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Editorial office:

National Institute of Public Health - National Institute of Hygiene

24 Chocimska Street, 00-971 Warsaw, Poland

e-mail: roczniki.pzh@pzh.gov.pl

Editor-in-Chief phone: +48 22 54 21 266; fax +48 22 849 35 13, e-mail: cwludwicka@pzh.gov.pl

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100 years
of the National Institute of Public Health
- National Institute of Hygiene
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NATIONAL INSTITUTE OF PUBLIC HEALTH NATIONAL INSTITUTE OF HYGIENE

‘100 YEARS OF CARING FOR PEOPLE’S HEALTH IN POLAND’

The National Institute of Public Health – National Institute of Hygiene is the oldest public health institution in Poland.

We will be celebrating the centenary anniversary of its founding this year (2018). The Institute was originally established as the National Centre for Epidemiology, which in 1923 was renamed to the National Institute of Hygiene (*acronym in Polish* - PZH). Since 2007 it is called by its present name: The National Institute of Public Health – National Institute of Hygiene (*acronym in Polish* - NIZP-PZH).

Our Institute was created by the authorities of the reborn and newly independent Poland in response to health threats arising after the Russian October revolution and the German surrender in World War I within the old Russian and Austrian territories of partitioned Poland. The post-war ruins, poverty and mass movements of hundreds of thousands of refugees and repatriates resulted in many health epidemics; the gravest being typhus and typhoid fever.

The initial attempts to eliminate epidemics of infectious diseases and their prevention started in the newly independent Polish state in August 1919, when the Central Committee for Control of Typhus Epidemic was created. In March 1920, the Supreme Extraordinary Commissariat to Control Epidemics was established to whom we owe the organisation of sanitary cordons and effective control of epidemics at that time.

The National Institute of Hygiene was created as a scientific and laboratory base for the above activities, however from its inception, it was designed according to the farseeing vision of Dr Ludwik Rajchman, as a modern institute embracing the basic domains of public health with its expert activity.

Over the years, the scope of the Institute’s activity has been adapted to the changing needs and challenges in the field of public health. Its scientific, servicing and training activities have always dealt with basic threats of population health.

The initial range of such scientific research and services had covered issues of prevention, diagnoses and treatment of infectious diseases along with occupational health and safety, mental health, nutrition, testing of foodstuffs and articles of common use, sanitation engineering, sanitation administration and supervision over sera, vaccines and other drugs. Some of these areas have required separate scientific establishments to be set up. Our Institute has by such means thus given rise to the following:

- National Medicines Institute
- Institute of Food and Nutrition
- The Prof. J. Nofer Institute of Occupational Medicine in Łódź
- Institute of Maritime and Tropical Medicine in Gdynia
- Institute of Antibiotics

In the meantime our Institute has been dealing with new challenges.

At present there are two separate scientific divisions at the NIZP-PZH:

Analyses and Strategies in Public Health, which monitors and analyses public health status, performs economic and systemic healthcare analyses as well as deals with health promotion, prevention of chronic disease and with education and communication in public health.

Epidemiological and Environmental Safety, which performs surveillance of infectious diseases, their spread and risk factors. It also promotes vaccination and its safety, undertakes diagnostics, differentiates microorganism pathogens, performs health risk assessments along with identifying and assessing potentially harmful factors of a chemical, physical or microbiological nature, as found in food, water, air and selected areas of the environment.

The institute collaborates with many scientific centres both at home and abroad, like the WHO, European Commission, ECDC, EFSA.

The centenary celebrations of the NIZP-PZH are entitled '100 years of caring for people's health in Poland'. Their honorary patron is the President of the Republic of Poland Mr. Andrzej Duda.

As part of the celebrations, a Jubilee Gala will be held on the 9th April 2018 at the National Philharmonic.

Three scientific conferences are also planned:

'100 years of dealing with pathogens'- June 2018

This conference will be dedicated to infections arising from various pathogens and the role of NIZP-PZH in their diagnoses, monitoring and eradication with particular focus on the significance of immuno-prevention. Other issues covered will be the indispensability of microorganisms in assuring normal bodily function and also the possibilities of using pathogens for treating cancer.

'Poland's health – yesterday, today and tomorrow' – October 2018

This conference will focus on the changing health status of Poles, from the conditions prevailing between the world wars right through to the present time along with the projection of future trends, taking into account the changing demographics. Due account will also be given to the transformations seen in field of epidemiology, hygiene issues, health education and health promotion. In addition, the relationships between the health situation and the national health system will be considered.

'Food, water and air – do we live in a safe environment?' – December 2018

The conference will concentrate on issues regarding the identification of health hazards found in food, water, air and those arising from anthropogenic chemical stress in the environment together with climate change and their interrelated effects on health risk assessments.

We most cordially invite you to attend in these scientific conferences so organised, and to read our scientific journals as published by our Institute.

The Institute Management Board, the Scientific Committee and the Organising Committee for the Centenary Anniversary Celebration of NIZP-PZH



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THE OCCURRENCE OF RESVERATROL IN FOODSTUFFS AND ITS POTENTIAL FOR SUPPORTING CANCER PREVENTION AND TREATMENT. A REVIEW

Ewa Dybkowska, Anna Sadowska, Franciszek Świdorski, Rita Rakowska, Katarzyna Wysocka

Warsaw University of Life Sciences - SGGW, Faculty of Human Nutrition and Consumer Sciences,
Department of Functional Food, Ecological Food and Commodities, Warsaw, Poland

ABSTRACT

Over recent years, there has been increasing interest noted in those active substances derived from plants that show potential for preventing cancer development. The most promising candidate is resveratrol which can be found in large amounts in the skin of grapes, tomatoes and in red wine. Its beneficial effects on the human body are seen both in prevention and therapy. The anti-carcinogenic action of resveratrol is linked with its ability to neutralise reactive oxygen species and to modulate cellular processes such as apoptosis, and both cancerous cell proliferation and differentiation. This article presents the characteristics of resveratrol as a bioactive compound derived from natural sources exhibiting anti-cancer properties, which, because of a wide spectrum of biological activities may be used in the prevention of cancer. Many *in vitro* and animal-based studies have demonstrated such preventative anti-cancer action in the colon, prostate, breast and lungs. The beneficial effects of resveratrol are also presented when adopted as a support to conventional treatments of cancer using chemo- and radio-therapy.

Key words: *resveratrol, foodstuffs, chemo-prevention, cancer*

STRESZCZENIE

W ciągu ostatnich lat wzrosło zainteresowanie biologicznie czynnymi związkami pochodzenia roślinnego, które mogłyby ograniczyć rozwój chorób nowotworowych. Do najważniejszych z nich zalicza się resweratrol, występujący w większej ilości w skórcie winogron, pomidorów i czerwonym winie. Resweratrol wykazuje korzystne oddziaływanie profilaktyczne i terapeutyczne na organizm. Przeciwnowotworowe działanie reswerastrołu związane jest z jego zdolnością do neutralizowania reaktywnych form tlenu oraz modulującym oddziaływaniem na procesy apoptozy, proliferacji i różnicowania komórek nowotworowych. Niniejsza praca przedstawia charakterystykę reswerastrołu jako związku bioaktywnego pochodzenia naturalnego o działaniu przeciwnowotworowym, który dzięki szerokiemu spektrum działania biologicznego może mieć zastosowanie w chemoprewencji nowotworów. Przeciwnowotworowa aktywność tego związku została dowiedziona w licznych badaniach *in vitro* oraz na zwierzętach, w których wykazano działanie zapobiegawcze reswerastrołu w stosunku do nowotworów: jelita grubego, gruczołu krokowego, piersi, a także płuc. Przedstawiono również jego korzystne oddziaływanie wspomagające standardowe leczenie nowotworów przy zastosowaniu chemioterapii i radioterapii.

Słowa kluczowe: *resweratrol, żywność, chemoprewencja, nowotwór*

INTRODUCTION

Cancer constitutes the main threat to global health. Effective methods for reducing such disease risks, or in ameliorating and slowing down the disease course is constantly sought for because of the significant social problems arising from its high morbidity and mortality rates; most frequently from cancers of the lungs, breast, colon, prostate, stomach and liver. Within the

last decade, there have been great advances made in understanding the molecular biology of cancer, nonetheless there have been no drugs discovered hitherto that can definitively cure/combat this disease. One the most promising avenues for reducing cancer disease is in chemo-prevention; a form of prophylactic measures based on employing natural or pharmacological agents for preventing, inhibiting or regressing carcinogenesis [75]. Many cell-based

Corresponding author: Ewa Dybkowska, Warsaw University of Life Sciences - SGGW, Faculty of Human Nutrition and Consumer Sciences, Department of Functional Food, Ecological Food and Commodities, Chair of Functional Food and Commodities, Nowoursynowska 159 C, 02-776 Warsaw, Poland, tel./fax +48 22 5937040, e-mail: ewa_dybkowska@sggw.pl

or animal-model based studies, along with clinical trials, have enabled natural bioactive substances to be selected from a normal diet which are potentially able to prevent or inhibit many cancers from developing [31, 54, 77]. These compounds are both able to neutralise carcinogens and their harmful effects brought about on the cell. Examples of secondary plant metabolites that possess anti-carcinogenic action are: carotenoids, glucosinolates, monoterpenes, phytosterols, polyphenols, saponins and lectins. Resveratrol is classified under polyphenols and is found in a number of fruit, and in copious amounts in the skin of grapes, tomatoes and mulberry fruit extracts. For many years, resveratrol has been studied *in-vitro* and in laboratory animals. Epidemiological and clinical studies have also been performed. This work presents a review on the properties, occurrence, dosages and mechanisms of resveratrol action against various types of cancer.

STRUCTURE OF RESVERATROL

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a naturally occurring stilbenoid [19], consisting of two phenolic rings linked by an ethylene bridge, and is considered as belonging to the phytoestrogens. It is a white powder, insoluble in water but soluble in ethanol [2]. Resveratrol exists as two geometrical isomers: *trans*- and *cis*- (Figure 1) [24, 47]. The *trans*-resveratrol form has the greater stability and biological activity [80], however the *cis*-form arises from isomerisation of the *trans*-form following breakdown of the resveratrol polymer molecule due to the action of UV light during the fermentation of grape skins, or by being under high pH conditions. Both forms are present together in wine along with gallic acid and other antioxidants. [38]. A number of naturally occurring or synthetic resveratrol analogues are also recognised that differ in the type, number and the positions of their substitutions [2].

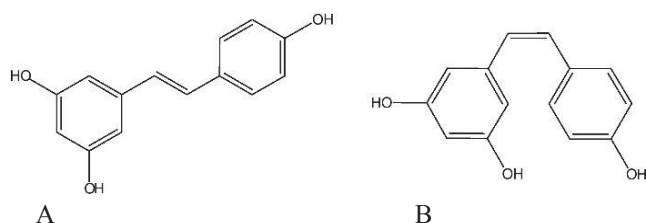


Figure 1. Chemical structure of resveratrol: A - *trans*-form, B - *cis*-form [24, 53]

OCCURRENCE OF RESVERATROL

Resveratrol is phytoalexin which up till now has been detected in 72 types of plants. It was first isolated in 1940 from the root of the white opuntium plant (*Veratrum grandiflorum* O. Loes) [2]. A rich source has been found to be the root of the knotweed, as cultivated

in China and Japan. Another source are grapes which contain varying amounts of this compound; Table 1, [53, 60]. Black grapes are the best source of resveratrol, whilst red grapes have more of this substance than the green varieties. Resveratrol is particularly abundant in grape skins [5, 49, 53, 61]. The highest amounts of the *trans*-3,5,4'-trihydroxystilbene form are found in the red Pinot Noir grape varieties [50]. In similar fashion to grapes, levels of this substance vary greatly in grape juice and wine, (Table 1) [21, 66], according to grape variety and the place of cultivation [77]. UV light stimulates the formation of resveratrol as well as do other factors such as the agrotechnical and the climatic, together with any stressful conditions that affect plants like pathogenic attack (bacterial or fungal) or by mechanical damage [21, 59]. Red grape wines contain more resveratrol than rosé or white wines, mainly resulting from the different types of manufacturing and the diverse varieties of grape [1]. Higher resveratrol concentrations are observed in organic wine [16]. In Poland, resveratrol concentrations vary from 0.39 to 4.45 mg/L in commercially available wines [62]. This compound can also be found in fruit berries, breadfruit, apples, nuts and in some herbs, (Table 1) [88]. It is also present in cocoa and chocolate [33, 49, 63]. The resveratrol composition measured in selected foodstuffs by various studies is presented in Table 1, [42, 53, 62, 66, 68, 80]. Exceptionally high levels are found in mulberry fruit extracts along with the seed extract from the exotic Jamun fruit [73]. Resveratrol has been found in the leaves and flowers of plants like orchids, scots pine and rhubarb [18], and furthermore it is synthesised by some trees such as eucalyptus and the spruce.

DOSAGE AND HEALTH SAFETY

Resveratrol is absorbed into the body via the small intestine and then becomes rapidly metabolised in the hepatocytes. Indeed, *in vitro* studies have shown that practically all resveratrol is metabolised in this way by humans [60, 62, 67]. It is recognised as being a safe and well tolerated substance at intakes of 5 g daily [58], however at levels of 2.5 and 5 g it can lead to mild to moderate symptoms appearing in the gastrointestinal system [11, 76]. A study by Patel et al. [57] suggests that 0.5 and 1.0 g of daily resveratrol is sufficient to elicit pharmacological action in the human gastrointestinal system. No side effects at intake levels below 1 g daily were observed in another study [76]. A multiple dose study demonstrated mild side effects, chiefly headaches, when 25, 50 100, 150 mg were given every 4 hours over a 48 hour period [4].

Resveratrol originating from the Japanese knotweed (*Polygonum cuspidatum*) has been used over many years throughout countries of the EU,

where dietary supplements are commercially available as derived from grapes and grape seeds, containing single 200-500 mg portions of resveratrol. Since 2012, permission was granted for the market placement of resveratrol manufactured from genetically modified yeast (*Saccharomyces cerevisiae*) as a novel food containing a maximum allowable dose of 500 mg/24 hours [20]. Over the last 10 years, there have been around 40 types of dietary supplements introduced onto the market in Poland that contain resveratrol. The main source of resveratrol in such products are grapes in the form of extracts from the fruit skin or seeds, but also from the roots of the Japanese knotweed; the resveratrol content in these supplements ranging between 50-250 mg. A study by *Ortuno et al.* [56] however showed a six-fold higher bioavailability of resveratrol when originating from natural grape products as compared to the resveratrol found in dietary supplements. It is estimated that the daily dietary intake of resveratrol per person in the EU from naturally occurring sources is on average 0.46 mg, whereas the highest of such daily intakes was recorded in elderly patients at 2.93 mg [20].

Table 1. Resveratrol content in chosen foodstuffs according to published studies

Foodstuff	Resveratrol content	Literature source
Lingonberry	5.88 µg/g d.m.	[66]
Grapes	6.47 µg/g d.m.	[66]
Fresh grapes	0.16-3.54 µg/g	[53, 61]
Peanuts and pistachios	0.02-1.79 µg/g	[62, 80]
Raw peanuts	0.09-0.30 µg/g	[42]
Roasted peanuts	0-0.13 µg/g	[42]
Grape skin	50-100 µg/g	[5]
Dried grape skin	24.06 µg/g	[53, 61]
Plum skin	0.1-6.2 µg/g	[68]
Tomato skin	18.4±1.6 µg/g d.m.	[63]
Mulberry fruit extract	50.61 µg/g d.m.	[73]
Grape seed extract	5.89 µg/g d.m.	[73]
Grape skin extract	3.54 µg/g d.m.	[73]
Cranberry juice	0.2 mg/L	[53]
Red grape juice	0.5 mg/L	[61]
Grape juice	0.45-2.60 mg/L	[81]
Grape juice	0.003-14.50 mg/L	[65]
Wine	0.1-14.3 mg/L	[21]
Cocoa	1.85±0.43 µg/g	[33]
Bitter chocolate	0.35±0.08 µg/g	[33]
Milk chocolate	0.10±0.05 µg/g	[33]
Milk chocolate	0.35±0.08 µg/g	[33]
Peanut butter	0.27-0.70 µg/g	[42]

ANTI-CANCER ACTION

Resveratrol is a compound that demonstrates a comprehensive range of chemo-preventative and chemo-therapeutic actions. Its anti-cancer properties arise from anti-oxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic and anti-proliferative characteristics [19, 77]. The anti-cancer action of resveratrol is, above all else, achieved by inhibiting the proliferation of cancerous cells, initiating apoptosis and modulating the action of a range of pro- and anti-apoptosis factors which affect apoptosis, differentiation of cancerous cells, reducing inflammatory reactions and neutralising free radicals [15, 19, 31, 54].

The mechanism underlying the chemo-preventative action occurs in two ways and is divided into two categories: blocking factors (acting at the initiation stage) and suppression factors (modulating stages of cancer promotion or progression) [5]. Because of its wide spectrum of biological activity, resveratrol is able to block every stage in the process of cancer development, ie. initiation, promotion and progression by means of modulating the signalling transduction pathways [5, 31, 74, 75]. Resveratrol can block the initiation phase of the tumour formation process; primarily through its ability to remove free radicals. Resveratrol exerts a protective effect, preventing lipid peroxidation and DNA damage by reactive oxygen species, thus in turn limiting cell mutation processes [3, 30]. In the promotion phase, resveratrol reduces the activity of cytochrome P-450 (CYP) compounds and inhibits their transcription. Cytochromes are involved in free oxygen radical formation which thereby cause increased activity of carcinogenic factors [67]. Resveratrol reduces the expression and activities of the CYP1A1, CYP1A2 and CYP1B1 enzymes and thus affords cellular protection against the action of many carcinogens; for example by polycyclic aromatic hydrocarbons [9, 47]. As an antioxidant, resveratrol prevents normal cells from being transformed into cancerous ones [50]. In the last and third phase of tumour development, resveratrol silences various cancer cell lines by partially inhibiting both DNA polymerases (an enzyme catalysing DNA synthesis) and ribonucleotide reductase (an enzyme vital for DNA synthesis in dividing cells). In addition, resveratrol inhibits the proliferation of tumour cells and DNA replication, inducing cellular apoptosis, thereby helping the removal of damaged or spent cells [88].

Throughout many years, resveratrol has been the subject of numerous biochemical, clinical and epidemiological studies which have confirmed its chemo-preventative action. Studies that confirm the anti-tumour action of resveratrol are mainly on the colon, prostate, lungs, breast and ovaries. Emerging

outcomes from clinical studies on human subjects confirm those findings, based on *in-vitro* studies on laboratory animals within the last ten years, which show that resveratrol also has a significant potential for improving health and preventing chronic disease in humans [77].

CANCER OF THE COLORECTAL CANCER

Many studies on animals and humans demonstrate the chemo-preventative action of resveratrol on tumours of the colon. An *in vitro* study by Wolter et al. [85] showed that resveratrol is able to inhibit the colonic cancer cell cycle. The anti-cancer action of resveratrol has also been demonstrated in animal models as well as in human clinical studies [47]. Experiments on animals predisposed to developing intestinal cancers have shown that this compound inhibits the proliferation of tumour cells [71]. The effect of grape extracts on blocking cancer has been studied in humans. Patients diagnosed with colorectal cancer after successful tumour resection were given a grape extract over 14 days containing relatively low doses of resveratrol ranging 0.073-0.114 mg. Nonetheless, those genes responsible for cancer development became inhibited in such patients. Further studies were performed using higher resveratrol doses of around 500 mg to 1 g, where decreases in cancerous cell proliferation of the colon was shown to be dose dependent [62]. In another study, healthy subjects received 500 mg to 5 g doses of resveratrol for 29 days where blood testing demonstrated, regardless of the administered dose, decreased production of IGF-1 (insulin-like-growth-factor 1) and IGFBP-3 (insulin-like-growth-factor-binding protein 3) growth factors, which are associated with the development of cancer [15]. A recent clinical study by Yang et al. [87] however confirmed that resveratrol inhibited colorectal cancer cell proliferation in a time and dose dependent fashion. Resveratrol's protective role in colorectal cancer is linked to modulating gene expression through its effects on p21 and Bax proteins [47]. Resveratrol induces cell apoptosis with the participation of the p53 tumour suppressor [17]. Ji et al. [35] demonstrated that resveratrol inhibits metastasis of colorectal cancer, both *in vitro* and *in vivo* in mice. Upon studying the effect of micronized resveratrol (SRT501) in patients with colorectal cancer metastasised to the liver, Howels et al. [32] showed that the micronisation increased the absorption of this compound, thereby increasing its bioavailability. They demonstrated the usefulness of this form of resveratrol as a potential agent for preventing cancer in those organs distant from the site of absorption.

PROSTATE CANCER

In vivo studies have shown that resveratrol blocks tumour growth in athymic mice by inhibiting

angiogenesis and increasing apoptosis in xenograft LNCaP cells. It also prevents cancer progression and growth in TRAMP mice (a prostate cancer animal model) and reduces the expression of androgen receptors in tumour cells [69]. It was shown that resveratrol's antitumor activity was elicited through its effects on the cell cycle, apoptosis, angiogenesis, metastasis, tumour invasion, as well as on other signal transduction pathways; eg. NF-kappaB, MAPK EGF in the prostate cancer cell [2, 64]. Resveratrol blocks the cell cycle and induces cytotoxicity and apoptosis in prostate cancer cells [93]. Studies by Ganapathy et al. [25] on prostatic cancer cells have confirmed that resveratrol may enhance the proapoptotic potential of TRAIL (TNF-Related Apoptosis Inducing Ligand); a cytokine capable of inducing apoptosis in cancer cells which is of low toxicity to untransformed cells. In the cell lines investigated, 60% of tumour cells were sensitive to TRAIL-induced apoptosis. Upon subjecting PC-3 cells (prostate cancer cell line) to resveratrol action in mice, angiogenesis became blocked in similar fashion to the effect of TRAIL. Fewer numbers of capillaries were found in tumour tissue and there was decreased expression of VEGF and VEGFR2 (vascular endothelial growth factor) as well as the metastatic matrix metalloproteinase markers (MMPs); MMP-2 and MMP-9. Elevated MMPs expression is related to an increased potential of metastases occurring in various types of cancerous cells. Resveratrol also inhibits cytoplasmic phosphorylation of the transcription factor FKHRL 1 (Forkhead Homolog Rhabdomyosarcoma Like 1), thus causing its activation and raising its ability to bind with DNA [52]. These aforementioned studies suggest that resveratrol may block the growth of prostatic cancer, the development of metastases and angiogenesis, along with perhaps being effective in the actual treatment of this condition. A study by Kumar et al. [39] confirmed the anti-tumour effect of resveratrol and its analogues on prostate cancer arising from their regulation of the chromatin modifier MTA1 (Metastasis-associated protein 1) and microRNA (miRNA). Anti-cancer effects were demonstrated on prostate cancer by both natural and synthetic analogues of resveratrol: pterostilbene, trimethoxy-resveratrol, piceatannol, diacetyl-resveratrol (2AC-Res, *trans*-3,5-diacetylstilbene), triacetyl-resveratrol (3AC-Res, *trans*-3, 5,4'-triacetylstilbene) and dimethoxystyryllaniline (DMSA, *trans*-4-(3,5-dimethoxystyryl) aniline).

LUNG CANCER

Animal studies have shown a significantly reduced proliferation of lung cancer cells transplanted into mice upon oral administration of resveratrol glycosides [37]. Other studies on the lymphocytes of both healthy and lung cancer patients have revealed

that resveratrol reduces the expression of cytochrome P450 (CYP1) isoenzymes in a dose-dependent manner under basic conditions, as well as after induction with benzo[a]pyrene and an extract of tobacco smoke. Such findings suggest that resveratrol may prevent or delay cancer development in tobacco smokers and to also confirm both its inhibition of cytochrome P450 isoenzymes which participate in phase I reactions, as well in stimulating the phase II metabolizing enzymes [50]. An *in-vitro* study by Zhang et al. [89] found that the 3,4'-trihydroxy-trans-stilbene resveratrol analogue induces apoptosis and autophagy in lung cancer cells.

BREAST CANCER

Studies by Gehm et al. [29] concluded that appropriate concentrations of resveratrol are needed to block oestradiol binding to oestrogen receptors in human breast cancer cells. In some cells, this compound functions as a 'super-agonist', where it has a higher activity than oestradiol, whilst in other cell types it exhibits an equal or lowered agonist activity as compared to oestradiol. It was suggested that resveratrol, which displays phyto-oestrogenic properties, regulates the expression of many genes directly associated with breast cancer development, amongst which are included the BRCA1 suppressor gene [41], as well as decreasing carcinogen DMBA-induction (7,12-Dimethylbenz[a]anthracene) of preneoplastic lesions in cultures of murine breast cells [48]. According to Laux et al. [40] resveratrol induces apoptosis of breast cancer cells by means of p-53 protein dependent signalling pathways. The anti-tumour effect can be linked to the inhibition of COX-2 and MMP-9 (matrix metaloproteinase-9) enzymes involved in the formation of metastases and NFκB, which mediates proliferation [8]. The chemotherapeutic properties of resveratrol in breast cancer were confirmed in murine studies showing reduced tumour growth, decreased angiogenesis and an increased apoptosis rate, achieved through reducing levels of VEGF endothelial growth factor and modulating progesterone receptor expression [28]. A study investigating the effect of resveratrol on the course of DMBA induced tumours in mice showed that dietary resveratrol is particularly beneficial. Indeed, when giving a resveratrol enriched diet to animals, cancer morbidity becomes reduced [10]. Whitsett et al. [84] also showed that DMBA treated rats when given dietary resveratrol at 1 g/kg inhibited cancer by reducing the number of tumours developing and extending the time of their suppression.

OVARIAN CANCER

Numerous studies have been conducted on the anti-tumour properties of resveratrol on ovarian

cancer cells which have demonstrated its inhibitory effects on tumour cell growth. The biochemical and morphological changes underway in tumour cells suggest more of an autophagocytosis process than apoptosis. The former may constitute a second mechanism of cell death induced by resveratrol which is apoptosis independent [55, 70]. Zhong et al. [90] investigated the effect of resveratrol treatment on two human ovarian cell lines, OVCAR-3 and Caov-3, which clearly demonstrated a marked inhibition of accumulation in the G1 phase, increased apoptosis and a simultaneous suppression of STAT3 (Signal transducer and activator of transcription 3). It was concluded that resveratrol is a promising candidate for treating ovarian cancer. Resveratrol inhibited glucose uptake in murine studies and significantly affects anti-tumour action through inhibiting ovarian tumour growth [78]. The latest *in-vitro* studies have shown a beneficial effect of a new derivative of resveratrol, 3,3',4,4'-Tetrahydroxy-trans-stilbeneon, on ovarian cancer by increasing apoptosis rates, inhibiting proliferation but accelerating cancer cell aging. It may thereby prove to be an invaluable supporting tool for combating ovarian cancer [51].

OTHER CANCERS

Apoptosis in cancer cells has been shown to be induced by resveratrol in cases of neuroblastoma, which is a very aggressive cancer suffering from a particularly poor prognosis at its advanced stages [45]. Other studies have observed a beneficial effect of resveratrol in treating B-cell lymphoma [12], non-Hodgkin's lymphoma [34], medulloblastoma - medulloblastoma [82], prostate cancer [27] and gastric cancer [26]. In a study on rats receiving N-nitrosomethylbenzamine (an oesophageal cancer provoking factor), the applied resveratrol doses of 1 or 2 mg/kg reduced both the number of tumours and their size [43]. Kalra et al. [36] indicated that resveratrol induces apoptosis in mouse skin tumours, suggesting chemo-preventive action through modulating those proteins involved in the mitochondrial pathway of apoptosis. Resveratrol induces the expression of pro-apoptotic Bax and p53 proteins, contemporaneously reducing anti-apoptotic Bcl-2 protein. Reducing the melanoma tumour growth due to resveratrol was confirmed in a murine study by Carletto et al. [14], which demonstrated a higher effectiveness when using a nanocapsule formulation. Resveratrol was observed to afford modulated protection against UVB radiation; this being the main cause of non-melanoma skin cancer [6]. It also diminishes the number of liver cancer cells and reduces the incidence of hepatitis B in HCC-infected mice (hepatocellular carcinoma) [47]. Resveratrol suppresses the formation

of reactive oxygen species and stimulates the growth of hepatocytes which replace damaged liver cells and supports their regeneration [44]. *Cai et al.* [13] have shown resveratrol to inhibit proliferation and induce apoptosis in a nasopharyngeal cancer cell line. Studies on the expression of 2,059 genes with potential links to the development of renal cell cancer examined the effect of giving resveratrol and showed that this compound may have very powerful and dose dependent anti-tumour action [72]. Recent studies indicate that resveratrol inhibits proliferation of pancreatic cancer cells, eliciting apoptosis and halting the cell cycle. It also stops metastasis, and thereby can be regarded as a potential anti-tumour agent in the treatment of pancreatic cancer [19, 86]. *Gao et al.* [46] found that resveratrol inhibits the Hh (hedgehog) and EMT (Epithelial mesenchymal transition) signalling transduction pathway and also inhibits tumour invasion and metastasis of gastric cancer *in-vitro*. The latest studies on the synthetic analogs of resveratrol have demonstrated their powerful anti-cancer action which may be used in the future treatment of leukaemia arising from their inhibitory effect on the polymerisation of tubulin.

RESVERATROL, A SUPPORTIVE FACTOR FOR CHEMO- AND RADIO- THERAPIES

Fulda and Debatin [23] found resveratrol to be a potent factor for sensitising cells to chemotherapeutic agents used in oncological treatment; for example in lung cancer. Initial treatment with resveratrol appears to inhibit the cell cycle during the S phase, which promotes induction of cellular apoptosis caused by the given medication. Such findings were also confirmed in studies where three malignant melanoma cell lines were isolated, with different levels of resistance to chemotherapeutics, and then subjected to resveratrol. Significant inhibition of tumour cell growth was observed together with cell cycle disruption, resulting in a sensitisation to standard treatment. It was suggested that this compound may become one of the adjuvant drugs used in treating advanced cases of melanoma [22]. When used at high doses, resveratrol sensitises cancer cells to X-rays when treating cases of cervical cancer, in chronic myeloid leukaemia and erythema in multiple myeloma. It was also shown to decrease the formation of free radicals in pancreatic cancer cells when administered together with radiation. Nevertheless in this last case, mitochondrial function became compromised when only resveratrol was given without any exposure to radiation [7]. Other studies found that resveratrol causes apoptosis by depolarising the mitochondrial membrane potential in the those β -cells of acute lymphoblastic leukaemia resistant to radiotherapy [67].

SUMMARY

As a natural ingredient in foodstuffs, numerous studies have demonstrated that resveratrol possesses a very high anti-oxidant potential, exhibits anti-tumour action as well as being a likely candidate agent for the prevention and treatment of several types of cancer. Its anti-cancer properties have been confirmed by many *in vitro* and *in vivo* studies which show that resveratrol is able to inhibit all stages of carcinogenesis; ie. initiation, promotion and progression. The anti-tumour action arises mainly from effects such as anti-inflammatory, anti-oxidant, pro-apoptosis and anti-proliferative actions. Many studies have provided much evidence that resveratrol not only acts a chemo-preventative agent, but also possesses chemo-therapeutic properties. *In vitro* work has shown that resveratrol enhances the effectiveness of chemotherapy through inactivating NF- κ B protein (a transcription factor) that is formed by cancer cells and which controls the expression of certain genes. When this factor is present, cancer cells become resistant to chemotherapy which then allows them to multiply. Resveratrol blocks this transcription factor, thereby enabling chemo-therapeutics to act at their targeted sites [88].

Animal experiments, along with clinical studies have shown the anti-carcinogenic action of resveratrol in instances of the following cancers: colon, prostate, lungs, breast and ovaries [77]. Apart from its preventative effects, many workers have demonstrated chemo-therapeutic action by increasing the efficacy of traditionally used chemotherapy and radiotherapy. Studies have found that resveratrol is a powerful agent for sensitising cells to chemotherapy used for oncological treatment that includes cancers of the lungs, cervix, chronic myeloid leukaemia, multiple myeloma and the prostate. Resveratrol is a natural product commonly found in food supplements which can be singly used or in supporting treatment. It is a natural and biologically active substance, safe to use, and thus should be much more widely consumed in those natural products rich in this ingredient and/or as dietary supplements, where it occurs in a concentrated form at higher levels (1 g/day). There is therefore a need to promote knowledge of resveratrol's beneficial pro-health and anti-tumour effects. Conventional cancer treatment with chemotherapy should be supported by an increased consumption of this substance from foodstuffs that contain natural amounts as well as via dietary supplements.

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INFLUENCE OF SELECTED DIETARY COMPONENTS ON THE FUNCTIONING OF THE HUMAN NERVOUS SYSTEM

Agnieszka Wendolowicz, Ewa Stefańska, Lucyna Ostrowska

Medical University of Białystok, Department of Dietetics and Clinical Nutrition, Białystok, Poland

ABSTRACT

The diet is directly connected not only with the physical status but also with the functioning of the brain and the mental status. The potentially beneficial nutrients with a protective effect on the nervous system function include amino acids (tryptophan, phenylalanine, tyrosine, taurine), glucose and vitamins C, E, D and *beta*-carotene, B group vitamins (vitamin B₁₂, vitamin B₆, vitamin B₄, vitamin B₁) and minerals (selenium, zinc, magnesium, sodium, iron, copper, manganese, iodine). The presence of antioxidants in the diet protects against oxidative damage to nervous system cells. Biochemical data indicate that polyunsaturated fatty acids such as arachidonic acid (AA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and gamma-linolenic acid (GLA) as structural components of the nervous system play a key role in its function. The nutrition of the entire body also influences the production of neurotransmitters in the brain. A diet without an appropriate supply of protein, mineral nutrients or vitamins may result in a failure to form appropriately balanced numbers of neurotransmitters, which, as a result, may lead to neurotransmission dysfunction. This is the reason why proper nutrition is based on vegetables, fruits, whole-grain cereal products supplemented with products providing full-value protein (dairy products, fish, lean meat) and high-quality fat products (vegetable oils, fish fats).

