]. Focusing on the situation in Poland only, the study WOBASZ II shows that in the years 2013 – 2014, in comparison with the years 2003 – 2005, there was an increase in body mass index – BMI. It was also noted that the percentage of adults with proper waist circumference significantly decreased in case of both sexes. In the study the incidence of excessive weight reached 43.2% in men and 30.5% in women, whereas obesity was recognized in 24.4% and 25% men and women, respectively. Abdominal obesity (defined as waist circumference larger than 94 cm in men and 80 cm in women) was noted in 27.2% men and 21.7% woman [34]. Etiopathogenesis of this chronic non-infectious disease relates to the excessive accumulation of adipose tissue in a body. Primary obesity, which is the most common type of obesity, occurring mainly in developed countries and less common in developing ones, results from a positive energy balance, caused by many environmental factors contributing to unhealthy lifestyle, including nutrition [36]. Accumulation of fatty tissue is also facilitated by irregular consumption of meals with long intervals between them, leading to disturbances in the function of endocrine system, especially in the release of insulin and leptin, resulting in insulin- and leptin-resistance [14]. Additionally, it was proved that eating foods at irregular intervals has a negative effect on satiety and hunger centre due to frequent consumption between the meals and at night [21]. This, in turn, increases the accumulation of energy storage in form of adipocytes. A crucial role in the formation of primary obesity is also played by improper thermal treatment of food products (frying) and low physical activity [3], which is related to sedentary lifestyle, common use of transport and developing urbanization [36]. Another factors contributing to increasing body mass are also the lack of time on the preparation of homemade meals, positively correlated with civilisation development [36], and increasing level of stress, which is a factor releasing the behaviours related to excessive consumption of food to lower emotional tension [17; 21]. Important aspect linked to the development of obesity is the imbalance in the composition of gut microflora, termed as dysbiosis. A typical "western" diet – high in protein, saturated fatty acids and simple sugars and lacking in dietary fibre, contributes to lowering the number of *Bifidobacterium* species. Additionally, in obese people the domination of Gram-positive bacteria is observed, e.g. *Clostridium* and *Mollicutes* species. *Mollicutes* especially have the ability to obtain energy from food waste, which is then stored in fatty tissue. A compromised intestinal barrier and the translocation of the bacteria to bloodstream lead to a generalized inflammatory reaction in the body [23].

Excessive body mass, including fatty tissue, significantly lowers the health and functionality of the whole organism. Obesity increases the risk of dyslipidaemia, hyperinsulinemia, insulin resistance, heart attack, ischemic stroke and numerous tumours, including digestive tract and hormone-dependent tumours [18]. Additionally, the most common diseases accompanying obesity are type 2 diabetes, ischemic heart disease, atherosclerosis, high blood pressure and arthritis and bone and joints diseases [36], gout, non-alcoholic fatty liver disease (NAFLD) and cholelithiasis [26; 32]. All the complications of obesity mentioned above have a negative effect on psychic functions and may strengthen low self-esteem often observed in obese people. Numerous complexes and the tendency to isolate oneself from the environment and to avoid social contacts may trigger the development of extreme obesity [33].

The methods used to treat obesity include the modification of lifestyle, involving balanced diet and intermediate physical activity, and increased self-acceptance through expanding own interests and hobbies. Moreover, pharmacological treatment and in many cases also psychological or psychic support are used, whereas surgical treatment is offered in case of patients with extreme obesity [11-12; 20]. Bariatric surgery is available for use in patients with BMI exceeding 40 kg/m^2, or 35 kg/m^2 with accompanying diseases, and includes restrictive, adsorption limiting and hybrid techniques. The effects of the treatment are visible shortly after the procedure and include, besides body mass reduction, an improvement in the course of, or even complete recovery from, accompanying diseases, e.g. type 2 diabetes. When making a decision whether to use this method of treatment one should consider that the surgery is just one part of the complex obesity treatment process, and the therapeutic group, both before and after the procedure, should include a bariatric surgeon, a general practitioner, a dietician, a psychologist and a nurse [22]. It seems that due to numerous health consequences of bariatric procedures and the risk of developing/strengthening nutritional deficiencies [20] the decision to use this method should be well-justified.

Due to increasing epidemic of obesity the frequency of bariatric procedures increases. The aim of the work was to assess of the frequency of consumption of selected food products among the candidates for surgical obesity treatment, together with the analysis of the potential effect of the diet on the development of obesity in examined group of people.

MATERIAL AND METHODS

Test group. The study involved 57 patients (30 women and 27 men) of Bariatric Clinic of the Independent Public Voivodeship Complex Hospital in Szczecin – Zdunowo. Anthropometric characteristics of examined people (the measurements with the accuracy 0.1 kg and 0.5 cm) are presented in Table 1. The majority of patients (58%) were the citizens of large cities, with secondary education (46%) and worked full time (54%) (Table 2). As much as 80.7% of examined people confirmed that they did not undertake any physical activity and led a sedentary lifestyle.

Table 1. Anthropometric data from examined people

Parameter	Women	Men	Total	p
n	30	27	57	
Age [years]	46.3 ±11.8	47.4 ±10	47 ±10.9	0.78
Body mass [kg]	131.6 ± 17.1	141.8 ±22.9	136.4 ±20.5	0.06
BMI [kg/m^2]	46.6 ± 5.8	44.4 ±6.6	45.5 ±6.2	0.18
WHR	0.97 ±0.05	1.09 ±0.06	1.03 ±0.08	0.0001

Table 2. Socioeconomic status vs sexes

Parameter	Women [%]	Men [%]	Total [%]	p
Place of living				
City > 100 000 citizens	65	48.15	57.9	0,31
City from 10 000 to 100 000 citizens	20	37.04	28.1	
Country/small town up to 10 000 citizens	15	14.8	14.04	
Education				
Primary	2.5	7.4	5.3	0,89
Secondary	47.5	48.1	46.6	
High	25	18.5	22.8	
Vocational	25	25.9	26.3	
Source of income				
Full-time job	56.67	51.9	54.4	0,42
Part-time job	0	7.41	3.51	
Unemployed	20	14.8	17.5	
Pension	20	25.93	22.8	
Other, e.g. civil contract	3.33	0	1.75	

Questionnaire. A standardized food frequency questionnaire (FFQ) was used in the study. Ranks from the questionnaire were translated into the frequency of consumption of selected groups of products according to the following scheme:

- Never or hardly ever – 0 times a day
- Once a month or less frequent – 0.025 times a day
- Several times a month – 0.1 times a day
- Several times a week – 0.571 times a day
- Every day – once a day
- A few times a day – twice a day

Statistical analysis

Obtained results were statistically analysed using StatView 5.0 (SAS Institute Inc. Cary, NC, USA). The normality of the distribution was tested with Shapiro-Wilk test. Statistical significance of anthropometric data depending on the sex was analysed using variance analysis ANOVA. Pearson's χ^2 test was used to analyse the relations between sociodemographic variables. Continuous variables were characterized by mean and standard deviation and in case of qualitative variables the results were given in percentage. In case of, correlations between frequency of eating food products and place of residence, education, source of income variance analysis ANOVA was used. Correlations between frequency of eating food products and age, BMI analysed using *Pearson* method. Correlations between frequency of eating and body mass and WHR analysed using *Spearman* method. The level of significance was p<0,05.

RESULTS

The frequency of consumption of food products is given in Table 3. In this study the frequency of products' consumption was not dependent on the sex (p>0.05). It was, however, observed that the consumption of salty snacks (r= -0.322; p=0.01) was correlated with age. It was also noted that along with the increase of BMI the intake of cheeses decreased (r= -0.333; p=0.01) and, at the same time, the frequency of consumption of vegetable and fruit - vegetable juices were higher (r= 0.316; p=0.02). Additionally, it was observed that with increased frequency of consumption of animal fats (lard, bacon, r=0.331; p=0.01) and beer (r=0.322; p=0.02) the value of WHR increased. The frequency of consumption of flavoured quarks (p=0.01) was directly proportional to body mass of examined people. The relations between the frequency of consumption of products and sociodemographic conditions of examined group of people are presented in Tables 4-6.

Table 3. Frequency of consumption of selected food products [%]