Key words: *nutrition, central nervous system*

STRESZCZENIE

Sposób żywienia ma bezpośredni związek nie tylko ze stanem fizycznym ale również z funkcjonowaniem mózgu i kondycją psychiczną. Wśród potencjalnie dobroczynnych składników pożywienia działających protekcyjnie na funkcjonowanie układu nerwowego wymienia się między innymi aminokwasy (tryptofan, fenyloalanina, tyrozyna, tauryna), glukozę oraz witaminy C, E, D i *beta*-karoten, witaminy z grupy B (witamina B₁₂, witamina B₆, witamina B₄, witamina B₁) oraz składniki mineralne (selen, cynk, magnez, sód, żelazo, miedź, mangan, jod). Obecność w diecie przeciwutleniaczy chroni przed oksydacyjnym uszkodzeniem komórek układu nerwowego. Dane biochemiczne wskazują, że wielonienasycone kwasy tłuszczowe takie jak kwas arachidonowy (AA), kwas dokosaheksaenowy (DHA), kwas eikozapentaenowy (EPA) oraz gamma-linolenowy (GLA) jako składniki strukturalne układu nerwowego odgrywają kluczową rolę w jego funkcjonowaniu. Odżywienie całego organizmu wpływa na wytwarzanie w mózgu neurotransmiterów. Brak w diecie odpowiedniej podaży energii, białka, związków mineralnych czy witamin może spowodować niewykształcenie odpowiednio zbilansowanych ilości neurotransmiterów, co w efekcie może prowadzić do dysfunkcji neurotransmisyjnej czego konsekwencją mogą być różne zaburzenia stanu psychicznego. Dlatego, tak ważne jest prawidłowe żywienie opierające się na warzywach, owocach, produktach zbożowych z pełnego przemiału uzupełnione produktami dostarczającymi pełnowartościowego białka (produkty mleczne, ryby, chude mięso) oraz produktami tłuszczowymi o wysokiej jakości (oleje roślinne, tłuszcze pochodzące z ryb).

Słowa kluczowe: *żywienie, centralny układ nerwowy*

INTRODUCTION

The diet influences human health to a large extent. Proper functioning of the central and peripheral nervous system depends on the diet [8, 9]. The function of the nervous system depends on the proper supply of

the necessary nutrients. The brain does not have the ability to store nutrients, so it requires a continuous supply of easily accessible sources of energy, carbohydrates, protein and fats. Nervous system cells need nutrients to build and keep their structure and also to maintain their function and avoid premature

Corresponding author: Agnieszka Wendolowicz, Department of Dietetics and Clinical Nutrition, Medical University of Białystok, Mieszka I-go 4B, 15-054 Białystok, Poland, Tel. +48 85 7328225, Fax +48 85 6865302; e-mail: agnieszka.wendolowicz@umb.edu.pl

ageing [5, 17]. The brain is a priority organ in the body and necessary nutrients are delivered to it first, before other organs, and, if necessary, the brain can use the reserves of other organs, thus weakening them. The consumption of too low or too high amounts of food may have an enormous influence on the human mental condition as individual nutrients cause significant changes in the biochemistry of the brain which has an effect on thinking processes and behaviour [17]. The potentially beneficial nutrients with a protective effect on central nervous system function include amino acids (tryptophan, phenylalanine, tyrosine, taurine), glucose, polyunsaturated fatty acids and vitamins C, E, D and *beta*-carotene, B group vitamins (vitamin B₁₂, vitamin B₆, vitamin B₄, vitamin B₁) and minerals (selenium, zinc, magnesium, sodium, iron, copper, manganese, iodine). An appropriate supply of these nutrients or their deficiencies can influence nervous system function. All that we feel, our emotions, desires and awareness of our existence depends on our brain and the neurotransmitters functioning in it. Deviations from their proper function influence all systems and organs in the human body, but the nervous system is the main victim. Diseases such as schizophrenia, recurrent mood disorders, *Parkinson's* disease, *Alzheimer's* disease, epilepsy, anxiety result from incorrect neurotransmitter function [17].

MACRONUTRIENTS AND THE NERVOUS SYSTEM

The human brain is a very “costly” organ in terms of energy. It constitutes approx. 2% of body weight but, under resting conditions, it uses as much as 20-23% of basic metabolism [5]. As opposed to other tissues, glucose is almost exclusively an energy substrate for neurons. It is transported through the blood-brain barrier with the participation of insulin-independent glucose transporters GLUT1 with high density and medium affinity to glucose. The transport to neurons, on the other hand, takes place by means of GLUT3 transporters, which show high affinity to glucose. These transporters are always saturated with glucose, even in mild hypoglycaemia conditions, which ensures the supply of an appropriate amount of substrates to neurons. Glucose uptake does not only depend on GLUT transporters but also on glucose concentrations on both sides of the blood-brain barrier [23, 31]. Brain activation leads to a reduction in intracellular glucose and ATP concentrations, which stimulates glucose uptake by neurons. Glucose concentrations in cerebrospinal fluid constitute 2/3 of its concentration in general circulation. Glucose transformation occurs in the process of glycolysis of pyruvate, which is the key precursor of acetyl-CoA. A higher amount of this substrate (acetyl-CoA) causes

an increase in acetylcholine production, which is a neurotransmitter with a proven effect on the memory, amongst other things [5, 10, 31]. In ketone acidosis or hypoxia conditions, the brain may use alternative sources of energy such as beta hydroxybutyrate or lactate. Lactate can be a source of energy in states of increased energy production with lower glucose availability [10, 31]. However, these sources of energy are not capable of fully replacing glucose. Neurons constitute 10% of all brain cells and use approx. 80% of the total glucose and oxygen supplied. As neural cells of the brain are not capable of storing high-energy compounds, their proper function depends on the supply and use of equivalent quantities of oxygen and glucose. Energy reserves accumulated in the CNS are very low, and it is estimated that they are enough for only 10 minutes of brain functioning [13, 23]. In numerous in vitro and in vivo studies, it was found that the majority of energy produced by neurons (60-70%) is used to keep and restore appropriate resting potentials on their pre- and post-synaptic membranes after functional depolarization. Appropriate gradients of Na⁺/K⁺/Ca⁺ ion concentrations between inter-neuron and extracellular spaces as well as in their intracellular partitions are kept. It is necessary to keep these gradients for proper neurotransmitter activity both in glutamatergic neurons as well as in the neurons of other neurotransmitter systems (GABA-ergic, cholinergic, serotonergic) [13, 19].

Foodstuffs that provide carbohydrates (including cereal products, fruits) are willingly eaten when one is in a depressed mood as they increase endorphin secretion and have a calming effect. In numerous studies, carbohydrates were proven to increase tryptophan and serotonin synthesis in the CNS, which may contribute to mood improvement [8, 10, 31].

Fats contained in food — apart from proteins and carbohydrates — belong to basic nutrients. Depending on their source, they contain a lot of other ingredients, especially vitamins A, D, E, K and the necessary unsaturated fatty acids. In the past, fats were thought to be unhealthy so they were not desirable in the human diet. At present, they are considered to be necessary for the proper function of the human body and especially the nervous system [19].

Polyunsaturated fatty acids are compounds of proven importance for proper development and function of the brain and the peripheral nervous system. The central nervous system contains several necessary fatty acids including arachidonic acid (AA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and gamma-linolenic acid (GLA) [25]. Biochemical data show that Omega-3 fatty acids (EPA and DHA) and Omega-6 fatty acids play a key role in the function of the nervous system as its structural components [1]. Necessary unsaturated fatty acids

constitute approx. 15-20% of the dry mass of the brain and over 30% of all fatty acids in the nervous system. EPA is part of phospholipids of cell membranes in which it is the precursor of eicosanoids necessary for ongoing regulation of the function of the central nervous system and transfer of information between brain cells [30]. Elevated EPA concentrations reduce the activity of phospholipase A2 (PLA2), the enzyme responsible for releasing DHA from nerve cell membranes [22, 25]. *Omega-3* DHA is a structural fatty acid and a building material for neurons. DHA acid is metabolized to compounds with neuroprotective action such as docosahexaenoylethanolanid (DEA) and neuroprotectins 1 (NPD1). *Omega-6* GLA is metabolized to dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (AA) [11]. The former fulfils an anti-inflammatory function while the latter plays a role in the function of the central nervous system, including processes responsible for memory. Arachidonic acid is also a substrate for the production of eicosanoids, physiologically and pharmacologically active compounds known as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and lipoxins (LX) [16, 22, 26]. In recent years, it has been found in a large number of studies that reduced DHA and AA concentrations occur in the blood serum and erythrocyte membranes in some people. This applied to patients suffering from depression, schizophrenia and also dyslexia, dyspraxia, ADHD and autism [1, 27, 30]. *Omega-3* EPA and DHA acids influence the reduction in the production of inflammatory cytokines from arachidonic acid. This is caused by the competition of these acids with arachidonic acid for incorporation into phospholipid membranes, as a result of which the level of arachidonic acid is reduced both in the cells and in the plasma. EPA, on the other hand, competes with arachidonic acid in the cyclooxygenase pathway by inhibiting the production of proinflammatory eicosanoids, including prostaglandines E2 and thromboxane B2. DHA and EPA inhibit the secretion of proinflammatory cytokines such as interleukins 1 (IL-1), IL-2, IL-6, interferon gamma (IFN) and the tumour necrosis factor (TNF alpha) [3, 16, 19]. A lot of data show that fatty acids are related to the activity of the serotonergic system [15, 19, 22]. Research conducted on people and animals has shown the influence of *omega-3* acids on the receptor function, the neurotransmitter level and its metabolism [15]. The relationship between a low concentration of *omega-3* DHA and a low concentration of 5-hydroxyindolacetic acid (5-HIAA) in the cerebrospinal fluid, which is the main metabolite of serotonin. Low concentrations of 5-HIAA in the cerebrospinal fluid are observed with people after violent suicide attempts, in the state of aggression, weakened impulse control, especially in people suffering from depression, schizophrenia,

alcohol addiction and adjustment disorders. This may imply that low concentrations of *omega-3* DHA acids is related to impaired serotonergic neurotransmission [25]. Fatty acids influence intracellular transmission related to secondary transmitters such as cyclic adenosinomonophosphate (cAMP) and the phosphatidylinositol. The influence of *omega-3* on mood stabilization can occur through mechanisms of inhibiting the phosphatidylinositol system [16]. Research on animals also shows that *omega-3* acids have a modulatory and inhibitory influence on ion channels depending on calcium [19]. EPA and DHA have an inhibitory effect on the activity of protein kinase C (PKC), which is dependent on calcium and phospholipids. PKC plays an important role in pre- and postsynaptic regulation of signal transmission by releasing neurotransmitters, receptor function, ion channels, neuronal excitation and gene expression [16, 25]. Docosahexaenoic acid induces the synthesis of neurotrophic factor (BDNF), thus extending the life of neurons. Antidepressants may increase BDNF concentrations, while a diet rich in saturated fatty acids and stress inhibit the production of this neurotrophin [16]. Quantitative changes of this acid in cell membranes of neurons may impair the function of ion and receptor channels such as dopaminergic, GABA-ergic and cholinergic ones [1, 18, 30]. Polyunsaturated fatty acids in nervous system cells influence the release and uptake of neurotransmitters and enzymatic processes. Their deficiency may contribute to the development of depression, anxiety, aggression [30, 31].

The only real source of necessary *omega-3* polyunsaturated fatty acids is fat from fish and seafood. The EPA and DHA content and their mutual proportions depend on the fish species, season, physiological condition of fish and the fishing area. Fish from cold northern seas contain more EPA while those from southern seas - more DHA. Also, some species of freshwater fish contain high levels of *omega-3* acids - mostly salmon. Various types of seafood (oysters, shrimps, crabs) and algae also contain *omega-3* acids, however, oil extracted from the liver of sharks living near New Zealand and Tasmania is still considered to be the most valuable source these acids [22]. More cases of depression are observed in countries with low consumption of fish that are a source of *omega-3* acids [17]. Research shows that the consumption of *omega-3* fatty acids is recommended in *Alzheimer's* disease prevention [21]. Eating fish once a week reduces the risk of *Alzheimer's* disease by 60% [18, 25]. Necessary *omega-6* fatty acids are present in oilseeds. *Gamma*-linolenic acid occurs in primrose oil and arachidic acid is present in the bodies of various animals [21].

A high content of saturated fatty acids and trans fatty acids in the diet may reduce the flexibility and elasticity of nerve cells. Excessive amounts of these ingredients reduce the oxygen supply and the ability to remove metabolites from the brain. The main source of saturated fatty acids are milk and meat products; they also occur in large amounts in coconut oil and in palm oil. A source of trans isomers are hardened vegetable oils and products in which they are used, mostly solid margarines, confectionery and frying fat and, as a result, confectionery products, candy bars, cookies and fast foods [8, 17, 21].

A diet containing an appropriate amount of protein, which is a source of exogenous amino acids for our body, is necessary for proper function of the nervous system [8, 17]. Meat, fish, eggs and dairy products contain protein that contains exogenous amino acids that are necessary for the proper function of the brain, which is the basis of mental performance [19]. A protein-rich diet stimulates the brain function. High-quality protein that provides sulphur amino acids stimulates a higher uptake of tryptophan and also branched-chain amino acids [8, 19, 21]. In the digestive process, proteins are transformed into peptides, amino acids and biogenic amines, which fulfil the function of neurotransmitters. Peptides formed from milk proteins, such as endorphins, enkephalin and dynorphins are important for the brain function. These are neurotransmitters with opioid action (similar to morphine and codeine), which stimulate brain activity in adults and have a sedative effect in small children [29]. Another protein that is very precious for the function of the nervous system is glutathione - a tripeptide containing cysteine, glutamic acid and glycine. Together with superoxide dismutase and catalase, it is the main detoxication factor of the brain that captures and neutralizes free radicals [13, 21, 29]. The glutathione level depends on the quantity of sulphur amino acids in the diet and the degree of saturation of the body with vitamins B₆, B₁₂ and B₉ (folic acid). Amino acids released from food proteins in enzymatic digestion processes in the gastrointestinal tract show a stimulating effect on the function of the central nervous system [4]. Glutamate is the main neurotransmitter from which γ -aminobutyric acid - GABA is formed in the brain. GABA-ergic synapses constitute approx. 20% of all synapses in the brain. Through glutamatergic pathways, visual, auditory and sensory information reaches the brain cortex from the peripheral nervous system. Glutamatergic pathways also participate in remembering and forgetting information in learning processes. Taurine also plays an important role in neurotransmission and neuromodulation in the central nervous system [18], which is confirmed by its high concentrations in the cortex, hippocampus and hypothalamus. Moreover, taurine also shows antioxidative and anti-inflammatory

action protects nuclear DNA from free radicals, which is very important, in particular, for long-living cells of the brain, heart and skeletal muscles. A lot of research shows that taurine may limit the development of neurodegenerative diseases and its best sources within the human diet include tuna, beef and poultry. A broad spectrum of biological action of taurine also makes daily consumption of milk and dairy products advantageous to health, especially if sulphur amino acids are absent from the diet (methionine and cysteine) [32]. Endogenous synthesis of taurine does not fully meet the demand of the human body [29]. Catecholamines: adrenaline and noradrenaline are formed from tyrosine, which intensify mental activity, i.e. shorten the reaction time and also dopamine, the so-called 'happiness hormone' whose deficiencies may cause schizophrenia, *Parkinson's* disease and *Tourette syndrome*. Serotonin is formed from tryptophan, which has a pain-killing, relaxing and slowing-down action. When tryptophan concentrations go down in the brain, serotonin synthesis also decreases [12, 13, 26]. Phenylalanine is particularly valuable for the brain amongst amino acids. It is a chemical carrier of impulses between the peripheral nerve cells and the brain. It is present in soy, dairy products, legumes, sesame seeds [17].

NERVOUS SYSTEM AND ANTIOXIDANTS

The nervous system is particularly exposed and sensitive to free radicals, hence, at present particular attention is paid to a proper supply of antioxidants. Antioxidants are collected together with food and they include vitamins C, E and β -carotene, zinc, copper, manganese, glutathione, co-enzyme Q10, selenium, which are active in cells and prevent oxidative stress, damaging a large number of cell components including DNA [15, 18]. The best sources of antioxidants include vegetables (onion, broccoli, spinach, carrot, cabbage) and fruit (oranges, grapes, strawberries, cherries, black currants, avocado) [9]. In numerous biochemical and psychological studies, it was found that oxidative molecules may contribute to the development of neurodegeneration diseases such as *Parkinson's* disease and *Alzheimer's* disease and oxidative modification of lipoproteins from the LDL fraction is an important factor in the atherosclerosis process. It results from literature data that regular consumption of fruits and vegetables is the best protection against oxidative stress. It has been found that enriching the diet with vegetables and fruits for 14 days improves the DNA and lipid condition in the human body [15]. The human brain contains high levels of oxidized lipids, which should be protected against oxidative stress. The accumulation of antioxidants in the brain and in the nervous tissue is much slower than in other

tissues [2, 10]. Low vitamin E concentrations in the cerebrospinal fluid are often observed in patients suffering from *Alzheimer's* disease. It has also been found that the rate of formation of peroxidation products is correlated with vitamin E levels, which is related to daily magnesium consumption. Vitamin E, apart from its antioxidative function, may additionally inhibit the development of atherosclerosis by influencing the immune system function [9, 10]. In direct epidemiological research, a relationship was found between the level of antioxidants in the plasma and metal activity. A correlation was observed between low vitamin C consumption and a simultaneous low level of this vitamin in the plasma and an increased risk of ischaemia and reduced mental performance [2]. In other studies, a significant correlation was shown between components of the diet and mental performance. From among the variables, tested, the consumption of vitamin C, β -carotene, folic acid and iron was positively correlated and the consumption of monounsaturated fatty acids, saturated fatty acids and cholesterol - negatively [2].

Over the past decade, there has been a growing interest in vitamin D, which allowed for discovering new action of calcitriol within the central nervous system. On the basis of epidemiological and molecular research, calcitriol was recognized as neurosteroid whose deficiency plays a key role in etiopathogenesis of dementia, depression, schizophrenia, epilepsy and autism [28, 29].

Proper function of the central and peripheral nervous system depends directly on the mechanism of action of group B vitamins. Vitamin B₁ in the active form of thiamine triphosphate plays an important role in the nervous tissue function [9, 23]. This compound influences the serotonin- and adrenergic system and participates in impulse transmission [13, 23]. Vitamin B₂ is necessary to keep glutathione in its reduced form as it protects against harmful action of free radicals [24]. The deficiency of vitamin B₆ may cause catecholamine synthesis disorders, reduced quantities of g-aminobutyric acid (GABA) and changes in the amino acid composition in certain areas of the brain. Disorders caused as a result of vitamin B₆ deficiency can, as a result, cause premature ageing of neurons [16, 24]. Niacin takes part in the synthesis of cortisol, thyroxine, as a result of which it is necessary for the proper function of the brain and the peripheral nervous system. The cobalamin content influences the amount of reduced glutathione in erythrocytes and in the liver and, as a result, its deficiency may weaken the antioxidant system. Choline, which is called vitamin B₄, takes part in the transport of important nutrients in the body and in the function of all cells. As a precursor of acetylcholine, a neurotransmitter in the cholinergic system, choline takes part in the control of

the function of muscles, the respiratory system and the heart action and also in brain functions connected with memory. It is very important in the diet of pregnant and breastfeeding women as it influences the prenatal and postnatal development of the brain and the spinal cord (the central nervous system) [24]. It was found that choline uptake improves long-term memory and learning functions. Research on animals has shown that ageing-related memory deterioration can be delayed if the mother's diet is supplemented with choline during pregnancy. There are also reports that claim that a diet containing large quantities of easily assimilable choline can reduce the risk of dementia that is characteristic of Alzheimer's disease. Deficiencies of group B vitamins in the body, such as B₁, B₂, B₆, B₁₂ and folic acid, may result in the development of depression and myelin degeneration and the lack of vitamins B₆ and B₁₂ to peripheral neuropathy [17, 20, 24]. The deficiency of these vitamins may also cause disorders in homocysteine metabolism. The level of synthesized homocysteine in the plasma is negatively correlated with the level of vitamins B₁₂, B₆ and folic acid. Vitamin B₆, as a co-factor of cystathionine β -synthetase catalyses the process of cystathionine formation from homocysteine and serine, while vitamin B₁₂ and folic acid participate in the process of changing homocysteine back to methionine. Homocysteine, which is a precursor of cysteine and homocystein acid, takes part in neurodegeneration, regardless of atherosclerosis. In studies involving older people with depression, the homocysteine level was higher among those with vascular diseases, but a significant correlation between homocysteine concentrations and the impaired function was only obtained among people in whom no vascular diseases were found [21]. A relationship was shown between a very low level of folic acid and depression that may, to a certain extent, be explained by changes in the level of S-Adenosyl methionine and serotonin in the brain. S-Adenosyl methionine is formed as a result of homocystein transformation to methionine in the presence of folic acid. In the acetylation process, serotonin is transformed into acetyl-5-hydroxytryptamine from which, with the participation of hydroxy-indolo-O-methyltransferase and S-adenosyl methionine, N-acetyl-5-methoxytryptamine, i.e. melatonin is formed [20]. Melatonin neutralizes free radicals, thus protecting tissues from damage and premature ageing. Melatonin is also a factor that improves physiological sleep, and a form of S-Adenosyl methionine also participates in the synthesis of catecholamines [2].

Bioelements such as iron, calcium magnesium, copper, manganese, zinc, iodine and selenium are very important for the proper function of the brain and the entire nervous system. Many neurological and mental diseases are caused by changes in the distribution and

action of neurotransmitters. Iron, as a component of monooxygenase, participates in the transformation of tryptophan to 5-hydroxy-tryptamine, a serotonin precursor.

Iron, zinc, magnesium and copper are important modulators of glutamatergic transmission related to etiopathogenesis of depression. Physiological concentration of copper and zinc plays an important role in the activation of enzymes dependent on these elements that are involved in catecholamine transmission [7]. Copper participates in the metabolism of biogenic amines participating in myelinogenesis. Magnesium regulates the action of glycoprotein P, which is one of transport proteins responsible for proper permeability of the blood-brain barrier for many substances. Magnesium deficiencies in the daily food ration are frequent nutritional problems of various population groups [6, 19]. The zinc level in the serum is closely related to inflammatory processes that are activated during depression. Zinc absorption is enhanced by the presence of vitamins A and E. Zinc is necessary for the proper development and function of the brain. The important role of this element results from the fact that the transmission of nervous signals is modulated in synapses in the central nervous system [23]. Neurons which contain zinc cations in a complex with glutamate, which are also called glutamatergic, occur in brain areas such as: the cortex, limbic system structures which are responsible for managing emotions, receiving and processing sensory impressions, i.e. smell, taste, hearing, vision or pain and also learning processes and memory formation [7, 10]. Calcium and sodium also take part in the transmission of neural stimuli. Another element that plays a key part in the proper brain function is iodine. It is an element necessary for the production of triiodothyronine T_3 and thyroxine T_4 in the thyroid gland. Appropriate differentiation and maturation of the brain cells and nervous system cells depend on proper concentrations of thyroid hormones in the blood. Iodine deficiencies during the foetal life are particularly dangerous as they may cause congenital defects and the development of neurological cretinism [19].

CONCLUSIONS

Synthesis of neurotransmitters responsible for maintaining well-being in the brain is conditioned by the quantity and quality of the consumed food. Deficiencies, even moderate, especially if they persist for a long time, have a disadvantageous effect on human health, including, in particular, the functioning of the nervous system. Some nutrients such as amino acids, necessary unsaturated fatty acids and carbohydrates take part in neurotransmitter synthesis, which may influence the human mood. The presence

of antioxidants in the diets, including vitamin C, E, β -carotene, protects against oxidative damage to nervous system cells. Deficiencies of B group vitamins may result in memory and concentration disorders, emotional balance disorders, symptoms that frequently occur in patients suffering from depression. A properly composed diet, based on vegetables, fruits, whole-grain cereal products supplemented with products providing full-value protein (dairy products, fish, lean meat) and high-quality fat products (vegetable oils, fish fats) may become the key to solving a large number of health problems.

Conflict of interest

The authors declare no conflict of interest.

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NUTRITION PRACTICES IN NURSERIES IN POLAND - INITIAL RESULTS OF NATIONWIDE STUDY

Anna Harton, Joanna Myszkowska-Ryciak

Chair of Dietetics, Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw
University of Life Science - SGGW, Warsaw, Poland

ABSTRACT

Background. Rational nutrition of infants and toddlers is essential for their normal growth and development, and for the development of proper nutritional habits. It should be preceded by proper planning.

Objective. The aim of the study was to evaluate the planning and organization of nutrition in nurseries.

Material and methods. In the research conducted within the program “*Eating healthy, growing healthy*” (EHGH), 128 crèches from all over Poland participated. The nurseries were attended by 8182 children under the age of 3. The research was carried out between 2015 and 2016. Data on the organization and quality of nutrition were collected through direct interviews with directors and / or staff responsible for feeding in crèches. In addition, analysis of the decade’s menu of the participating institutions (128 menus) and daily inventory reports (1280 documents) were analyzed. The data were analyzed for the total number and the type (public and non-public) of institution. The program Statistica Version 13.1 was used.

Results. Half of the surveyed DCCs planned in the menu whole grains, nearly all of them served fresh vegetables and fruits to the children, and every third added them to every meal. The most common drink during the meal was compote. Access to water between meals was offered to children in majority of the DCCs. The quality of diet was differentiated by the type of nursery: depending on the type (public vs non-public), the differences in salting and sweetening meals have been shown. Public DCCs had a much lower average amount of money allocated per day to feed a child compared to non-public, most of these managed own kitchens and did not employ a dietitian. Despite the higher nutritional rates in non-public crèches, some errors in nutrition planning have been observed.

Conclusions. Higher average amount of money allocated per day to feed a child in non-public nurseries did not provide adequate nutrition. There is a need to publish standardized, understandable and practical recommendations in nutrition of children in nurseries. Adopting such recommendations in daily practice in all public and non-public crèches should improve the nutrition of children.

Key words: *nurseries, child care, early age, nutrition*

STRESZCZENIE

Wprowadzenie. Racjonalne żywienie niemowląt i małych dzieci jest kluczowe dla ich prawidłowego wzrostu i rozwoju oraz kształtowania prawidłowych nawyków żywienia. Powinno być poprzedzone jednak prawidłowym zaplanowaniem.

Cel. Celem badania była ocena planowania i realizacji żywienia dzieci w żłobkach.

Material i metody. W badaniach w ramach programu „Zdrowo jemy, zdrowo rośniemy” (ZJZR) uczestniczyło 128 żłobków z całej Polski. Do placówek tych uczęszczało 8182 dzieci w wieku poniżej 3 lat. Badania realizowano w latach 2015-2016. Dane dotyczące organizacji i jakości żywienia zbierane były drogą bezpośredniego wywiadu z dyrektorami i/ lub personelem odpowiedzialnym za żywienie w żłobkach. Dodatkowo poddano analizie jadłospisy dekadowe placówek uczestniczących w badaniach (128 jadłospisów) oraz dzienne raporty magazynowe (1280 dokumentów). Dane analizowano dla placówek ogółem oraz w podziale na rodzaj placówki (publiczna i niepubliczna). Do analizy statystycznej wyników zastosowano program Statistica ver. 13.1.

Wyniki. Wykazano, że połowa badanych placówek planowała w jadłospisach udział produktów pełnoziarnistych, prawie wszystkie podawały dzieciom świeże warzywa i owoce, a co trzecia podawała te produkty w każdym posiłku. Najczęściej do picia podczas posiłków był kompot. Dostęp do wody między posiłkami oferowała dzieciom większość badanych placówek. Jakość diety była zróżnicowana ze względu na rodzaj placówki (publiczna, niepubliczna), w tym w zależności od jej typu wykazano zróżnicowanie w zakresie dosalania i dosładzania potraw. Placówki publiczne miały dużo mniejszą stawkę żywieniową niż niepubliczne, w większości prowadziły własną kuchnię i nie miały dietetyka. Pomimo wyższej stawki żywieniowej w żłobkach niepublicznych, obserwowano pewne błędy w planowaniu żywienia dzieci.

Corresponding author: Anna Harton, Warsaw University of Life Science - SGGW, Faculty of Human Nutrition and Consumer, Chair of Dietetics, Department of Dietetics, Sciences, Nowoursynowska 159c Street, 02-776 Warsaw, Poland, phone: +48 22 5937022, fax: +48 22 5937018, e-mail: anna_harton@sggw.pl

Wnioski. Wyższa stawka żywieniowa w żłobkach niepublicznych nie zapewniała prawidłowego żywienia. Istnieje potrzeba opublikowania ujednoliconych, zrozumiałych i praktycznych zaleceń w zakresie żywienia dzieci w żłobkach. Stosowanie takich zaleceń w codziennej praktyce we wszystkich żłobkach, zarówno publicznych jak i niepublicznych, powinno wpłynąć na poprawę żywienia dzieci.

Słowa kluczowe: *żłobki, opieka nad dziećmi, wczesne dzieciństwo, żywienie*

INTRODUCTION

Parents are the most responsible for the nutrition of young children. An important role has also a care and education institution, where a child usually spends many hours during the day. Many studies prove that planning and balancing of meals used in both homes and care/education settings are wrong [5, 10, 11, 13, 15, 22, 24]. Many child care facilities often do not employ specialized staff to plan nutrition, especially for children requiring elimination diets. Some nurseries resign from maintaining their own kitchen for catering. In addition, there is a big problem with the legal regulations on nutrition. There are norms and some quantitative and qualitative nutritional recommendations for planning and organization of nutrition [9, 23, 25] but they are not mandatory. Lack of uniform and mandatory legal regulations results in a large degree of discretion in their application. Such practices can affect the quality of nutrition of young children, which is extremely important, especially in this period of life. This is the moment to shape certain eating habits and period critical for the prevention of many diet-related diseases. Nutrition of toddler should be particularly well planned, and implemented on the basis of uniform nationwide recommendations.

The purpose of this study was to analyze and evaluate selected quality factors of diets used in crèches and to collect information on nutrition practices in these centers from all over Poland.

MATERIAL AND METHODS

The study covered 128 daily care centers (DCCs) attended by 8182 children under the age of 3, including public nurseries ($n = 65$), and non-public nurseries ($n = 63$) which came from all over Poland. All examined DCCs participated in a research and education program “*Eating healthy, growing healthy*” (EHGH) [8]. Recruiting process lasted from November 2015 to April 2017. Data on selected aspects of nutrition practices came from a direct interview with the director or authorized staff of the facility. Interviews were conducted by EHGH educators during the on-site meeting at the facility. As a tool for collecting data a specially prepared and validated questionnaire was used. First, the data has been collected in paper form, then carefully analyzed and checked, and

entered into the computer database. An additional source of data that was used to validate nutrition data were decade’s menu and inventory reports. For this purpose, 128 decade’s menu and 1280 daily inventory reports were analyzed. All data have been processed statistically using Statistica Version 13.1. The collected data were further analyzed considering the type of facility (public vs. non-public DCCs). We used chi-squared quality assays and quantitative *Mann-Whitney* U tests with significance levels of $p < 0.05$.

RESULTS

In the general pool of randomly selected DCCs that joined the EHGH program and were presented in the article, half were public crèches. More than 8,000 children attended, including most of them aged 1-3; infants accounted for only 5% of the total group (Table 1).

The selected aspects of nutrition quality are presented in Table 2. It was found that half of the DCCs in daily diets used whole grains products, all nurseries planned fresh vegetables and fruits and every third included them to every meal. As a drink for a meal, most often compote and tea were served, in the smallest percentage - juice and other fruit drinks. Water for meals was planned in more than half of the crèches, and access to water between meals was declared by a high percentage of DCCs. In every second institution, frying was used 1-2 times a week, and complete absence of this cooking method was recorded in half of the crèches. The correct assortment of fat for frying was found in 8 out of 10 DCCs. Salting and sweetening, including using salt while preparing food, was more popular than after preparation. Some of the quality aspects of nutrition practices were determined by the type of nursery (Table 2). Better balanced diets were reported in public institutions compared to non-public crèches. But in the same time, salting and sweetening occurred there more frequently.

The selected aspects of nutrition practices are presented in Table 3. It has been shown that in over half of the surveyed nurseries, a kitchen run by institution and internal catering were present. This type of organization of nutrition was found more frequently in public institutions, where the financial rate for nutrition (average amount of money allocated per day to feed a child/PLN) was twice lower than in

non-public crèches. The diet planning was made by different specialists, and the dietician was noted only in non-public DCCs. The dietitian was not employed by nursery, but by the catering company, which the

DCC actually cooperated. In all nurseries, a decade's menu included breakfast and dinner consisting of two dishes (soup and second course). In public DCCs, meals were served earlier (Table 3).

Table 1. General characteristics of children in DCCs (number / % of children)

Children	DCCs		
	Total (n=128)	Public (n=65)	Non-public (n=63)
Total number of children	8182/100	5723/70	2459/30
Children <1 year of age	407/5	297/5.2	110/4.5
Children on a special diets	654/8	491/8.6	163/6.6
Children on a non-dairy diet	393/4.8	266/4.6	127/5.2
Children on a gluten-free diet	30/0.4	14/0.2	16/0.7
Children on other diets*	238/2.9	217/3.8	21/0.9

*Including dietary supplements, used in the various, co-existing problems of the digestive tract or other diseases

Table 2. Selected aspects of nutrition quality (total/% of DCCs)

Category	DCCs			p*
	Total (n=128)	Public (n=65)	Non-public (n=63)	
Occurrence daily				
Whole grain products	63/49	43/66	20/32	0.00010
Vegetables and / or fruits in every meal	45/35	37/57	8/13	0.00000
Fresh vegetables and / or fruits	121/97	63/92	58/95	NS
An assortment of drinks for meals / between meals				
Compote	116/91	61/94	55/87	NS
Tea	94/73	50/77	44/70	NS
Water	85/66	45/69	40/63	NS
Natural juices 100%	47/37	34/52	13/21	0.00020
Other fruit drinks, not juices	27/21	18/28	9/14	0.06307**
Water between meals	120/94	60/92	60/95	NS
Culinary techniques and the type of fat used				
Food frying 1x / week	40/31	24/37	16/25	NS
Food frying 2x / week	23/18	11/17	12/19	NS
No frying food	65/51	30/46	35/56	NS
Olive / rapeseed oil for frying	81/63	44/68	37/59	NS
Butter for frying	19/15	9/14	10/16	NS
Sweetening dishes / beverages				
Sweetening dishes	107/84	64/98	43/68	0.00000
Sweetening during preparation	96/75	57/88	39/62	0.00076
Sweetening after preparation	31/24	20/31	11/17	0.07889**
Sweetening dishes with white sugar	48/38	32/49	16/25	0.02071
Sweetening beverages with white sugar (compote/tea)	64/50	41/63	23/37	0.00265
Salting dishes / type of salt or its replacement				
Salting dishes	109/85	62/95	47/75	0.00095
Salting dishes with salt/sea salt	54/49	27/44	27/58	0.06253***
Salting with diet salt	27/25	18/29	9/15	
Salting with different types of salt	25/23	17/27	8/17	
No data	3/3	0/0	3/6	
Salting during preparation	100/78	58/89	42/67	0.00202
Salting after preparation	23/18	13/20	10/16	NS

p – level of significance for $\alpha=0.05$; *statistically significant Chi-squared test, NS – not statistically insignificant, **tendency, ***tendency, taken into account and converted into DCC which salting (n = 109)

Table 3. Selected aspect of nutrition practices

Category	DCCs		
	Total number (n=128)	Public (n=65)	Non-public (n=63)
Type of kitchen in the DCCs*	Number / % of DCCs		
Own kitchen	77/60	62/95	15/24
Internal catering	5/4	2/3	3/5
Own kitchen and external catering	4/3	0/0	4/6
External catering	42/33	1/2	41/65
DCC having a person responsible for planning nutrition**	Number / % of DCCs		
	84/66	62/95	22/35
Dietician	9/7	0/0	9/14***
Cook	14/11	8/12	6/9
Commissary	50/39	46/71	4/6
Nurse	7/5,5	7/11	0/0
Director	2/1.6	1/1.5	1/1.6
No person indicated	14/11	13/20	1/1.6
Nutritional rate (PLN)****	X \pm SD / median (min-max)		
	7.34 \pm 2.90/6 (4-15)	5.28 \pm 1.25/5 (4-12)	9.35 \pm 2.62/10 (4-15)
The type of meals served in DCCs	Number / % of DCCs		
Breakfast	128/100	65/100	63/100
II breakfast	93/73	55/85	38/60
Dinner – soup	128/100	65/100	63/100
Dinner – second course	128/100	65/100	63/100
Dessert	108/84	47/72	61/97
The time of meals served in DCC	Hours: minutes (median)		
Breakfast	8:30	8:15	8:30
II breakfast	10:00	10:00	10:00
Dinner – soup	11:45	11:45	11:40
Dinner – second dishes	12:00	11:30	13:00
Dessert	14:30	14:30	15:00

*statistically significant, Chi-square test $p=0.0000$, **Chi-square test $p = 0.0000$, in few cases indicated several people, ***dietitian from catering, ****U Mann-Whitney test, $X \pm SD$ – mean and standard deviation; nutritional rate - average amount of money allocated per day to feed a child

DISCUSSION

The number of children under the age of 3 using various forms of care has grown in Poland in recent years (in 2013 it was nearly 60 thousand children) [3]. According to Central Statistical Office data in 2013 [3] the proportion of children in DCCs was 4.8%. For comparison, in 2012 it was 3.8%. There are also a number of such DCCs, including mainly non-public nurseries. Considering the age structure of children in care facilities, the biggest groups are children aged 2 (about 50%) and least infants (4.1%). A slightly higher percentage of infants were observed in present study and lower in the England study [14].

Nursery is a place where a child spends more than 5 hours a day, and one third of such centers work more

than 10 hours a day. Due to this fact, the DCC should to a large extent meet the needs of a small child for energy and all nutrients. Therefore, the supply of food should be adequate, and the diet properly balanced. Such a diet cannot lack vegetables and fruits, including the best in the fresh form. Many studies, however, prove that the menu is not properly balanced in the DCC, not only in terms of vegetables and fruits [13, 24]. In our study most of the institutions in their daily menu served fresh fruit and vegetables to children. The satisfactory performance of the model ration for vegetables and fruits was also demonstrated in another study [6]. Daily diet should also include whole grains, however, these products are not very popular in feeding small children. There is still a misconception that better choice is a wheat bread. In present study

it was shown that every second DCC placed whole grain products in the decade menu. Better balance of diets for the supply of vegetables and fruits, and whole grains was reported in public institutions compared to non-public ones. Larger quantities of cereal products in the menu of children from private or non-private institutions were reported in the study of preschoolers from Croatia [12]. However, in this case, the authors did not analyze the type of products but only their total supply. The same authors did not note differences in the supply of vegetables and fruits depending on the type of institutions. The lower share of fruits and vegetables in the daily menu of crèches, and higher weekly grains was noted in another large England study [14]. However, it is worth noting that vegetables and fruits were evaluated as separate groups, and cereal products converted per week, which makes comparison of data difficult. Similarly, as in the own study, the results on the supply of vegetables and fruits in the menu of DCCs was recorded in US research [26]. In those study institutions implemented the “*Head Start*” obesity prevention program, which could have resulted in better awareness and implementation of nutritional recommendations for children.

Another issue are drinks. In many studies of infants and young children, the authors point to their abnormal assortment, including too many sweetened beverages [19] with the lack of water. In present study, access to water was provided to children in a very high percentage of crèches, but it was not the most commonly served beverage for meals. Compote according to traditional recipe is prepared with added sugar. Drinks of beverages, including compote, were recorded in half of the surveyed DCCs. Excessive consumption of sugar, including sweetened beverages, is raised as a risk factor for obesity. WHO data from 2014 show that 41 million children worldwide over the age of five are overweight [4]. Thus, in programs to prevent obesity in children and adolescents, the WHO puts special emphasis on reducing the consumption of sweetened beverages [2]. In the latest WHO recommendations [4] the consumption of sugar highlights the importance of free sugars in the prevention of many diet-related diseases - as the authors point out most often come from sweet drinks. The recent recommendations of the American Academy of Pediatrics (AAP) on infant feeding propose a complete resignation from juices [7]. In present study juices were only planned in every third nursery, including a greater percentage of public DCCs. The higher proportion of planned juices for children from crèches with slightly lower water was reported in a British study [14]. Such a situation may be due to the fact that juices continue to be perceived as valuable in the general public opinion, and are often used in feeding young children. The AAP emphasizes that parents and caregivers should be educated in

order of proper share of juices in children’s nutrition because consumed in the right amount of 100% juice produce health benefits, and do not necessarily have to associate with obesity [1].

The use of salt is a separate issue in nutrition of infants and toddlers. As recommended in infant feeding, salt should be not use [20, 21], just as in feeding children above a year. It is recommended to limit the use of this ingredient with the indication of other alternatives e.g. in the form of appropriate herbs. The excess of salt is often noted in infant and young child nutrition [19, 24]. Salt comes not only from it addition during preparation food, but also from the high proportion of salty snacks often eaten by children. To reduce the supply of salt in diet planning, it is a good idea to replace regular salt with low sodium salt.

The diet of young children should not only be well-balanced but also easy digestible, including the technological processing of food. Very common way of preparing dishes is frying also in the case of the youngest age group. However, in present study, every second institution did not use frying. Another issue is the assortment of fat - here the choice is not always correct. An insufficient supply of fat of different types in the diets of young children has been demonstrated in other nutritional studies [27].

In the light of the changing dietary recommendations for infants and young children in recent years, there is an urgent need for them to continuously disseminate and update knowledge among staff of DCCs. In addition, some of these recommendations are not mandatory in the whole country, resulting in their selective use. To make matters worse, many people involved in diet planning in DCCs do not have specialized education. The scale of errors and other irregularities in this area is also not well known because of the lack of large research on this subject. Existing research concerns only individual institutions, selected regions of the country or only selected issues of this area. Such studies also have a very different methodology for both data collection and development so they cannot be compared. On this basis, no binding conclusions can be drawn. In the light of the above there is an urgent need for continuous staff training, including planning and organization of nutrition, taking into account the latest scientific reports. However, that should be preceded by the issuance of uniform national recommendations, which should be obligatory. Such recommendations concerning the requirements to be fulfilled by foodstuffs used in the framework of collective nutrition for children and young people in the Regulations of the Minister of Health were published in 2015 [17] then corrected in 2016 [18]. It is worth pointing out that they do not refer to nurseries but only to older age groups of children, including preschool age. These recommendations are

often based on childcare facilities. However, they are not targeted to this age group and should therefore not be the basis for crèche staff. This situation shows that there is a need to publish similar recommendations for the feeding of young children in care and educational institutions. The crèche staff declare such a need, which is most often communicated during individual meetings in institutions. These data are not the subject of this study, however.

The overall quality of nutrition in selected nurseries varies, however. Based on the study, which compared the quality of feeding DCCs from Warsaw and other Polish cities [6] it was showed that it was better for the first ones - it was characterized by smaller fluctuations in the supply of selected groups of food products. According to the authors of the study, this could be attributed to the fact that feeding in Warsaw is governed by internal regulations [28] including model rationale and purchase specifications of products. In addition, this nursery team takes care of a dietician who is not listed in many other state agencies. Such a state of affairs is confirmed by the presented data. The different situation is in non-public institutions - in this case the dietician comes from a catering company and is not employed to plan child nutrition. Other authors also point out that child nutrition is not always planned by professionals in this area, and knowledge is derived from various sources. [14]. The results of these and other studies [14] argue for the need to create uniform, mandatory recommendations that will help to plan the nutrition of young children.

In conclusion, it can be stated that nutrition of young children should be preceded by proper planning. It is not easy, however, when there are no standardized and practical recommendations, as well as the child care facility most often does not employ professionals in this area. On the basis of the results, after considering the type of facility (public vs non-public), there was variation in nutritional quality. Although, in the non-public DCCs the average amount of money allocated per day to feed a child was two times higher than the rates in public DCCs, some errors in planning child nutrition have been observed. So funding is not decisive here. As present and other studies show, there is an urgent need for publication of standardized, understandable and practical recommendations for child nutrition. Adopting such recommendations in daily practice in all public and non-public crèches should improve the nutrition of children. Some limitations in the conclusions of this study result from the analysis of only selected aspects of nutrition, the lack of a representative group of DCCs, and the use of the tool that was dedicated to this study.

CONCLUSIONS

1. Nutrition practices in nurseries in Poland did not meet all nutrition recommendations.
2. Higher average amount of money allocated per day to feed a child in non-public nurseries did not provide adequate nutrition.
3. There is a need to publish standardized, understandable and practical recommendations in nutrition of children in nurseries. Adopting such recommendations in daily practice in all public and non-public crèches should improve the nutrition of children.

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Conflict of interest

The authors declare no conflict of interest.

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NUTRITION-RELATED PRACTICES IN KINDERGARTENS IN THE CONTEXT OF CHANGES TO LEGAL REGULATIONS ON FOODSTUFFS USED IN CANTEEN MENUS FOR CHILDREN

Joanna Myszkowska-Ryciak, Anna Harton

Warsaw University of Life Science - SGGW, Faculty of Human Nutrition and Consumer Sciences,
Chair of Dietetics, Department of Dietetics, Warsaw, Poland

ABSTRACT

Background. In order to improve the quality of nutrition in care and educational institutions, in year 2015 a restrictive law regulation was introduced. The following year this regulation was withdrawn, and a new legal policy has been introduced. However, data on the impact of these legislations on nutrition in kindergartens are lacking.

Objective. The aim of the study was to evaluate the compliance with mandatory nutrition recommendations in preschools.

Material and methods. The study included 706 institutions. Data were collected during the periods when two consecutive Regulation of the Minister of Health were in force (I - 01.09.2015 - 31.08.2016 and II - 01.09.2016 to 30.05.2017). Data were obtained by face-to-face interviews with staff with a validated questionnaire. Parameters characterizing the quality of nutrition were analyzed with focus on compliance with legal regulations. 706 decade's menus and 7060 daily inventory reports were analyzed to verify information on vegetables and fruit supply, frequency of frying and type of fat used, sweetening and salting practices. Statistical significances were determined in *Statistica 13.1* with *t-Student* and *Chi²* tests ($p < 0.05$).

Results. The preschools have implemented regulations concerning the supply of fresh fruit and vegetables (~93%), the amount of fried dish per week (~75%), and the use of suitable frying fat (~95%). About half of institutions managed to serve vegetables and/or fruits in every meal. Compliance with the regulations on sweetening and salting was unsatisfactory: about 1/4 of institutions used banned sugar, and 46% regular salt.

Conclusions. Legislations influenced nutrition practices in preschools, but additional support is needed to bring all institutions into compliance with the current policies. The current law regulations are general, and allow a great flexibility in planning menus, which might have an adverse effect on menus quality. Therefore, nutrition education and more detailed mandatory nutrition standards for kindergartens are crucial.

Key words: preschool, legislation, food, nutrition, children

STRESZCZENIE

Wprowadzenie. W celu poprawy jakości żywienia w placówkach opiekuńczo-wychowawczych i edukacyjnych, w 2015 r. wprowadzono restrykcyjne rozporządzenie w tym zakresie. W kolejnym roku uchylono obowiązujące rozporządzenie poprzez wprowadzenie kolejnego. Brakuje jednak danych o wpływie tych regulacji na żywienie w przedszkolach.

Cel. Celem pracy była ocena zgodności parametrów jakości żywienia z obligatoryjnymi zaleceniami w przedszkolach.

Material i metody. Badaniem objęto 706 przedszkoli. Dane zbierano w okresie obowiązywania dwóch kolejnych rozporządzeń Ministra Zdrowia (I - 01.09.2015 - 31.08.2016 i II - 01.09.2016 do 30.05.2017). Dane uzyskano na podstawie bezpośredniego wywiadu z personelem przedszkoli za pomocą walidowanego kwestionariusza. Dla weryfikacji informacji przeanalizowano 706 dekadowych jadłospisów i 7060 dziennych raportów magazynowych pod kątem zawartości warzyw i owoców, częstotliwości serwowania potraw smażonych i rodzaju tłuszczu, słodzenia i solenia. Analizę statystyczną wykonano w *Statistica 13.1* z wykorzystaniem testu *t-Studenta* oraz *Chi²* ($p < 0,05$).

Wyniki. Przedszkola wprowadziły przepisy dotyczące podaży świeżych owoców i warzyw (~ 93%), liczby smażonych potraw na tydzień (~ 75%), a także stosowania odpowiedniego tłuszczu do smażenia (~ 95%). Około połowa przedszkoli serwowała warzywa i / lub owoce w każdym posiłku. Przestrzeganie regulacji dotyczącymi słodzenia i solenia było niezadowalające: około 1/4 instytucji stosowało niedozwolony cukier, a 46% sól kuchenną.

Wnioski. Wprowadzenie regulacji prawnych wpłynęło na praktyki żywieniowe w placówkach przedszkolnych, ale konieczne jest dodatkowe wsparcie, aby wszystkie ośrodki przestrzegały obowiązujących zasad. Obecne przepisy są bardzo ogólne i pozwalają na dużą elastyczność w planowaniu żywienia dzieci, co może powodować pogorszenie jakości żywienia w przedszkolach. Dlatego kluczowe znaczenie ma edukacja żywieniowa i obowiązkowe, bardziej szczegółowe zalecenia żywieniowe dla przedszkoli.

Słowa kluczowe: przedszkola, prawo, żywność, żywienie, dzieci

Corresponding author: Joanna Myszkowska-Ryciak, Warsaw University of Life Science (SGGW), Faculty of Human Nutrition and Consumer Sciences, Chair of Dietetics, Department of Dietetics, Nowoursynowska 159c Street, 02-776 Warsaw, Poland, phone: +48 22 5937022, fax: +48 22 5937018, e-mail: joanna_myszkowska_ryciak@sggw.pl

INTRODUCTION

In Poland, in the school year 2015/2016 there were 21.1 thousand day care centers (DCCs) for children aged 3-5, including 11.3 thousand kindergartens. Pre-school education covered 1140.6 thousand children, which represents 84.2% of children in the age group of 3-5 years in Poland [2]. In kindergarten, child spends up to 10 hours a day and receives at least 3 meals, including two main meals, which covers up to 75% of the daily energy and nutrient intake [3]. Proper nutrition in preschool setting is therefore crucial for adequate child diet: prevention of nutrients deficiencies and oversupply, as well as shaping correct eating habits [3, 5, 11, 16]. The role of feeding in DCCs is also becoming increasingly important due to the increasingly widespread problem of children overweight and obesity [28], also in Poland [14, 17].

Due to the above, in year 2015 a restrictive legislation of the Minister of Health was introduced, which among others regulated the supply of vegetables and fruit, salt and sugar, fried foods and the type of fat used for frying. Especially restrictive was the legal regulation on sweetening of beverages: no sugar, but only honey for selected beverages was allowed (except e.g. very popular compote) [24]. The regulation was widely commented and met with a great criticism in media. Consequently, it was repealed, and a new regulation was introduced from the 1st of September 2016. In the current regulation, restrictions on sugar and salt supply, as well as required amounts of fresh vegetables have been significantly reduced [23]. There are no data available on the effect of introducing these legislations, changes in institutions, compliance with recommendations, and effects on nutrition in kindergartens.

The purpose of the study was to evaluate nutrition-related practices and the compliance with mandatory nutrition recommendations in preschool settings in Poland.

MATERIAL AND METHODS

The study included 706 kindergartens localized throughout Poland which took part in the education and research program “*Healthy eating, healthy growing*” (HEHG)¹ [9]. The analysis included the data collected during the period when two consecutive Regulation of the Minister of Health were in force: I - between 01.09.2015 and 31.08. 2016 [24], and II - between 01.09.2016 to 30.05.2017 [23]. Data were obtained by face-to-face interviews with

DCCs directors and/or staff responsible for nutrition. All interviews were conducted by specially trained interviewers using a validated author’s questionnaire consisted of closed-ended questions (e.g. presence of vegetables and/or fruits in every meal, presence of fresh vegetables and/or fruits in daily menu, frequency of frying, sweetening practices), and open-ended questions (e.g. others sweeteners used in DCC). In addition, 10-day menu and inventory reports were analyzed to verify selected information. In total, 706 decade’s menus and 7060 daily inventory reports were examined. The parameters characterizing the quality of nutrition were analyzed. Particularly we focused on the parameters regulated by the mentioned above legal regulations: serving of vegetables and/or fruits in every meal (including fresh), sugar and salt supply, the frequency of frying, and the type of fat used. The basic characteristics of the participating institutions were also collected (e.g. number of children, the daily food allowance², and the type of kitchen). All data has been processed statistically using *Statistica 13.1*. The collected data were analyzed by the type of institution (public vs. non-public DCCs), and separately for the two legislative periods (public in I vs. II; non-public in I vs. II period). For statistical analysis *Chi-squared* quality assays and quantitative *t-Student* tests with significance levels of $p < 0.05$ were performed.

RESULTS

The general characteristic of DCCs participating in the study is presented in Table 1. In the analyzed institutions, meals were distributed to nearly 80,000 children. The average number of children in public preschool was 123, while in non-public 74, respectively. Children on special diets accounted for less than 3 %, and most often a non-dairy diet, then gluten free diet were listed. Among the “others” special diets reported by the preschools managers were: diabetic, vegetarian, low-calorie diet, eliminating selected products and related to religion (e.g. pork meat free). The daily food allowance was significantly higher in non-public DCCs; with no statistical difference in the rate between the first and the second study period. However, for public DCCs downward trend in the second period was observed ($p=0.07$). In both analyzed periods, the organization of nutrition differentiated public institutions from non-public. Public kindergartens more often managed own kitchen, and in private institutions catering system was more popular. In contrast, no difference in the organization of nutrition between institutions during the two study periods was observed.

¹ education and research program granted by Danone Ecosystem [http://www.zdrowojemy.info]

² the amount of money allocated per day to feed a child in a DCC

Table 1. The general characteristic of DCCs including the information on nutrition organization (n = 706)

Factor	I period (n = 349)		II period (n = 357)	
	Public n=266	Non-public n= 83	Public n = 255	Non-public n = 102
Number of children (total)	32 638	5 420	31 575	8 277
Children on special diets:	663 (2.0%)	264 (4.9%)	865 (2.7%)	242 (2.9%)
gluten-free	82	47	110	55
non-dairy	502	188	695	173
others	150	59	167	53
Budget in PLN/child/day menu* mean \pm SD min - max	5.8 \pm 1.26 ^a 3.4 - 9.5	8.4 \pm 2.14 ^b 4.0 - 15.0	5.7 \pm 1.28 ^a 3.0 - 11.0	8.1 \pm 1.97 ^b 4.5 - 16.0
Type of kitchen in the DCCs**:				
DCC's kitchen***	249 ^a (93.6%)	47 ^b (56.6%)	238 ^a (93.3%)	55 ^b (53.9%)
internal catering****	8 (3.0%)	4 (4.8%)	4 (1.6%)	8 (7.8%)
catering	8 ^a (3.0%)	27 ^b (32.5%)	13 ^a (5.1%)	36 ^b (35.3%)
mixed	1 (0.4%)	5 (6.0%)	0 (0%)	3 (2.9%)

* t-Student test; ** Chi-square test: ^{a,b} public vs. non-public; *** kitchen managed and run by DCC; **** kitchen placed in DCC but managed and run by catering company or kitchen co-run in a collaborating institution

Table 2. The aspects of nutrition related-practices and quality of the diet in DCCs (n = 706)

Factor	I period (n = 349)		II period (n = 357)	
	Public n = 266	Non-public n = 83	Public n = 255	Non-public n = 102
Occurrence daily:				
Vegetables and/or fruits in every meal	140 (52.6%)	37 (44.5%)	141 ^c (55.3%)	36 ^d (35.3%)
Fresh vegetables and/or fruits	249 (93.6%)	76 (91.6%)	238 (93.3%)	98 (96.0%)
Frequency of frying and the type of fat used*:				
Frying \leq 2 x / week	206 (77.4%)	61 (73.5%)	194 (76.1%)	77 (75.5%)
Frying $>$ 2 x / week	60 (22.6%)	22 (26.5%)	61 (23.9%)	25 (24.5%)
Olive oil for frying	17 (6.4%)	10 (12.0%)	15 ^c (5.9%)	13 ^d (12.7%)
Canola oil for frying	258 ^a (97.0%)	74 ^b (89.2%)	245 (96.1%)	95 (93.1%)
Other type of vegetable fats	12 (4.5%)	6 (7.2%)	19 (7.5%)	9 (8.8%)
Butter, lard, coconut oil	24 ^A (9.0%)	8 ^C (9.6%)	50 ^{cB} (19.6%)	33 ^{dD} (23.4%)
No data	2 (0.8%)	7 (8.4%)	1 (0.4%)	3 (2.9%)
Sweetening tea, cocoa, coffee substitute, fruit/herbal tea*:				
Sugar	59 ^A (22.2%)	23 ^C (27.7%)	76 ^{cB} (29.8%)	47 ^{dD} (46.1%)
Honey	178 ^a (66.9%)	38 ^{bC} (45.8%)	153 ^c (60.0%)	28 ^{dD} (27.4%)
Others	3 (1.1%)	4 (4.8%)	2 (0.8%)	2 (2.0%)
Sweetening compote*:				
Sugar	69 ^A (25.9%)	23 ^C (27.7%)	131 ^B (51.7%)	63 ^D (61.8%)
Honey	68 (25.6%)	20 ^C (24.1%)	72 ^c (28.2%)	8 ^{dD} (7.8%)
Others	19 (7.1%)	2 (2.4%)	5 (2.0%)	0 (0%)
Sweetening juices, milk shakes*:				
Sugar	45 ^A (16.9%)	11 ^C (13.3%)	65 ^{cB} (25.5%)	37 ^{dD} (36.3%)
Honey	69 (25.9%)	16 (19.3%)	61 ^c (23.9%)	12 ^d (11.8%)
Others	8 (3.0%)	2 (2.4%)	4 (1.6%)	0 (0%)
Type of salt / salting practices*:				
Regular salt	124 ^A (46.6%)	38 ^C (45.8%)	152 ^{cB} (59.6%)	76 ^{dD} (74.5%)
Low sodium salt	160 ^{aA} (60.2%)	33 ^{bC} (39.8%)	127 ^{cB} (49.8%)	20 ^{dD} (19.6%)
Salting during preparation	259 ^{aA} (97.4%)	61 ^{bC} (73.5%)	230 ^B (90.0%)	90 ^D (88.2%)
Salting after preparation	14 ^{aA} (5.3%)	12 ^b (14.5%)	59 ^B (23.1%)	15 (14.7%)

* - possible multiply answers statistically significant Chi-squared test:

^{a, b} - public vs. non-public (I period), ^{c, d} public vs. non-public (II period)

^{A, B} - public in I vs. II period, ^{C, D} - non-public in I vs. II period

The examined aspects of nutrition related-practices are presented in Table 2. Vegetables and/or fruits occurred at every meal in more than half of the public kindergartens (both in 1 and 2 analysis); whereas in case of the private institutions these numbers were lower. The percentage of private vs. public DCCs serving fruit and/or vegetables in every meal was significantly different during the second analysis. However, there was no such difference in the first analysis, and no differences between the two analyzed periods. Fresh vegetables and/or fruits were served every day in more than 90% of DCCs, no significant differences were found depending on the type of facility or study period. Practise of serving fried food more than 2 times per week was observed in about one quarter of preschools. There were also no significant differences in frying frequency between public and private DCCs, and in both analyzed periods. The type of fat used was significantly differentiated by the type of facility, but in all DCCs canola oil was the most frequently used for this purpose. For sweetening tea, cocoa, coffee substitute, fruit/herbal tea, honey was the most often used, but during the second analysis the percentage of DCCs using sugar increased (especially in non-public DCCs). Similar tendency was observed in case of sweeteners for compote: the percentage of DCCs adding sugar increased significantly in second analysis. For sweetening juices, milk shakes in the first period honey was the most popular, whereas in the second period sugar was the most frequent choice. There were also differences in the type of salt used: in the first analysis, low sodium salt was more commonly used in public institutions but its share decreased in the second period. Additionally, there was an increase in the frequency of “regular” salt usage in both public and private institutions in the second analysis. The salt was added usually during preparation, but in the second period the frequency of salting after the preparation increased significantly in public preschools.

DISCUSSION

By September 2015 there was no mandatory legislation on nutrition in pre-school facilities. When setting up the menu, DCCs used the various recommendations available, such as nutrition guidelines, SANEPID recommendations, children’s food pyramids or other publications [3, 10, 22]. However, studies on nutrition in these institutions conducted by many authors pointed existing abnormalities, e.g. insufficient supply of vegetables, fruit, as well as often too high share of sugar and sweets in the diets [8, 12, 19, 20, 21]. The introduction of legal regulations was aimed at improving the quality of nutrition situation in kindergartens. Unfortunately, due to the lack of relevant information and education campaign, the regulation met

with great social criticism and was repealed after a year. The current law regulations are very general, and allow great flexibility in planning menus. Unfortunately, there are no data available to illustrate the effect of the above regulations, no information on the nature and extent of the changes introduced in DCCs. The question is also raised, whether these institutions that made “good” changes required by the first regulation, would retain them, despite the absence of such a need at present. In present paper we try to answer these questions.

The study covered 706 kindergartens from all over Poland; pre-school units located in schools and part day care facilities were excluded because they usually do not provide a full board. This represented about 6% of all kindergartens in Poland in 2015-2016 [2]. Most of analyzed DCCs were public facilities, characterized by higher number of children, lower financial rate for nutrition and managing the kitchen. “Own” kitchen gives the ability to fully influence the planning and preparing menus, which can result in faster and more complete adaptation to legal requirements. However, the limitation may be the lower budget, which may limit the assortment of purchased food products, especially those more expensive. It also seems that large institutions may be less flexible with respect to individual children’s diets: in public kindergartens there were 2.3% of children on special diets, while in non-public 3.4%, respectively. The catering system was used more often in private DCCs. In this case, the facility has a limited influence on the planning and preparation of meals for children, as well as less control over their quality.

The aspect regulated by both legislations was the addition of vegetables and/or fruits to every meal. Approximately half of public institutions failed to implement this recommendation; in the case of private DCCs this percentage was even higher (especially in the second analysis). It seems that the problem with required supply of vegetables and fruits is not due to financial constraints. Previous studies on children nutrition specially indicated a low intake of vegetables [8, 19, 25]. The first legislation also required fresh vegetables in the menus at least 3 times a week in lunch meal. Over 90% of facilities served fresh vegetables and fruits every day, which is even more than was required. This number did not decrease in the second analysis, when the legal legislation was repealed. However, it is still a challenge to provide vegetables or fruits in every meal served, which is an important element in shaping correct eating habits in children. It could be concluded that, despite the existence of mandatory regulations, education is still needed to increase the amount of vegetables and/or fruits in the pre-school diet.

The method of culinary process influences the quality and nutritional value of meals. Frying causes an increase in the amount of fat in the final product, thus increasing its energy value. When frying in inappropriate

fat, compounds with harmful effects on health can be formed [7]. The current review suggested that more frequent consumption of fried foods (i.e., four or more times per week) was associated with a higher risk of developing diabetes type 2, heart failure, obesity and hypertension [6]. Both regulations [23, 24] restricted the number of fried dishes to 2 servings per weeks, as well as allowed to use only canola oil and olive oil for this preparation. In the present study, nearly a quarter of the facilities served fried foods more than twice a week. There also have been no changes in this frequency over time or depending on type of institution. On the other hand, some changes have been observed with regard to fat used for frying. The most commonly used was canola oil, which is in line with the requirements. The other of permitted fats, olive oil was actually used more often by private institutions. The reason for this might be higher oil prices compared to canola oil. It is alarming to see a significant increase in the number of facilities using prohibited fats for frying. This may be due to a lack of knowledge in this regard: frequently listed butter may be suitable for children nutrition but not for frying. Conversely, coconut oil or lard should not be used in pre-school children diets [3, 10].

In the prevention of excessive weight in children, it is very important to limit the intake of sugar and sweets [4, 6, 27]. Studies show that young children's main sources of added sugar are sweetened beverages and desserts; the majority of daily beverages are consumed during children's meals [18]. Thus, policy change is the one approach for improving healthy beverage practices in child care settings. The first legislation completely forbade sweetening fruit juices or often served compotes (compote according to traditional recipe is prepared with added sugar). Instead, only honey was allowed to other drinks e.g. tea, cocoa or coffee substitute [24]. In this respect, many facilities did not follow the recommendations. Approximately, one quarter of the DCCs sweetened the compote with sugar or honey, a slightly lower percentage of facilities inappropriately sweetened fruit juices and milk shakes. In case of sweetening tea, cocoa or coffee substitute, also approximately one quarter of DCCs used banned sugar. In the second legislation [23], the requirements for sweetening were considerably reduced, allowing the addition of 10 g of sugar to 250 ml of any beverage prepared for children. It can be observed that facilities have benefited from this opportunity, and increased the frequency of sweetening beverages, especially by using sugar. With regard to the general recommendations for reducing sugar supply, specially so called "free" sugars coming from beverages, [10, 22, 27] this is not beneficial and needs improvement.

The excessive salt intake is a widespread problem, resulting in an increased risk of developing hypertension in adulthood [15]. High content of sodium was also

reported in preschool menus in Lublin [13]. Strategies to reduce sodium intake include replacement of regular salt with low sodium salt, as well as lack of salt at the table. The legislation from 2015 [24] used both options: allowed to use only low sodium salt (so called dietetics or potassium salt) during cooking, but not after preparation of meals. Most DCCs limited salting after preparation of meals, but nearly half of the kindergartens used the regular salt. Interestingly, despite the higher price of "diet" salts, a greater number of public establishments have introduced such salt to the menu. The legislation from 2016 has not regulated the type of salt, its quantity or way of addition [23]. In response, the facilities increased the usage of regular salt and reduced the low-sodium salt. There was also a significant increase in the frequency of salting meals after preparation. The increased of salt supply is unfavourable, and contrary to the principles of proper nutrition [10, 22, 26], so education in this regard seems to be crucial.

This study is not without limitations. The selection of DCCs was not representative, but the strength of the study is a large number and nationwide range. The great advantage of the study is the way of collecting data: with a validated questionnaire, in a face-to-face interview conducted by trained interviewers. Unique on such a scale is the verification of data based on 10-day menus and inventory reports, which significantly increases the reliability of the results. Finally, this is the first study to examine the effects of nutrition legislation policies in DCCs in Poland.

Our findings suggest that policies may be effective in changing nutrition in kindergartens, but policies alone may be insufficient to obtain a clear and permanent improvement. Similar observations were made by *Benjamin Neelon et al.* [1].

CONCLUSIONS

Policy might affect nutrition-related practices in child care centers, but additional support is needed to bring all preschools into compliance with the regulations. Lack of mandatory regulations might have an adverse effect on nutrition quality. Therefore, nutrition education and more detailed mandatory nutrition standards for kindergartens are crucial. It is worth stressing that proper nutrition of children must be treated as an investment in their healthy development, and there is a urgent need for a systematic solution to improve nutrition in pre-schools.

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Conflict of interest

The authors declare no conflict of interest.

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EFFECT OF CALORIC RESTRICTION ON LIVER FUNCTION IN YOUNG AND OLD APOE/LDLR^{-/-} MICE

Renata B. Kostogrys¹, Magdalena Franczyk-Żarów², Angelika Manterys², Iwona Wybrańska¹

¹ Department of Clinical Biochemistry, Faculty of Medicine,
Jagiellonian University Collegium Medicum, Kopernika 15, 31-501 Kraków, Poland

² Department of Human Nutrition, Faculty of Food Technology,
University of Agriculture H. Kołłątaja in Cracow, Balicka 122, 30-149 Kraków, Poland

ABSTRACT

Background. Caloric restriction (CR) leads to decrease metabolic intensity, which results in a reduction of oxygen consumption and the amount of free radicals. This can affect the function of the liver. Studies show that caloric restriction does not alter or significantly increase the enzyme activity associated with gluconeogenesis, but the effect was different according to the age of the model animals.

Objective. The aim of the study was to determine the effect of caloric restriction on liver function in young and old ApoE/LDLR^{-/-} mice.

Material and methods. Dietary experiments were performed on 2 and 5 month old male ApoE/LDLR^{-/-} mice. Animals were divided into 3 experimental groups (n=6) and fed AIN⁹³G diet for 8 and 5 weeks, respectively. Control animals were fed *ad libitum* (AL) and housed in a colony cages. These animals were checked for dietary intake. The second group were also fed *ad libitum* but the animals were kept individually in cages (stress AL- sAL). Similarly to sAL group, the animals from the CR group were kept individually but received a 30% less diet compared to AL group. At the end of the experiment animals were euthanized and the blood, liver and adipose tissue have been collected. Alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST) were measured in plasma. Fatty acid profile was evaluated (relative %) in adipose tissue (GC-MS). Liver's steatosis was assessed. Results were analyzed statistically (ANOVA, STATISTICA v.10.0).

Results. CR ApoE/LDLR^{-/-} mice showed significantly lower body weight compared to animals, both AL and sAL. There were no significant differences between ALT and AST in both younger and older animals. However, negative tendencies were more pronounced in younger animals. In young animals CR significantly increased liver weight compared to AL (4.14 vs 3.73g/100g). In adipose tissue fatty acid profile differed in CR mice compared to control in young animals.

Conclusions. Caloric restriction did not affect liver enzymes in mice. Caloric restriction showed similar but not identical metabolic activity in young and old mice.

Key words: caloric restriction, liver, ApoE/LDLR^{-/-} mice

STRESZCZENIE

Wprowadzenie. Restrykcje kaloryczne (CR) prowadzą do spadku intensywności metabolizmu, co wiąże się ze zmniejszeniem zużycia tlenu i ilości powstających wolnych rodników. Tym samym mogą mieć wpływ na funkcjonowanie wątroby. Badania wykazują, że ograniczenia kaloryczne nie zmieniają lub znacząco zwiększają aktywność enzymów związanych z glukoneogenezą, w tym kluczowego enzymu jakim jest aminotransferaza alaninowa (ALT). Obserwowany efekt był różny w zależności od wieku modelowych zwierząt.

Cel badań. Celem pracy było określenie wpływu restrykcji kalorycznej na czynność wątroby u młodych i starszych myszy ApoE/LDLR^{-/-}.