Products	Never or hardly ever	Once a month or less frequent	Several Times a month	Several Times a week	Every day	A few Times a day	Products	Never or hardly ever	Once a month or less frequent	Several Times a month	Several Times a week	Every day	A few Times a day
Sugar for drinks	56.14	3.51	1.75	1.75	12.3	24.6	Apples and pears	7.02	5.26	7.02	57.9	14.03	8.77
Honey	61.4	21.1	5.3	7.02	1.75	3.51	Avocado	71.93	12.28	3.51	8.77	3.51	0
Chocolates, candies and chocolate bars	31.58	15.79	22.81	17.54	8.77	3.51	Olives	64.91	14.03	8.77	10.53	1.75	0
Non-chocolate sweets	54.39	10.53	8.77	14.04	8.77	3.51	Dry fruits	54.39	17.54	10.53	17.54	0	0
Biscuits and cookies	42.7	11.1	25.93	14.8	3.7	3.7	Sweet fruit preserves	43.86	19.3	21.05	15.79	0	0
Ice-cream and puddling	31.58	24.5	22.81	17.54	1.75	1.75	Vegetables, all kinds	0	0	5.26	21.05	50.88	22.81
Salty snacks	47.37	21.1	17.54	14.04	0	0	Vegetables of the cabbage family	8.78	10.53	43.86	35.09	1.75	0
Milk and natural milk drinks	10.53	19.3	19.3	19.3	28.1	3.51	Yellow and orange vegetables	0	1.75	21.1	64.91	12.28	0
Sweetened milk drinks	21.1	12.3	15.8	29.8	21.1	0	Leafy green vegetables	3.51	10.53	26.32	47.37	10.53	1.75
Natural quarks	8.78	10.53	22.81	47.37	10.5	0	Tomatoes	1.75	3.51	1.75	61.4	31.58	0
Sweetened quarks	82.5	5.3	1.8	10.5	0	0	Vegetables of the gourd family	7.02	1.75	15.79	64.91	10.53	0
Cheeses	24.6	10.53	29.8	33.3	1.8	0	Root vegetables	0	1.75	22.81	70.18	5.26	0
Eggs and meals from eggs	3.51	12.3	36.8	43.9	3.51	0	Fresh seeds of legumes	19.3	14.04	45.61	21.06	0	0
Wholemeal bread	10.53	5.3	3.51	36.8	21.1	22.81	Dry legumes	45.61	14.03	36.84	3.51	0	0
Refined bread	49.1	5.3	1.8	24.6	8.8	10.5	Potatoes	5.26	8.77	15.79	57.89	12.28	0
Coarse ground grits	12.3	12.3	33.3	38.6	3.51	0	Nuts	47.37	21.06	15.79	12.28	3.51	0
Finely ground grits	22.81	17.54	33.3	26.32	0	0	Grains	49.12	14.04	12.28	15.79	8.77	0
Ready breakfast cereal products	73.7	8.8	8.8	5.3	3.51	0	Sausages	12.28	8.77	21.06	38.6	15.79	3.51
Oil	3.51	0	15.8	50.9	29.8	0	High quality meats	5.26	3.51	5.26	56.14	26.32	3.51
Butter	47.37	3.51	3.51	10.5	26.32	8.77	Offal and cold meats	21.06	17.54	35.09	26.32	0	0
Margarine	45.6	0	0	21.1	22.81	10.51	Poultry and rabbit meat	0	1.75	15.79	68.42	14.04	0
Cream	35.1	14.03	15.8	33.3	1.75	0	Lean fish	1.75	17.54	43.86	36.84	0	0
Other animal fats	70.17	8.77	12.9	8.77	0	0	Fatty fish	21.06	19.3	31.58	28.07	0	0
Mayonnaise and dressings	31.58	21.05	33.33	14.03	0	0	Fruit juices and nectars	52.63	8.77	7.02	19.3	10.53	1.75
Fruits, all kinds	1.75	0	3.51	24.56	54.39	15.79	Vegetable and fruit-vegetable juices	56.14	7.02	12.28	21.06	3.51	0
Stone fruits	10.53	10.53	15.79	49.12	12.28	1.75	Energy drinks	84.21	7.02	5.26	3.51	0	0
Kiwi and citrus fruits	7.02	3.51	31.58	50.88	5.26	1.75	Sweetened drinks	45.61	14.04	14.04	14.04	12.28	0
Tropical fruits	21.05	26.32	17.54	29.82	3.51	1.75	Beer	61.4	7.02	19.3	10.53	1.75	0
Berry fruits	3.51	10.53	22.81	52.63	7.02	3.51	Wine and drinks	61.4	17.54	12.28	7.02	1.75	0
Bananas	26.32	15.79	17.54	38.6	0	1.75	Vodka and spirits	56.14	21.05	14.04	8.77	0	0

Table 4. Relations between the place of living and the frequency of consumption expressed as a mean value and standard deviation

Frequency of consumption	DM	MM	W	p
White bread	0.27 ±0.44	0.67±0.85	0.71±0.64	1) 0.034
Fruits, all kinds	0.94±0.54	1.18±0.6	1.5±0.54	2) 0.013
Kiwi and citrus fruits	0.31±0.3	0.53±0.46	0.51±0.29	1) 0.048
Tropical fruits	0.18±0.29	0.45±0.5	0.25±0.27	1) 0.017
Bananas	0.2±0.27	0.43±0.49	0.27±0.25	1) 0.033
Dry legumes	0.5±0.5	0.11±0.18	0.01±0.04	1) 0.044 3) 0.030
Potatoes	0.44±0.3	0.43±0.33	0.68±0.2	2) 0.047
Lean fish	0.23±0.23	0.24±0.23	0.43±0.26	2) 0.03
Other products – NS (p>0,05)				

DM – city above 100 000 citizens; MM – city from 10 000 to 100 000 citizens; W – small town up to 10 000 citizens or a village;
Statistical significance (p):
1) DM vs MM
2) DM vs W
3) MM vs W
NS – statistically insignificant.

Table 5. Relations between the education and the frequency of consumption expressed as a mean value and standard deviation

Frequency of consumption	Primary	Secondary	Higher	Vocational	p
Honey	0.0±0.0	0.076 ±0.18	0.39±0.77	0.073±0.26	4) 0.024 6) 0.041
Ice-cream and pudding	0.33±0.06	0.24±0.29	0.29±0.58	0.03±0.04	6) 0.044
Sweetened milk drinks	0.53±0.49	0.28±0.37	0.58±0.38	0.45±0.38	4) 0.027
Eggs and meals from eggs	0.38±0.33	0.31±0.26	0.49±0.33	0.24±0.24	6) 0.028
Margarine	0.52±0.5	0.56±0.63	0.25±0.45	0.81±0.75	6) 0.026
Cream	0.38±0.33	0.28±0.31	0.08±0.16	0.22±0.26	4) 0.033
Stone fruits	1.05±0.83	0.48±0.34	0.36±0.26	0.44±0.54	1) 0.03 2) 0.014 3) 0.025
Coarse ground grits	0.38±0.33	0.2±0.23	0.49±0.26	0.25±0.32	4) 0.003 6) 0.024
Olives	0.0±0.0	0.07±0.22	0.25±0.29	0.02±0.04	4) 0.014 6) 0.004
Sweet fruit preserves	0.41±0.27	0.12±0.2	0.11±0.22	0.05±0.15	1) 0.0163 2) 0.0162 3) 0.004
Potatoes	0.41±0.27	0.47±0.3	0.3±0.34	0.62±0.25	6) 0.007
Grains	0.22±0.31	0.12±0.22	0.44±0.46	0.12±0.28	4) 0.004 6) 0.009
High quality meats	0.57±0.0	0.58±0.34	0.9±0.63	0.63±0.15	4) 0.023
Lean fish	0.57±0.0	0.24±0.24	0.28±0.26	0.2±0.23	1) 0.026 3) 0.017
Fatty fish	0.38±0.33	0.17±0.22	0.32±0.26	0.1±0.19	6) 0.017
Beer	0.0±0.0	0.07±0.15	0.24±0.35	0.1±0.2	4) 0.031
Wine and drinks	0.01±0.01	0.03±0.11	0.22±0.32	0.05±0.15	4) 0.005 6) 0.017
Other products – NS (p>0.05)					

Statistical significance (p):
1) Primary vs Secondary
2) Primary vs Higher
3) Primary vs Vocational
4) Secondary vs Higher
5) Secondary vs Vocational
6) Higher vs Vocational
NS – statistically insignificant

Table 6. Relation between the source of income and the frequency of consumption expressed as a mean value and standard deviation

Source of income	Food products	Frequency of consumption	p
Part-time job vs civil contract	Stone fruits	0.34±0.33 vs 2.0	0.001
	Sweet fruit preserves	0.05±0.07 vs 0.57	0.04
Part-time job vs unemployed	Milk and natural milk drinks	1.29±1.01 vs 0.48±0.46	0.04
Part-time job vs full-time job	Milk and natural milk drinks	1.29±1.01 vs 0.41±0.42	0.02
	Flavoured quarks	0.3±0.39 vs 0.04±0.15	0.048
	Butter	1.5±0.71 vs 0.43±0.46	0.02
	Fruit juices and nectars	1.00±1.41 vs 0.25±0.35	0.01
Part-time job vs pension	Butter	1.5±0.71 vs 0.39±0.76	0.02
	Fruit juices and nectars	1.00±1.41 vs 0.05±0.16	0.002
Civil contract vs unemployed	Ready breakfast cereals	0.57 vs 0.1±0.01	0.01
	Stone fruits	2.0 vs 0.40±0.34	0.00
Civil contract vs full-time job	Ready breakfast cereals	0.57 vs 0.06±0.21	0.02
	Stone fruits	2.0 vs 0.41±0.31	0.00
	Sweet fruit preserves	0.57 vs 0.09±0.17	0.02
Civil contract vs pension	Natural quarks	1.0 vs 0.33±0.32	0.049
	Stone fruits	2.0 vs 0.58±0.55	0.001
	Sweet fruit preserves	0.57 vs 0.11±0.21	0.03
Unemployed vs full-time job	Finely ground grits	0.34±0.27 vs 0.17±0.23	0.03
	Berry fruits	0.7±0.48 vs 0.37±0.30	0.03
	Potatoes	0.63±0.35 vs 0.41±0.29	0.04
	Offals and cold meats	0.33±0.28 vs 0.15±0.22	0.03
Unemployed vs pension	Finelt ground grits	0.34±0.27 vs 0.14±0.19	0.04
	Root vegetables	0.57±0.20 vs 0.35±0.31	0.03
	Fruit juices and nectars	0.44±0.43 vs 0.05±0.16	0.02
Full-time job vs pension	Fresh seeds of legumes	0.12±0.16 vs 0.31±0.28	0.01
Other products – NS (p>0.05)			

DISCUSSION

Performed study clearly shows that the diets of obese patients, expressed as the frequency of consumption of specific food products, are different from the assumptions of balanced nutrition. It turns out that depending on the place of living of the patients there are significant differences in frequency of consumption of specific products. Among the people living in smaller towns and in the country, there was significantly higher intake of starchy foods and lean fish species in comparison to citizens of large agglomerations. This seems to be in agreement with the data of the Central Statistical Office (CSO) [27] which stated that at the end of 2014 in Poland, irrespective of the sex, an excessive body mass was relatively more often observed in people living in the country than in the cities. In NHANES study performed in USA (2005 – 2008) it was proved that the incidence of obesity was higher among the people living in the country – 39.6% than in the people living in the cities – 33.4% [2]. High consumption of refined grain products among the people of smaller agglomerations undoubtedly contributes to the occurrence of obesity. The stage of grains refinement shows a negative correlation with their content of B group vitamins and magnesium, iron, copper or zinc [15]. Additionally, it was proved that the consumption of refined grains is directly proportional to the accumulation of subcutaneous and visceral adipose tissue [24].