Material i metody. Doświadczenie żywieniowe przeprowadzono na 2. i 5. miesięcznych samcach myszy ApoE/LDLR^{-/-}. Zwierzęta podzielono na 3 grupy doświadczalne (n=6) i żywiono dietą AIN⁹³G przez okres 8 i 5. tygodni. Zwierzęta z grupy kontrolnej (AL) żywione były *ad libitum* i przetrzymywane zbiorowo w klatkach. Spożycie diety było sprawdzane. Grupa druga otrzymywała dietę *ad libitum* przy czym zwierzęta przetrzymywano w klatkach indywidualnie (sAL). Analogicznie do grupy drugiej zwierzęta z grupy restrykcji kalorycznych (CR) były trzymane indywidualnie, jednak otrzymywały 30% mniej diety w porównaniu do grupy kontrolnej AL. Po zakończeniu doświadczenia zwierzęta poddano eutanazji

Corresponding author: Renata B. Kostogrys, Jagiellonian University Collegium Medicum, Faculty of Medicine, Department of Clinical Biochemistry, Kopernika 15, 31-501 Kraków, Poland, tel: +48 12 662 48 21, e-mail: renata.kostogrys@uj.edu.pl

i pobrano wątrobę w celu oceny histologicznej (barwienie H&E i ORO) oraz krew, w której metodą spektrofotometryczną przeprowadzono oznaczenie enzymów aminotransferazy alaninowej (ALT), jak również aminotransferazy asparaginowej (AST). W tkance tłuszczowej oznaczono profil kwasów tłuszczowych (GC-MS). Wyniki poddano analizie statystycznej (ANOVA, STATISTICA v.10.0).

Wyniki. Zarówno młodsze, jak i starsze myszy ApoE/LDLr^{-/-} poddane restrykcji kalorycznej wykazały istotnie niższą końcową masę ciała w porównaniu do zwierząt z grup kontrolnych, zarówno AL, jak i sAL. Nie stwierdzono istotnych statystycznie różnic pomiędzy grupami w poziomie ALT, jak również AST zarówno u zwierząt młodszych, jak i starszych, przy czym negatywne tendencje były wyraźniej widoczne u zwierząt młodszych poddanych restrykcji kalorycznej. Również u młodszych zwierząt CR miało istotny niekorzystny wpływ na masę wątroby w porównaniu do kontroli (4,14 vs 3,73 g/100g). Zaobserwowano istotny wpływ CR na profil kwasów tłuszczowych w tkance tłuszczowej u zwierząt młodszych.

Wnioski. Restrykcje kaloryczne nie miały wpływu na próby czynnościowe wątroby u myszy. Restrykcje kaloryczne wykazały podobną, ale nie identyczną aktywność metaboliczną u młodszych i starszych zwierząt.

Słowa kluczowe: restrykcje kaloryczne, wątroba, myszy ApoE/LDLr^{-/-}

INTRODUCTION

A balanced diet can provide proper proportions of nutrients to organisms. In 1935 McCay [13], for the first time documented the hypothesis that in rodents the increase in life expectancy is correlated with the reduction of the consumption of a diet called caloric restriction (CR) [7]. Caloric restriction (CR) is defined as restricting the diet of an organism to fewer calories (20–50%) than *ad libitum* feeding without altering the levels of vitamins, minerals and amino acids in order to include all essential nutrients. Initially it was speculated that caloric restriction prolongs life, because it causes a slowdown of growth and development, and thereby contributes to the inhibition of “gene age”. This theory, however, was rejected. Recent studies suggest that caloric restriction causes the body’s low stress levels and thus stimulates intracellular signaling pathways. This protects cells and tissues against the effects of aging. In addition, CR regulates the metabolism of glucose, fats and proteins in a way that increases the chances of survival under stress conditions [21]. However, the actual mechanism that CR can effectively lengthen lifespan remains controversial. Many of the early hypotheses to explain this effect were based on it being a passive alteration in metabolism, however recent data support the idea that CR is not simply a passive effect. It has therefore been hypothesized that the beneficial effects of the CR diet are due to increased activity or efficiency of cellular antioxidant defenses. Some studies have supported this hypothesis [14, 17], other studies have failed to demonstrate consistent enhancement of antioxidant defenses in association with the CR diet [4, 19, 22]. There is evidence that CR is an active, highly conserved stress response that evolved early in life’s history to increase organism’s chance of surviving adversity [21].

CR has also been proven to be an effective treatment for NAFLD [1, 23]. Nonalcoholic fatty liver disease (NAFLD) is a disease spectrum that includes

hepatic steatosis, steatohepatitis, fibrosis and liver cirrhosis. The fatty liver has been shown to be insulin resistant and to overproduce glucose, VLDL, CRP, and coagulation factors leading to hyperglycemia and lipid disorders.

Tauriainen et al. [24] reported that CR almost completely prevented fatty liver formation in mice. The mechanisms underlying the beneficial effects of CR is not well understood.

Therefore, CR is the most potent and reproducible intervention demonstrated to not only extend lifespan but also delay the negative physiologic consequences of chronic diseases associated with aging. However, *Harisson* et al. [6] has shown, that 40% CR increases mortality in C57BL/6J mice when started just after weaning (i.e. 4 weeks of age), but increases lifespan when started in middle age. Unfortunately, little is known about effect CR on mice in different age.

The aim of the study was to determine the effect of caloric restriction on liver function in young and old ApoE/LDLr^{-/-} mice.

MATERIAL AND METHODS

Animals and feeding

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. ApoE/LDLr^{-/-} male mice were originally obtained from Jackson Lab (USA) and bred in house. Animals were housed in a temperature-controlled environment (22–25°C) with a 12 hour light/dark cycle.

Two and five month old male ApoE/LDLr^{-/-} mice were divided into 3 experimental groups (n = 6) and fed AIN⁹³G diet (Table 1) for 8 and 5 weeks, respectively. Control animals were fed *ad libitum* (AL) and housed in a cages. Daily dietary intake were monitored. Stressed AL group (sAL) received *ad libitum* diet, but animals were kept individually in cages. Similarly to sAL group the animals from the CR group were kept

individually but received a 30% less diet compared to the AL group. Body weight of mice were monitored weekly. At the end of the experiment animals were euthanized. The mice were injected intraperitoneally with 1000 IU of heparin (Sanofi-Synthelabo; Paris, France) and after 10 min, anesthetized with ketamine/xylazine given intraperitoneally. Finally mice have been sacrificed by cervical dislocation.

Table 1. Composition of experimental diet (%)

Corn starch	53.2486
Caseine	20
Sucrose	10
Soybean oil	7
Celulose powder	5
Mineral mixture *	3.5
Vitamin mixture *	1
Choline	0.25
Tert-butylhydrochinone	0.0014

* According to Reeves, 1993

Blood sampling and measurements of biochemical markers

Blood samples were taken from the vena cava and were collected into test tubes and centrifuged (13 000 x g, 4 min) to obtain plasma samples. The plasma were deep frozen (-80°C) and stored until further analysis using commercially available kits for alanine transaminase (ALT), aspartate aminotransferase (AST) (Pentra 400, Horiba).

Determination of fatty acids composition in adipose tissue

Adipose tissues were collected from each mice. The samples (10 mg) were placed in vials and treated with 2 ml solution of 0.5 M KOH in methanol. The samples were heated at 57°C for 15 min. Next, 2 ml of 14% BF₃ in methanol was added and heated at 57°C for 15 min. After cooling, 2 ml of hexane and 2 ml saturated sodium chloride was added. The mixture was vortexed. The upper n-hexane layer was transferred to eppendorf tubes and dried with anhydrous Na₂SO₄. The analysis of fatty acids methyl esters (FAME) was performed on a SHIMADZU GC-MS- QP 5050A equipped with a SP-2560 capillary column (100 m x 0.25 mm i.d. x 0.25 µm film thickness,

Supelco). Helium was the carrier gas and operated at flow rate of 1.8 ml/min. Injector temperature was maintained at 245°C, detector temperature was 200°C. The total FAME profile in a 1 µl injection at a split mode was determined. The oven temperature was operated at 60°C for 5 min, then the temperature programmed at 5°C/min to 180°C, held for 16 min, programmed at 5°C/min to 220°C, held for 7 min. FAME identification was validated and based on electron impact ionization mode.

Histological analysis

The livers were excised and weight. Part of liver from each mice was formalin fixed for routine histopathological examination. Tissues were placed in sucrose for 24 h, dried and frozen in OCT. Slides (7 µm sections) were stained with hematoxylin and eosin (H&E) and oil red O (ORO).

Statistical analysis

Results are expressed as mean ± SEM. Where appropriate, the data were subjected to analysis of variance calculated in the STATISTICA 10 package (StatSoft Inc., USA), followed by *post-hoc* Tukey multiple range test. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of CR on body weight

Regardless of age, body weight in ApoE/LDLr^{-/-} caloric restricted mice significantly decreased compared to the AL and sAL animals (Table 2). Young animals have reduced body weight by 6.52 g, while old animals by 5.97 g. Both in young as well as old animals the highest decrease in body weight were observed after first week of experiment (5.9 g in young mice and 7 g in old mice, data not shown).

Effect of CR on liver

In young animals CR significantly affected the liver. Liver weight (g/100g b.w.) significantly increased (by 11%) in mice fed CR diet. However, in old animals no significant differences in liver weight were observed (Table 2).

Table 2. Effect CR diet on ApoE/LDLr^{-/-} mice in different age ($p < 0.05$. ^{a,b} young animals, ^{A,B} old animals)

	Experimental groups					
	AL _y	sAL _y	CR _y	AL _o	sAL _o	CR _o
% fatty acids						
Body weight (g)	26.20 ± 1.24 ^a	26.26 ± 0.45 ^a	19.68 ± 0.85 ^b	27.96 ± 0.37 ^A	27.59 ± 0.80 ^A	21.99 ± 0.92 ^B
Liver weight (g/100g)	3.73 ± 0.08 ^a	3.77 ± 0.04 ^a	4.16 ± 0.16 ^b	4.11 ± 0.12	4.08 ± 0.28	3.62 ± 0.11
ALT(U/L)	47.15 ± 11.93	31.85 ± 11.50	90.45 ± 11.84	53.86 ± 8.60	53.75 ± 7.31	66.42 ± 8.18
AST(U/L)	147.59 ± 37.65	128.37 ± 46.16	322.94 ± 46.30	234.16 ± 52.76	145.86 ± 14.39	346.93 ± 75.76

* – różnica istotna statystycznie przy $p < 0.05$

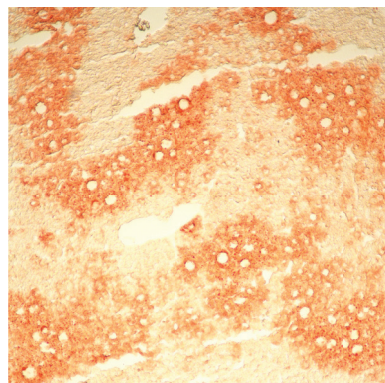
In old mice ALT as well as AST levels were higher compared to young animals (by 14% for ALT and by 59% for AST). After CR treatment no significant differences between ALT and AST both in young and old animals were observed, however negative tendencies (i.e. increase) were more pronounced in

young animals compared to old (ALT: 91% vs 23% AST: 118% vs 48%).

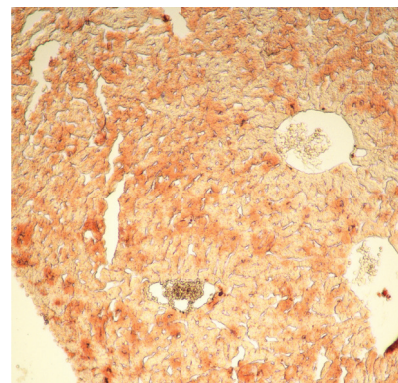
No significant differences were observed in livers stained ORO. However, compared to AL slightly more fat in liver has been observed in young animals after CR treatment.

Table 3. Fatty acid profile in adipose tissue in ApoE/LDLr^{-/-} mice ($p < 0.05$, ^{a,b} young animals, ^{A,B} old animals)

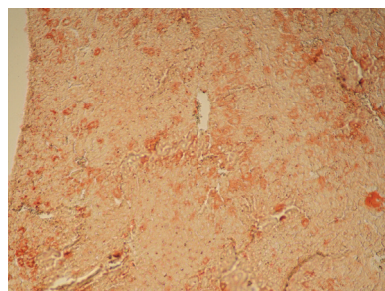
Fatty acids	Experimental groups					
	AL _y	sAL _y	CR _y	AL _o	sAL _o	CR _o
Fatty acids (%)						
C14:0	0.52 ± 0.02 ^a	0.62 ± 0.03 ^a	0.91 ± 0.03 ^b	1.83 ± 0.09	1.77 ± 0.07	2.12 ± 0.14
C14:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.09 ± 0.01	0.11 ± 0.01	0.15 ± 0.02
C15:0	0.06 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.20 ± 0.05
C16:0	14.16 ± 0.21 ^a	15.04 ± 0.41 ^a	20.52 ± 0.51 ^b	17.16 ± 0.37 ^{AB}	15.94 ± 0.32 ^A	17.89 ± 0.85 ^B
C3-16:1	0.40 ± 0.04 ^a	0.34 ± 0.02 ^{ab}	0.18 ± 0.07 ^b	0.24 ± 0.09	0.32 ± 0.14	0.21 ± 0.07
C9-16:1	3.57 ± 0.31	3.09 ± 0.29	3.87 ± 0.38	6.76 ± 0.47	6.20 ± 0.17	8.27 ± 0.47
C17:0	0.13 ± 0.02 ^a	0.09 ± 0.01 ^{ab}	0.07 ± 0.01 ^b	0.38 ± 0.03	0.34 ± 0.01	0.28 ± 0.02
C8-17:1	0.20 ± 0.03 ^a	0.07 ± 0.01 ^b	0.05 ± 0.02 ^b	0.73 ± 0.05	0.71 ± 0.02	0.64 ± 0.07
C18:0	1.82 ± 0.07 ^a	1.83 ± 0.08 ^a	4.54 ± 0.49 ^b	3.99 ± 0.34	3.91 ± 0.17	4.28 ± 0.55
C9-18:1 n-9	33.84 ± 0.20	33.55 ± 0.25	34.63 ± 0.41	30.87 ± 0.33	31.32 ± 0.38	30.32 ± 0.43
C9.12-18:2 n-6	41.73 ± 0.22 ^a	41.72 ± 0.33 ^a	32.32 ± 1.18 ^b	30.89 ± 0.46 ^A	31.90 ± 0.44 ^A	28.93 ± 1.62 ^B
C20:0	0.14 ± 0.04	0.12 ± 0.04	0.25 ± 0.02	0.60 ± 0.04	0.68 ± 0.03	0.57 ± 0.11
9.12.15-18:3 n-3	3.40 ± 0.11 ^a	3.49 ± 0.12 ^a	2.63 ± 0.19 ^b	5.29 ± 0.28	5.77 ± 0.26	6.01 ± 0.35
C6.9-18:2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
C8.11-20:2 n-9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.11 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
other (e.g.C20:3)	0.06 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.58 ± 0.03	0.63 ± 0.05	0.94 ± 0.13



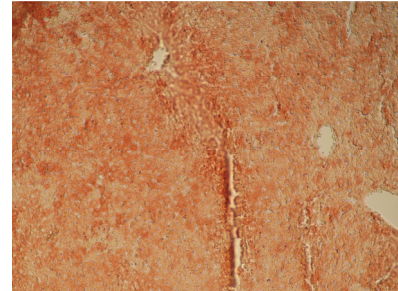
AL_y



CR_y



AL_o



CR_o

Photo 1. Livers of young (AL_y, CR_y) and old (AL_o, CR_o) ApoE/LDLr^{-/-} mice stain ORO

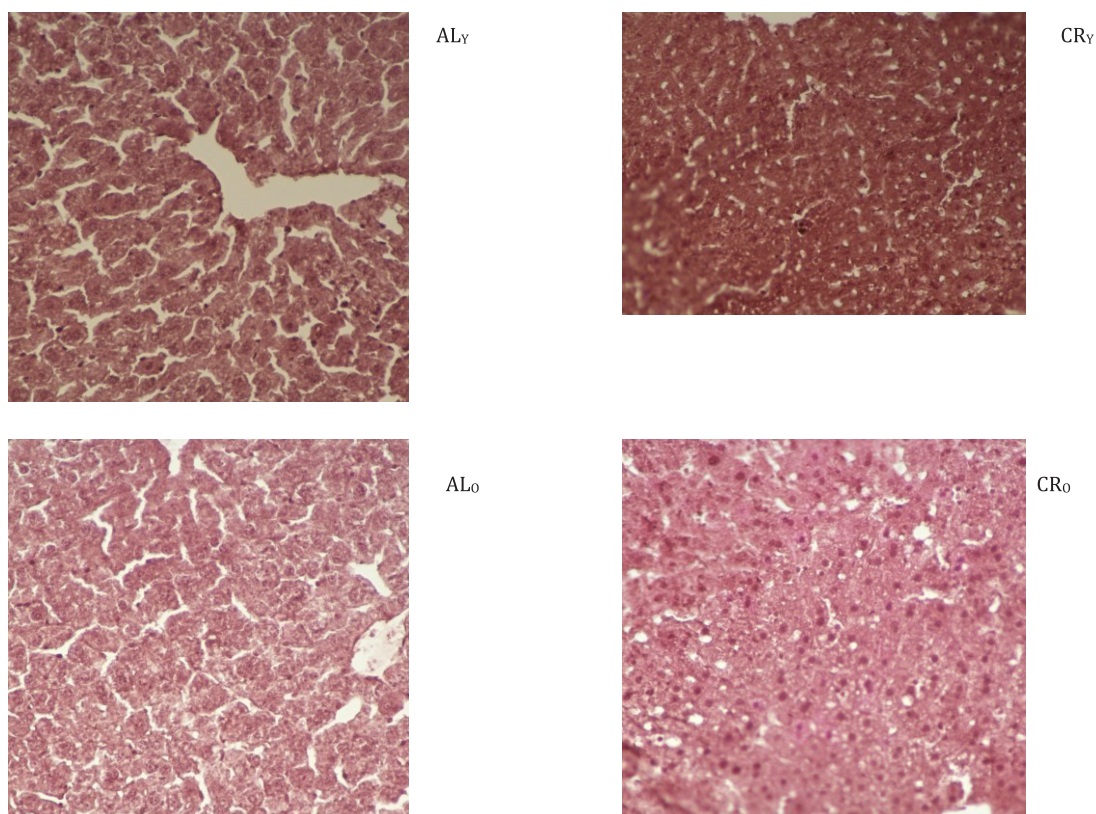


Photo 2. Liver of young (AL_y , CR_y) and old (AL_o , CR_o) ApoE/LDLr^{-/-} mice stain H&E

Effect of CR on fatty acid profile in adipose tissue

Fatty acid profile in adipose tissue changed significantly after caloric restriction. In young animals negative changes in individual fatty acids levels were observed. Compared to control SFA level has

significantly increased, whereas PUFA level was significantly decreased. In old animals significant difference was observed only in linoleic acid level (Table 4). No differences in SFA level in old animal have been observed.

Table 4. Saturated fatty acids, monounsaturated fatty acid and polyunsaturated fatty acids in ApoE/LDLr^{-/-} mice adipose tissue ($p < 0.05$, ^{a,b} young animals, ^{A,B} old animals)

Fatty acids	Experimental groups					
	AL_y	sAL_y	CR_y	AL_o	sAL_o	CR_o
Fatty acids (%)						
Σ SFA	16.83 ± 0.24^a	17.73 ± 0.49^a	26.30 ± 1.0^b	24.23 ± 0.79	22.90 ± 0.49	25.22 ± 1.35
Σ MUFA	38.00 ± 0.29^b	37.04 ± 0.35^a	38.72 ± 0.52^b	38.44 ± 0.81^A	38.34 ± 0.29^{AB}	39.38 ± 0.85^B
Σ PUFA	45.13 ± 0.19^a	45.21 ± 0.25^a	34.95 ± 1.32^b	36.26 ± 0.44^{AB}	37.78 ± 0.22^A	34.27 ± 1.99^B

DISCUSSION

While excessive calorie intake are associated with several health problem also NAFLD, caloric restriction (CR) with adequate nutrition ameliorates metabolic disturbances. A strong connection between caloric intake and aging has been developed. Caloric restriction significantly increases lifespan and decreases the rate of occurrence of most age-associated degenerative diseases in rodents. CR leads to a decrease in metabolic intensity. This is associated with a reduction in oxygen consumption and the

amount of free radicals produced, which can affect the liver's function. *Tauriainen* et al. [24] has shown that CR almost completely prevented fatty liver formation in mice. However, the impact of caloric restriction on liver is not fully understood. Therefore, the aim of the study was to determine the effect of caloric restriction on liver function in young and old ApoE/LDLr^{-/-} mice.

Presented study confirmed, that CR affect the body weight both in young and old animals. *Omodei* et al. [15] review that caloric restriction is one of the most effective nutritional interventions that protects against obesity. The use of caloric restriction causes

a reduction in body fat and thus losing weight, thereby lowering leptin synthesis and simultaneously an increase in adiponectin. The recent evidence suggests that even when implemented over a short period, caloric restriction is a safe and effective treatment.

In our study we observed that CR had no significant effect on liver's enzymes. However, ALT and AST levels tended to increase mostly in young animals after CR treatment. Additionally, CR significantly increased liver weight in young animals, whereas no differences were observed in old animals. ORO staining showed that hepatic steatosis was similar in old animals regardless of the type of nutrition. In young animals liver histology was slightly impaired by CR.

Caloric restriction (CR) is commonly recommended for improvement of obesity-related diseases such as NAFLD [9]. Kim et al [9] found that CR reverted hepatic steatosis of db/db mice. H&E and ORO staining showed that hepatic steatosis in db/db mice was reduced by CR administration. Additionally, they found that hepatic enzymes (ALT and AST) were significantly decreased by CR treatment. On the contrary, Hagopian et al. [5] observed that short-term (1 month) and long-term (28 months) calorie restriction diet in mice significantly increased liver transaminases including ALT and AST as well as multiple other gluconeogenic enzymes, e.g. pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1-6-biphosphatase, and glucose-6-phosphatase.

Many authors have proved, that the transaminases are important to effectively utilize amino acids for gluconeogenesis during caloric restriction. ALT plays a significant role in gluconeogenesis and amino acid metabolism. Therefore, it is not just a marker of cellular injury. Liver ALT will increase in response to dietary protein as well as fasting [20]. This suggests some ALT elevations may reflect a metabolic adaptive response and not necessarily indicate hepatocellular injury. Thus should not be considered adverse [18]. However, in our study in young animals liver's weight significantly increased after CR treatment. The same was true for liver histology which indicated slightly more fat (i.e. intensified steatosis).

The mechanisms underlying the accumulation of fat in the liver may include excess dietary fat, increased delivery of free fatty acids (FFA) to the liver, inadequate fatty acid oxidation and increased de novo lipogenesis [26]. Plasma FFA normally originate from adipose tissue lipolysis and are the major circulating lipid fuel.

Because the vast majority of the fatty acids stored in adipose tissue originate from dietary fat, studying this aspect of adipose tissue physiology is warranted [8].

Fatty acid profile in mice's adipose tissue fed caloric restricted diet were analysed. Linoleic acid (18:2 n-6) significantly decreased after CR treatment in both young and old animals. Therefore PUFA

decreased after CR treatment. However, this effect was more visible in young animals.

This may reflect lower dietary intake, where soybean oil is a source of fat in AIN93G diet. Composition of soybean oil is: linoleic acid (51%), oleic acid (23%), palmitic acid (10%), linolenic acid (7%) and stearic acid (4%). When examining the effect of CR on fatty acid composition in young mice, it was observed that most of the fatty acid changes occurred. SFA level statistically increased in young animals after CR treatment (16,83 vs 26,30%). In old animals no effect on SFA has been observed.

Phinney et al. [16] found that there was a decrease of EPA and linolenic acid (18:3 n-3) in the adipose tissue of subjects consuming weight-reducing diets for 3-5 months. These observations suggest that essential fatty acid content in weight-reducing diets may be inadequate.

When investigating the effect of CR (CR-soy vs. control) on mitochondrial phospholipid fatty acid composition, increase of ($P < 0.05$) total n-6 fatty acid content was observed [2]. CR also resulted in a decrease in monounsaturated fatty acid (MUFA) content due to decreases in the content of 16:1n-7 and 18:1n-7.

It has been reported that CR increases the content of 18:2n-6 and decrease long chain polyunsaturated fatty acids of membrane phospholipids in liver [10, 11], spleen [25] and heart [12]. However, we found no evidence that CR increases the level of 18:2n-6. The results of the present study are consistent with the idea that CR decreases long chain polyunsaturated fatty acids. However, it is possible that additional changes in fatty acid composition may occur with age and/or duration of CR. In support of this idea, it has been reported that both, level of CR and length of CR influence skeletal muscle phospholipid fatty acid composition [3, 6].

Thus, additional studies at multiple time points are likely needed to completely characterize the influence of CR on mice model.

CONCLUSIONS

1. Caloric restriction did not affect liver enzymes in mice.
2. Caloric restriction showed similar but not identical metabolic activity in young and old mice.

Conflict of interest

The authors declare no conflict of interest.

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THE COMBINED EFFECTS OF ALCOHOL AND TOBACCO USE DURING PREGNANCY ON BIRTH OUTCOMES

Jadwiga Hamulka, Monika A. Zielińska, Katarzyna Chądzyńska

Warsaw University of Life Sciences (SGGW-WULS), Faculty of Human Nutrition and Consumer Sciences, Department of Human Nutrition, Warsaw, Poland

ABSTRACT

Background: Exposure to alcohol and tobacco during pregnancy may lead to developmental disorders in the foetus and can also adversely impact pregnancy outcomes and the newborn's health status.

Objective: Assessment of alcohol consumption and tobacco use during pregnancy as well as their impact on pregnancy outcomes and birth parameters of the newborn in a selected group of mothers with children aged ≤ 2 years.

Material and methods: The study was conducted in 104 mothers with children aged ≤ 2 years living in the Mazowiecki Voivodeship. A questionnaire was used for assessing selected lifestyle parameters tobacco and alcohol use, dietary supplements use, physical activity during pregnancy, socio-demographic characteristics as well as children's birth parameters (birth weight, length, head circumference, chest circumference). Data were analyzed statistically using logistic regression analysis, *Spearman's* rang correlation, the *Mann-Whitney U*, *Kruskal-Wallis*, *Student-t*, *Tukey* and *Chi²* tests ($p \leq 0.05$; STATISTICA 13.1 PL).

Results: 22% of the participants solely smoked cigarettes, 13% solely drank alcohol while 7% smoked cigarettes and drank alcohol. Both cigarette smoking and alcohol use increased the risk of preterm birth (OR 9.6, 95% CI 2.9 – 31.4, $p=0.00015$; OR 5.3 95% CI 1.3 – 22.3, $p=0.0199$, respectively) as along with a low birth weight (OR 6.9, 95% CI 1.9 – 25.2, $p=0.0028$; OR 7.6, 95% CI 1.7 – 34.3, $p=0.007$). Cigarette smoking and alcohol use by the pregnant women also adversely impacted on children's health status (8.7 ± 1.4 vs. 9.7 ± 0.8 , $p=0.001$ and 8.5 ± 1.9 vs. 9.6 ± 0.8 , $p=0.027$, respectively). Simultaneous use of alcohol and cigarettes exacerbated the adverse impact of such substances on birth parameters and health status (birth weight, body length, Apgar score) as well as on pregnancy duration.

Conclusions: The introduction of educational programs for high risk groups as well as prenatal obligatory consultations for mothers about the negative effects of substance use on fetus and the development of the child are warranted.

Conclusions: Introducing educational programs are thus warranted for high risk groups as well as prenatal obligatory consultations for mothers about the adverse effects of such substance use on foetal and child development.

Key words: pregnancy, alcohol use, smoking, birth parameters, pregnancy outcomes

STRESZCZENIE

Wprowadzenie: Ekspozycja na używki (alkohol, papierosy, kofeinę) w okresie ciąży może prowadzić do zaburzeń rozwoju płodowego, jak również może negatywnie wpływać na przebieg ciąży i stan urodzeniowy dziecka.

Cel pracy: Ocena powszechności picia (spożywania) alkoholu i palenia tytoniu w ciąży oraz ich wpływu na przebieg ciąży i parametry urodzeniowe noworodka w wybranej grupie matek dzieci do 2 roku życia.

Material i metody: Badanie przeprowadzono wśród 104 matek dzieci poniżej 2 roku życia zamieszkujących województwo mazowieckie. Do oceny wybranych parametrów stylu życia (palenie papierosów, spożywanie alkoholu, kawy oraz herbaty, aktywność fizyczna), charakterystyki socjo-demograficznej oraz parametrów urodzeniowych dziecka (wykorzystano kwestionariusz ankiety. Wyniki zinterpretowano za pomocą analizy regresji logistycznej, korelacji rang *Spearmana*, oraz testów *U* *Manna-Whitneya*, *Kruskala-Wallisa*, *t-Studenta*, *Tukeya* i *Chi²* ($p \leq 0.05$; STATISTICA 13.1 PL).

Wyniki: W badanej grupie 22% kobiet w trakcie ciąży paliło papierosy, 13% spożywało alkohol, natomiast jednoczesne stosowanie obu używek deklarowało około 7% badanych. Palenie tytoniu, jak również spożywanie alkoholu zwiększało ryzyko wystąpienia porodu przedwczesnego (odpowiednio: OR 9.6, 95%CI 2.9 – 31.4, $p=0.00015$; OR 5.3 95% CI 1.3 – 22.3, $p=0.0199$) oraz niskiej masy urodzeniowej (OR 6.9, 95%CI 1.9 – 25.2, $p=0.0028$; OR 7.6, 95% CI 1.7 – 34.3, $p=0.007$), jak również predysponowało do gorszego stanu urodzeniowego dziecka (odpowiednio 8.7 ± 1.4 vs. 9.7 ± 0.8 , $p=0.001$ oraz 8.5 ± 1.9 vs. 9.6 ± 0.8 , $p=0.027$). Jednoczesne spożywanie alkoholu i palenie tytoniu zwiększało negatywne oddziaływanie stosowanych używek zarówno na parametry urodzeniowe, jak i stan zdrowia dziecka (masa urodzeniowa, długość ciała, punkty w skali *Apgar*) oraz czas trwania ciąży.

Wnioski: Konieczne jest wdrożenie programów edukacyjnych skierowanych do grup ryzyka, jak również wprowadzenie obowiązkowej rozmowy z matkami (przyszłymi rodzicami) na temat szkodliwego działania używek na płód i rozwój dziecka.

Słowa kluczowe: ciąża, spożycie alkoholu, palenie tytoniu, parametry urodzeniowe, przebieg ciąży

Corresponding author: Jadwiga Hamulka, Department of Human Nutrition, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (SGGW-WULS), Nowoursynowska 159C, 02-776 Warsaw, Poland, phone: +48 22 5937112, e-mail: jadwiga_hamulka@sggw.pl

INTRODUCTION

Recent studies have shown the importance of lifestyle on pregnancy outcome, child's health both during childhood and adulthood as well as on maternal health [28, 31]. This is all related to the concept of intrauterine programming, which states that exposure to certain environmental factors during pregnancy induces adaptation changes in the fetus, which may lead to its developmental disorders and an increase in the risk for the occurrence of non-communicable chronic diseases at later life [8, 29, 31]. Exposure to stimulants or substance use such as drinking alcohol as well as cigarette smoking, both actively and passively, are among environmental factors that induce adaptation changes to human body [29, 36]. Alcohol crosses placental barrier and is metabolized 2-fold slower by the fetus as compared to the mother and its stronger teratogenic effect occurs between the 1st and 8th week of gestation. However, it should be mentioned that alcohol has negative effects throughout the period of pregnancy and depends on the ingested dose and frequency of intoxication. Alcohol increases the risk of abortion, occurrence of fetal alcohol syndrome (FAS), alcohol-related birth defects (ARBD) as well as alcohol-related neurodevelopmental disorders [6, 33]. On the other hand, tobacco use, both active and passive, increase the level of carbon monoxide and nicotine in the blood of the mother and fetus. Nicotine decreases blood flow with the maternal-placental circulation, which increases the risk of IUGR and premature separation of the placenta. There is a clear correlation between the number of smoked cigarettes or exposure to cigarette smoke and the occurrence of low birth weight (<2500 g). Women who smoke cigarettes during pregnancy are more likely to experience ectopic pregnancy, preterm placental

abruption, and particularly, cesarean birth and preterm birth, which alone causes more infant deaths than any other known cause. The most severe consequences of smoking tobacco during pregnancy are intrauterine fetus death as well as increase risk SIDS during the first months of life [1, 33, 36]. Compounds present in cigarette smoke inhaled by the mother get into fetal circulation and lead to many disorders, including tachycardia, tachyarrhythmia and a decline in immunity. Children, who have been exposed to negative effects of smoking during fetal life are at higher risk for the development of cardiovascular disorders, asthma and allergies. In addition, substance use by mothers during pregnancy has been demonstrated to increase the risk of psychiatric disorders, attention deficit hyperactivity disorder (ADHD) and poor school performance in their children [33].

Studies have indicated that a large proportion of women smoke tobacco and drink alcohol in their reproductive years [25, 30, 37], which can negatively affect the development of the fetus [6, 36]. Furthermore, despite the abundance of information about the adverse effects of drinking alcohol and smoking cigarettes on pregnancy outcome, a lot of women still use these substances when pregnant [19, 23, 36, 37, 38]. For this reason, the purpose of the present study was to assess the rates of this substance use during pregnancy as well as its impact on pregnancy outcome and birth parameters of the newborn in a selected group of mothers of children aged ≤ 2 years.

MATERIAL AND METHODS

The study was conducted among 104 mothers of children aged ≤ 2 years, patients of pediatric counseling centers from Warsaw and the surrounding area (Figure 1).

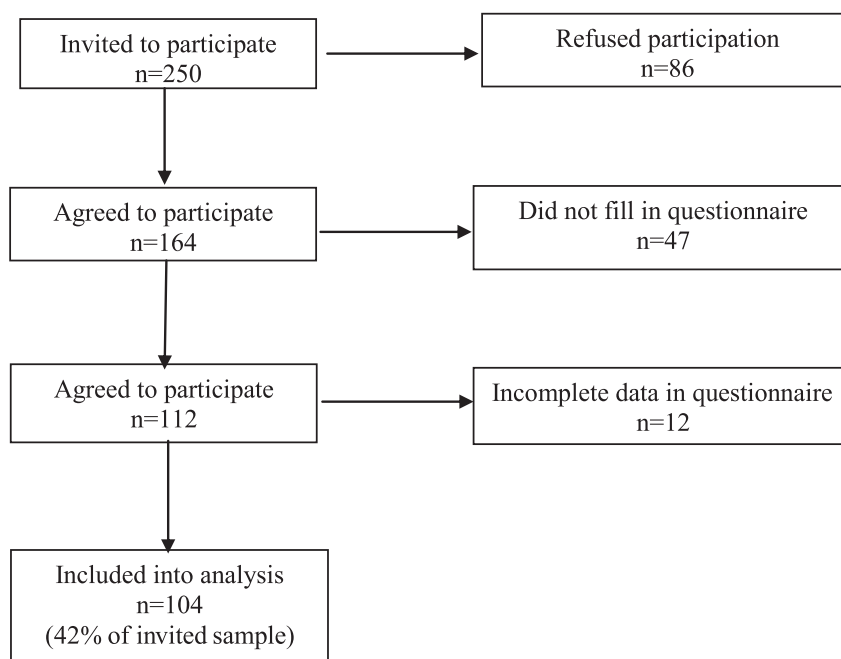


Figure 1. Flow chart of sample collection

Table 1. Maternal characteristics and alcohol and tobacco use during pregnancy

Variables	Alcohol and tobacco use during pregnancy n (%)					p-value
	Total n=104 (100)	Abstinent n=75 (72)	Alcohol n=6 (6)	Tobacco n=16 (15)	Alcohol and tobacco n=7 (7)	
Maternal age, y	28.46 ± 5.62 16 ÷ 42	29.7 ± 5.1 ^a 19 ÷ 42	27.0 ± 5.2 ^a 20 ÷ 34	26.1 ± 5.5 ^a 19 ÷ 39	21.9 ± 6.0 ^b 16 ÷ 31	0.0002*
Maternal education						
primary or vocational	26 (25)	8 (11)	1 (17)	11 (69)	6 (86)	
secondary	27 (26)	21 (28)	1 (17)	4 (25)	1 (14)	
university	51 (49)	46 (61)	4 (17)	1 (6)	-	<0.0001*
Occupation situation						
working	14 (13)	11 (15)	-	2 (13)	1 (14)	
employed and/or studying						
sick leave	3 (3)	5 (7)	-	-	-	
unemployed and/or no	58 (56)	47 (63)	4 (67)	7 (44)	-	
studying	27 (26)	12 (16)	2 (33)	7 (44)	6 (86)	0.0074**
Maternal place of residence						
rural area	10 (10)	10 (13)	-	-	-	
city <100.000 inhabitants	14 (13)	12 (16)	-	1 (6)	1 (14)	
city >100.000 inhabitants	80 (77)	53 (71)	6 (100)	15 (94)	6 (86)	0.31**
Economic situation						
poor	27 (26)	10 (13)	1 (17)	9 (56)	7 (100)	
average	43 (41)	34 (45)	2 (33)	7 (44)	-	
good	34 (33)	31 (41)	3 (50)	-	-	<0.0001**
Pre-pregnancy BMI category						
underweight	5 (5)	2 (5)	-	1 (6)	-	
normal	85 (82)	59 (79)	6 (100)	14 (88)	6 (86)	
overweight and obesity	14 (13)	12 (16)	-	1 (6)	12 (16)	0.81**
Weight gain during pregnancy, kg	13.07 ± 5.62 - 4 ÷ 27	13.4 ± 4.4 ^a - 4 ÷ 27	14.8 ± 3.4 ^a 10 ÷ 20	12.0 ± 3.2 ^{ab} 9 ÷ 22	10.1 ± 3.4 ^b 6 ÷ 16	0.0275***
Weight gain during pregnancy, kg/week	0.34 ± 0.11 - 0.14 ÷ 0.71	0.34 ± 0.12 - 0.14 ÷ 0.71	0.39 ± 0.08 0.27 ÷ 0.50	0.32 ± 0.07 0.23 ÷ 0.54	0.30 ± 0.14 0.17 ÷ 0.14	0.159***
Dietary supplements use						
no	46 (44)	27 (36)	3 (50)	9 (56)	7 (100)	
yes	58 (56)	48 (64)	3 (50)	7 (44)	-	0.0077**
Physical activity						
no	69 (66)	49 (65)	4 (67)	11 (69)	5 (71)	
yes	35 (34)	26 (35)	2 (33)	5 (31)	2 (29)	0.98**

*t-Student and NIR Tukey test; **Chi² test; ***ANOVA Kruskal-Wallis test, a, b – the values in lines with the same letters do not differ significantly (ANOVA, post-hoc Tukey's test, p<0.05).

Inclusion criteria were children age ≤24 months old and consent to participate in the study. All participants were inhabitants of Mazowiecki Voivodship and were within the age range of 16 to 42 years (28.5 ± 5.6). Most of the subjects were characterized by normal BMI before gestation (82%; 22.2 ± 3.1; 15.6 ÷ 33.5) and were mainly dwellers of big towns (77%) with population of above 100 000. About half of the

subjects (49%) attended higher institutions, 41% were characterized by an average income and most of them (63%) had only one child. A detailed study sample characteristics was shown in Table 1.

Retrospective assessment of selected elements of lifestyle of the participants and birth parameters of the newborn were carried out with the use of questionnaire during a visit to pediatricians. The

questionnaire consisted of questions pertaining to age, education, financial situation, dwelling place, profession, selected elements of lifestyle, including tobacco and alcohol use, coffee, tea and supplements consumption, and physical activity during pregnancy as well as anthropometric parameters before and after giving birth to the newborn. Furthermore, data related to birth parameters (body weight, length, head circumference, chest circumference, and points for evaluation of baby's condition according to *Apgar* scale) were accessed from the children's health book and certificate. *Ponderal* Index was calculated using birth body measurements ($[\text{birth weight (g)/birth length (cm}^3\text{)}] \times 100$) and then tercile was determined as low (<10 percentile), average (10-90 percentile) and high (>90 percentile) [15]. Also, the questionnaire consisted of questions about infant anthropometric development in the first year of life.

Statistical analysis was performed using the software STATISTICA ver. 13.1. The normal distribution for quantitative variables was determined by *Shapiro-Wilk* test, but for the evaluation of statistical significance t-test, U *Mann-Whitney* test as well as *Kruskal-Wallis* test were applied depending on the type of data. For certain set of data, post hoc analysis with the use of *Tukey* test had to be applied. *Chi*² test was used for qualitative variables. *Spearman* rang correlation was also performed. Logistic regression analysis was conducted for dichotomic variables (occurrence of preterm birth as well as low birth weight), where substance use was treated as independent variables and the results were presented as odd ratios (OR) with 95% confidence interval (95%CI). A statistical significance of $p \leq 0.05$ was set for all statistical analyses.

Table 2. Tobacco use during pregnancy and infant birth parameters

Variables	Tobacco use during pregnancy $\bar{x} \pm \text{SD}$ min ÷ max			p-value
	Total n=104 (100)	No n=81 (78%)	Yes n=23 (22%)	
Gestational age, weeks	38.4 ± 2.5 27 ÷ 42	38.8 ± 2.2 28 ÷ 42	36.8 ± 2.8 27 ÷ 41	0.0001*
Preterm birth, n (%)	16 (15)	6 (7)	10 (43)	<0.0001**
Risk of premature birth, OR (95% CI)	-	-	9.6 (2.9 – 31.4)	0.0002
Birth weight, g	3238.9 ± 591.1 800 ÷ 4960	3346.5 ± 536.8 1180 ÷ 4960	2860.2 ± 628.9 800 ÷ 3900	0.000*
Low birth weight (<2500g), n (%)	9 (9)	4 (5)	5 (22)	0.01143**
Risk of low birth weight, OR (95% CI)	-	-	5.3 (1.3 – 22.3)	0.02
Birth length, cm	54.3 ± 3.7 35 ÷ 62	54.9 ± 3.3 36 ÷ 62	52.2 ± 4.5 35 ÷ 57	0.001*
Ponderal Index	2.0 ± 0.2 1.5 ÷ 3.0	2.0 ± 0.2 1.6 ÷ 2.8	2.0 ± 0.2 1.5 ÷ 2.5	0.31***
Ponderal Index category				
low	14 (13)	11 (14)	3 (13)	0.93**
average	74 (71)	57 (70)	17 (74)	
high	16 (15)	13 (16)	3 (13)	
Head circumference at birth, cm	34.4 ± 2.0 25 ÷ 37	34.5 ± 1.8 25 ÷ 37	33.9 ± 2.5 26 ÷ 37	0.44*
Chest circumference at birth, cm	33.6 ± 2.1 24 ÷ 37	33.8 ± 2.0 25 ÷ 37	33.0 ± 2.4 24 ÷ 36	0.082*
Apgar score	9.5 ± 1.0 5 ÷ 10	9.7 ± 0.8 6 ÷ 10	8.7 ± 1.4 5 ÷ 10	0.001*
Birth disorders, n (%)	36 (35)	21 (26)	8 (35)	0.00047**
Passive tobacco smoke exposure, n (%)	31 (30)	13 (16)	18 (78)	<0.0001**
Passive tobacco smoke exposure, h	1.2 ± 2.2 0 ÷ 10	0.4 ± 1.2 0 ÷ 5	3.9 ± 1.2 0 ÷ 10	<0.0001**
Number of smoked cigarette per day	-	-	6.1 ± 4.0 1 ÷ 15	-

* - U *Manna-Whitney* test; ***Chi*² test; ****t-Student* test

Table 3. Alcohol use during pregnancy and infant birth parameters

Variables	Alcohol use during pregnancy $\bar{x} \pm SD$ min ÷ max		p-value
	No (n=91) 87	Yes (n=13) 13	
Gestational age [weeks]	38.7 ± 2.2 28 ÷ 42	36.4 ± 3.5 27 ÷ 40	0.010*
Preterm birth, n (%)	10 (11)	6 (46)	0.001**
Risk of premature birth, OR (95% CI)	-	6.9 (1.9 – 25.2)	0.0028
Birth weight [g]	3304.8 ± 528.5 1180 ÷ 4960	2778.1 ± 801.0 800 ÷ 3890	0.010*
Birth length [cm]	54.6 ± 3.2 36 ÷ 62	51.7 ± 5.9 35 ÷ 58	0.036*
Low birth weight (<2500g), n (%)	5 (5)	4 (31)	0.0024**
Risk of low birth weight, OR (95% CI)	-	7.6 (1.7 – 34.3)	0.007
Ponderal Index	2.0 ± 0.2 1.5 ÷ 2.8	2.0 ± 0.2 1.6 ÷ 2.3	0.4***
Ponderal Index category			
low	12 (13)	2	0.71**
average	64 (70)	10 (77)	
high	15 (16)	1 (8)	
Head circumference at birth [cm]	34.5 ± 1.7 25 ÷ 37	33.3 ± 3.0 26 ÷ 37	0.24*
Chest circumference at birth [cm]	33.8 ± 1.9 25 ÷ 37	32.7 ± 3.4 24 ÷ 36.5	0.34*
Apgar score	9.6 ± 0.8 7 ÷ 10	8.5 ± 1.9 5 ÷ 10	0.027*
Birth disorders, n (%)	29 (32)	7 (54)	0.1192**

* U *Manna-Whitney* test; ** *Chi*² test; *** *t-Student* test

RESULTS

This paper presents the impact of drinking alcohol and smoking during pregnancy on the birth parameters of the studied group of children.

Among all the participants, 15% were found to have given birth to premature babies and the average gestation period was 38.4 ± 2.5 (27÷42) weeks (Table 1). The mean birth weight of the newborns was 3238.9 ± 591.1, but a big inter-individual difference was observed being in the range of 800 g to 4960 g. 9% of the newborns were characterized by low birth weight (<2500 g) while 3% of them were found to be macrosomic (>4500 g). The mean value of points within the *Apgar* scale was high, being 9.5 ± 1.0 with high inter-individual variation from 5 to 10 points. ≤7 points in the *Apgar* scale was found in 5.3% of the newborns. Furthermore, the occurrence of different birth disorders, including hyperbilirubinemia,

circulatory and respiratory failure, heart defects, apnea, Down syndrome, retinopathy, HCV infection, weak muscular tone, skin pallor as well as the presence of the 6th finger on the hand were noticed in 34.6% of the newborns. No statistical significance was observed between the evaluated birth parameters and socio-demographic features as well as the mother's BMI.

It was found that most of the participants (72%) abstained from drinking alcohol during pregnancy, but 6% stated they consumed alcohol. 15% of the subjects declared smoking tobacco during, while 7% of them both consumed alcohol and smoked cigarettes during pregnancy. Substance use was more frequent among younger women, women with lower level of education, unemployed as well as women with lower socio-economic status. Additionally, women, who smoked tobacco and drank alcohol during pregnancy were characterized by lower weigh gain and very seldom used dietary supplements (Table 1).

Table 4. Alcohol and tobacco use during pregnancy and infant birth parameters

Alcohol and tobacco use during pregnancy					
Variables	$\bar{x} \pm SD$				p-value
	min ÷ max				
	Abstinent n=75(72)	Alcohol n=6 (6)	Tobacco n=16 (15)	Alcohol and tobacco n=7 (7)	
Gestational age [weeks]	38.9 ± 2.2 ^a 28 ÷ 42	38.2 ± 2.3 ^a 34 ÷ 40	37.7 ± 1.8 ^a 35 ÷ 41	34.9 ± 3.8 ^b 27 ÷ 39	0.0005*
Preterm birth, n (%)	5 (7)	1 (17)	5 (31)	5 (71)	<0.0001**
Birth weight [g]	3359.5 ± 533.6 ^a 1180 ÷ 4960	3183.3 ± 601.3 ^a 2300 ÷ 3890	3048.1 ± 430.7 ^a 2480 ÷ 3900	2430.7 ± 822.7 ^b 800 ÷ 3300	0.0011*
Low birth weight (<2500g), n (%)	3 (4)	1 (17)	2 (13)	3 (43)	0.004**
Birth length [cm]	54.8 ± 3.3 ^a 36 ÷ 62	55.0 ± 3.1 ^a 50 ÷ 58	53.7 ± 2.4 ^a 50 ÷ 57	48.9 ± 6.5 ^b 35 ÷ 55	0.0021*
Ponderal Index	2.0 ± 0.2 1.6 ÷ 2.8	1.9 ± 0.2 1.6 ÷ 2.2	2.0 ± 0.3 1.5 ÷ 2.5	2.0 ± 0.2 1.66 ÷ 2.3	0.6*
Ponderal Index category					
low	10 (13)	1 (17)	2 (13)	1 (14)	0.96**
average	52 (69)	5 (83)	12 (75)	5 (71)	
high	13 (17)	-	2 (13)	1 (14)	
Head circumference at birth [cm]	34.5 ± 1.8 25 ÷ 367	34.2 ± 1.2 32 ÷ 35	34.5 ± 1.2 33 ÷ 36	32.6 ± 4.0 26 ÷ 37	0.6543*
Chest circumference at birth [cm]	33.8 ± 2.0 25 ÷ 37	33.6 ± 2.2 31 ÷ 36.5	33.4 ± 0.8 32 ÷ 35	31.9 ± 4.2 24 ÷ 36	0.3192*
Apgar score	9.7 ± 0.6 ^a 7 ÷ 10	9.3 ± 1.6 ^a 6 ÷ 10	9.1 ± 1.0 ^a 7.0 ÷ 10	7.7 ± 1.0 ^b 5 ÷ 10	0.0002*

* ANOVA *Kruskal-Wallis* test; ***Chi*² test, a, b – the values in lines with the same letters do not differ significantly (ANOVA, post-hoc *Tukey's* test, $p < 0.05$).

Statistical analysis revealed that 30% of the respondents were at risk to the damaging effects of cigarette smoke, out of which 22% actively smoked cigarettes during pregnancy with the mean number of smoked cigarettes standing at 6.1 ± 4.0 (1÷15) daily. The number of smoked cigarettes correlated negatively with the child's birth weight ($r = -0.447$; $p \leq 0.05$). Smoking tobacco was found to shorten gestation period, caused lower birth weight, body length on average by 15% and 4%, respectively as well as lower points in the *Apgar* scale. No statistical significance was found for the other evaluated parameters (Table 2). Cigarette smoking had also negative impact on the child's health status; abnormalities were noticed among 65% of the newborns from smoking mothers vs. 25% of non-smoking mothers ($p = 0.0005$), which included heart defects, skin pallor, hypotrophy, hypoxia and different types of infections. Smoking tobacco was found to increase the risk of premature birth OR 9.6 (95% CI 2.9 – 31.4; $p = 0.0002$) as well as lower birth weight OR 5.3 (95% CI 1.3 – 22.3; $p = 0.02$).

Alcohol ingestion during pregnancy was found in 13% of the participants with beer being the most frequently consumed alcoholic beverage ($n = 11$). One respondent declared consuming wine once every 3 months, while another participants stated she ingested

vodka once per week (Table 3). Gestation period in women, who drank alcohol during pregnancy, was shorter by 2.3 weeks and their children were characterized by lower birth weight and length by 19% and 6%, respectively as well as lower points in the *Apgar* scale. Alcohol intake also increased the risk of premature birth OR 6.9 (95% CI 1.9 – 25.2; $p = 0.0028$) as well as low birth weight OR 7.6 (95% CI 1.7 – 34.3; $p = 0.007$). Alcohol consumption during pregnancy did not significantly affect the occurrence of birth defects among the investigated newborns.

In addition, it was found that simultaneous use of alcohol and tobacco during pregnancy had a synergistic negative effect on pregnancy duration as well as the child's birth parameters, such as body weight, body length as well as the number of points in the *Apgar* scale – differences were statistically significant (Table 4). Women, who used both alcohol and tobacco smoked on average more cigarettes daily than women, who used only tobacco (9.0 vs. 4.9; $p = 0.025$). Furthermore, more birth abnormalities, including heart defects, hypoxia and infections were found in children from women within this group as compared to women, who did not use any of these substances (86% vs. 27%; $p = 0.002$).

DISCUSSION

The result of the present study has demonstrated negative effects of alcohol ingestion and smoking tobacco during pregnancy on gestation period, birth weight as well as health status of the newborn. Furthermore, the adverse effects of the use of these substances were stronger when they were taken simultaneously. Despite the abundance of information about the negative effects of drinking alcohol and smoking cigarettes on pregnancy outcome, it was found that 22% of the respondents smoked tobacco and 7% of them used both alcohol and tobacco during pregnancy.

Studies conducted by *Caleyachetty* et al. [2] have shown the proportion of women, who actively smoke during pregnancy is small in low and middle income countries and stand at the level of just 2.5% (95%CI 0.00 – 6.40). The results of the present investigation showed a higher percentage of women (22%), who smoked during pregnancy, which is in concordance with the results of studies from different countries, where researchers demonstrated that 15-30% of women - 13% [20], 20% [7, 9], 22% [14], 25% [17, 36], 27% [23]. It is worth mentioning that women often do not disclose active smoking during pregnancy [17, 26]. Passive smoking, to which many pregnant women are exposed to, has also been shown to be hazardous [9, 14, 17, 26, 35].

Globally, it has been estimated that about 10% (95%CI 8.9 – 11.1) of women ingest alcohol during pregnancy. Countries with high percentage of women, who drink alcohol during pregnancy, have been found to be: Ireland (60%), Belarus (47%), Denmark (46%) and Russia (37%). The percentage of women, who ingest alcohol during pregnancy in Poland, has been estimated to be between 15 and 25% [27], which is higher than the number found in the present study (13%). Studies conducted in Poland by different authors have yielded variable results for women, who drink alcohol during pregnancy: 15% reported by *Wojtyła* et al. [37], 24% by *Żuralska* et al. [38], 44% by *Godala* et al. [9], and 60% by *Gacek* et al. [7]. It is worth underlying that these data are fragmentary and there is scarcity of representative research in this area. It is alarming to mention that only 43% of gynecologists and obstetricians recommend to their patients total abstinence during prenatal visits and consultations, and even 2% of these specialists recommended ingestion of small amounts of alcohol during pregnancy. 55% of physicians have been shown not to even mention about the harmful effects of alcohol consumption on the fetus and child [37].

Results from different studies have shown alcohol ingestion and smoking tobacco during pregnancy is practiced mainly by young women with the lowest

level of education and worst socio-economic status, which were also confirmed in the present study. Young pregnant women are significantly more likely than older pregnant women to smoke cigarettes, use illicit drugs, and drink alcohol during pregnancy [7, 14, 19, 20, 32, 36, 37]. Women, who do not plan or think of pregnancy as well as women, who not experienced any positive reaction or support from family members were more often found to smoke tobacco or exposed to cigarette smoke. Generally, the absence of social support may be a particularly important determinant of which young women drink, smoke, or use drugs during pregnancy [19, 32]. Teenage girls belong to high risk group for substance use, which further increases the risk for the occurrence of pathologies of pregnancy [3, 25].

The negative effect of smoking tobacco on birth weight, including the risk for giving birth to a child with low birth weight has been demonstrated in this study and confirmed the results of previous investigations from different countries [4, 13, 16, 34, 35, 37]. The number smoked cigarettes seems to be the most important determinant in causing these birth defects.

The present study revealed a negative correlation between the number of smoked cigarettes and the child's birth weight. Children of mothers, who smoked tobacco during pregnancy were found to be smaller in weight by about 170-377 g in comparison to children of non-smoking mothers [1]. Studies by *Jaddoe* et al. [13] also showed the negative impact of smoking during pregnancy on head and chest circumference of the newborns. Smoking tobacco increases the risk of bleeding and preterm birth, which is related to preterm placental abruption [4, 16, 18, 35]. Furthermore, smoking tobacco negatively affects the newborn health status expressed as points in the *Apgar* scale and increases the risk of hypoxia and infections in the newborn [36]. Smoking cigarette during pregnancy also increases the risk of the occurrence of birth defects, including heart defect, which was demonstrated in the present study as well as by other investigations [1, 10, 36].

Alcohol ingestion is one of the anti-health behaviors practiced by some women during pregnancy. The exact effects of alcohol on the fetus remain to be elucidated, which is related to lack of evidence of the existence of safe dose of alcohol that could be ingested during pregnancy. However, it clear that even the smallest amount of alcohol can be transferred from the mother to fetus via the placenta and therefore, the blood level of alcohol in the fetus is the same as in the mother. The consequences of alcohol ingestion include premature birth, IUGR and intrauterine fetus death [6, 22, 24]. However, a meta-analysis conducted by *Patra* et al. [24] as well as systematic review

performer by *Henderson et al.* [12] have indicated that sporadic alcohol consumption or the ingestion of 10 g ethanol/day does not increase the risk of giving birth to a baby with low birth weight. The present study demonstrated that ethanol intake in the amount of >18 g/day did increase the risk of the occurrence of low birth weight, which is supported by the results of studies by *Henderson et al.* [12] and *Patra et al.* [24]. Results from some investigations have shown that alcohol ingestion during pregnancy increases the risk of the development of birth defects [6, 33, 37], but this was not confirmed in the present study. One of the severe and well-known consequences of alcohol intake during pregnancy is the development of FAS, which frequency of occurrence in Poland has been estimated to be 900 children annually, but many other cases may remain undisclosed or undiagnosed [33, 38].

Children born to mothers, who both ingest alcohol and smoke tobacco are at a higher risk of preterm birth, developing or having worst birth parameters as well as poorer health status in comparison to children born to mothers, who only ingest alcohol or only smoked cigarettes [21]. This may be related to the similar biochemical action of alcohol and tobacco that leads to perturbations in nutritional status as well as health status of the mother, including low folate status, which contributes to elevation of blood level of homocysteine and subsequently to oxidative stress [21]. For this reason, it is worth considering the increase of the intake of dietary supplements, for example folic acid and antioxidants by pregnant women, who smoke tobacco and drink alcohol [5]. However, but the result of the present study indicate the contrary that pregnant women, who smoke and drink alcohol very seldom use dietary supplements.

The major strength of this study was the investigation of the effect of alcohol and tobacco use, as well as its combined effect on birth outcomes in children of mothers from Mazowiecki Voivodship. Our research can be the basis for designing further research on a larger and representative group of mothers and their children.

This study has a number of limitations. First, the study sample is small and the rate of refused participation is high (34%) with no data about the reasons for refusal or socio-demographic characteristic of these patients. Second, the data about alcohol and tobacco use during pregnancy were self-reported by participants, so we cannot exclude the possibility of false reports which may led to an underestimation of the real rate of alcohol and tobacco use during pregnancy. Third, all the data was collected retrospectively which may increase the risk of error. Fourth, because this study is cross-sectional, not prospective, we can investigate only associations, not the causations between alcohol and tobacco use and birth parameters. Further prospective studies in larger samples would be required.

CONCLUSIONS

1. Tobacco use during pregnancy was declared by 22.1% of respondents, whereas alcohol use was declared by 12.5% of participants.
2. In the group of alcohol users there were significantly higher rates of preterm birth ($p \leq 0.001$) and low birth weight ($p \leq 0.01$) observed, as well as lower birth length ($p \leq 0.05$) and *Apgar* score ($p \leq 0.05$).
3. Among tobacco users during pregnancy significantly higher rates of preterm birth ($p \leq 0.001$) and low birth weight ($p \leq 0.01$) were observed. Also, in this group birth length and *Apgar* score were significantly lower compared with non-smokers ($p \leq 0.001$ for both measurements).
4. In group of both tobacco and alcohol users the rates of preterm birth and low birth weight were significantly higher compared with only tobacco or alcohol users ($p \leq 0.001$ and $p \leq 0.01$, respectively), as well as birth length ($p \leq 0.01$) and *Apgar* score ($p \leq 0.001$).

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Conflict of interest

The authors declare no conflict of interest.

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PHYSICAL ACTIVITY AND ASSOCIATED SOCIO-DEMOGRAPHIC FACTORS IN ADOLESCENTS FROM THE EASTERN REGION OF POLAND

Małgorzata Wasilewska, Józef Bergier

Pope John Paul II State School of Higher Education, Faculty of Health and Social Sciences,
Department of Physical Culture and Physiotherapy, Biała Podlaska, Poland

ABSTRACT

Background. The introduction and maintenance of healthy habits in the field of physical activity at the early stage of life is particularly important for public health. With increasing of public awareness in physical activity, researchers are increasingly questioning its determinants in different age groups. In this paper we focus on variables such as age, sex and place of residence.

Objectives. To determine what selected socio-demographic factors influence the level of physical activity of school youth from the eastern region of Poland in different domains of daily life.

Material and Methods. A random sample of 916 Polish school youth from Secondary Schools from Eastern Region of Poland aged 16-18 was interviewed in spring 2016 by diagnostic survey method with the use of the official Polish long version of the International Physical Activity Questionnaire (IPAQ).

Results. Analysing the relationship between gender and the level of physical activity statistically significant differences were found in all domains in favour of boys. The level of total physical activity in girls was 5345.5 MET-min./week, whereas in boys – 6556.6 MET-min./week. In the area of total physical activity, the highest values were observed in pupils from small towns and villages, with lower values in the youth living in large and medium-sized cities. When the relationship of age with the level of physical activity in particular domains was examined, only the activity in the work/school domain ($p = 0.0129$) spoke in favour of the youngest pupils (2578.7 MET-min./week) when compared to the oldest ones (2226.4 MET-min./week).

Conclusions. The level of physical activity in young people from the Eastern region of Poland is decreasing with age, both in girls and boys. It is therefore important, especially for the group emerging adulthood, to offer a wide variety of updated, involving, age and gender-oriented school physical activity classes that could be easily practised outside the school environment to improve the level of physical activity in leisure domain.

Key words: *physical activity, adolescents, IPAQ questionnaire, determinants, Poland*

STRESZCZENIE

Wprowadzenie. Szczególnie ważne dla zdrowia publicznego jest wprowadzenie i utrzymanie zdrowych nawyków w obszarze aktywności fizycznej już we wczesnym okresie życia człowieka. Wraz ze zwiększoną świadomością społeczeństwa w obszarze zachowań zdrowotnych uwzględniających aktywność fizyczną, badacze coraz częściej stawiają sobie pytania o czynniki je determinujące w różnych grupach wieku. W niniejszej pracy z uwagi między innymi na grupę badawczą skupiono się na zmiennych takich jak wiek, płeć i miejsce zamieszkania.

Cel pracy. Poznanie jakie wybrane czynniki socjodemograficzne warunkują aktywność fizyczną młodzieży ponadgimnazjalnej ze wschodniego regionu Polski w różnych domenach życia.

Material i metoda. Badania przeprowadzono wiosną 2016 roku na losowo wybranej grupie 916 uczniów ponadgimnazjalnych, będących w przedziale wieku 16-18 lat. Badania przeprowadzono metodą sondażu diagnostycznego, przy wykorzystaniu oficjalnej polskiej wersji Międzynarodowego Kwestionariusza Aktywności Fizycznej (IPAQ-wersji długiej).

Wyniki. Analizując związek płci z poziomem aktywności fizycznej stwierdzono istotne statystycznie zróżnicowanie z całkowitym poziomem aktywności fizycznej we wszystkich analizowanych obszarach na korzyść chłopców. Poziom całkowitej aktywności fizycznej u dziewcząt wynosił 5345,5 MET-min./tydz., podczas gdy u chłopców - 6556,6 MET-min./tydz. W obszarze całkowitej aktywności fizycznej zaobserwowano najwyższe wartości u uczniów z małych miast i wsi, z niższymi wartościami u młodzieży mieszkającej w dużych i średnich miastach. Gdy badano związek wieku z poziomem aktywności fizycznej w poszczególnych domenach życia, jedynie aktywność w domenie praca/szkola ($p = 0,0129$) przemawiała na korzyść najmłodszych uczniów (2578,7 MET-min./tydz.) w porównaniu do najstarszych (2226,4 MET-min./tydz.).

Corresponding author: Małgorzata Anna Wasilewska, Pope John Paul II State School of Higher Education, Faculty of Health and Social Sciences, Department of Physical Culture and Physiotherapy, 95/97 Sidorowska, 21-500 Biała Podlaska, Poland, tel.: +48 83 3446913, e-mail: m.wasilewska@dydaktyka.pswbp.pl

Wnioski. Poziom aktywności fizycznej młodzieży ze Wschodniego regionu Polski obniża się wraz z wiekiem, tak u chłopców, jak i u dziewcząt. Jest zatem niezmiernie ważne, szczególnie w grupie wieku stojącej u progu dorosłości, aby zaproponować młodzieży szeroką ofertę interesujących zajęć ruchowych w szkole. Powinny one być nowoczesne, dopasowane do wieku, płci i z łatwością oraz chęcią przenoszone poza obszar szkoły, aby zwiększać niski poziom aktywności fizycznej w czasie wolnym.

Słowa kluczowe: *aktywność fizyczna, młodzież, kwestionariusz IPAQ, determinanty, Polska*

INTRODUCTION

One of the main factors influencing lifestyle is physical activity, which can be considered both in the context of health-promoting lifestyle activities as well as disease prevention measures. The increase in physical activity level is one of the activities that has the greatest positive impact on the health of the population. If everyone followed the recommendation on physical activity on a daily basis, the population health would definitely improve and the health care costs would drop significantly [17]. Systematic exercises, which have already been well documented in scientific research, have preventive and therapeutic effects on many different diseases, including: diabetes, cardiovascular diseases, colorectal cancer and depression [12, 26]. Unfortunately, the transition from childhood to adulthood is characterized by a significant decline in physical activity [6, 24]. Having this in mind, a number of countries have introduced their own guidelines or recommendations regarding the recommended dose of physical activity in adolescence [4, 10]. The introduction of the guidelines helps researchers to assess the scale of changes in the physical activity level of the examined age group as well as helps governmental institutions and individuals to work together to promote physical activity. All activities should be focused on sport activities conducted in schools, as children and young people spend a significant part of the day on school duties [27]. There are many techniques that serve to assess the physical activity of the population. However, the lack of standardisation between them is the major limitation in the study of this factor. This is due to, the use of different methodological instruments and to the fact that research into participation in sport or recreation is conducted independently of one another. Consequently, this can lead to many differences in research design, questionnaires, methodologies, or term definitions. [23]. Therefore, the tool used in this study in form is the long version of the IPAQ questionnaire, adapted in many countries, which allows for comparisons of the obtained results in an international scale. Despite the fact that Poland belongs to the group of developed countries, its Eastern macro-region, including Lublin Voivodeship, is one of the poorest regions in Poland and one of the weakest in the European Union. The problems adversely affecting

the socio-economic situation and the development prospects of this area have a largely structural backing, which is historically conditioned. They are additionally negatively strengthened by the effects of the peripheral position of the macro-region on the external frontier of the European Union, which as a whole is largely underdeveloped [20]. Therefore, the need to monitor the level of physical activity of school youth in this region of Poland is the primary criterion of the choice of the presented research problem.

The following paper focuses on the level of physical activity of the research group and the socio-demographic factors that determine it, out of age, gender and place of residence were taken into account.

MATERIAL AND METHODS

The study was conducted in the spring of 2016 on a randomly selected group of pupils attending public secondary schools from the Eastern Region of Poland (Lublin Voivodship), aged 16-18. Schools were drawn in a simple, dependent way. Surveys were completed independently by the students during classes at school. The average time to complete the questionnaire was 40 minutes. The interviewees were explained what the purpose of the survey was and how to complete the procedure. Participation in the study was voluntary and did not involve any form of gratification. The study protocol was approved by the Bioethical Committee of the Medical University of Lublin in Poland (KE-0254/295/2015).

According to the IPAQ [8] data cleaning protocol, part of the test material had to be abandoned due to its incompleteness. As a result, out of the 916 completed questionnaires, 109 were excluded from further analysis, so that the final sample concerned 807 pupils. The efficiency index was 88%. 51% of the girls and 49% of the boys participated in the study. Almost 22% of the respondents were at the age of 16, 37% were 17 years old and 41% - 18. As the place of residence, pupils most often indicated a village - 40% and the medium-sized city - 31%. The categories of a small town and a large city recorded smaller values (14% and 15% respectively). We used the following four categories of settlements: village (fewer than 1000 inhabitants); small city (1001-30.000 inhabitants); medium-sized city (30.001-100.000 inhabitants); large city (100.000 inhabitants and more).

For estimating the level of physical activity, the official long form of the Polish version of the IPAQ was used. The questionnaire consists of 27 questions that cover 4 domains of physical activity (work, transport, domestic and garden, and leisure-time) as well as time spent sitting. The items in IPAQ are structured so that they provide separate domain specific scores for walking, moderate-intensity, and vigorous-intensity activity. All questions refer to the previous 7 days. The results were presented as the estimation of energy expenditure in metabolic equivalent-minutes per week (MET min./week). According to the IPAQ scoring protocol [8], MET min./week of specific activity (walking or moderate intensity activity or vigorous intensity activity) is computed by multiplying MET value of a particular activity (3.3 for walking, 4.0 for a moderate intensity activity, and 8.0 for a vigorous intensity activity) with minutes spent in that particular activity. To calculate physical activity scores only the activities lasting at least 10 minutes at the time were taken into account. Algorithms for calculating the continuous physical activity scores were used to estimate physical activity based on pupils' answers [8]. Total physical activity score was calculated, as well as separate scores for each of the 4 physical activity domains [8]. The choice of the questionnaire was determined by its high diagnostic value [5] and widespread use in a number of international studies [1]. To investigate the parameters associated with physical activity, the following variables were additionally used: place of residence, age and gender.