In this study the majority of the patients were living in big cities. This might result from higher awareness of the citizens of larger agglomerations of the negative health implications of obesity, as well as from their higher economic status, which causes that they more often seek medical help than people living in the country. CSO data suggest that higher education correlates with higher economic status and that the citizens of the cities have higher income than people living in the country [5, 29]. In this study it was noted that the people with higher education significantly more often consumed wholegrain products, grains and fatty fish species (containing omega 3 fatty acids and vitamin D) in comparison to people with vocational training. It seems that highly educated people made better choices due to their more accurate knowledge on pro-healthy properties of food. Wholegrain food products selected by them are the source of dietary fibre, which shortens the intestinal passage of food, prevents constipation and the development of some diseases [25]. Dietary fibre present in wholegrain products is also the nutrient for natural intestinal microflora [16], thus its consumption is potentially linked to increased concentration of short-chained fatty acids (SCFAs) [10; 37], vitamins K, B12, B6, B1 and folic acid, and also to proper function of intestinal barrier [10; 16].

More frequent intake of wholemeal bread, rice and pasta and coarse ground grains contributes to lowering the levels of LDL cholesterol and triacylglycerols [9]. Moreover, it regulates the release of insulin and thus supports the maintenance of proper glycaemia and prevents the development of type 2 diabetes [28] and obesity [35].

Interestingly, in this study the highest consumption of fatty fish species was noted in group of people with primary education. Fatty fish supply precious polyunsaturated fatty acids (PUFA). The studies on animals and people showed that the consumption of PUFA is in inverse proportion to the risk of visceral obesity [4]. These acids affect the reduction of appetite and improve the circulation, which helps to maintain proper level of nutrition for skeletal muscles. They also cause changes in genes expression leading to increased energy expenditure, potentiated fats oxidation processes, decreased fatty tissue deposits and increased fat-free body mass [4]. It should be, however, remembered that the majority of patients obtained secondary education, which was related to lower intake of both wholegrain products and those rich in omega-3 fatty acids, which, together with simultaneous low consumption of vegetables and fruits (the consumption of vegetables a few times a day was declared by only 23% respondents, and fruits – 16% respondents) can be regarded as a significant factor related to both pathogenesis and development of obesity. It should also be stressed that people with higher education consumed significantly higher amounts of sweets and sweetened dairy products, which had, in this study, an important influence on patients' body weight. Due to a high glycaemic index and high content of sugar and trans fatty acids (TFA), sweets and salty snacks should be consumed sporadically or totally eliminated from the diet. Their frequent consumption contributes to excessive body weight, increased level of glucose and triglycerides in blood and higher risk of the development of type 2 diabetes, cardiovascular diseases and metabolic syndrome [13]. TFA increase the ratio of total cholesterol to HDL cholesterol and the level of lipoprotein in blood [19].

In this study, an important correlations were noted regarding alcohol consumption. Wine and drinks were consumed significantly more often by people with higher education, which is correlated with higher income [29]. Similar relation was observed for beer, whose frequency of intake additionally proportionally increased with increased WHR of examined people. Studies show that moderate consumption of red wine may protect against cardiovascular diseases, atherosclerosis, high blood pressure, selected tumours, metabolic syndrome and type 2 diabetes. It is caused by the presence of polyphenols, such as resveratrol, and anthocyanins, flavonols and catechins [1]. Thus, the effect depends on the frequency of consumption, the amount and quality of consumed drink. In case of beer and spirits the negative results of their consumption definitely dominate. Beer, due to its very high glycaemic index, increases the appetite and thus facilitates the development of obesity. Moreover, chronic and excessive consumption of alcohol may lead to the loss of control over the habit due to addiction, as well as contribute to the damage of internal organs, especially chronic pancreatitis and liver steatosis, inflammation or even cirrhosis [6].

When discussing the frequency of consumption of food products among the candidates for surgical obesity treatment one should also consider dairy products. Undoubtedly, they are a good source of protein and easily absorbable calcium, which are essential for proper function of the body. *Singh* et al. described a positive effect of dairy protein on the microbiome [31]. Fermented milk drinks, as they contain lactic acid bacteria, have a positive effect on the body. Their consumption is negatively correlated with the presence of pathogenic *Bacteroides fragilis* and *Clostridium perfringens* and positively correlated with the numbers of Bifidobacterium and Lactobacillus. Appropriate contents of these bacteria in the intestines determine proper function of intestinal barrier and immunity against pathogens, as well as prevent against inflammation and gastroenteritis [7, 31]. Unfortunately, only 28% of the respondents declared they consume dairy products every day. At the same time it was noted that as many as 30% of people choose sweetened milk drinks several times a week, and 21% - every day. Such products are highly processed and often contain glucose-fructose syrup. Fructose is a simple sugar, which in glucose metabolism bypasses the stage catalysed by phosphofructokinase. This in turn leads to elevated synthesis of fatty acids and higher release of VLDL, which can increase the concentration of triacylglycerols and LDL cholesterol in blood. Studies on rats also indicate on harmful results of excessive intake of this sweetening agent. It facilitates the accumulation of visceral fatty tissue [8], development of insulin resistance, increases the risk of type 2 diabetes, contributes to the formation of lipids disorders and increases the risk of non-alcoholic liver disease [30].

CONCLUSIONS

1. Numerous dietary mistakes made by the patients contributed to a high extent to their problem with obesity.
2. The patients preparing for the surgery should especially resign from the consumption of beer and spirits, sweetened dairy products, sweets and animal fats with high melting temperature (lard, ba-

con) to facilitate the reduction of liver volume, which is necessary before the procedure.
3. Frequency of consumption of selected products is correlated with socioeconomic status of the patients. It also depends on age, body mass and anthropometric indexes (BMI and WHR).
4. Obese patients qualified for the procedure should obtain the support of a dietician and a psychologist in order to introduce a balanced diet. It is necessary on every stage of preparation for the procedure and also for maintaining reduced body mass after the surgery, later in life.

Ethical approval

The study received the acceptance of the Bioethical Commission at Pomeranian Medical University, Szczecin, Poland

Disclosure

Authors report no conflict of interest.

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ORIGINAL ARTICLE

IDENTIFICATION OF METABOLIC INDICATORS FOR CARDIOVASCULAR RISK IN SCHOOLCHILDREN

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ABSTRACT

Objective. CardioVascular Disease (CVD) is one of the most important causes of death worldwide affecting people at younger ages every year. The purpose of this study was to identify the metabolic indicators for cardiovascular risk factors in primary school students from Mexico and Colombia.

Methods. A clinical, prospective, cross-sectional and comparative study was conducted in Mexico and Colombia to contrast anthropometric measurements, biochemical and dietetic determinations and physical activity.

Results. The Waist-Hip Ratio (WHR) and the Waist-to-Height Ratio (WtHR) showed significant differences ($p \leq 0.001$) between Mexico and Colombia (0.8 ± 0.1 versus 0.5 ± 0.1) and (0.4 ± 0.06 vs. 0.78 ± 0.04) respectively. The Automatic Linear Modeling showed that the main predictors for cholesterol levels were WtHR, MonoUnsaturated Fatty Acids (MUFA) and lipids ingestion. For glucose there were four main predictors: WHR, carbohydrates, MUFA and Saturated Fatty Acids (SFA). For triglycerides the pedictors were Products of Animal Origin (PAO), BMI, waist circumference, lipids and cholesterol ingestion and Mean Arterial Pressure (MAP). The Weight Estimation tests weighted per gender showed that for glucose levels the main determinants were carbohydrates, MUFA and oils; for cholesterol these were MUFA, PUFA and oils; and for LDL the significant variables were proteins, SFA, PAO and sugars; and last, for triglycerides the main variables were BMI, cholesterol and vegetables.

Conclusions. Mexico has higher values in almost all items of cardiovascular risk in children, but both countries have significant percentages of obesity and the population free of cardiovascular risk is minimal.

Key words: *cardiovascular risk, children, cholesterol, Colombia, glucose, Mexico, triglycerides*.

INTRODUCTION

Cardiometabolic risk is that which carries a predisposition to a chronic disease, such as atherosclerosis and diabetes; it can comprise a single factor or can be associated with Metabolic Syndrome (MetS) [1, 2]. Despite its being a topic widely studied in adults, in pediatric population it has not had the same impact [2]. Fortunately, there has been a major effort currently in the prevention of CardioVascular Disease (CVD) in childhood. Indeed, the adoption of novel indexes, apart from the traditional ones, has been proof of this growing interest. However, the identification of a high-risk subject when symptoms are absent remains a challenge [3].

In addition to the new markers for CVD, such as like homocysteine and fibrinogen, there are some other anthropometric indices with greater specificity for children, such as waist-to-height index (WtHR), which has completely replaced the Waist-Hip Ratio (WHR). This is a very useful tool to know the adiposity, which is one of the first steps in the atherosclerotic process [4].

Previous studies have confirmed that anthropometric indicators are associated with selected cardiometabolic risk factors in early childhood [5] but measurement of their risk factors beyond Body Mass Index (BMI) and waist circumference may provide evidence in defining cardiometabolic risk in early childhood [6].

The purpose of this study was to identify the main metabolic indicators for cardiovascular risk factors in primary school students from Mexico and Colombia.

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MATERIALS AND METHODS

This clinical, prospective, and comparative study was carried out in the cities of Toluca, Mexico, and Bogota, Colombia, from September 2015 to August 2016. Children aged 6–13 years from Primary Schools "Horacio Zuñiga" (Toluca) and "Venustiano Carranza" (San Mateo Atenco) both in Mexico, and "Gimnasio Yacard" (Bogota) in Colombia were matched by gender and age. We excluded all children whose parents did not authorize their participation by signing informed consent or those who did not accept to participate with informed assent. Children with phobias to venous sampling were excluded, and non-viable samples were not included in the final analysis.