The statistical analyses of the collected data were performed by STATISTICA software, version 10. In

the IPAQ – the long form, for 4 domains of physical activity and total physical activity and time devoted to sitting, arithmetic (arithmetic mean, median, minimum and maximum values, and variance measures (standard deviations) were calculated. Then, the significance tests using t-student and a variance analysis (ANOVA) were performed. In the case of non-normality of distributions of the analysed variables (*Schapiro-Wilk* test), non-parametric tests were used: *Mann-Whitney U* and *Kruskal-Wallis* ANOVA. *Chi-square* independence tests were performed for qualitative variables. In all analysed cases, we assumed the significance level $p=0.05$.

RESULTS

The total level of physical activity of the researched pupils was 5935.5 MET-min./week. The relationship between gender and the level of physical activity was analysed, statistically significant differences in all analysed physical activity domains were found in favour of boys. The level of total physical activity of girls was 5345.5 MET-min./week. The highest score was found within work/school domain (2119.0 MET-min./week.) and leisure domain (1240.5 MET-min./week), while the lowest in the domestic and garden domain (859.6 MET-min./week). In the case of boys, the total physical activity level was 6556.6 MET-min./week, and the highest score was also noted within work/school domain (2611.7 MET-min./week) and leisure domain (1640.8 MET-min./week) and the lowest, as in the case of girls, in domestic and garden domain (1084.5 MET / min) (Figure 1.).

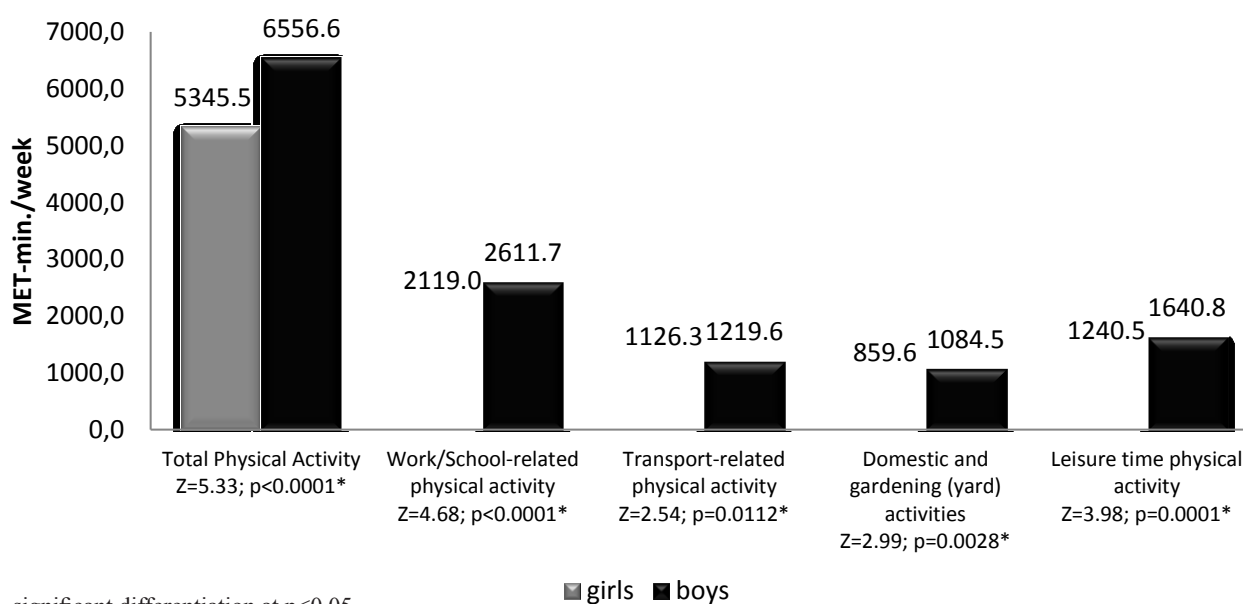
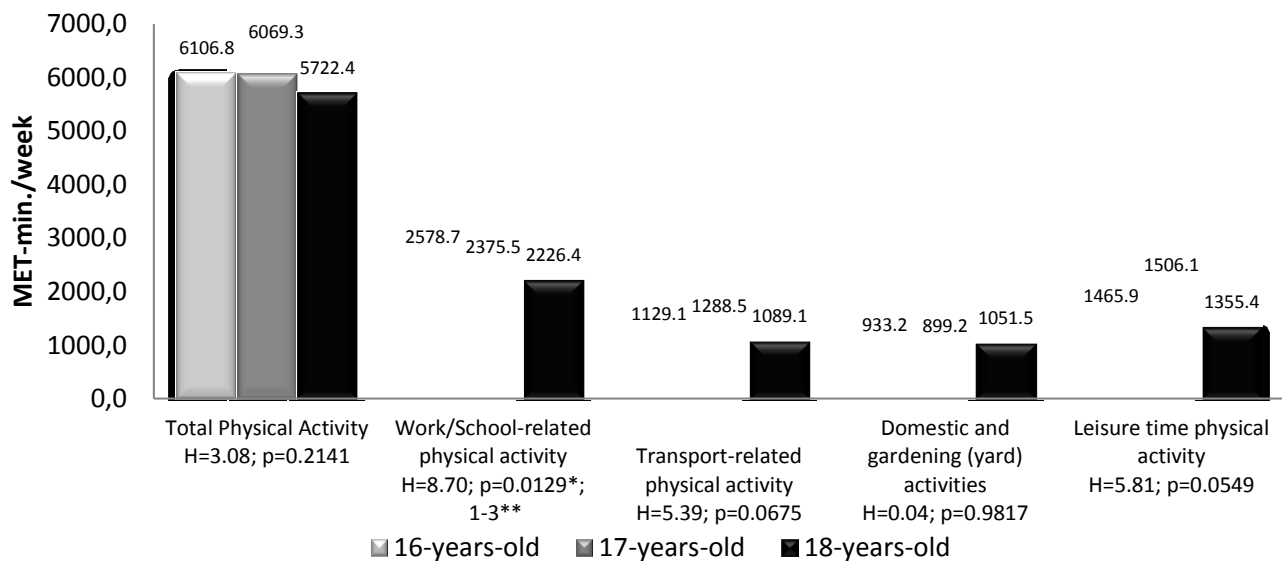


Figure 1. Physical activity in metabolic equivalents-minutes per week (MET-min./week) and the gender-related difference

When the relationship between age and the total level of physical activity was analysed, there were found no significant differences ($p=0.2141$), with a decrease in total physical activity of students with age. When the relationship of this variable with the level of physical

activity in 4 individual domains was contrasted, such a relationship was found only in work/school domain ($p=0.0129$) and in favour of the youngest pupils (2578.7 MET- min./week) when compared to the oldest (2226.4 MET-min./week) (Figure 2.).



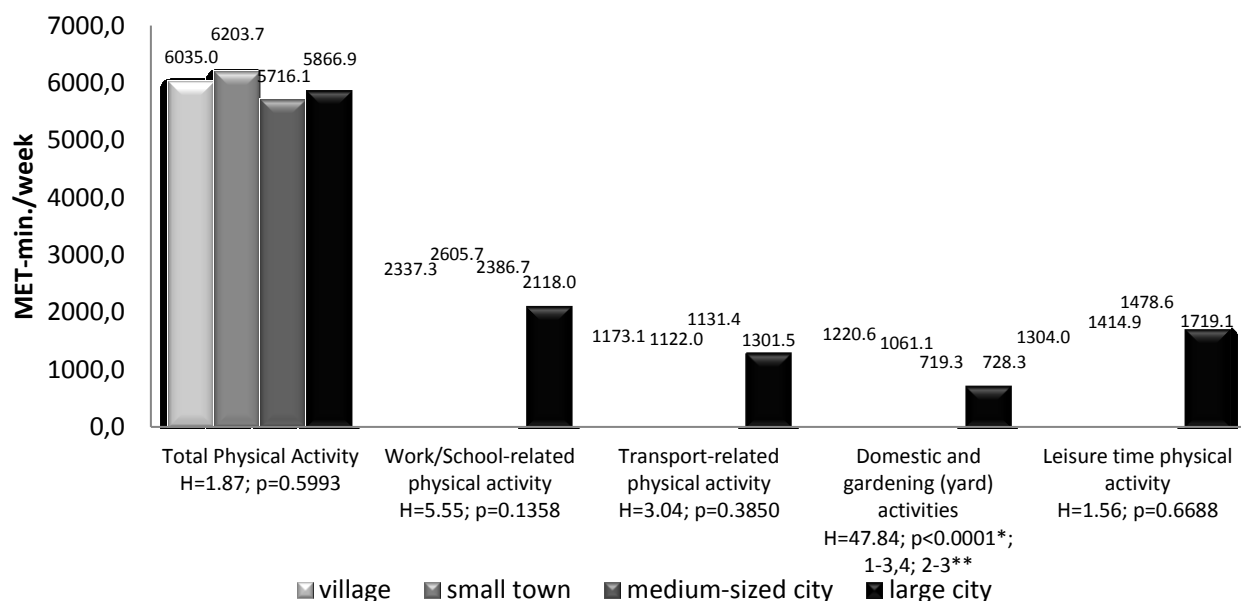
*- significant differentiation at $p<0.05$

** - age groups between which there were found statistically significant differences significant differentiation at $p<0.05$

Figure 2. Physical activity in metabolic equivalents-minutes per week (MET-min./week) the age group-related difference

In the case of the place of residence of pupils and the total level of physical activity no significant relationship was found. The only statistically significant difference was observed in domestic and garden domain ($p < 0.0001$). The highest score in this domain was obtained by residents of village (1220.6 MET- min./week.) and the residents of small town (1061.1 MET- min./week.), and the lowest by

pupils living in the medium-sized city (719.3 MET-min./week) and large city (728.3 MET-min./week). Significant differences in physical activity within domestic and garden domain between village residents and residents of medium-sized and large city have also been demonstrated, as well as between inhabitants of small and medium-sized town in favour of pupils from smaller settlements (Figure 3.).



*- significant differentiation at $p<0.05$

** - places of residence between which there were found statistically significant differences

Figure 3. Physical activity in metabolic equivalents-minutes per week (MET-min./week) and the place-related differences

DISCUSSION

In the study, conducted on a representative number of youth ($n=807$) from Eastern part of Poland, the total level of physical activity was 5935.5 MET-min./week. The majority of pupils (77.4%) presented a high level of physical activity where their total energy expenditure exceeded 1500 MET-min./week and resulted in at least 3 days or more of vigorous-intensity activity or 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities of at least 3000 MET-min./week.

Gender and age differentiated the level of the undertaken physical activity. Girls were less active than boys. The level of total physical activity in girls was 5345.5 MET-min./week. If we want to compare the results of this research with similar ones conducted in European adolescents we will not find many done with the use of the long form of the IPAQ. In Lithuanian girls, the level of activity was also lower than in boys and amounted to 4.404 MET-min./week [3]. Also, the Czech girls achieved lower scores than boys, and the least active girls were 15-year-olds (2.372 MET-min./week) [11]. Another study in which lower scores were obtained in the area of total physical activity by females is the research project on adolescents from the Visegrad Countries (V4) [2]. The Polish girls participating in that project (5862.0 MET-min./week) achieved almost identical results in total physical activity score as the girls from this study (5345.5 MET-min./week).

With age, the level of physical activity of school youth decreased. A similar example that would confirm the thesis is the one conducted on the international junior group of teenagers (12-14 years) from the HELENA study [7], who presented a higher total physical activity score – 4.963 MET-min./week than the older group (15-17 years) – 4.144 MET-min./week. The results of the HBSC study in Poland also confirmed the hypothesis about a decreasing level of physical activity with age and a lower level of activity in girls. For both genders, a linear decrease in the percentage of adolescents who followed the recommendations on health-enhancing physical activity was observed [14]. A confirmation of the decisive role of gender and age in influencing the level of physical activity can also be found in other international studies conducted within this age group [9, 13, 15, 18, 21, 22, 24, 25].

The debate about greater physical activity in boys than girls may be enriched by ethnographic research involving teenagers that have shown that boys receive more encouragement for physical activity from family and society than girls [16]. Boys are particularly encouraged to perceive sports as consistent with the conceptualisation of their masculinity. Through activity and competition in sport they can establish

their position and increase their popularity in the group. On the other hand, doing a sport is rarely seen as feminine [18]. Furthermore, in the qualitative research done among teens, girls reported the following reasons for low participation in physical activity: practising sports is seen as something masculine and not ‘cool’ for girls who prefer other forms of socializing, such as shopping together or having a coffee with friends. Another reason may be, an awareness of the value that comes from studying and working in school, which is revealed in girls earlier than in boys, as well as a greater variety of sports activities directed to boys. Finally, there is a fear of looking unattractive in a sports outfit, which is expressed by girls [19].

Pupils’ place of residence is another socio-geographic variable that was used to analyse the level of physical activity in school youth. While analysing the teenagers’ place of residence and the total physical activity score we did not find any significant relationship. Statistically significant differences were observed only in the domestic and garden domain. The highest score in this domain was observed in inhabitants of rural areas and small towns, while the lowest score in the medium-sized and large cities inhabitants. It can only be assumed that young people from rural areas and smaller urban centres spend far more time than their peers from larger urban centres to help their parents with field or garden work. In this case, it is difficult to find comparable data from other international research due to a huge variation in the authors’ accepted criteria for the size of the surveyed settlements as well as a lack of numbers of inhabitants living in the area.

Our study also had several limitations. Although, there are more precise physical activity measures as activity monitors, questionnaires are most often used in population based studies of physical activity. Our survey was conducted in spring, so another possible limitation might result from a different amount of activity done in different seasons. Further studies of physical activity in the eastern region of Poland should investigate differences concerning education and of parents’ income, in order to develop more adequate physical activity promoting strategies.

CONCLUSIONS

The level of physical activity of young people from the eastern region of Poland is decreasing with age, both in girls and in boys. It is therefore important, especially for the age group emerging the adulthood to offer a wide variety of involving, age and gender-oriented school physical activity classes that could be easily practised outside the school environment to improve the level of physical activity in leisure domain. Since girls present lower level of physical activity in all

domains of life and physical activity at school has the greatest impact on the total value of physical activity of pupils, PE classes should become more attractive to girls to help them find favourite types of physical activity. It might be helpful to create a selection of especially designed blocks for girls within PE classes, such as e.g. fitness, aqua aerobics, dancing to instil lifelong love for physical activity, outside the school environment. Secondary school seems to be the last moment for young people to implement healthy habits and learn how to spend leisure time actively in their adult lives.

Conflict of interest

The authors declare no conflict of interests.

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EVALUATION OF FOOD STORAGE RACKS AVAILABLE ON THE POLISH MARKET IN THE HYGIENIC CONTEXT

Wiesława Grzesińska, Marzena Tomaszewska, Beata Bilska, Joanna Trafiałek, Michał Dziadek

Warsaw University of Life Sciences (SGGW), Faculty of Human Nutrition and Consumer Sciences,
Department of Food Gastronomy and Food Hygiene, Warsaw, Poland

ABSTRACT

Background. Providing safe food products to the consumer depends on the material and technology used and adherence to hygienic practices, throughout the production process. The degree of microbial contamination of a surface is an important indicator of equipment cleanliness and effectiveness of cleaning and disinfection. Used material, construction solutions and quality of the applied devices also have an effect on hygienic status.

Objective. The objective of the present study was to evaluate the influence of the design and construction material of selected food storage racks, available on the Polish market, on their hygienic status.

Material and methods. The study was based on determination of the capability of microbial growth on the surface of the racks and the effectiveness of their cleaning. Microbiological cleanliness on the surface of the racks was monitored by the contact plates which are able to estimate the total number of microorganisms. Examination of effectiveness of cleaning was conducted by the use of ATP bioluminescence method.

Results. This experiment has proven a significant influence of adopted construction solutions on the hygienic status of the examined racks. Presence of antibacterial layer and a choice of the appropriate construction material characterized by a low surface roughness impedes the microbial growth and increases the effectiveness of cleaning.

Conclusions. Design solutions have significant impact on the hygienic status of shelves. Selection of a suitable material for the construction of racks can greatly reduce the possibility of the development of microorganism, despite the low efficiency of the cleaning. The application of antimicrobial coatings inhibits microbial growth.

Key words: storage racks, microbiological cleanliness of surfaces, effectiveness of cleaning processes, ATP bioluminescence, hygiene of racks

STRESZCZENIE

Wprowadzenie. Dostarczanie konsumentowi produktów spożywczych o jak najwyższej jakości, w tym bezpiecznych, uzależnione jest od wykorzystanych surowców, zastosowanej technologii oraz zachowania warunków higienicznych w całym procesie wytwórczym. Stopień zanieczyszczenia mikrobiologicznego powierzchni jest ważnym wskaźnikiem czystości urządzeń oraz prawidłowości procesów mycia i dezynfekcji. Wpływ na higienę urządzeń mają takie czynniki jak zastosowany materiał oraz rozwiązania konstrukcyjne, a także jakość wykonania urządzenia.

Cel. Celem pracy była ocena wpływu konstrukcji i użytego materiału na jakość higieniczną wybranych regałów magazynowych, dostępnych na polskim rynku.

Materiał i metody. W badaniu sprawdzono zdolność wzrostu drobnoustrojów (ogólna liczba drobnoustrojów) na powierzchniach wybranych regałów. Czystość mikrobiologiczną na powierzchni regałów monitorowano za pomocą płytek kontaktowych do oznaczania ogólnej liczby drobnoustrojów. Oznaczenia wykonano zgodnie z normą PN-ISO 18593. Badanie skuteczności procesu mycia przeprowadzono z wykorzystaniem metody bioluminescencyjnego pomiaru ATP.

Wyniki. Doświadczenie wykazało znaczący wpływ zastosowanych rozwiązań konstrukcyjnych na higienę ocenianych regałów. Badania wykazały istotny wpływ rodzaju regału na poziom zanieczyszczenia mikrobiologicznego odnotowanego na powierzchni roboczej regałów oraz na powierzchni połączeń półek ze słupkami nośnymi. Analiza statystyczna wykazała, że w przypadku każdej z prób rodzaj regału miał wpływ na osiągnięte wyniki.

Wnioski. Rozwiązania projektowe mają istotny wpływ na higieniczną jakość półek. Dobór odpowiedniego materiału do budowy regałów może znacznie ograniczyć możliwość rozwoju mikroorganizmów, pomimo niskiej efektywności czyszczenia. Zastosowanie powłok antybakteryjnych hamuje wzrost drobnoustrojów.

Słowa kluczowe: regały magazynowe, mikrobiologiczna czystość powierzchni, efektywność procesu mycia, bioluminescencyjny pomiar ATP, higiena regałów

Corresponding author: Wiesława Grzesińska, Warsaw University of Life Sciences (SGGW), Faculty of Human Nutrition and Consumer Sciences, Department of Food Gastronomy and Food Hygiene, Nowoursynowska 159 C, 02-776 Warsaw, Poland, tel. +48 22 5937075, e-mail: wieslawa_grzesinska@sggw.pl

INTRODUCTION

Meals consumed at catering establishments are a common cause of food poisoning [4, 16]. The data published by the European Food Safety Authority (EFSA) [10] show that in 2015 there were 36,4% of verified sources of food poisoning associated with the services provided by the catering establishments, such as restaurants, cafés, pubs, bars, and by the hotel gastronomy. Less frequently, food poisoning was caused by meals served in canteens.

Responsibility for food safety rests with the food producers [22, 26]. Obtaining safe products of the highest quality depends not only on the material and technology used, but also on the adherence to hygienic practices, throughout the production process [7, 20, 27]. According to Scott [24], nearly 39% of recorded food poisonings was the result of cross-contaminations. The source of the contaminations can be, among others: employees at food establishments [27, 28] construction of technological internal and external surfaces [19, 23], inadequate planning of a production line and the functional layout of a building, as well as carelessly conducted cleaning and disinfection [12, 15].

The degree of microbial contamination of a surface is an important indicator of equipment cleanliness and effectiveness of cleaning and disinfection [18]. Used material, construction solutions and quality of the applied devices also have an effect on its hygienic status [1, 8, 9, 11].

The objective of the present study was to evaluate the influence of the design and construction material of selected food storage racks available on the market on their hygienic status. The study was based on determination of the capability of microbial growth on the surface of food storage racks and the effectiveness of their cleaning.

MATERIALS AND METHODS

For the purposes of the study, an evaluation of three types of food storage racks, available on the Polish market, was made. Detailed characteristics of the these racks are presented in Table 1. These characteristics include elements such as: construction materials, way of installation shelves on support and shelves construction. The racks were set in a separate room, in which the following conditions were ensured: humidity 80-85%, room temperature (20-22°C).

Table 1. Characteristics of the evaluated racks

	<i>Rack 1</i>	<i>Rack 2</i>	<i>Rack 3</i>
Construction materials	support and shelves: carbon steel coated with epoxy resin and antibacterial coating*	support and shelves: steel coated with a layer of chrome-nickel, secured an additional protective lacquer	support: anodized aluminum, shelves: rack made of anodized aluminum, modular inserts made of polyethylene
Way of installation shelves on support	clamping system, the possibility of adjusting the height as 2.5 cm	clamping system, the possibility of adjusting the height as 2.5 cm	rack twisted with support behind assistance of screw, support have holes for assembling shelves at 15 cm
Shelves construction	wire shelves	wire shelves	inserts with ventilation holes, surface of rough to prevent slippage of stored materials

Own study based on [11]

* Composition of the antibacterial coating is reserved by the producer; it is built into the molecular structure of the shelf what provides even distribution in the cross-section and on the surface

Material preparation for testing and sampling

In order to simulate conditions characteristic for catering industry, the racks soiled with a mixture containing organic compounds. The mixture was prepared from the following ingredients: stock prepared from bouillon cubes (the number of cubes in 1000 cm³ of water according to manufacturer's recipe), butter (115 g), cream with 18% of fat content (115 g), flour (400 g) and egg yolk (190 g) [13]. The ingredients were mixed until homogenous. The mixture was prepared directly before its use.

Racks 1, 2 and 3 soiled with the sponge and with the prepared mixture (temperature of emulsion about 20 °C) in an amount of 0.02 g/cm² and left for 2 hours

at room temperature. After this time, the racks were dismantled, washed by the use of professional cleaner kitchen surfaces (concentration 2,5%) having the following composition: secondary alkylosufonians, glycols - glycol ethers, and then rinsed under running water (10 min). Cleaner was apply onto clean surface and let it work for 15 min. Application of the mixture and the cleaning were each time performed by the same person in order to make sure that all activities were carried out under comparable conditions. After cleaning the racks were visually clean.

The samples for examination cleanliness and adenosine triphosphate (ATP) measurement were collected on "day 0" from clean racks (test 0), and

from the washed areas which had been earlier dirtied with the mixture (test 1), (Figure 1). Measurements were performed on the operational (A1, A2, A3) and on the one connecting shelves and their support (B1, B2, B3), (Figure 2). In each shelf, two samples were collected according to the scheme shown in Figure 3. Field markings were determined using disinfected with alcohol line. Sampling of area B were taken alternately: in one day of testing - right side of rack (front, rear connection), the second day of testing - left side (front, rear connection).

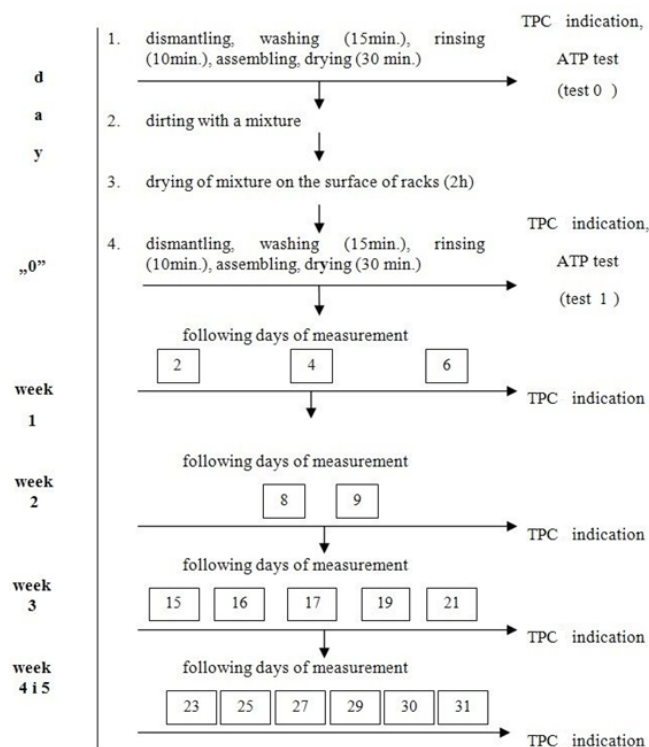


Figure 1. Scheme of experience. TPC (Total Plate Count)

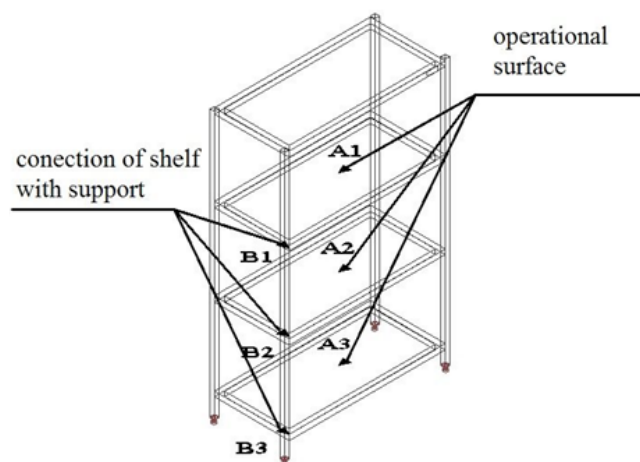


Figure 2. Areas of measurement of the total count of microorganisms (total plate count – TPC) and the ATP (adenosine triphosphate). Source: Own study based on [17]

	15	23	31		15'	23'	31'	
2	9	21	30		9'	21'	30'	2'
1	8	19	29		8'	19'	29'	1'
0	6	17	27		6'	17'	27'	0'
	4	16	25		4'	16'	25'	

Explanatory note: The numbers are in accordance with successive days markings shown in Figure 1

Figure 3. Scheme of sampling on operational surface

The choice of area A was dictated by the fact that it directly contacts the master packages or surfaces of products themselves, which can cause cross-contamination of raw materials and food products. Whereas, the surface connecting shelves and their support (area B) is difficult to reach during cleaning. Thus, they are potentially dangerous areas in terms of growth of microorganisms.

The cleaning of food equipment in catering establishments depends on the protocols of that facilities. Factors influencing the choice the choice of hygiene practice methods include cost, time, staff, ease of use, management needs, and nature of the food contact surfaces [5].

Duration of the experiment and the sampling frequency were determined on the basis of long experience of the research team (frequent visits to the backrooms of catering establishments caused by various renovations and modernizations as well as implementation and auditing of food safety management systems). Interviews conducted among employees of catering establishments and people engaged in HACCP audits show that in most cases, during preparation of GMP / GHP procedures, cleaning and disinfection of racks is planned 2 - 4 times a month. For this reason, due to generally accepted GMP/GHP procedures, frequency of cleaning and disinfection, which is up to every 14 days, the experiment samples in week 1 and 2 were performed with less frequency. Analysis performed in weeks 3, 4 and 5 were intensified to check the hygienic status of the racks in whose cases the proper cleaning and disinfection procedures were not obeyed.

Methods of microbiological determinations

Determination of the total count of microorganisms (Total Plate Count – TPC) was performed by the contact method in accordance with ISO 18593 [14], by the use of contact plates Rodac ConTact Test with neutralizers. The area of a plate equaled 25 cm². The

plates were incubated for 24 hours at the temperature of 37 °C. Subsequently counting of the number of grown colonies was performed. The result was reported as the count of CFU per 25 cm².

TPC obtained in the experiment was compared with the indicators in Polish Standard PN-A-82055-19 [21], (Table 2). This document corresponds to determination of microbial contamination of surfaces as well as evaluation of the effectiveness of disinfectants and detergents in case of meat processing plants. However, due to the fact that the investigated storage racks are designed to store all food products, including meat, it was assumed that they should comply with the requirements of the norm.

Table 2. The indicators and evaluation of microbiological contamination of surface depending on the total count of oxygen microorganisms on 25 cm²

Number of microorganisms CFU/25cm ²	Indicator	Evaluation
$0 \div 2,0 \times 10^0$	0	Excellent
$3,0 \times 10^0 \div 9,0 \times 10^0$	1	Very good
$1,0 \times 10^1 \div 2,9 \times 10^1$	2	Good
$3,0 \times 10^1 \div 9,9 \times 10^1$	3	Sufficient
$1,0 \times 10^2$ and above	4	Insufficient

Source: PN-A-82055-19 [21]

Methods of bioluminescent ATP measurement

ATP measurement was performed by the use of Uni-Lite Xcel apparatus by Biotrace and the swabs in the form of pens in test tubes which are compatible with the device. After the swabbing, each pen was placed in a test tube for the reaction to be performed. Subsequently, the tube was placed in the apparatus and after 15 - 20 s, the results could be read as relative light units (RLU).

Statistical analysis

Statistical analysis was done by the use of STATGRAPHICS 5.1. Data analysis was performed by the use of one-way analysis of variance (one-way ANOVA). Testing was performed at a significance level of $P < 0,05$.

RESULTS AND DISCUSSION

Conducted assay showed that on the day of cleaning ("day 0"), the lowest values of RLU/25 cm² (Figure 4) and cfu/25 cm², both on the surface of the shelves and on the surface connecting shelves and their support (Tables 3 and 4), were found in case of the rack 1. Whereas, the highest values were characteristic for the rack 3.

Table 3. Average value of TPC [cfu/25cm²] noted on the operational surfaces of evaluated racks

Sample number	Kind of racks		
	Rack 1	Rack 2	Rack 3
0	2.33 ± 1.21	7.50 ± 2.43	23.50 ± 5.50
1	4.83 ± 2.48	8.50 ± 3.619	12.83 ± 0.98
2	3.00 ± 0.63	6.50 ± 3.56	9.50 ± 1.05
4	3.50 ± 1.05	8.67 ± 1.75	11.50 ± 2.43
6	7.67 ± 1.21	19.67 ± 4.03	35.17 ± 7.39
8	8.83 ± 1.47	23.83 ± 5.60	51.17 ± 8.03
9	7.50 ± 1.38	23.33 ± 3.33	51.50 ± 6.22
15	8.33 ± 1.51	35.00 ± 7.07	91.00 ± 15.26
16	8.17 ± 2.79	42.50 ± 5.79	115.33 ± 16.03
17	13.67 ± 1.21	59.67 ± 6.53	137.00 ± 14.67
19	9.00 ± 2.61	59.83 ± 6.11	154.67 ± 17.91
21	9.00 ± 2.37	60.83 ± 7.28	166.67 ± 20.41
23	9.50 ± 2.81	82.33 ± 11.15	270.83 ± 43.06
25	10.67 ± 1.21	87.67 ± 6.89	354.17 ± 29.23
27	8.33 ± 1.03	87.67 ± 13.31	337.50 ± 37.91
29	12.17 ± 1.47	91.00 ± 6.23	345.83 ± 45.87
30	14.17 ± 1.60	101.00 ± 17.32	391.67 ± 14.43
31	14.00 ± 2.28	103.50 ± 17.33	TN

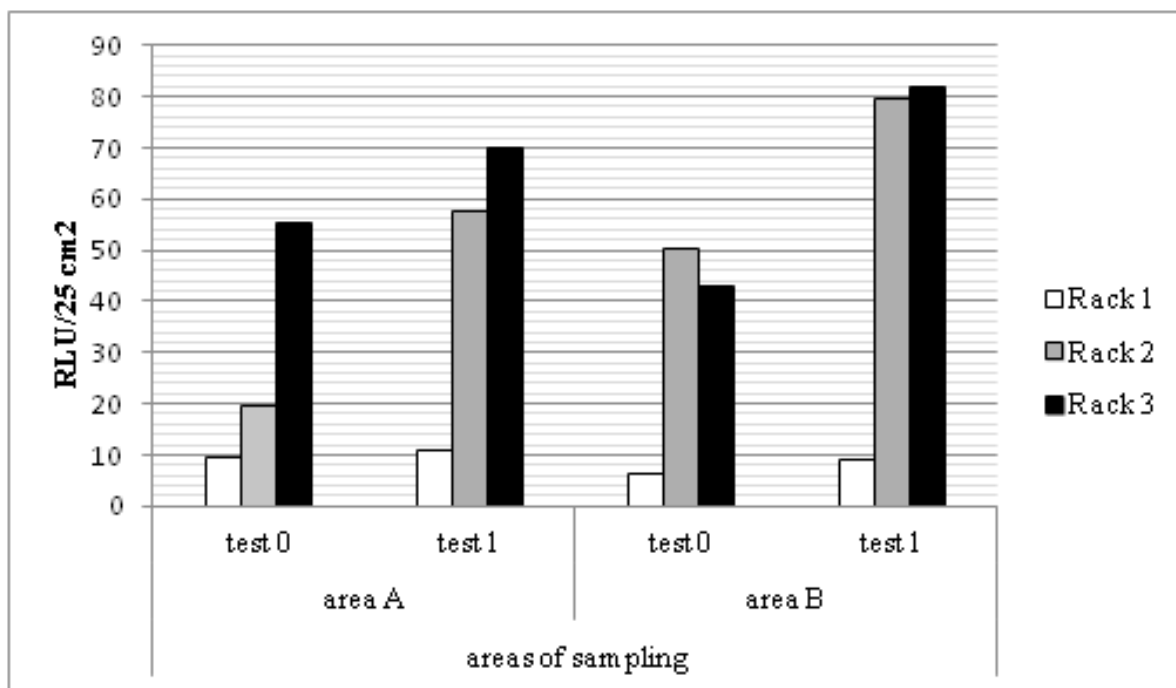
Explanatory note: TN – too numerous to count;

Table 4. Average value of TPC [cfu/25cm²] noted on the surfaces connecting shelves and supports

Sample number	Kind of racks		
	Rack 1	Rack 2	Rack 3
0	13.17 ± 3.37	22.50 ± 5.24	20.17 ± 5.60
1	9.00 ± 2.53	12.00 ± 3.63	10.00 ± 4.19
2	4.00 ± 1.09	7.00 ± 1.79	4.83 ± 2.04
4	3.50 ± 1.38	6.17 ± 1.33	5.00 ± 1.26
6	3.50 ± 1.05	8.50 ± 2.81	8.17 ± 2.99
8	2.83 ± 1.72	7.17 ± 2.64	8.33 ± 2.58
9	3.33 ± 1.37	6.33 ± 2.42	8.67 ± 2.25
15	2.50 ± 1.52	8.33 ± 2.87	8.83 ± 5.74
16	2.83 ± 1.47	8.67 ± 3.01	8.00 ± 3.29
17	3.00 ± 1.09	9.83 ± 2.48	9.33 ± 3.88
19	1.33 ± 1.21	7.50 ± 2.07	8.00 ± 3.35
21	1.83 ± 1.47	7.83 ± 2.40	8.33 ± 2.58
23	1.83 ± 0.75	8.50 ± 3.94	9.17 ± 1.94
25	2.00 ± 0.89	8.00 ± 4.73	13.67 ± 4.46
27	2.83 ± 1.17	7.33 ± 3.08	12.83 ± 3.43
29	2.50 ± 1.87	7.50 ± 2.59	13.50 ± 2.74
30	2.33 ± 1.51	7.83 ± 3.06	18.83 ± 4.96
31	2.33 ± 1.63	8.33 ± 3.93	18.00 ± 4.77

The analysis of the ATP measurements on the surfaces of racks 2 and 3 present after cleaning (test 0), and after cleaning the surfaces dirtied with the mixture (test 1) proves the low effectiveness of the cleaning of the operational surfaces (areas A) and of the surfaces connecting shelves and their support (areas B). In case of the rack 2, the value of RLU/25 cm² found on the operational surface of the shelves after the second

cleaning (test 1) was on average about 38 units higher than in the survey carried out after the first cleaning (test 0), while in case of the surfaces connecting shelves and their support the value was 29 units higher. In case of the rack 3, differences between the second and the first cleaning of areas A and B were respectively 14,5 and 39 RLU/25 cm². The same assay performed in case of the rack A showed high effectiveness of cleaning of areas A as well as B (Figure 4).