Taking into account a previous work that showed that for Colombian children only 13% had an altered metabolic parameter [7] while in the population of Mexican children this percentage was 45%; accepting an alpha risk of 0.05 and a beta risk of 0.2 in a bilateral test, 30 subjects per group were required to find a statistically significant difference in proportion in the presence of an SM parameter.

Clinical and sociodemographic data were obtained from the child's clinical history. Food-consumption habits were evaluated with the 3-day reminder as gold standard (2 days from Monday to Friday, and 1 day for the weekend). All of the nutrients were calculated on Nutrimind® nutritional software, and equivalents per day were measured based on the groups from the Mexican model "Plato del Buen Comer". Physical activity was evaluated with the short form of the International Physical Activity Questionnaire (IPAQ), which has three levels: 1) Low level (no physical activity); 2) Moderate (3 or more days with intense activity, taking into consideration intense activity or at least 30 min), and 3) Intense level (intense activity 3 days per week, seven, or more days with any combination).

Weight and height were measured to one decimal place while wearing the child was wearing light clothing and without shoes, using a calibrated digital scale (Microlife AG 9435 model) and a stadiometer (Seca 213 model). Waist Circumference (WC) and Hip Circumference (HC) were measured as anthropometric indicators for cardiovascular risk utilizing a fiberglass tape measure to the nearest 0.1 cm, and for WC taking the midpoint between the last rib and the iliac crest, considering the average of both measures. The WtHR was determined by dividing WC (cm) by height (cm) [5]. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Blood Pressure (BP; mmHg) was checked by auscultation employing a pediatric sphygmomanometer and an appropriately sized cuff. Measurement was performed with the child at rest and by a single experienced professional. Percentiles for systolic and diastolic pressure by age and gender were determined [8].

Blood samples were collected to measure glucose (mg/dL), Total Cholesterol (TC) (mg/dL), High-Density Lipoprotein cholesterol (HDLc) (mg/dL), Low-Density Lipoprotein cholesterol (LDLc) (mg/dL), and TriGlycerides (TG) (mg/dL) (RX Monza CH200 Randox for Mexico and Mindray BS200 Annar Diagnostics for Colombia), according to standardized procedures. The TG-HDLc ratio (TG-HDLc) was calculated as a possible risk iondicator of organ damage. All measurements followed standardized procedures according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Malnutrition (underweight, overweight, and obesity) was defined according to the BMI percentiles of the World Health Organization (WHO) [9]. Cut-off points were defined with percentiles for WC, for the waist/hip index (0.85 for girls and 0.94 for boys) [10] and for WtHR equations from *Marrodán* et al. [4] High BP was defined as BP >95th percentile according to the National High Blood Pressure Education Program Working Group on Children and Adolescents (NHBPEP) [6].

Impaired Fasting Glucose (IFG) was defined by a value of fasting plasma glucose of >100 mg/dL according to the recommendations of the American Diabetes Association (ADA) [11]; for lipid profile and MetS, we utilized Adult Treatment Panel (ATP) III criteria as follows: TC < 170 mg/dL; LDLc < 110 mg/dL; HDLc > 45 mg/dL, TG from 0–9 years < 75 mg/dL, and TG from 10–19 years, < 90 mg/dL. For Tg-HDLc, a cut-off value of < 2.2 was obtained [12].

We utilized the SPSS ver. 22 statistical software package (IBM Corp., Armonk, NY, USA). Continuous data were expressed as means ± Standard Deviation (SD). Student *t* test or *Mann-Whitney U* test were used depending whether the variables were or were not normally distributed when doing comparisons per Country or per gender. The degree of association between variables was evaluated using the *Pearson* correlation. A P value of ≤ 0.05 was considered statistically significant in all tests. The Multivariate General Linear Model, Automatic Linear Modeling and Weight Estimation were used to analyze several variables to predict glucose, cholesterol and triglycerides levels.

The study was approved by the Ethics and Research Committees of the Medical Sciences Research Center (CICMED), Autonomous University of the State of Mexico (UAEMex), Toluca, Mexico (code: 2015/10), Universidad Antonio Nariño, Bogota, Colombia (Acta No. 001 of 2015, clause z), and Universidad de la Sabana, Bogota, Colombia (Act 50-2015). All of the procedures were conducted in accordance with the Declaration of Helsinki and the General Health Law of Mexico. Informed consent was obtained from the children's parents and informed assent, from the students.

RESULTS

Ninety students were included in the study: 57 from Mexico (22 boys and 35 girls), and 33 from Colombia (21 boys and 12 girls). Anthropometric and clinical characteristics are presented in Table 1. From the obtained data, there are three significant aspects between both studied groups. First, weight was higher in Mexican children (37.2 ± 12.5 kg) than in Colombian children (34.3 ± 8.9 kg). Second, BMI was also higher in Mexican young population (19 ± 5.5 vs. 18.4 ± 3.07). Third, in relation to both, the WHR and the WtHR, there were significant differences ($p \leq 0.001$) between Mexico and Colombia (0.8 ± 0.1 versus 0.5 ± 0.1) and (0.4 ± 0.06 vs. 0.78 ± 0.04).

Table 1. Anthropometric and clinical characteristics of the population (mean ± Standard Deviation - SD)

Variables	Mexican children			Colombian children			*p*
	Mean	SD	Range	Mean	SD	Range	
Age (years)	10.6	1.31	6.9 - 13.4	9.1	2.1	6 - 12.7	0.333
Height (cm)	141	9.5	108.5 - 160	135.9	12	119.9 - 163.3	0.065
Weight (kg)	37.2	12.5	18.2 - 80.9	34.3	8.9	21.5 - 51.5	0.182
BMI (kg/m^2)	19	5.5	12 - 38.7	18.4	3.0	13.2 - 24.5	0.525
Waist (cm)	68.6	12.1	53 - 113	65	10.2	51 - 97	0.523
Hip (cm)	77.5	10.3	60 - 116	83	11.1	64.5 - 110.1	0.51
WHR	0.8	0.1	0.14 - 1-07	0.5	0.1	0.4 - 0.8	≤0.001
WtHR	0.4	0.06	0.4 - 0.7	0.78	0.04	0.73 - 1	≤0.001
SBP (mmHg)	96.9	13.5	65 - 130	114	21.2	78 - 171	≤0.001
DBP (mmHg)	69	9.8	40 - 90	67.2	12.3	26 - 88	0.518
Physical activity (m/d/w)	1,510	1832.5	200 - 11,300	2,507.1	1,847	720 - 8760	0.001

BMI: Body Mass Index; DBP: Diastolic Blood Pressure; SBP: Systolic Blood Pressure; WHR: Waist-Hip Ratio; WtHR: Waist-to-Height Ratio; m/d/w: minutes per day per week.

According to our results, for the nutritional diagnosis, 10% of children were found with obesity, 68% with malnutrition and 8.7% with overweight in Mexico, while in Colombia these same percentages were 24.24%, 12.12% and 23.1%. To be more emphatic, while in Colombia 18% of children were eutrophic, in Mexico, this percentage was only 13.3%.

Regarding the biochemical parameters (Table 2), for Mexican population, we found that 3.5% of the children registered high glucose concentrations (>100 mg/dL), and regarding cholesterol, 31.5% of the population was found with numbers > 170 mg/dL, 28% with numbers < 45 mg/dL for HDLc, 50.8% exhibited numbers above desirable limits for TG, and 71.9% demonstrated an altered high TG/lipoprotein index. The results obtained for the Colombian population for these were 1.7, 42.4, 0, 57.5, and 45.4%, respectively. When comparing by country, it is possible to observe that Mexico has higher numbers in virtually all items except total cholesterol.

Table 2. Biochemical data (mean ± Standard Deviation [SD])

	Mexican children			Colombian children			
	Mean	SD	Range	Mean	SD	Range	*p*
Glucose (mg/dL)	90.1	10.1	73 – 112	78.2	10	51 - 106	0.518
HDLc (mg/dL)	43.1	7.9	24 - 56.2	60.5	10.3	43 - 78	0.158
LDLc (mg/dL)	94.6	21.5	41.8 - 133	102.4	31.2	46.4- 169.6	0.155
TC (mg/dL)	161.4	20.4	103 - 205	186.7	37.6	130 - 268	0.082
TG (mg/dL)	126.5	48.8	48 – 274	118.5	38.2	60 - 196	0.023
TG/HDLc index	3.1	1.2	1.1 – 6.6	2	0.8	0.8 - 4.4	≤0.001

HDLc: High Density Lipoprotein cholesterol; LDL: Low-Density Lipoprotein cholesterol; TC: Total Cholesterol; TG: TriGlycerides; TG/HDLc: TriGlycerides/High-Density Lipoprotein cholesterol index.

Nutritional information is illustrated in Table 3. Total caloric ingestion was higher in Mexican than in Colombian children (1,889.14 ± 300.78 calories vs. 1,850.9 ± 369.6), and this was similar with proteins (62.5 ± 14.16 vs. 56.33 ± 13.46 grams). On the other hand, fat consumption was higher in Colombian than in Mexican population (71.29 ± 23.91 vs. 66.29 ± 17.58 g/day). The distribution of fiber was similar for both countries (8.4 ± 2.82 vs. 10 ± 4.01 g/day). For the vitamin consumption, the same pattern was maintained with an intake below the daily recommendations for both countries. In the case of fatty acids (monounsaturated) and polyunsaturated, the higher consumption was registered in Colombian children (26.7 ± 5.3 vs. 16.2 ± 5.8 g/day) and (265.4 ± 326.1 vs. 184.8 ± 107.0 g/day). For food groups and equivalents

per day, the mean difference was between oils and fats, with an increased consumption in the Colombian group (5.24 ± 1.77 vs. 2.21 ± 0.92 equivalents); the remainder of the food groups remained homogeneous.

Classification of nutritional intake and percentage of nutritional recommendation is presented in Table 4. In both countries, only two groups covered the full recommendation for protein (100%) and dairy products (84% in Colombia and 88% in Mexico). For the carbohydrate group, consumption level is above the recommendation in both countries (110 and 120% for México and Colombia, respectively). The groups that were found at the lower end with a very low percentage of consumption in the Mexican group were those of fiber, vegetables, and sugars (1.7, 2, and 5%), while in the Colombian group, sugars make the difference with an increase of 10% on comparison with the Mexican group.

Table 3. Nutritional information (mean ± Standard Deviation - SD)

	Mexican children			Colombian children		
	Mean	SD	Range	Mean	SD	Range
Energy (calories/day)	1,889.1	300.7	1,300 - 2,741	1,850.9	369.6	912 – 2507
Carbohydrates (g)	218.2	119.7	98 - 745	240.3	47.1	115 – 317
Fat (g)	66.2	17.5	33 - 101	71.2	23.9	16 – 107
Protein (g)	62.5	14.1	33 - 89	56.3	13.4	17- 87
Fiber (g)	8.4	2.8	03 21	10.0	4.0	3 17
Pyridoxine B6 (mg/dL)	0.61	0.4	0 - 1.7	0.9	0.3	0 - 1.7
Folic acid B9 (µg/d)	115.7	18.9	50 - 165	116.5	33.1	34 – 178
Cobalamin B12 (µg/d)	1.41	0.6	0.5 - 3.6	2.2	1.0	0 - 4.3
Dietary cholesterol (µg/d)	202.1	69.8	76 - 380	175.6	97.8	0 – 373
MUFA (g/d)	16.2	5.8	04- 36	26.7	5.3	0 – 260
PUFA (g/d)	184.8	107.0	9 - 541	265.4	326.1	1 – 887
Saturated fatty acids (g/d)	16.2	10.4	02 62	11.3	9.3	1 43
Animal products (eq/d)	2.1	1.0	1 - 5.5	1.7	1.0	0 - 6.3
Cereals (eq/d)	7.4	1.5	04 11	7.4	1.8	03 12
Fruits (eq/d)	2.5	1.2	1 - 5.4	1.9	1.3	0 - 5.2
Oils and fat (eq/d)	2.2	0.9	0 - 5	5.2	1.7	1 8
Vegetables (eq/d)	1.2	0.8	0 - 4	0.4	0.3	0 - 1
Dairy products (eq/d)	1.1	0.4	0.4 - 2.7	1.2	0.6	0 - 2.8
Sugars (eq/d)	5.7	1.5	1.4 - 9	5.1	1.8	1 - 8.7

MUFA: MonoUnsaturated Fatty Acids; PUFA: PolyUnsaturated fatty acids.

Table 4. Classification of nutritional intake and percentage of nutritional recommendation (mean ±Standard Deviation - SD)

	Mexican children				Colombian children			
	A	I	≤RDA	>RDA	A	I	≤RDA	> RDA
Energy (calories/day)		X	19	81		X	8.8	91.2
Carbohydrates (g/day)		X	0	100		X	0	100
Fat (g/day)		X	7	93		X	3	97
Protein (g/day)	X		100	0	X		100	0
Fiber (g/day)		X	98.3	1.7		X	2.2	97.8
Pyridoxine B6 (mg/dL)		X	59.7	40.3		X	68.9	31.1
Folic acid B9 (µg/d)		X	68.1	31.9		X	80	20
Cobalamin B12 (µg/d)		X	82	18		X	75.7	24.3
Dietary cholesterol (mg/d)		X	10	90		X	5	95
Animal products (eq/d)		X	28	72		X	33	67
Saturated fatty acids (g/d)		X	30	70		X	33	6 7
Cereals (eq/d)		X	22.8	77.2		X	24.2	75 8
Fruits (eq/d)		X	5.2	94.8		X	9	91
Vegetables (eq/d)		X	98	2		X	97.3	2.7
Dairy products (eq/d)	X		84	16	X		88	12
Oils and fat (eq/d)		X	20	80		X	21 .2	78. 8
Sugars (eq/d)		X	5	95		X	15.1	84.9

A: Adequate; I: Inadequate, RDA: Recommended Dietary Allowance.

The Pearson test revealed a positive relationship between WtHR and WHR for the two countries and both rates were higher for Mexican population (0.89 ± 0.07 vs. 0.78 ± 0.04) and (0.48 ± 0.07 vs. 0.47 ± 0.07) (Table 5).

The Multivariate General Linear Model, showed that the levels of cholesterol are influenced by the WtHR (p=0.019), and glucose levels are influenced by the grams ingestion of carbohydrates (p=0.019), MonoUnsaturated Fatty Acids (MUFA) (p ≤ 0.001), and PolyUnsaturated fatty acids (PUFA) (p=0.008). Performing the same model but weighted by gender showed that a statistical significance was kept for the same intereactiones with the next values of p: 0.045, 0.021, ≤ 0.001 and 0.008. In a third approach, weighting per Country, carbohydrates, MUFA and PUFA determined the values of glucose (p=0.017, 0.001 and 0.013, repectively).

Table 5. Pearson correlation between Body Mass Index (BMI) and atherogenic indices

	Mexico		Colombia		Both countries	
	r	*P*	*r*	*P*	*r*	*P*
BMI vs. WHR	0.385	0.003	1.000	≤ 0.001	0.999	≤ 0.001
BMI vs. WtHR	0.681	≤ 0.001	1.000	≤ 0.001	0.999	≤ 0.001
BMI vs. TG/HDLc	0.420	≤ 0.001	0.002	≤ 0.990	0.001	0.995

BMI: Body Mass Index; TG/HDLc: triglycerides/high density lipoprotein cholesterol index, WHR: Waist Hip ratio; WtHR waist-height ratio.

Using the Automatic Linear Modeling it was obtained that the main predictor for cholesterol were WtHR, MUFA and lipids ingestion (grams/day) (Figure 1A). For glucose there were four main predictors: WHR, carbohydrates, MUFA and Saturated Fatty Acids (SFA) (Figure 1B). Finally, for triglycerides the pedictors were Products of Animal Origin (PAO) (equivalents/day), BMI, waist circumference, lipids ingestion (grams/day), cholesterol ingestion and Mean Arterial Pressure (MAP) (mm Hg) (Figure 1C).

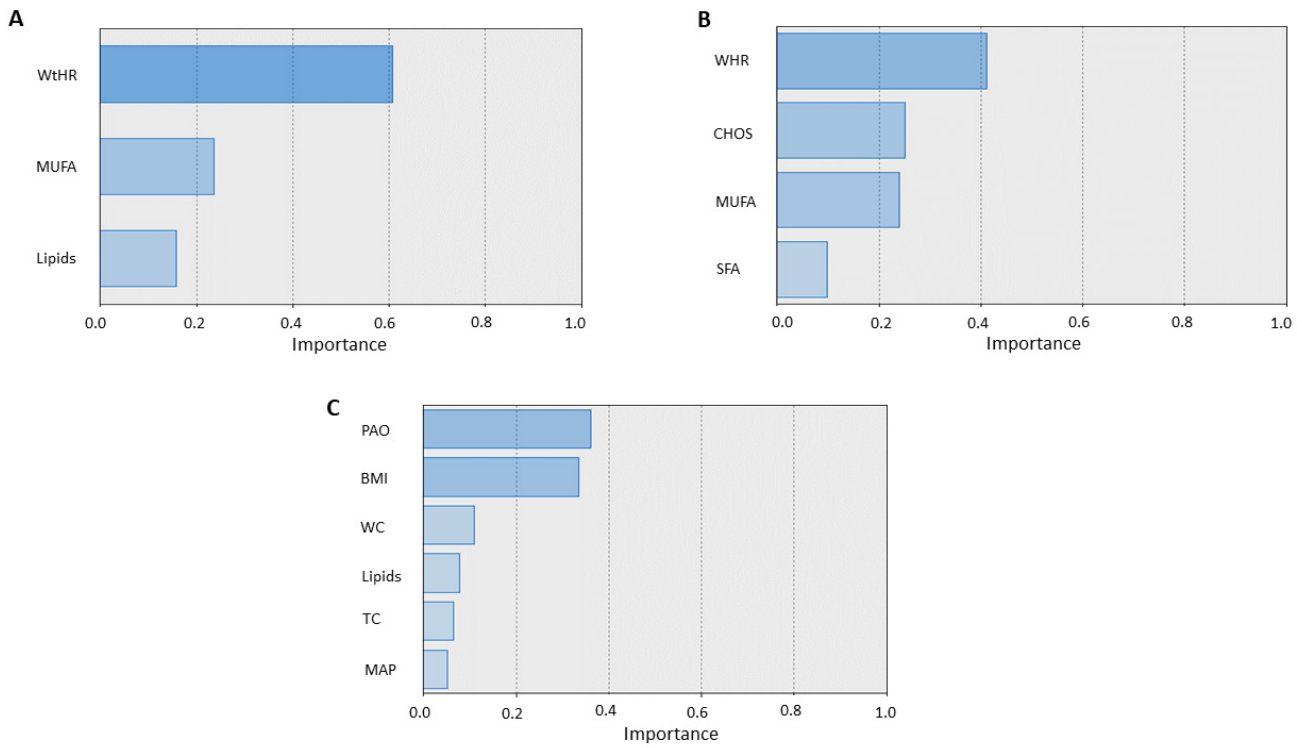

Figure 1. Main predictors for cholesterol (A), for glucose (B), for triglycerides (C).

Finally, the significant Weight Estimation tests weighted per gender are listed in Table 6, showing that for glucose levels the main determinants are carbohydrates, MUFA and Oils; for cholesterol these are MUFA, PUFA and Oils; in third place for LDL the significant variables are proteins, SFA, PAO and sugars; and last, for triglycerides the main variables are BMI, cholesterol and vegetables. When we contrast the same variables weighing per Country, the only difference was found for BMI (p≤0.001).