Explanatory notes: area A – operational surfaces; area B – surfaces connecting shelves and their support; test 0 – measurement after first cleaning (day “0”); test 1 – measurement after cleaning the surfaces dirtied with the mixture (day “0”)

Figure 4. Results of the bioluminescent ATP measurement on the surfaces of racks

The microbiological assay taken on “day 0”, on the surfaces connecting shelves and their support, in case of every rack surveyed, prove that the cleaning caused reduction of TPC (Table 4). Whereas, the ATP measurement performed in areas B shows an increase of value of RLU/25cm² after cleaning of the surfaces previously dirtied with the special mixture. The results show that the materials used for construction of racks as well as their design itself, particularly in case of racks 2 and 3, hinder complete removal of the organic contamination deriving from raw materials or food of plant and animal origins under the cleaning.

It should be emphasized that, under the conditions of the present experiment, the rack 1 complied with the requirements imposed on equipment used in hospitals, which were defined by *Anderson et al.* [2] at the level not higher than 100 RLU/100 cm².

On the basis of TPC assay, it was concluded that the differences between the examined racks, due to the level of microbial contamination, were statistically significant ($P < 0.05$). This relation was observed in case of the TPC determined on the operational

surfaces, as well as the areas connecting shelves and their support (Table 4).

The lowest number of microorganisms during the whole experiment, was determined on the shelves of the rack 1, whose characteristic is the presence of antibacterial coating.

Performed assay (Table 3) show that during the study period, on the operating surface of the rack 1 there was a 4 - fold increase in the number of microorganisms (from 3.5 to 14 cfu/25cm²), more than a 10 - fold one in case of the rack 2 (from 8, 5 to 104 cfu/25cm²) and an over 40 - fold one in case of the rack 3 (from 12 to more than 400 cfu/25cm²). On the last day of the experiment (day 31), on the operational surface of the rack 3, the number of microorganisms was reported to as too numerous to count (TN), and therefore the value is not included in the Table 3.

The design solution of the shelves applied in case of racks 1 and 2 (wire shelves) resulted in the fact that the contact surface of raw material with shelves is much weaker compared to the solution proposed in case of the rack 3 (polyethylene plate with ventilation

holes), which was the cause of finding the lower total number of microorganisms (Table 3). In addition, the shelves of the rack 3 are characterized by a rough surface intended to prevent slipping of stored materials. According to *Donlan* [8] the adhesion to the surface is dependent upon among others the physicochemical properties of the surface such as texture (rough or smooth) or their hydrophobicity.

In favorable circumstances, bacteria are capable of forming a biofilm which is defined as an aggregation of microorganism attached to and growing on a surface [6]. Biofilms form a reservoir of contamination that persist where cleaning of the manufacturing plant is ineffective [25].

Assay of the TPC performed in case of areas B (Figure 2) indicate a significantly lower level of microbial contamination (maximum value: 22.5 cfu/25 cm²) in comparison to the operational surface of shelves (maximum value: 392 cfu/25 cm²). When it comes to the surfaces connecting shelves and their support the lowest value of TPC was stated in case of the rack 1, whereas the highest one was found in case of the rack 3 (Table 4).

In case of the surfaces connecting shelves and the support of the racks 1, 2 and 3, on “day 0”, a reduction of TPC after the second cleaning process was noted. The assay of microorganisms show the effectiveness of the process. In every three of the analyzed variants, after 48 hours subsequent to the cleaning the decrease of TPC was noticed. This may indicate that microbial cells that remained on the examined surfaces after

performance of a double washing on the “day 0”, began to die out due to environmental shortages of nutrients necessary for the their development . In the following days of the experiment, it was found that in case of the rack 1 as well as 2, the changes of TPC were not statistically significant ($p > 0.05$). Whereas, in case of the rack 3, an increase in the population of microorganisms was observed in the last week of the experiment (from day 23). The TPC found on the surfaces connecting the examined shelves and their support show that the use of antimicrobial coating, as part of the structural material, effectively hinders the growth of micro flora, which allows cleaning to be performed less frequently. The least desirable solution, due to the greatest increase of TPC (Table 4), was proposed in case of the rack 3, whose aluminum frame with polyethylene cartridges was attached to the supports with bolts. This design solution impedes performance of effective cleaning, due to the hindered access to fissures, which in turn might have accelerated the formation of biofilm on the surface.

After comparing the assay of TPC with the indicators and evaluation of the microbiological contamination of surfaces mentioned in the Polish Standard PN-A-82055-19 (Table 2), it was stated that during the first three weeks of the experiment, shelves of the rack 1 were characterized by very high microbiological cleanliness, i.e. ≤ 9 cfu /25 cm² (Figure 5). Whereas, the lowest rating was obtained by the shelf 3.

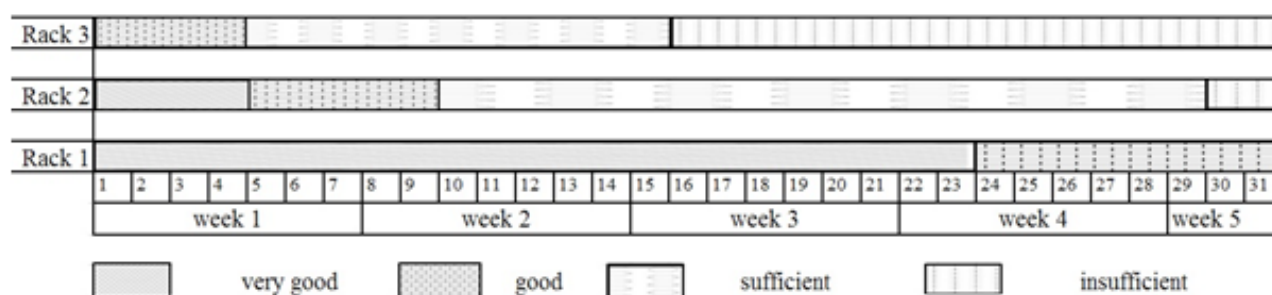


Figure 5. Evaluation of operational surfaces of evaluated racks according to the Polish Standard PN-A- 82055-19 [21]

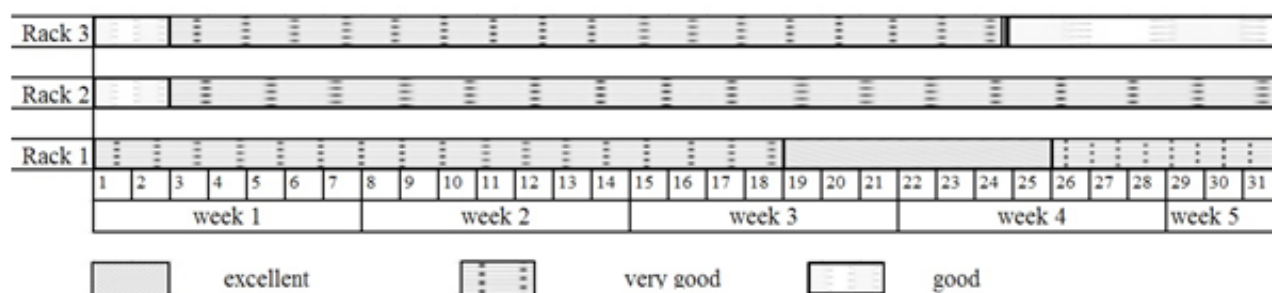


Figure 6. Evaluation of the surfaces connecting shelves and their support of evaluated racks according to the Polish Standard PN-A- 82055-19 [21]

Also in case of the surfaces connecting shelves and their support, the highest rating, in accordance with the PN-A-82055-19 was obtained by the rack 1 (Figure 6).

According to the decision No. 2001/471/EC of the European Commission on the cleaned and disinfected surfaces in meat processing plants, TPC should be in the range of 0 - 10 cfu/10 cm² [3]. The required number of cfu, during the entire experiment, was found only in case of assay performed for the rack 1.

CONCLUSIONS

1. Design solutions have significant impact on the hygienic status of shelves. Rounded and smooth edges greatly enhance cleaning and reduce the possibility of microbial growth. Sharp edges, complicated design of jointing and increased roughness facilitate development of microorganisms and reduce the effectiveness of cleaning.
2. Selection of a suitable material for the construction of food storage racks can greatly reduce the possibility of the development of microorganism, despite the low efficiency of the cleaning. Rack 2 with the lowest performance indicators of wash, thanks to the chrome-nickel steel, had a satisfying values of the total number of microorganisms. The rough surface of the material of rack 3 had the greatest extent conducive to microbial growth, despite the fact that the washing process proved to be effective against it.
3. Area of rack 1 with the antibacterial coating, was characterized by the lowest microbial contamination and the ATP measurement showed the highest cleaning efficiency among the examined racks.

Conflict of interest

The authors declare no conflict of interest.

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ASSESSMENT OF NUTRITIONAL BEHAVIOURS OF CHILDREN AGED 7-12 ATTENDING TO PRIMARY SCHOOLS IN BIALA PODLASKA, POLAND

Anna Galczak-Kondraciuk, Paweł Stempel, Jan Czezelewski

Human Nutrition Laboratory, Faculty of Physical Education and Sport in Biala Podlaska,
Jozef Pilsudski University of Physical Education, Warsaw, Poland

ABSTRACT

Background. Children and youth constitute a group that is particularly prone to the effects of improper diet. In the puberty period, numerous significant lifestyle choices are made, for instance regarding particular nutritional behaviours which may be repeated later in life and may determine the state of health.

Objective. The aim of the study was to assess nutritional behaviours of children aged 7-12 attending to primary schools in Biala Podlaska county (Eastern Region of Poland).

Material and methods. The study included 1643 children aged 7-12 from primary schools in Biala Podlaska county. The research was carried out in 2015. The questions included in the questionnaire prepared by the authors regarded the number and regularity of meals, the types of products (mainly fruit and vegetables, sweets, fizzy drinks, fast food, milk and dairy products) and the frequency of consuming them during these meals and between them. Differences between the responses to the questions in groups divided according to gender were analysed with the use of χ^2 test (G function).

Results. It was revealed that 672 girls (81.7%) and 633 boys (71.9%) had at least four meals a day, while as many as 23% of the girls and boys did not have breakfast before going to school and 33.5% of the girls and 37.4% of the boys did not take lunch to school. The main meal (dinner) was eaten every day by 652 girls (78.5%) and 605 boys (74.5%). Products most commonly eaten between the main meals were fruit and vegetables (51% of the girls and 42.4% of the boys), while sweets were consumed 2-3 times a week by 37.2% of the girls and 36.1% of the boys. The consumption of dairy products (milk, natural yoghurt and/or kefir) once a week or less frequently was declared by 53% of the girls and boys.

Conclusions. Assessment of nutritional behaviours revealed numerous mistakes which may negatively affect the development of the study participants. In the light of the above findings, it seems necessary to provide children and their parents with nutritional education in order to develop proper nutritional behaviours and correct the mistakes.

Key words: *nutritional behaviours, frequency of consumption, children*

STRESZCZENIE

Wprowadzenie. Dzieci i młodzież to grupa szczególnie podatna na skutki niewłaściwego żywienia. W okresie dojrzewania dokonuje się wiele istotnych wyborów dotyczących stylu życia, w tym określonych zachowań żywieniowych, które utrwalone mogą funkcjonować w późniejszym okresie życia i decydować o stanie zdrowia.

Cel pracy. Celem badań była ocena zachowań żywieniowych dzieci w wieku 7-12 lat ze szkół podstawowych powiatu bialskiego.

Material i metody. Badaniem objęto grupę 1643 dzieci w wieku 7-12 lat ze szkół podstawowych powiatu bialskiego. Badania zrealizowano w 2015 roku. Pytania w autorskiej ankiecie wykorzystanej w badaniach dotyczyły liczby i regularności spożywania posiłków, rodzaju i częstości spożywania produktów spożywanych podczas tych posiłków oraz między posiłkami, w tym przede wszystkim spożycia warzyw i owoców, słodczych, napojów gazowanych, produktów typu fast-food oraz mleka i produktów mlecznych. Analizę różnicowania odpowiedzi udzielonych w ankiecie w grupach wyodrębnionych na podstawie płci przeprowadzono testem χ^2 w postaci funkcji G.

Wyniki. Wykazano, że 672 dziewcząt (81,7%) i 633 chłopców (71,9%) spożywało co najmniej cztery posiłki w ciągu dnia, przy czym aż 23% dziewcząt i chłopców wychodząc do szkoły nie spożywało I śniadania, a 33,5 i 37,4% odpowiednio: dziewcząt i chłopców nie zabierało do szkoły II śniadania. Główny posiłek – obiad – spożywało codziennie 652 dziewczęta (78,5%) i 605 chłopców (74,5%). Najczęściej spożywanymi produktami pomiędzy głównymi posiłkami były owoce i warzywa; spożywało je 51% dziewcząt i 42,4% chłopców, natomiast po słodczych od dwóch do trzech razy w tygodniu

The study was conducted within the county health prevention and promotion programme titled "Together for heart" aimed at reducing cardiovascular diseases morbidity and mortality rate and financed from the resources of the Norwegian Financial Mechanism 2009-2014 and the national budget.

Corresponding author: Anna Galczak-Kondraciuk, Pracownia Żywienia Człowieka, Wydział Wychowania i Sportu w Białej Podlaskiej, Akademia Wychowania Fizycznego J. Piłsudskiego, ul. Akademicka 2, 21-500 Biała Podlaska, tel. +48 83 3428734, e-mail: anna.galczak-kondraciuk@awf-bp.edu.pl

sięgało odpowiednio 37,2% dziewcząt i 36,1% chłopców. Spożycie produktów mlecznych (mleka, jogurtu naturalnego i/ lub kefiru) raz w tygodniu lub rzadziej deklarowało 53% dziewcząt i chłopców.

Wnioski. Ocena zachowań żywieniowych wykazała liczne błędy, które mogą wpłynąć negatywnie na rozwój badanych dzieci oraz ich stan zdrowia obecnie i w przyszłości. W świetle powyższych spostrzeżeń koniecznym wydaje się prowadzenie edukacji żywieniowej wśród dzieci, ale również ich rodziców w celu kształtowania odpowiednich zachowań żywieniowych i korygowania popełnianych w tym zakresie błędów.

Słowa kluczowe: zachowania żywieniowe, częstotliwość spożycia, dzieci

INTRODUCTION

Children and youth constitute a group that is particularly prone to the effects of improper nutrition. In the puberty period, numerous significant lifestyle choices are made, for instance regarding particular nutritional behaviours which lead to many disorders and diseases at school age and, when repeated later in life, may determine the state of health.

Epidemiology studies conducted in recent years in the population of children and youth [2, 9, 12, 15, 17, 18] have pointed to numerous nutritional mistakes such as leaving home for school without breakfast, not taking lunch to school, too few meals eaten a day, irregular consumption of meals and not diversified composition of meals. Avoiding the main meals as well as their irregularity may lead to the consumption of high-calorie products with low nutritional value between these meals. The research results indicated that leaving home without breakfast and at the same time eating products with low nutritional value between meals are factors which increase the risk of overweight and obesity [18]. On the other hand, apart from health effects, not eating the main meals has consequences regarding everyday functioning, e.g. fatigue, apathy or disorders of cognitive processes, which may lead to difficulties in concentrating and worse school results [20].

The aim of the study was to assess nutritional behaviours of children aged 7-12 from primary schools in Biała Podlaska county.

MATERIAL AND METHODS

The research included 1643 children, i.e. 831 girls (50.6%) and 812 boys (49.4%), aged 7-12 from 16 primary schools from 13 towns of Biała Podlaska county (Konstantynów, Rossosz, Drelów, Janów Podlaski, Leśna Podlaska, Wisznice, Terespol, Szóstka, Dołha, Miedzyrzec Podlaski, Małaszewicze, Woskrzenice Duże, Swory) situated in the northern part of Lubelskie Province. The study was conducted in 2015 within the county health prevention and promotion programme titled "Together for heart" aimed at reducing cardiovascular diseases morbidity and mortality rate and financed from the resources of the Norwegian Financial Mechanism 2009-2014 and the national budget. A questionnaire prepared by the authors was applied in the study. The questions

included in the questionnaire concerned the frequency and number of consumed meals, types of products eaten between meals and the frequency of consuming fruit and vegetables, sweets, fizzy drinks, fast food as well as milk and dairy products (natural yoghurt, kefir, cottage cheese). Differences between the responses to the questions in groups divided according to gender were analysed with the use of χ^2 test (G function).

RESULTS

Table 1. includes data regarding the number and regularity of eating meals. The obtained data revealed that 672 girls (81.7%) and 633 boys (77.9%) had at least 4 meals a day. Three meals a day were eaten by 127 girls (15.3%) and 151 boys (18.6%), while a maximum of two meals were eaten by 32 girls (3.9%) and 28 boys (3.4%). Differences between girls and boys regarding the declared number of meals per day were statistically significant ($G=7.31$; $p\leq 0.05$). The data also revealed that 640 girls (77%) and 626 boys (77.1%) had breakfast before going to school, while the remaining 191 girls (23%) and 186 boys (22.9%) did not have breakfast at all. Similarly, lunch was taken to school every day by 553 girls (66.5%) and 508 boys (62.6%), while 278 girls (33.5%) and 304 boys (37.4%) did not take lunch to school. The main meal, i.e. dinner, was eaten every day by 652 girls (78.5%) and 605 boys (74.5%). In turn, 3.0% of the girls and 4.2% of the boys had dinner only at the weekend and no more than once a week. The declared consumption of dinner in a week differed significantly between the girls and boys ($G=4.43$; $p\leq 0.05$). Having supper every day was declared by 736 girls (88.6%) and 741 boys (91.3%). Supper was not eaten regularly by 95 girls and 71 boys, which constituted 11.4% and 8.7% of the subjects, respectively.

Table 2 demonstrates data regarding the types of products eaten between the main meals. The respondents could choose more than one answer, so the calculated percentage results do not add up to 100%. Fruit and vegetables were eaten between meals by 424 girls (51%) and 344 boys (42.4%). Sweets and ice-cream were considerably less popular, as they were eaten by 24.7-25.5% of the study participants. Crisps and salty breadsticks were eaten least frequently, i.e. by 72 girls (8.7%) and 79 boys (9.7%). Differences between the girls and boys were statistically significant ($G=4.89$; $p\leq 0.05$).

Table 1. Number and regularity of meals

Question	Categories	Girls n [#] =831		Boys n [#] =812		G function
		n ^{##}	%	n ^{##}	%	
How many meals do you have a day?	More than 5 meals	51	6.1	49	6.0	7.31*
	5 meals	324	39.9	271	33.4	
	4 meals	297	35.7	313	38.5	
	3 meals	127	15.3	151	18.6	
	Fewer than 3 meals	32	3.9	28	3.4	
Do you have breakfast before going to school?	Yes	640	77.0	626	77.1	0.00
	No	191	23.0	186	22.9	
Do you take lunch from home?	Yes	553	66.5	508	62.6	2.85
	No	278	33.5	304	37.4	
How often do you have dinner?	Every day	652	78.5	605	74.5	4.43*
	5-6 times a week	86	10.3	102	12.6	
	2-4 times a week	68	8.2	71	8.7	
	Only at the weekends	9	1.1	11	1.4	
	Once a week	16	1.9	23	2.8	
Do you have supper every day?	Yes	736	88.6	741	91.3	3.28
	No	95	11.4	71	8.7	

Note: *p≤0.05 – statistically significant differences between the number of responses provided by girls and boys (χ^2 test – G function); #number of interviews, ##number of declared responses

Table 2. Types of products consumed between the main meals

Question	Categories	Girls n [#] =831		Boys n [#] =812		G function
		n ^{##}	%	n ^{##}	%	
What products do you eat between meals?	Fruit and vegetables	424	51.0	344	42.4	4.89*
	Sweets, ice cream	205	24.7	207	25.5	
	Crisps, salty breadsticks	72	8.7	79	9.7	

Note: *p≤0.05 – statistically significant differences between the number of responses provided by girls and boys (χ^2 test – G function); #number of interviews, ##number of declared responses; respondents were allowed to choose more than one answer or not to choose any answer at all, so the percentage results do not add up to 100%

Data concerning the frequency of consuming sweets, fizzy drinks and fast food are presented in Table 3. It was revealed that most respondents had sweets 2-3 times a week. It was declared by 309 girls (37.2%) and 293 boys (36.1%). Every day sweets were consumed by 19.4% of the girls and 17% of the boys, while once a week or less frequently they were eaten by 15.5% of the girls and 18.3% of the boys. Sweet fizzy drinks were consumed more than 4 times a week by 426 girls (51.3%) and 408 boys (50.3%). In turn, 184 girls (22.1%) and 193 boys (23.8%) had sweet fizzy drinks once a week or less frequently. The consumption of fast food once a week or less frequently was declared by 557 girls (67%) and 502 boys (61.8%). However, 139 girls (16.7%) and 138 boys (17%) had it at least four times a week, while 135 girls (16.2%) and 172 boys (21.2%) consumed fast food products

2-3 times a week. The declared consumption of fast food differed significantly between the girls and boys ($G=7.14$; $p\leq 0.05$).

Table 4 illustrates data on the frequency of consuming fruit and vegetables. According to the data, 162 girls (19.5%) and 120 boys (14.8%) had vegetables every day. The consumption of vegetables no more than once a week was declared by 199 girls (23.9%) and 235 boys (28.9%). Differences between the girls and boys regarding the consumption of vegetables were statistically significant ($G=9.25$; $p\leq 0.05$). Everyday consumption of fruit was declared by 290 girls (34.9%) and 225 boys (27.7%). In turn, 73 girls (8.8%) and 108 boys (13.3%) had fruit no more than once a week. Differences between the girls and boys concerning the consumption of fruit were statistically significant ($G=14.95$; $p\leq 0.05$).

Table 3. Frequency of consuming sweets, fizzy drinks and fast food

Question	Categories	Girls n [#] =831		Boys n [#] =812		G function
		n ^{##}	%	n ^{##}	%	
How often do you eat sweets (chocolate, cakes, candies)?	Every day	161	19.4	138	17.0	3.42
	4-6 times a week	232	27.9	232	28.6	
	2-3 times a week	309	37.2	293	36.1	
	Once a week or less frequently	129	15.5	149	18.3	
How often do you have sweet fizzy drinks?	Every day	215	25.9	206	25.4	0.62
	4-6 times a week	211	25.4	202	24.9	
	2-3 times a week	221	26.6	211	25.9	
	Once a week or less frequently	184	22.1	193	23.8	
How often do you have fast food?	Every day	50	6.0	51	6.3	7.14*
	4-6 times a week	89	10.7	87	10.7	
	2-3 times a week	135	16.2	172	21.2	
	Once a week or less frequently	557	67.0	502	61.8	

Note: *p≤0.05 – statistically significant differences between the number of responses provided by girls and boys (χ^2 test – G function); #number of interviews, ##number of declared responses

Table 4. Frequency of consuming fruit and vegetables

Question	Categories	Girls n [#] =831		Boys n [#] =812		G function
		n ^{##}	%	n ^{##}	%	
How often do you eat vegetables?	Every day	162	19.5	120	14.8	9.25*
	4-6 times a week	219	26.4	211	26.0	
	2-3 times a week	251	30.2	246	30.3	
	Once a week or less frequently	199	23.9	235	28.9	
How often do you eat fruit?	Every day	290	34.9	225	27.7	14.95*
	4-6 times a week	278	33.5	285	35.1	
	2-3 times a week	190	22.9	194	23.9	
	Once a week or less frequently	73	8.8	108	13.3	

Note: *p≤0.05 – statistically significant differences between the number of responses provided by girls and boys (χ^2 test – G function); #number of interviews, ##number of declared responses

Table 5 presents data regarding the frequency of consuming milk, natural yoghurt, kefir and cottage cheese. According to the data, everyday consumption of milk, natural yoghurt and kefir was declared only by 78 girls (9.4%) and 71 boys (8.7%), while 444 girls (53.4%) and 436 boys (53.7%) had these products once a week or less frequently. The consumption of cottage cheese at least six times a week was declared by 25 girls (3%) and 27 boys (3.3%). In turn, 415

girls (49.9%) and 422 boys (52%) had cottage cheese once a week or less frequently. The consumption of Swiss cheese 2-3 times a week was declared by 333 girls (40.1%) and 307 boys (37.8), while only 74 girls (8.9%) and 80 boys (9.9%) had it every day. No statistically significant differences between the girls and boys regarding the consumption of milk and dairy products were revealed.

Table 5. Frequency of consuming milk and dairy products

Question	Categories	Girls n [#] =831		Boys n [#] =812		G function
		n ^{##}	%	n ^{##}	%	
How often do you have milk, natural yoghurt and/or kefir?	Every day	78	9.4	71	8.7	1.17
	4-6 times a week	91	11.0	79	9.7	
	2-3 times a week	218	26.2	226	27.8	
	Once a week or less frequently	444	53.4	436	53.7	
How often do you have cottage cheese?	Every day	25	3.0	27	3.3	1.72
	4-6 times a week	109	13.1	91	11.2	
	2-3 times a week	282	33.9	272	33.5	
	Once a week or less frequently	415	49.9	422	52.0	
How often do you have Swiss cheese?	Every day	74	8.9	80	9.9	1.13
	4-6 times a week	213	25.6	217	26.7	
	2-3 times a week	333	40.1	307	37.8	
	Once a week or less frequently	211	25.4	208	25.6	

Note: *p≤0.05 – statistically significant differences between the number of responses provided by girls and boys (χ^2 test – G function); #number of interviews, ##number of declared responses

DISCUSSION

Rational diet allows for making full use of genetically determined potential of physical and mental development. The first years of life are a period of many changes in a child's nutrition. These changes are closely related to the dynamic development of children and their achievement of subsequent development stages [1]. Thus, it is crucial to meet nutrition needs, not only in terms of quantity but also quality, i.e. the number and regularity of meals a day and the frequency of consuming certain products.

The analysis of the collected material regarding the number and regularity of meals produced similar results to previous studies [3, 4]. The rule of having 4-5 meals a day was not followed in the same manner by the study participants (Table 1). Although the majority of the respondents had four or more meals a day, it was noted that girls had them more regularly than boys (81.7% vs. 77.9%, respectively). Moreover, it was revealed that gender was an important differentiating factor in terms of the number of meals consumed. A significant value of G function ($G=7.31$; $p<0.05$) was determined mainly by the differences between the girls and boys regarding the consumption of three (15.3% vs. 18.6%) and five (39.9% vs. 33.4%) meals a day. The study also revealed that 77% of the girls and boys went to school without breakfast, while 33.5% of the girls and 37.4% of the boys did not take lunch to school (Table 1). These findings are alarming due to the risk of an insufficient amount of nutrients in a daily

diet of these individuals. A similar pattern of nutrition was noted among lower-secondary school students from Szczecin [6], primary school pupils and lower-secondary school students from Białostockie region [15], primary school pupils from Kalisz county [18] and 6-year pupils from several primary schools from Warsaw and Zamosc [5].

Eating between meals was a common phenomenon among primary school pupils from Biała Podlaska county. Despite the fact that fruit and vegetables were the most commonly chosen products (declared by 51% of the girls and 42.4% of the boys), sweets, ice cream, crisps and salty breadsticks were also popular. Such products were included in a daily menu of 33.4% of the girls and 35.2% of the boys (Table 2). Eating between meals (in particular highly processed food such as fast food or sweet fizzy drinks), which is becoming a serious nutritional problem, has been observed in Poland in the last few years [13, 16]. Our research revealed that 37.2% of the girls and 36.1% of the boys had sweets 2-3 times a week, and a further 47.3% of the girls and 45.6% of the boys consumed them 4-6 times a week (Table 3). The consumption of sweet fizzy drinks and fast food was equally high. Sweet fizzy drinks were consumed more than 4 times a week by 51.3% of the girls and boys, while fast food was eaten at least once a week by 67% of the girls and 61.8% of the boys. However, 6% of the girls and 6.3% of the boys had it every day. The research conducted by Jeżewska-Zychowicz [8] among slightly older youth revealed that 4-12% of 13-15-year-olds had hamburgers, hot-dogs or chips at least once a day, and

40-60% of these individuals had sweet fizzy drinks. In turn, *Kośmider* and *Gronowska-Senger* [11] revealed that, depending on the place of living, the consumption of fast food 1-2 times a week was declared by between 40 and over 80% of the youth up to 14 years of age.

Products which should be consumed by children and youth every day include fruit and vegetables. They facilitate maintaining proper body mass and make it possible to supplement potential deficits of vitamins, minerals and fibre [19]. The research indicated that as many as 23.9% of the girls and 28.9% of the boys had vegetables once a week or less frequently (Table 4). However, fruit was consumed every day more often by girls (19.5%) than boys (14.8%).

In turn, *Stefańska* et al. [15] noted that the recommended frequency of consuming raw vegetables was declared by 15% of the girls and 12% of the boys aged 10-12 and 13% of the girls and 7% of the boys aged 13-15. Another significant observation, made only in a younger age group, was a significantly lower consumption of raw vegetables by boys than girls.

Fruit consumption among children was much higher (Table 4). More girls than boys had fruit every day (34.9% vs. 27.7%). Its consumption once a week or less frequently was reported by 13.3% of the boys and 8.8% of the girls. These findings are in line with the results of the study by *Wojtyła-Buciora* et al. [18], who revealed that 10% of the examined primary school pupils declared having fruit once a week or less frequently. Different results were revealed by *Głębocka* and *Kęska* [5] in their research on children from Warsaw and Zamosc. The consumption of fruit by boys from Warsaw did not differ significantly from the consumption of fruit by girls from this city. However, male pupils from Zamosc declared much higher consumption of fruit than their female counterparts (41% vs. 26%, $p < 0.05$).

Intensive growth of a young body requires everyday consumption of milk or dairy products due to their high nutritional value and digestibility and because of the fact that they include easily absorbed nutrients [14]. Children from Biala Podlaska county consumed milk, natural yoghurt and/or kefir less frequently than recommended [19]. The consumption of these products once a week or less frequently (Table 5) was declared by 53.4% of the girls and 53.7% of the boys, which, combined with the fact that only 9.4% of the girls and 8.7% of the boys had them every day, may lead to such diseases as osteoporosis, arterial hypertension or coronary artery disease [14]. Equally alarming observations were made by *Stefańska* et al. [15], who revealed that 19.6% of the girls and 20.5% of the boys from primary schools in Bialystok had milk less frequently than 2-3 times a week. In turn, *Wojtyła-Buciora* et al. [18] found out that milk and dairy products were consumed no more than once a week by 10% of the 5th- and 6th-year primary school pupils from Kalisz town and county.

A negative trend was also noted regarding the frequency of consuming cottage cheese and Swiss cheese (Table 5). The highest percentage of children (49.9% of the girls and 52.0% of the boys) had cottage cheese once a week or less frequently. Swiss cheese was consumed 2-3 times a week by 40.1% of the girls and 37.8% of the boys. Slightly different results were obtained by *Jeżewska-Zychowicz* and *Łyszkowska* [7] and *Kollajtis-Dolowy* et al. [10], who revealed that milk, milk drinks and cottage cheese were consumed every day by more than a half of the respondents. Similar results were obtained in the study on children and youth from primary and lower-secondary schools in Bialystok by *Stefańska* et al. [15], who revealed that most respondents had Swiss cheese 2-3 times a week and differences between younger and older girls were statistically significant. A low consumption of milk and dairy products noted in our study is a particularly unfavourable phenomenon due to a low supply of e.g. calcium, as the aforementioned products are a significant source of this element. Such a diet may result in problems connected with improper bone build. As research revealed, reaching peak bone mass within the first 20 years of life constitutes a significant factor that reduces the risk of osteoporosis in the future [20].

CONCLUSIONS

1. The research revealed that, regardless of gender, children consumed certain meals irregularly, i.e. they left home without breakfast, did not take lunch to school or did not have supper.
2. A considerable percentage of children had sweets, fizzy drinks and fast food every day, while a relatively low percentage of children had vegetables, fruit, milk, natural yoghurt and/or kefir every day.
3. The development of bad nutritional habits may lead to their continuation in adult life and may exert a negative influence on the proper development as well as current and future health state.
4. In the light of the aforementioned conclusions, it seems necessary to provide children and their parents with nutritional education in order to develop pro-health nutritional behaviours.

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Conflict of interest

The authors declare no conflict of interest.

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COMPARISON OF DIET AND PHYSICAL ACTIVITY OF CHILDREN AND ADOLESCENTS WITH PATIENTS AFTER CANCER TREATMENT

Joanna Zapolska¹, Katarzyna Witczak-Sawczuk¹, Maryna Krawczuk-Rybak²,
Małgorzata Sawicka-Żukowska², Joanna Smarkusz¹, Lucyna Ostrowska¹

¹Department of Dietetics and Clinical Nutrition, Faculty of Health Sciences,
Medical University of Białystok, Poland

²Department of Pediatric Oncology and Hematology, Faculty of Health Sciences,
Medical University of Białystok, Poland

ABSTRACT

Background. Properly balanced diet and exercise are an essential element of healthy living for children and adolescents. Particular attention should be paid to nutrition and physical activity among juniors after cancer treatment, which is one of the most important elements of the convalescence period.

Objective. The aim of the study was a comparative analysis of diet, physical activity of healthy children and adolescents with patients after cancer treatment.

Material and methods. The study involved 60 children and adolescents; 30 healthy juniors and 30 patients after treatment for cancer. An analysis of diets based on a 3-day 24-hour nutrition diary. The questionnaire surveyed collected data about participation and physical activity preferences. Statistical program-Statistica 12.0, published by StatSoft, was used to develop the results.

Results. Both groups were characterized by increased consumption of proteins and carbohydrates. Insufficient fat intake was shown in comparison with the recommended amounts in all study groups. It was observed that in the group of patients after treatment, vitamins B1, A, E and D intake was higher than in their healthy peers. Determinants of the choice of physical activity among children after cancer treatment was pleasure and fun, while among their healthy peers, aesthetic considerations (taking care of appearance).