Table 6. Significant Weight Estimation tests weighted per gender

Variable		Coefficients				t	Sig.
		Non-standardized coefficients		Standardized coefficients			
		B	Standard error	Beta	Standard error		
Glucose	(Constant)	57.622	19.672			2.929	0.005
	CHOS	0.03	0.011	0.28	0.105	2.663	0.01
	MUFA	0.114	0.042	0.337	0.124	2.714	0.008
	Oils	-8.117	2.91	-0.337	0.121	-2.789	0.007
Cholesterol	(Constant)	116.203	49.539			2.346	0.022
	MUFA	-0.457	0.139	-0.381	0.116	-3.295	0.002
	PUFA	-0.034	0.017	-0.236	0.12	-1.972	0.053
	Oils	24.906	8.135	0.384	0.125	3.062	0.003
LDL	(Constant)	125.963	481.508			0.262	0.795
	Proteins	4.175	1.763	0.323	0.136	2.369	0.022
	SFA	6.084	2.135	0.37	0.13	2.85	0.006
	PAO	-110.572	30.294	-0.458	0.126	-3.65	0.001
	Sugars	152.142	62.479	0.282	0.116	2.435	0.018
TG	(Constant)	249.129	70.917			3.513	0.001
	BMI	3.694	0.954	0.381	0.098	3.874	0
	CHOS	-0.089	0.039	-0.221	0.096	-2.301	0.024
	Vegetables	-37.077	14.877	-0.243	0.098	-2.492	0.015

CHOS: carbohydrates, BMI: Body Mass Index; LDL: Low-Density Lipoprotein cholesterol, MUFA: MonoUnsaturated Fatty Acids; PAO: Products of Animal Origin, PUFA: PolyUnsaturated fatty acids, SFA: Saturated Fatty Acids, TG: Triglycerides.

DISCUSSION

CVD involves a complex phenomenon due to numerous causes that may be involved in including of the genetic predisposition and lifestyle, where the latter can be observed as the primary determinant. This is because it can be influenced by assuming a positive or a negative connotation according to each individual [13].

If one takes into consideration BMI values for cardiovascular risk, there is a difference between both populations. For example, in the case of Mexico, the majority of students had malnutrition, while in Colombia, recommended weight-per-age was the constant, with a small percentage with overweight. It should be noted that the socioeconomic context might exert an important influence, in that BMI is considered a weak marker when employed alone as a cardiovascular risk factor. Moreover, BMI is associated with age, not so with body fat; this is in agreement with previous studies in Germany by *Bohn* et al. and, with respect to Latin America, with a similar population in Argentina [14].

Recently, the relationship of WC with CVD and MetS has been studied in children and adolescents, determining its epidemiological clinical usefulness and notoriety, as it is sufficient to recall that this indicator is a prerequisite for the diagnosis of obesity. In this study, we observed significant figures when we related the WtHR as a cardiovascular risk factor in both populations studied. It is noteworthy that the figures obtained in both Colombian and Mexican children coincide with other Latin-American references, such as *Mederico* et al. [8] in Argentina and Chile. This reinforces the idea that it is better to use WtHR and WC in isolation for school population. Our results in this area are also similar to those referred by Cabrera in a study conducted in Cuba in a similar population [15].

WC/H is an index with a strong predictive value of cardiovascular risk compared with BMI or WC and even with the percentage of body fat, despite that the potential of this indicator has not been studied in its entirety [4]. There is a direct relationship with lipid profile and even with blood pressure figures. As in similar studies, it was observed that schoolchildren with a WC above the references correlated significantly with high blood pressure (above the 50th percentile for age).

No association was found between lipid profile and anthropometric indicators, except perhaps with that of serum concentrations of TG; despite what was found in the literature and with regard to metabolic alterations, in this study we do not report anything other than hypertriglyceridemia. This could be explained by the sample size and the areas monitored in both countries. Another factor to consider is the positive relationship found between the TG/HDLc index and cardiovascular risk factors, confirming its importance as a clinical sign of damage at the level of tissues and organs. In addition, in the sole study of *Di Bonito* et al. [16] conducted in Italy, measurement this indicator exceeds the measurements of another type of lipoprotein, with

the only limitation that in African population, it does not exert the same effect in this study, but where the author has worked exclusively with Latino population, it is entirely valid.

In a previous study with very similar population, *González Devia* et al. in 2014 [12] observed a positive correlation between atherogenic indices and BMI only for Mexican population; in our case, we found significant figures for both countries. As previously mentioned, both the WHR as well as the WtHR have a clinical interpretation and, combined with the traditional BMI, can enhance a more accurate interpretation of metabolic status in childhood.

With regard to the nutritional aspect in terms of food groups, this is similar for both countries, which was not expected, in that we found in the literature that Latin-American countries have a greater tendency toward greater consumption of vegetables and fruits. However, this is not so in the case of Colombia, where we can clearly observed consumption below the recommendations and one that, of course, also has to do with socioeconomic factors [17]. The fact that the intake of protein is the only one where they have an intake less than the recommended intake may be explained by the cost of meat in both countries resulting from difficult acquisition, and not the amount of carbohydrates that, as reported in the literature, comprises a completely excessive consumption. The latter is directly related with metabolic alterations such as hyperinsulinemia and dyslipidemia.

The ingestion of MUFA influences the serum levels of cholesterol and glucose. This has been previously stated by *Park* and *Lee*, both of whom demonstrated that the MUFA to SFA ratio modulates the genetic effects of Glucokinase regulator (GCKR) on serum lipid levels in children [18, 19].

In relation to the effect of Products of Animal Origin (PAO) (equivalents/day), lipids and cholesterol ingestion on the levels of triglycerides, it has been previously proven en several animal models that dietary proteins act synergistically with dietary lipids to regulate cholesterol metabolism, being described that dietary proteins and lipids exerted a separate effect on serum total cholesterol, very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol to high density lipoprotein cholesterol (LDL-C/HDL-C) ratio [20, 21].

The clinical picture of physical activity is disheartening when it comes to describing the findings for Mexican population, where the majority of the children were assigned a moderate degree up to null; undoubtedly, the entry of technology and advertising via television or the Internet have exacerbated the situation. Nonetheless, this is not the case in Colombia, where children engage in some type of physical activity more frequently. Despite not performing a physical activity, neither of the two populations had representative numbers of obesity, which is well known as the point of departure in the pathophysiology of CVD. This is important because, despite what has been reported in the most recent national surveys [22, 23], malnutrition and its metabolic alterations continue to comprise a current problem.

This study has some limitations that should be considered. Our sample is not representative of schoolchildren in Mexico and Colombia; therefore, the results cannot be generalized to a larger population. However, the results may be relevant to the extent that the authors have worked with indices of recent integration into the cardiovascular risk profile and by the amount of components discussed in the diet and physical activity, as well as feasibility and practicality for the use of anthropometric and metabolic markers proposed within the daily routine for comprehensive assessment of children-at-risk because they do not always have the opportunity to include biochemical markers due to the high cost of the gaps-in-evidence. Further studies are required to explore afterward both biological and environmental aspects and their effect on the diet as well as on the lipid profile in this age group.

It is important to promote programs and policies at the school level and at home that foster a healthy lifestyle in the school, and it is certainly important to include these as the priority group in terms of the prevention of CVD.

CONCLUSIONS

1. The use of anthropometric markers such as the WHR and the WtHR, in addition to already known indicators such as BMI and lipid profile, is a useful and accessible tool for the early diagnosis of cardiovascular risk factors in children.
2. This study points out that in children the levels of cholesterol are influenced by the WtHR, MUFA, PUFA, oils and lipids ingestion; glucose levels are influenced by WHR, carbohydrates, MUFA, PUFA, SFA and oils; for triglycerides the main pedictors are PAO, BMI, waist circumference, lipids, cholesterol and vegetables ingestion and MAP.

Conflict of interest

All of the authors declare that there are no competing interests regarding the publication of this paper.

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FOOD DIGESTION IN IVAN PETROVICH PAVLOV STUDIES ON 115 ANNIVERSARY OF HIS NOBEL PRIZE AND PRESENT AVENUES

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ABSTRACT
Story of *Pavlov's* studies on food digestion including his Nobel Prize in 1904 are presented. Some new ideas and concepts are also viewed based on 3th International Conference on Food Digestion held in Wageningen (the Netherlands) in 2014.

Key words: *food digestion, fistulas, saliva, gastric juice, pancreatic juice, intestinal juice, conditioned and non-conditioned biological reflexes, enzymes*

STRESZCZENIE
Przedstawiono historię badań *Pawłowa* nad trawieniem żywności, w tym jego Nagrodę Nobla w 1904 roku. Dokonano przeglądu niektórych nowych idei i koncepcji na podstawie 3. Międzynarodowej Konferencji na temat Trawienia Żywności, która odbyła się w Wageningen (Holandia) w 2014 r.

Słowa kluczowe: *trawienie żywności, przetoki, ślina, sok żołądkowy, sok trzustkowy, sok jelitowy, warunkowe i bezwarunkowe odruchy biologiczne, enzymy*

INTRODUCTION

Food digestion is prerequisite process for its further utilization by human body. Consumed food products containing proteins, fats and carbohydrates, must be split into amino acids, fatty acids, glycerol or fatty acids, and monosaccharides before dietary tract can absorb these nutrients into blood or lymphatic circulation for further metabolism. Catalytic activities of various enzymes and emulsifiers provide crucial mechanisms starting with ca. 6.8 pH in the mouth through 1.0 pH in the stomach and 7.0 in duodenum up to 8.0 pH in the rest of the intestines [3].

The knowledge of food digestibility is very useful in the final evaluation of all nutrients bioavailability to our body. Therefore respective digestion coefficients both in humans and especially animals are used both in health and diseases. It is also important that undigested fragments (e.g. fibre) of food and "waste" products are eliminated this way through the gastrointestinal tract. Also age and/or diseases related changes in this tract are often linked with xerostomia, dysphagia, atrophic gastritis or achlorhydria. Often changes of sensory perception are also linked (usually decreasing) with age having the risk of nutrient deficiency or digestibility due achlorhydria, liver and pancreas diseases or lactose intolerance.

STUDIES OF IVAN PETROVICH PAVLOV ON FOOD DIGESTION PROCESS

It seems to be very surprising that in the famous and interesting book "A History of Nutrition" by *Elmer Verner McCollum* [6] there is a chapter (12 pages) on "Ideas about food utilization derived from studies on digestion" no single sentence is devoted to *I.P. Pavlov's* studies. As a matter of fact this scientist was never mentioned in the whole book containing index of circa one thousand names. Similarly in "A Textbook of General Physiology" by *Mitchell* [7] there is a nice chapter on digestion (41 pages) but no studies related to *I.P. Pavlov* are mentioned in the literature list, however his name was quoted several times in the text.

Therefore the authors of this paper considered that the 115 anniversary of awarding *Ivan Petrovich Pavlov* (Figure 1) the Nobel Prize in Physiology or Medicine 1904 is a right opportunity to present his studies.

As seen from the Table 1, *I.P. Pavlov* was born in the priest family in Ryazan (Russia) in 1849 and obtained education at a cleric seminary. In these times the interest in life sciences scientific literature grew very quickly in Russia. For the first time Pavlov read about digestion and saw the digestive tract schema in

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an English philosopher *G.E. Lewes* book "Physiology of Common Life", published in Russian in 1861-62. He was also deeply influenced by the study of great Russian physiologist *Ivan M. Sechenov* (1829-1905) on brain reflexes, published in 1863. To pursue his interest *Pavlov* enrolled in 1870 at St. Petersburg University, in Faculty of Mathematics and Physics, the Department of Natural Science.

Table 1. *Ivan Petrovich Pavlov's* life – main events and achievements

	Event	Place	Date	Remarks
1.	Birth	Ryazan, Russia	14.IX.1849	Or 26.IX.1849
2.	Study at University of Saint Petersburg	St. Petersburg, Russia	1870-1875	Outstanding degree – Candidate of Natural Science
3.	Study and work at Academy of Medical Surgery, Veterinary Institute and Military Medical Academy (MMA)	St. Petersburg, Russia	1876-1879	Gold medal award for his research work in physiology at MMA
4.	Cooperation with famous clinician S.P. Botkin at MMA	St. Petersburg, Russia	1880-1883	Doctor's thesis: The centrifugal nerves of the heart (1883)
5.	Study with prof. Rudolf Heidenhain and prof. Carl Ludwig	Breslau and Leipzig, Germany	1884-1886	Food digestion in dogs – Heidenhain or Pavlov pouch
6.	Offers from University of Saint Petersburg, Tomsk University, University of Warsaw	St. Petersburg, Tomsk, Warsaw, Russia	1887-1889	No appointments
7.	Professor of pharmacology at the MMA	St. Petersburg, Russia	1890-1896	Chairmen of Physiological Department, up to 1896
8.	Director of the Department of Physiology, Institute of Experimental Medicine	Russia/Soviet Union	1987-1936	Most important center of physiological research
9.	Chairmanship of Physiology at the MMA	Russia/Soviet Union	1895-1925	
10.	Member of the Russian Academy of Sciences	St. Petersburg, Russia/ Soviet Union	1901* 1907**	*correspondent member ** academician
11.	The NOBEL PRIZE in Physiology or Medicine	Oslo, Norway	10 Dec 1904	"In recognition of his work on the physiology of digestion through which knowledge on vital aspects of the subject has been transformed and enlarged"
12.	Doctor Honoris Causa of Cambridge University	Cambridge, UK	19 July 1912	Story of artificial dog filled with fistulas from students
13.	Copley Medal of The Royal Society	London, UK	1915	On the ground of his investigations in the physiology of digestion and of the higher centres of the nervous system
14.	Order of the Legion of Honour	Medical Academy of Paris	1915	
15.	President of the XVth International Physiological Congress	Leningrad and Moscow, Soviet Union	9-17 Aug 1935	Monument of the Dog
16.	Passed away	Leningrad, Soviet Union	27 Feb 1936	

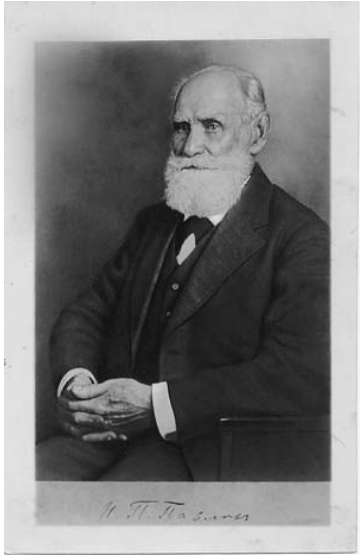

Figure 1. Portrait of *Ivan Petrovich Pavlov*
Source: http://www.iemrams.spb.ru:8100/english/pav-photo.htm

In 1875 *Pavlov* graduated from the University winning a gold medal for the first learned treatise on the physiology of the pancreatic nerves, developed in collaboration with *M.I. Afanasyev* (another student). *Pavlov* decided to continue studies in animal physiology and proceeded to the Academy of Medical Surgery (later Military Medical Academy) in St. Petersburg. He studied at professor *Ilia Cion* (1842-1912) and *Sergius Botkin* (1832-1880) laboratories and has learnt how to perform surgery operations.

In 1883 *Pavlov* received there PhD degree on "The centrifugal nerves of the heart". In the thesis *Pavlov* developed his idea of nervism, using as an example the intensifying nerve of the heart which he had discovered, and furthermore laid down the basic principles on the trophic function of the nervous system. Soon he received a foreign scientific internship in physiology and in the years 1884-1886 continued his research in Germany, in Breslau (Wrocław) in Professor *Rudolf Heidenhain's* laboratory, then in Leipzig (Lipsk) with Professor *Carl Ludwig* at the Institute of Physiology. Working with these two famous scientists *Pavlov* became acquainted with the functioning of the intestinal organs, including several aspects of food digestion from the mouth to the large intestine (e.g. saliva production or motility of the gastrointestinal tract), including the complex role of the nervous system, as suggested by *R. Heidenhain*. During his stay in Germany, *Pavlov* studied the digestion in dogs using an exteriorized section in the stomach perfecting the technique by overcoming the problem of maintaining the external nerve supply. This section of stomach became known as the *Heidenhain* and/or *Pavlov* pouch (pocket or small stomach). It was very helpful in explaining conditioning and involuntary reflex actions, using implanted fistulas (for collecting stomach juice). *Pavlov* knew and studied different points along whole the dog digestive tract, particularly stomach, where esophagostomy (Figure 2) was performed. Every day in the morning and the evening (sometimes also in the midday) *Pavlov* collected saliva or stomach juice. He was also using and perfecting an exteriorized section of the stomach improving technique by overcoming the problem of maintaining the external nerve supply.

Figure 2. *Pavlov* experiment: dog with esophagostomy and gastric fistula for collection of "pure" gastric juice during sham feeding (watercolor from the Pavlov exhibit at Koltushi)

Source: *Wood J.D.*, The First Nobel Prize for Integrated Systems Physiology: *Ivan Petrovich Pavlov*, 1904. 2004, *Physiology*, 19, 326-330. (http://physiologyonline.physiology.org/content/nips/19/6/326.full.pdf)

In 1890, after returning from Germany and unsuccessful applications at universities in St. Petersburg, Tomsk, and Warsaw he was appointed professor of pharmacology at the Military Medical Academy working there 5 years. But in 1895 by invitation of the Institute of Experimental Medicine *Pavlov* has organised and was director of Department of Physiology up to 1925.

In the Institute for over 45 year period *Pavlov* created the most important centre in the physiological studies and research. He developed the surgical method of the long-running experiment with extensive use of fistulas, which enabled the functions of various organs to be observed continuously under relatively normal conditions. With his method he opened the way for new advances in theoretical and practical medicine. He showed that the nervous system played the dominant part in regulating the digestive process, and this discovery is in fact the basis of modern physiology of digestion. In 1897 *Pavlov* published (in Russian) the results of his research in the book entitled "Lectures on the Function of the Principal Digestive Glands" (Figure 3). In recognition of his academic achievements he was elected a corresponding member of the Russian Academy of Sciences in 1901, and then – Academician in 1907.

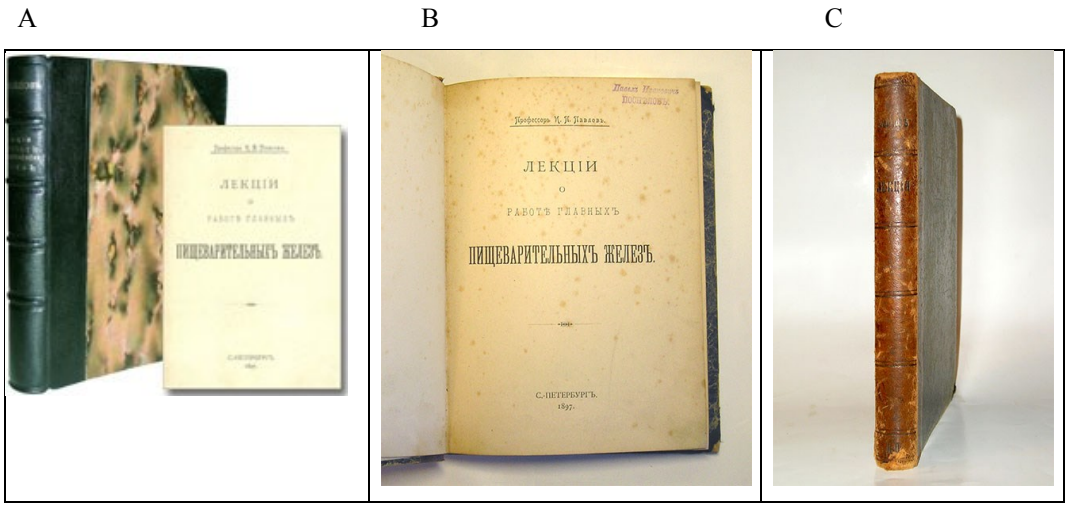

Figure 3. First edition of *I.P. Pavlov's* breakthrough book on digestion, St. Petersburg, 1897; discoveries presented in the book were the base for awarding *I.P. Pavlov* Nobel Prize (the title in Russian: И.П. Павлов. «Лекции о работе главных пищеварительных желез»)

Source A: http://www.raruss.ru/russian-thought/595-pavlov.html
Source B-C: http://knigirossii.narod.ru/html/18/224/1468458.html

Pavlov research was highly acclaimed in the world and therefore in 1901 he was nominated and in 1904 awarded the Nobel Prize in Physiology and Medicine "in recognition of his work on the physiology of digestion, through which knowledge on vital aspects of the subject has been transformed and enlarged" [12]. *Pavlov's* Nobel Prize was the first ever awarded for integrated systems physiology and the first for a Russian [11]. It is worth noting that in 1904 the Nobel prizes were awarded for the fourth time.

It is interesting to know that *Pavlov* investigated the gastric function of dogs and children by collecting, measuring and analysing juices especially from saliva glands for longer period of time. It was noticed that especially salivation process including psychic secretion and observing also conditioned and unconditioned reflexes e.g. in saliva secretion, which as we know is very much linked with cerebrum activity hormones or some physio-chemical change in the brain [7]. It is worthwhile to note that nervous system is very important in physiology of digestion, as reported by *Pavlov* during presenting his paper on International Congress of Physiologists held in Madrid in 1904. *Pavlov* continued studies on this phenomenon for the next 30 years up to the end of his life.

In 1912 he was given an honorary doctorate at Cambridge University. During the ceremony of nominating him the students participated in the ceremony offered *Pavlov* the pupped dog (a toy) with many fistulas attached. It was taken by professor *Pavlov* with great attention as a very precious present to his laboratory to memorialise this event of his career. The dog was placed among books, diplomas, and many other *Pavlov* souvenirs.

In the following years *Pavlov* received honorary membership of many scientific societies abroad and in 1915, upon the recommendation of the Medical Academy of Paris, was awarded the Order of the Legion of Honour.

Pavlov's achievements have also been highly appreciated by the communist government of the Soviet Union by nominating him as a chairman of the organising committee of the 15th International Physiological Congress which was held in Leningrad and Moscow in 1935. It was *Pavlov's* strong intention to build the monument of the dog as the friend of humanists servicing in science and to be finished just before the Congress (Figure 4).

Figure 4. The Monument to a Dog in the garden of the Institute of Experimental Medicine RAMS, St. Petersburg, on *I.P. Pavlov* initiative installed in 1935 to pay a tribute to the dog's unselfish service to biological science

Source: http://seaseas.livejournal.com/830261.html

I.P. Pavlov is recognised as a promoter or creator of the very important and classical now school of physiologists in Russia and the Soviet Union. In the experimental biological station in Koltushi (near St. Petersburg) he introduced a very interesting activity named as "Wednesday Meetings". It was the possibility to present and discuss his views on present and future scientific activity in the field of many topics, including physiology, among his co-workers since 1921 up to his death in Leningrad on February 27, 1936.

PRESENT STUDIES IN FOOD DIGESTION

After classical discoveries of *I. P. Pavlov* digestion of food was and is studied for many years by several researchers [9]. Recently new avenues in this field are investigated and created. The most current issues on digestion are presented and discussed at the International Conferences on Food Digestion (ICFD). Conferences are organised by INFOGEST, an international network focusing on "Improving Health Properties of Food by Sharing our Knowledge on the Digestive Process". The specific objectives of the network assign the most important research areas in food digestion which are [8]:

- Comparing the existing digestion models, harmonization the methodologies and suggestion of the guidelines for performing new experiments,
- Validation in vitro models towards *in vivo* data in animal and/or human organism,
- Identifying the beneficial/deleterious components that are released in the gut during food digestion,
- Demonstration the effect of these compounds on human health,
- Determining the effect of the matrix structure on the bioavailability of nutrients and bioactive molecules contained in food.

The audience of 3rd ICFD heard 27 oral presentations and from this number 4 were given by invited speakers [8]. They focused on methodology issues in digestion research and intestinal microbiome aspects. *Ourania Gouseti* (University of Birmingham, UK) stressed that the area of investigation in digestion has grown lastly significantly aiming to address the design criteria for functional food products, to assess energy density, and protein allergenicity. This resulted in the development of a large number of methodologies varying in the equipment and in the levels and types of chemicals. Current models represent also more complex elements of food digestion, incl. mechanical forces/shear. A new generation of models (e.g. DGM, TIM) is becoming very popular because of their ability to more accurately assess the effect of food matrix in digestion. Dr *Gouseti* team research has demonstrated the potential of food formulation/matrix in modulation digestion using a chemical engineering approach. The team has developed *in-vitro* and *in-silica* models to study human digestion and nutrient bioaccessibility.

Paul Singh (University of California, Davis, USA) presented novel tools using computational methods and *in vivo* trials to study food digestion. Professor *Singh's* team developed computational models based on average size and shape of a human stomach for understanding the role of physical properties of foods on digestion in the gastrointestinal tract. Viscosity of the digesta was identified as a key physical property: an increase in the viscosity reduced the strength of the fluid motion. During *in vivo* trials with growing pigs physical properties of brown and white rice gastric chyme were quantified to describe the food breakdown during an 8 hours postprandial period. The result of the experiment was that proximal and distal regions of the stomach had different inherent functionalities. On the base of these studies the researchers proposed a new classification system of food breakdown during gastric digestion for future trials on food digestion in a systematic manner.

Hauke Smidt (Wageningen University, The Netherlands) concentrated on identifying gastrointestinal (GI) tract microbes. He stressed that the using of culture-independent approaches is crucial to provide a comprehensive picture of the GI tract microbial functioning. GI tract ecology experiences a revival since the introduction of the approach based on 16S ribosomal RNA (rRNA) and its encoding gene. The culture-independent approaches gave insight into the temporal, spatial and inter-individual microbial diversity in the GI tract. These approaches offer great potential in finding significant correlations between microbiota compositional signatures and the health status of the body. Moreover meta-omics studies and stable isotope probing method allow studying the genetic potential and functional properties of the GI tract microbes. The application of these approaches to understand the role of intestinal microbiota in food components' digestion and health of the host can provide the necessary knowledge for the development of innovative dietary strategies.

Johan Garssen (Utrecht University and Nutricia-research, The Netherlands) started his presentation underling that the development of a healthy intestinal microbiome after birth plays an important role in immune development. Some of non-digestible oligosaccharides transferred through the breastmilk to the child affect the composition and/or activity of the GI tract microbiota leading to health benefits (prebiotic function). The awareness of the importance of these unique carbohydrates in immune-regulation is growing exponentially. The specific non-digestible oligosaccharides scGOS/IcFOS (ratio 9:1) induces a gut microbiome comparable to breastfed infants. Besides these indirect effects on the immune system

via microbiome changes prebiotic oligosaccharides can affect immune cells directly as well. The unique receptors such as lectins/galectins are responsible for these direct immune effects. Clinical trials indicated that scGOS/IcFOS impaired the incidence of infections and allergies, especially in infants and toddlers. However very recent research indicate significant effects on the immune system during HIV infections, cancer and asthma. More multicenter trials and long-term studies are needed in order to validate the uniqueness of non-digestible oligosaccharides in specialised and medical nutrition as well as pharma approaches.

In the frame of 3rd ICFD many posters were also shown. Among them 4 papers showing the results of the research curried out by *Wiesław Wiczkowski* team at the Institute of Animal Reproduction and Food Research of Polish Academy of Science were presented. The title of these posters were: Bioavailability of anthocyanins from fermented red cabbage; The bioaccessible reducing capacity of buckwheat-enhanced wheat breads estimated by electrochemical method; The Global Antioxidant Response (GAR) of buckwheat-enhanced wheat breads; Buckwheat-enhanced wheat breads ameliorate TNF-α-induced migration in an intestinal cell model (myofibroblasts of colon) [8].

FINAL REMARKS

Ivan Petrovich Pavlov's achievements as a pioneer in nutrition science are recognised in many publications, e.g. *Berger's* chapter entitled "Historia nauki o żywieniu" [History of nutrition science] in *Gawęcki* book (ed.) [1] or *Wood* paper [11].

It should be admitted here that recently some authors are often referring to *Pavlov's* pioneering research on the food digestion physiology. Among them *Clark* in his 15 pages paper [2] adopted the classical origins of *Pavlov's* conditioning. As a matter of fact it was originally introduced to the USA as "the *Pavlov* salivary reflex method" indicating that the term "conditioned" is used to show that the response is "learned".

In the very carefully analysis of *Pavlov* achievements and Nobel Prize Award procedure *Windholz* and *Kuppers* [10] made the statement that his works contributed significantly not only to digestion physiology, but also to the development in the discipline of psychology, and particularly by accepting new methodology and results of the study using conditional reflexes or responses as a paradigm in learning, and the role of the brain of the body interaction with the external environment.

Our paper covers most important activities and scientific achievements of the Nobel Laureate Professor *I.P. Pavlov* as well as some chosen facts of his private life. More information is presented in the very interesting and full of fascinating details book, published in Russian [5] and Polish [4], by great Russian physiologist, professor *Hachatur S. Kosztojanc* (1900-1961). These books are among several vivid publications which were of great help in preparation of this paper.

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