Conclusions. Children and adolescents after cancer treatment in a much greater percentage covered of daily intake of nutrients than healthy children, and more willingly spent time on physical activity. Greater interest in physical activity in this group was probably due to previous restrictions related to illness and therapy.

Keywords: children, diet, physical activity, cancer

STRESZCZENIE

Wprowadzenie. Odpowiednio zbilansowana dieta i ćwiczenia fizyczne są niezbędnym elementem zdrowego życia dzieci i młodzieży. Szczególną uwagę należy zwrócić na odżywianie i aktywność fizyczną wśród młodych po leczeniu raka, co jest jednym z najważniejszych elementów okresu rekonwalescencji.

Cel. Celem pracy była analiza porównawcza diety, aktywności fizycznej zdrowych dzieci i młodzieży z pacjentami po leczeniu nowotworu.

Materiał i metody. W badaniu wzięło udział 60 dzieci i młodzieży; 30 zdrowych juniorów i 30 pacjentów po leczeniu raka. Analizę diet oparto na 3-dniowym 24-godzinny dzienniku żywieniowym. Ankieta zgromadziła zebrane dane dotyczące preferencji uczestnictwa i aktywności fizycznej. Program statystyczny Statistica 12.0, opublikowany przez StatSoft, został wykorzystany do opracowania wyników.

Wyniki. Obie grupy badanych charakteryzowały się zwiększonym spożyciem białka i węglowodanów. Niewystarczające spożycie tłuszczu wykazano w porównaniu z zalecanymi ilościami we wszystkich grupach badanych. Zaobserwowano, że w grupie pacjentów po leczeniu raka spożycie witaminy B1, A, E i D było wyższe niż u zdrowych rówieśników. Determinantami wyboru aktywności fizycznej wśród dzieci po leczeniu nowotworowym była przyjemność i zabawa, a wśród zdrowych rówieśników - względy estetyczne (dbanie o wygląd).

Wnioski. Dzieci i młodzież po leczeniu nowotworowym w znacznie większym odsetku pokrywały dzienne spożycie składników odżywczych niż zdrowe dzieci, a chętniej spędzali czas na aktywności fizycznej. Większe zainteresowanie aktywnością fizyczną w tej grupie prawdopodobnie wynikało z wcześniejszych ograniczeń związanych z chorobą i terapią.

Słowa kluczowe: dzieci, dieta, aktywność fizyczna, rak

Corresponding author: Katarzyna Witczak-Sawczuk, Department of Dietetics and Clinical Nutrition, Faculty of Health Sciences, Medical University of Białystok, Mieszka I street 4B, 15-054 Białystok, Poland
tel./fax: +48 85 732 82 44, e-mail: katarzyna.witczak@umb.edu.pl

INTRODUCTION

The period of growth and maturation is the time in which eating habits and motivational preferences are formed. Properly balanced diet and exercise are an essential element of healthy living for children and adolescents. Improper diet and too little physical activity pose a serious health risk, increasing the risk of overweight, obesity and related diseases: type 2 diabetes, hypertension, cardiovascular disease [30].

Particular attention should be paid to the way of eating and physical activity among juniors after cancer treatment, which is one of the most important elements of the convalescence period. Cancer is a destructive disease which destroys the entire organism, and treatments such as radiotherapy, chemotherapy and surgery further damage organs and tissues, thereby causing significant damage to the heart, lungs, skeletal muscles and resulting in chronic fatigue [9].

Properly balanced diet and exercise are designed to improve cardiovascular performance, improve muscle strength and flexibility, and improve physical fitness and well-being. All of the aforementioned factors are conducive to the shortening of convalescence time, reduce the incidence of complications after oncological treatment and, above all, affect the psychosocial wellbeing of children [8, 9].

The aim of the study was a comparative analysis of diet, physical activity of healthy children and adolescents with patients after cancer treatment.

MATERIAL AND METHODS

The study was conducted among 60 children and adolescents - 30 patients after completed cancer treatment from Oncology and Hematology Clinic

in the Medical University of Białystok Children's Clinical Hospital (group I: 13 girls and 17 boys) and 30 healthy juniors who started exercise in a fitness club (control group - group II: 16 girls and 14 boys).

Nutritional analysis was performed using a 3-day 24-hour nutrition diary. The information obtained was developed using the computer program DIETA 5 (IZŻ). The questionnaire surveyed collected information about participation and physical activity preferences.

The results are summarized as average, median, range, and standard deviation. In this study we also calculated the percentage of protein, fat, and selected vitamins and minerals, according to the recommended intake (RDA), the amount corresponding to the coverage of the nutrient requirement [11].

Statistical program Statistica 12.0 was used to develop the results. Descriptive statistics have been assigned: median, quartiles, average, deviation (SD), range (minimum, maximum). The normality of the distribution was checked by the *Shapiro-Wilk* test. In the comparison of the studied groups, in the absence of normal distribution, the *Mann-Whitney U* test was used, while for the normal distribution *t-Student*. The *Chi²* test was used to compare the nominal characteristics. Statistically significant values was shown with $p < 0.05$.

RESULTS

The study involved 60 people (children and adolescents). The study group consisted of 13 girls (21.7%) and 17 boys after cancer treatment (28.3%) and 16 healthy girls (26.7%) and 14 boys (23.3%). Table 1 classifies the study group in terms of age and disease incidence.

Table 1. Division of study and control group on age and onset of disease

Respondents	Girls after illness n = 13	Healthy girls n=16	Boys after illness n=17	Healthy boys n=14
Me (Q ₁ , Q ₃)	13 (10-17)	14 (13.5-16)	16 (12-17)	14.5 (14-16)
Range	9-19	13-16	7-22	12-18

Among the surveyed groups most frequently consumed meals were: first breakfast, lunch and supper. The whole group declared eating breakfast. It was slightly less frequent to eat a second breakfast for young people after illness - 73.3%, compared to healthy children - 76.7%. Afternoon tea was more commonly consumed by children after oncological treatment - 76.7% than healthy children - 40%. Dinner was consumed by 100% of people after cancer treatment, while their healthy peers - 93.3%.

The daily nutritional rations of the studied youth were analyzed in terms of energy content, protein

content, fats and carbohydrates. The average of consumption and percentage of implementation of selected minerals and vitamins was calculated.

The mean energy value of children's diet after cancer was 2186 ± 696.1 kcal, while healthy youth provided an average of 1897.4 ± 997 kcal per day (Table 2). Differences between these values were statistically significant. There was also a statistically significant difference ($p = 0.042$) in average of the greater daily protein supply among children and adolescents (94 ± 46.5 grams) of patients after cancer treatment compared to healthy children who consumed $75\% 8 \pm 34.2$ g.

Table 2. Nutritional value of daily ration in both studied groups

Values	Children and adolescents after illness (group I)			Healthy children and adolescents (group II)			p
	Average±SD	Me	Range	Average ±SD	Me	Range	
Energy (kcal)	2186±696.1	2107.7	946-3611.8	1897.4±997	1584.2	734.2-4910	0.05
Protein (g)	94±46.5	81.6	40-258.5	75.8±34.2	66.8	36.5-195,0	0.04
Fat (g)	74±30.3	73.1	30-136.5	63.3±39.9	52.8	19.7-215,1	0.07
Carbohydrates (g)	305±101.5	298	112.8-512.2	274.6±160.2	222.8	93.3-669.0	0.06

There was also a statistically significant difference ($p = 0.042$) in the greater daily protein supply among children and adolescents after cancer, which was 94 ± 46.5 g on average, compared with healthy children who consumed 75.8 ± 34.2 g. The average daily intake of fats in group I was 74 ± 30.3 g while in group II 63.3 ± 39.9 g ($p=0,07$). The consumption of carbohydrates

were 305 ± 101.5 g in group I and in the second group- 274.6 ± 160.2 g.

The results for protein, fat and carbohydrate intake have been extended by comparing them with the recommended daily RDAs intake as a percentage of standard implementation (Table 3).

Table 3. Percentage of recommended daily intake of RDAs of essential nutrients in both study groups

Values	Children and adolescents after illness (group I)	Healthy children and adolescents (group II)	
	Average ±SD	Average ±SD	p
Protein (%)	172.5±81.7	130.7±58.7	0.006
Fat (%)	83.1±33.2	64±33.5	0.015
Assimilable carbohydrates(%)	218.7±74.2	195.2±119.3	0.043

The average of protein intake by group I was 172.5% and in the second group 130,7%. There was a statistically significant difference between this values ($p=0,006$). The coverage percentage of the recommended daily fat intake was insufficient in both groups. Consumption of carbohydrates assayed in the I study group was 218,7% and in II study group was 195.2% of the recommended daily intake. Differences in the dietary content of the individual components were significantly higher in group I.

Saturated fatty acid intake (SFA), mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were analyzed. In addition, the average intake of dietary cholesterol were studied. Children in I group consumed greater amounts of monounsaturated, polyunsaturated and saturated fatty acids and dietary cholesterol, but significant differences were only related to the amount of monounsaturated fatty acids consumed in group I. Average intake of MUFA in group I were 30.3 g whereas group II only 24 g ($p=0,037$). The data were presented in Table 4.

Table 4. Consumption of fatty acids and dietary cholesterol in both studied groups

Fatty acids	Children and adolescents after illness (group I)			Healthy children and adolescents (group II)			p
	Average ±SD	Me	Range	Average±SD	Me	Range	
SFA (g)	28.9±12	28.3	9.7-55.1	26±21.3	19	7.2-118.7	0.105
MUFA (g)	30.3±14.2	27.8	10.7-60.6	24±15.1	20	5.7-69.2	0.037
PUFA (g)	9.4±4.5	8.6	3.5-19	8.4±4.5	8	2.7-17.2	0.328
Dietary cholesterol (mg)	366.5±337.8	245.2	84-1401	223.4±123.7	207.2	80-663.5	0.109

The intake of selected vitamins and minerals was assessed and RDA analysis was performed in both groups.

Group I in their daily ration provided higher amounts of vitamin E - average 9.4 mg (Me = 8.9 mg)

and vitamin D - 2.6 mg (Me = 1.5 µg), compared to group II: vitamin E - 9.2 mg (Me = 7.8 mg), vitamin D - 1.7µg (Me = 1.2 µg). Minor differences can be seen in the intake of vitamin A and C in both groups (Table 5).

Table 5. Intake of selected vitamins in both studied groups

Vitamins	Children and adolescents after illness (group I)			Healthy children and adolescents (group II)			p
	Average ±SD	Me	Range	Average ±SD	Me	Range	
Vitamin B1 (mg)	1.8± 0.9	1.6	0.4-3.8	1.4± 0.9	1.1	0.4-4	0.029
Vitamin A (µg)	1085.6± 890	811	122.6-3867.3	1012.3± 653.3	886	114.6-2587	0.102
Beta-carotene (µg)	3656± 4036.7	2077.6	135.8-20203	4310.6± 3882	3624.8	66.5-14162	0.431
Vitamin E (mg)	9.4± 4.9	8.9	1.6-24	9.2± 5.6	7.8	2-26	0.622
Vitamin C (mg)	125.4± 103.7	100.2	3.6-391.3	132±108.4	91	2.6-414.5	0.854
Vitamin D (µg)	2.6± 3	1.5	0.36-15	1.7± 1.4	1.2	0.07-6.9	0.35

The statistically significant differences were only in the higher supply of vitamin B1 (p = 0.029) in children and adolescents following oncological treatment (on

average 1.8 mg vitamin B1 (Me = 1.6 mg), compared to their healthy peers of 1.4 mg = 1.1 mg).

The daily intake of selected minerals was assessed and the results are shown in Table 6.

Table 6. Evaluation of intake of selected minerals in both studied groups

Minerals	Children and adolescents after illness (group I)			Healthy children and adolescents (group II)			p
	Average ±SD	Me	Range	Average ±SD	Me	Range	
Magnesium (mg)	330±170	290.5	135-1051	283.9±116	287.6	81.7-551	0.241
Iron (mg)	12.6±5	11	5.3-24.6	10.1±4.4	9	2.5-21	0.065
Zinc (mg)	11.7±5.7	10	5.9-32.6	9.6±4.7	8.3	4-26.2	0.051
Copper (mg)	1.3±0.5	1.2	0.6-2.52	1.1±0.5	1.1	0.3-2.2	0.905

Children and adolescents after the oncological treatment consumed higher amounts of the analyzed minerals but the statistically significant differences were only in higher zinc intake (group I – 10 mg vs. group II - 8.3 mg). Near statistical significance was the higher iron intake (group I - 11 mg vs group II - 9 mg). The above data was compared to the recommended daily RDA (Figure 1).

The coverage of the RDA for selected minerals has shown a statistically significant difference between children and adolescents of group I and healthy juniors (group II).

Differences in the percentage of recommended intake of minerals are noticeable in the group of girls after treatment who consumed larger amounts of tested ingredients than their healthy peers. Girls after treatment covered on average the recommended copper intake of 147%, zinc 116%, iron 77% and magnesium

95%. Meanwhile, their healthy peers in 106% copper, zinc 84%, iron only 56% and magnesium 67%.

Similar differences in group of boys were observed. Boys after cancer treatment covered the average the recommended intake of copper in 157%, zinc 133%, iron 123%, and magnesium 104%. Meanwhile, healthy boys covered the recommended value of minerals in 153% (copper), 108% (zinc), 103% (iron) and 81% (magnesium).

Evaluation of participation in physical education classes, there was a statistically significant relationship between the youth of both groups.

It has been observed that nearly one third of children and adolescents after treatment have attended 1-2 times a week (33.4%) to additional sports activities, slightly fewer 3-4 times a week (30%). 20% participants of group I participated in physical exercise 5 and more times per week, while 16.6%

of children and adolescents in group I declared that they did not participate in additional physical activity. Similar frequency of physical activity occurred in group II, in which 40% of participants participated in classes 1-2 times a week, 26.6% 3-4 times a week, 13.4% exercised 5 or more times a week, 20% did not participate in them at all.

The most commonly chosen physical activity in both groups was cycling, with 66.7% among healthy children, and 80% after cancer. Children and adolescents after oncological treatment are the most frequent cause of physical activity mentioned pleasure and fun (43%), while their healthy peers (43%) taking care of appearance.

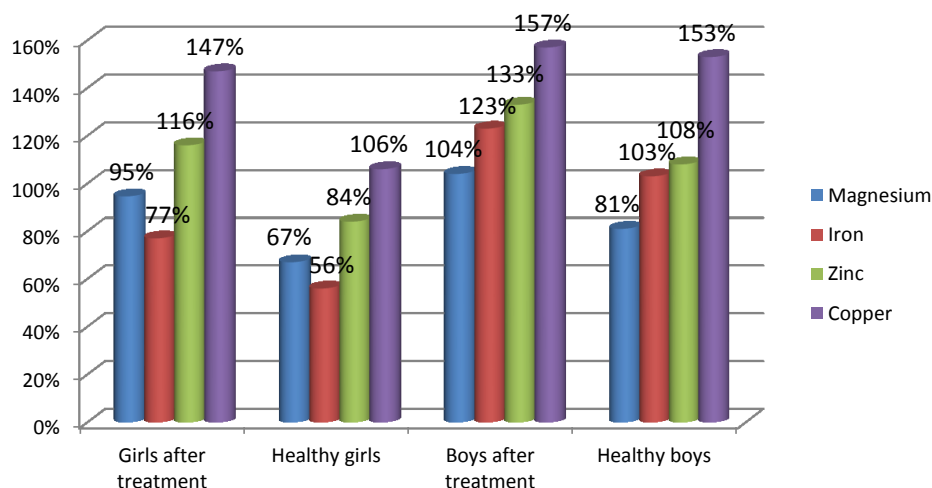


Figure 1. Percentage of recommended daily intake (RDA) of dietary minerals in study groups

DISCUSSION

Nutrition and physical activity among juniors after cancer treatment are one of the most important parts of the convalescence phase, after a long and exhausting treatment. However, there are not many publications about nutrition children with cancer, and after treatment, and to compare the results with a group of healthy children [9, 17].

A great emphasis is placed on the correct balance of the nutritional ration of patients treated in hematology and oncology clinics [22, 24]. At the time of diagnosis of cancer, it is important to remember that malnutrition is among the most significant and common complications. The diet should be individually selected, have a balanced composition, suitable for the patient and his condition [7].

In our study was no difference in the frequency of consumption first breakfast and lunch in the group of children after cancer treatment and healthy children. Little difference was observed in the rate of consumption of II breakfast (73% of children and adolescents after illness and in 76% of healthy children and adolescents). The opposite case was in the frequency of supper and afternoon tea consumption, where it was reported that the higher proportion of children in group I had consumed these meals.

Zimna-Walendzik et al. reported a healthy group of 100 children, of whom only 70% declared daily first breakfast. It was observed that similar as in own study 100% of respondents declared daily intake of dinner [30].

In studies conducted in a group of healthy Gdansk junior high school students, it was noted that the most commonly eaten meals were dinner (by 97.4%) and supper (by 93%). Moreover, the lower number of junior high school students, 83.5%, consumed the first breakfast. Gdansk's youth most often consumed afternoon tea - about 30% [28].

In our own studies it was observed that the average energy intake in the first group of children and adolescents after cancer was 2186 kcal, while among their healthy peers it was 1897 kcal. Differences were close to statistical significance. Similar results were obtained in a Jansen et al. study in group of 16 patients with acute lymphoblastic leukemia and 17 healthy children in control group. It was observed that the daily energy intake in the study group was on average 2125 kcal, while in the control group it was 1775 kcal. Both studies have confirmed the greater supply of energy in children with cancer history [10].

Analyzes of own studies showed significant differences in daily protein intake of children after oncological treatment (average 94 g/day) compared to healthy young (average 75.8 g/day). The protein supply in both groups was significantly higher than the reference values at the RDA level. Similar results in a study conducted in the group of healthy children and adolescents aged 10-12 were received by Falkowska et al. [5] in which excessive protein intake in relation to norms was observed. The average coverage of protein requirements in the group of girls was 182%

of the RDA, and 202% of the RDA in boys group. At the same time, it was noted that the study group consumed higher amounts of animal protein than plant. Similar results were obtained by *Kulińska-Szukalska et al.* [13] analyzing the daily nutrition ration of overweight children and adolescents. *Wolańska et al.* [25] in the study of healthy children aged 13-15 observed excessive protein intake, which exceeded the recommended intake of 35%. The amount of protein in the daily ration of the population of the developmental age should not exceed the recommended value. Increase in the consumption of this ingredient increases the ability to metabolize carcinogens, which may be a contributing factor to carcinogenicity [1, 18].

In our study oversupply carbohydrate intake was also observed in both groups. Children and adolescents after treatment were delivered average 305 grams carbohydrates per day, which covered 219% daily demand. Approximate results were obtained in group of healthy children (195.2% RDA and average intake was 274.6 g / day). Different results have been observed in a study of 617 healthy children and adolescents with normal body weight who were consumed less carbohydrates than recommended [5]. Also, *Kulińska-Szukalska et al.* [13] in the analyzes conducted in more than 75% of the respondents showed low intake of this ingredient among healthy school children. Similar results were also obtained in the study from 2011 [23].

In our own study, insufficient fat intake was observed in both groups. Children and adolescents after illness consumed 74 g/d of fat, which covered the RDA standard in 83.1%, while healthy children- 63.3 g/d (64% of the RDA). *Wolańska et al.* [25] observed lower fat intake in healthy children and adolescents, which in 80% covered the daily requirement for this ingredient. Similar results were obtained in works on similar topic [13, 29].

Own studies also evaluated the intake of selected vitamins and minerals in daily rations of the studied group of children and adolescents. The above-mentioned ingredients are responsible for the proper functioning of the immune system on many of its surfaces, providing adequate lymphocyte and phagocytic activity [12]. In our own study it was observed that the consumption of thiamine was significantly different in the studied groups. Children and adolescents after treatment consumed higher amounts of this vitamin (average 1.8 mg/day) compared to their healthy peers (1.4 mg/day). Similar results were obtained by *Lizoń et al.* In a group of 52 healthy children and adolescents thiamine intake was higher than the RDA. In the autumn and winter it was 1.5 mg/day and spring/summer 1.6 mg/day [15]. The consumption of vitamin B1, above the recommended standards was also demonstrated by *Błaszczuk et al.* [3] among children aged 10-13 years from Lodz.

In the own study, there were slight differences in average daily vitamin A intake. Children and adolescents in Group I consumed similar, slightly higher amounts of this vitamin (1085.6 µg/day), compared to group II (1012 µg / day). According to the Institute of Food and Nutrition RDA for vitamin A for the children in similar age as in study group is 700-900 µg/day [11]. *Błaszczuk et al.* [3] reported excessive vitamin A intake among healthy children and adolescents on average by more than 200% of the norm. In different study conducted by *Bączny et al.* [2], a slight retinol overdose was observed in children and adolescents from the Wielkopolska Region. The average daily vitamin A intake of the group boys was 982 µg/day and 920 µg/day for girls. *Goluch-Koniuszy et al.*, in their studies, observed that boys covered the demand for this vitamin in 106%, while girls were only 79% of the standard [6].

In our study children and adolescents of healthy children group provided 9,2 mg/day of vitamin E, while after treatment - 9,4 mg/day. *Bączny et al.* in their studies reported vitamin E deficiency among healthy children aged 10-12 years [2]. In addition, *Goluch-Koniuszy et al.* reported that in the during adolescence group, can be observed insufficient coverage of vitamin E (only 68% of RDAs), whereas in group of boys of the same age 102% of RDA [6]. Similar results were also obtained in work from 2012 [27].

Vitamin C intake among children and adolescents in Group I averaged 125.4 mg/day, while in healthy group 132 mg/day. *Bączny et al.* observed vitamin C deficiencies in their analyzes in the group of children aged 10-12, which in only 70% covered the RDA [2]. The deficiency of this vitamin is observed especially in middle school age [19].

When analyzing own research, there was insufficient supply of vitamin D by both groups. Children and adolescents from group I provided on average 2.6 µg/day this vitamin, while their healthy peers only 1.7 µg/day. *Błaszczuk et al.* reported that the daily intake of cholecalciferol in the study group was similar (93% of RDA) [6]. Deficiency of vitamin D and calcium is very common among children in this age group [4].

In analyzing our own studies significant differences in the percentage of recommended daily intake of magnesium were observed and the incidence of cancer in the past. Young people after the illness provided 100% of the recommended magnesium intake (330 mg/day), while their healthy peers were 73.6% of the RDA (283.9 mg/day). *Szczepanska et al.* also demonstrated lower magnesium intake by girls attending gymnasium [20]. Also, *Błaszczuk et al.* emphasize that this ingredient is poor in more than half of the children surveyed [3]. Magnesium deficiency is a common phenomenon in children and adolescents,

and low levels in the body result in increased free radical production [11].

Our own research has shown that the coverage of iron requirements among healthy children was 77.6% of the RDA (average 10.1 mg/day), while their peers after cancer treatment covered the RDA in 102% (12.6 mg/day). *Błaszczyk* et al. also reported insufficient intake of this ingredient, with particular attention being paid to the group of girls, whose coverage of this microelement was 71% of the RDA [3].

In our own study, there was a significant difference in the covering the RDA range for zinc among children and adolescents after cancer treatment and their healthy peers (124.4% RDA and 11.7 mg per day), whereas in healthy children and adolescents, at 95% RDA level (9.36 mg per day). *Goluch-Koniuszy* et al., in studies on healthy children and adolescents, noted significant deviations from the recommended daily intake of this element [6].

Copper excess was observed in daily ration, both children and adolescents after cancer, as well as their healthy peers. Healthy children covered the need for this microelement in 128% (1.1 mg per day), and the group of their peers after oncological treatment in 152% (1.3 mg per day). In *Goluch-Koniuszy* et al. [6] study, diets were characterized by copper deficiency; girls delivered 60% of the recommended standard, while boys were 71%. Both deficiency and excess copper in the diet is detrimental to the health of the young organism, excess can damage lipid DNA and proteins, and also result in metabolic changes in the organs [11].

Children who have been diagnosed with a disease are less likely to participate in physical activity and therefore, they are less likely to spend time actively. Children and adolescents commitment to physical exercise are characterized by improved well-being, slower fatigue, and less muscle atrophy due to prolonged illness [14].

In our own studies it was noted that the greater proportion of children and young people after treatment abandon their physical activity (13%) compared to their healthy peers (0%). Similar results were obtained by *Zimna-Walendzik* et al. examining healthy children and adolescents completing elementary school. It has been observed that young people are systematically attending physical education classes (80% of the respondents), and if they are absent from school, the cause is illness. The disturbing result is that only 38% of the respondents take extra physical activity after school activities [30].

People who suffer from cancer often fail despite the lack of opportunities and many inconveniences to participate in physical activities. This fact is confirmed by the study of *Malicka* et al., in which the interest

in physical activity of young people after cancer was examined. In the group of participants of Lower Silesian OnkoOlympics, there was a great interest in the physical activity of the participants, among which the disciplines such as table tennis and swimming were particularly popular.

Pawłowska et al. in their studies compared children after cancer and healthy young people, demonstrated a significant reduction in physical activity in patients after treatment in the hematology and oncology ward [18]. Low level of motor activity was also demonstrated by *Wolin* et al. in their studies, while at the same time referring to the detrimental effect on avoiding exercise of the convalescence process of persons affected by the disease [26].

It is important that young people, both healthy and suffering from cancer, maintain their physical activity, which affects not only their appearance, but also aspects of mental and social development and is an integral part of young people's lives [21].

CONCLUSIONS

1. Both groups were characterized by increased intake of proteins and carbohydrates. However, higher intake was reported in the group after cancer treatment.
2. Insufficient fat intake was shown in comparison with the recommended amounts in all study groups.
3. It was observed that in the group of convalescents the intake of vitamins B1, A, E, D was higher than their healthy peers. In contrast, healthy children consumed higher amounts of vitamin C and β -carotene compared to the group after the illness. The difference in thiamine supply was statistically significant.
4. Children and adolescents after cancer treatment covered the demand for magnesium, iron, zinc and copper, compared to healthy children. Differences in RDA coverage were statistically significant.
5. Determinants of the choice of physical activity among children after cancer treatment was pleasure and fun, while among their healthy peers, aesthetic considerations, like taking care of appearance.

Conflict of interest

The authors declare no conflict of interest.

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QUALITY OF DRINKING WATER FROM THE AGRICULTURAL AREA TREATED WITH PITCHER WATER FILTERS

Elżbieta Królak, Jolanta Raczuk, Danuta Sakowicz, Elżbieta Biardzka

Department of Environmental Studies and Biological Education, Institute of Biology,
University of Natural Sciences and Humanities, Siedlce, Poland

ABSTRACT

Background. Home methods of drinking water treatment through filtration have recently become quite popular.

Objective. The aim of the study was to compare chemical composition of unfiltered water with water filtered in households with pitcher water filters. Obtained results were discussed in view of the effect of analysed chemical components of water on human health.

Material and methods. Water samples were taken from water works supplies and from home dug wells from the agricultural area. Unfiltered water and water filtered through filters filled with active carbon and ion-exchanging resin and placed in a pitcher were analysed. Electrolytic conductivity, pH, hardness and the concentrations of calcium, magnesium, nitrate, phosphate and chloride ions were determined in water samples. Results of analyses were statistically processed.

Results. As a result of water filtration, the concentration of phosphates significantly increased and the concentrations of calcium, magnesium, electrolytic conductivity and pH decreased. No changes were noted in the concentration of chloride ions. Filtering water decreased the concentration of nitrates in dug wells samples.

Conclusions. Using water purification devices is justified in the case of water originating from home dug wells contaminated with nitrates when, at the same time, consumers' diet is supplemented with calcium and magnesium. Filtration of water from water works supplies, controlled by sanitary inspection seems aimless.

Key words: water filtration, calcium, magnesium, nitrates, phosphates, chlorides, acidity

STRESZCZENIE

Wprowadzenie. Sposoby uzdatniania wody pitnej w gospodarstwie domowym przez filtrację stały się ostatnio bardzo popularne.

Cel. Celem badań było porównanie składu chemicznego wody niefiltrowanej z wodą filtrowaną w gospodarstwach domowych za pomocą filtrów dzbankowych. Wyniki badań zostały omówione w aspekcie wpływu analizowanych składników chemicznych wody na zdrowie człowieka.

Material i metody. Próbkę wody pobierano z ujęć wodociągowych oraz ze studni kopanych na terenach rolniczych. Analizowano próbki wody niefiltrowanej i filtrowanej za pomocą filtrów dzbankowych, wypełnionych węglem aktywnym i żywicą jonowymienną. W próbkach wody oznaczano: przewodność elektrolityczną, pH, twardość i stężenie jonów wapnia, magnezu, azotanów, fosforanów oraz chlorków. Wyniki analizy opracowano statystycznie.

Wyniki. W wyniku filtrowania wody odnotowano znaczący wzrost stężenia fosforanów oraz obniżenie stężenia wapnia, magnezu, przewodnictwa elektrolitycznego i pH. Nie stwierdzono zmian w stężeniu jonów chlorkowych. W próbkach wody filtrowanej pochodzącej ze studni kopanych stwierdzono zmniejszenie stężenia azotanów w porównaniu z próbkami wody niefiltrowanej.

Wnioski. Wykorzystanie urządzeń do filtrowania wody jest uzasadnione w przypadku wody zanieczyszczonej azotanami, pochodzącej z studni kopanych, przy jednoczesnym uzupełnianiu diety konsumentów wapniem i magnezem. Filtracja wody z ujęć wodociągowych, kontrolowanych przez inspekcję sanitarną wydaje się bezcelowa.

Słowa kluczowe: filtracja wody, wapń, magnez, azotany, fosforany, chlorki, kwasowość

INTRODUCTION

Water and substances contained therein are essential for proper functioning of human organism. Drinking water should have a composition favourable for human health and be devoid of harmful substances. Basic

inorganic components in water are calcium, magnesium, sodium, potassium and bicarbonate ions. They are considered the main components in typical natural waters and constitute over 90% of dissolved substances [3]. Water quality and the content of inorganic components depend on the type of substratum. The following values

Corresponding author: Elżbieta Królak, Department of Environmental Studies and Biological Education, Institute of Biology, University of Natural Sciences and Humanities, Prusa street 12, 08-110 Siedlce, Poland, tel. +48 25 6431217, e-mail: elzbieta.krolak@uph.edu.pl

were adopted as a geochemical background for natural ground waters in Poland: pH 6.5 - 8.5, conductivity 200 - 700 $\mu\text{S}/\text{cm}$, Ca 2 - 200 mg/l, Mg 0.5 - 30 mg/l, NO_3^- 0 - 5 mg/l, Cl^- 2 - 60 mg/l, PO_4^{3-} 0.01 - 1.0 mg [22]. For 70% of people in Poland ground waters are the source of drinking water [26]. These are mainly deep waters situated under impermeable rocky formations and unaffected by anthropogenic pollution. Part of Poland's inhabitants, mainly from rural areas, use home dug wells fed with ground waters. In the year 2013, 76.6% of rural population were supplied in water from water works, the rest used water mainly from home wells [26]. Water from dug wells often situated near farm buildings is exposed to pollution and, compared with water from deep intakes, contains more nitrates, phosphates, chlorides and has a higher total hardness [2, 10, 20].

Water from water works in Poland is controlled by the State Sanitary Inspection and its consumption should not pose health risk. The order of the Minister of Health on the assessment of drinking water quality [24] imposes an obligation on the State Sanitary Inspection to issue the areal assessments of water quality to fulfil the demands determined in the order and to estimate health risk of the consumers. Legal regulations, being in effect since 2010 and agreed with the EU norms, guarantee that drinking water meets the highest standards [31]. Water from home wells, however, is not monitored by sanitary services and, in some cases, its systematic consumption may pose a risk for consumers' health, mainly due to nitrate concentrations that exceed the obligatory standards [2, 20, 27].

Various methods of water treatment through filtration are used to improve its quality [12, 21, 29, 30]. Filtration of water reduces both organic and inorganic (e.g. heavy metals) pollutants [1], removes pharmaceuticals [7, 21, 28] and decreases water hardness [12], but it also changes microbiological composition of water [30]. Home methods of drinking water treatment through filtration have recently become quite popular. The two most popular methods of water purification are reverse osmosis and pitcher filters equipped with replacement filters filled with active carbon and ion-exchanging resin. Studies on the quality of drinking water filtered in home intakes with reverse osmosis showed that the process decreased water hardness but did not efficiently remove nitrates [15].

This study was aimed at estimating the effect of water filtering in households though pitcher filters on changes in the chemical properties of water with main focus on such parameters as conductivity, pH, water hardness, concentrations of magnesium, calcium, chlorides, nitrates and phosphates. Water samples from water works and from home dug wells located in an agricultural area were analysed. The results obtained were used to assess the influence of water filtration on chemical properties of water and discussed in the context of human health.

MATERIAL AND METHODS

Water samples were randomly collected from 33 localities situated in an agricultural area in north-eastern Poland. Intakes of water works ($n=18$) were situated deeper than 100 m below ground. Samples from dug wells ($n=15$) were collected from the depth varying from 7 to 12 m. Each water sample was analysed before (sample A) and after (sample B) filtration. Filtered water samples were purified with filters filled with active carbon and ion-exchanging resin and placed in a pitcher. According to the information obtained in households, the filters had been used from 1 to 3 weeks.

Water samples were analysed for selected chemical parameters. Conductivity was measured with a conductivity meter and pH was determined with a digital pH meter. Water hardness and the concentrations of calcium and magnesium were determined complexometrically with EDTA as a titrant and eriochrome black and murexid as indicators. Concentrations of chloride ions were determined with the argentometric method. Nitrates concentration was determined with the use of disulfonic acid. Phosphates were measured with the molybdenum blue method. Nitrates and phosphates were determined spectrophotometrically at wavelengths of 410 and 700 nm, respectively. The applied methods of samples analyses were in accord with the recommendations given in the Regulation of the Minister of Environment [23]. All spectrophotometric analyses were performed with Shimadzu UV-VIS 1800 spectrophotometer (produced in Japan). All the analyses were repeated three times and the arithmetic mean of the three measurements was used for further interpretation of data.

The results of the analyses were statistically processed. Data distribution was checked with *Shapiro-Wilk* test, *t-Student* test or *Wilcoxon* test were used to compare means or medians of determined parameters. Comparisons of water components between the two types of water intakes were made with non-parametric *Mann-Whitney U* test. Statistical calculations were performed with Statistica 10 software.

RESULTS

Analysed water parameters varied significantly. Electrolytic conductivity varied from 52.0 to 1305.0 $\mu\text{S}/\text{cm}$, pH from 4.71 to 8.29, water hardness from 11.60 to 569.9 mg CaCO_3/l , calcium concentration from 2.40 to 168.3 mg/l, magnesium concentration from 0.0 to 36.47 mg/l, the concentration of nitrates from 0.040 to 104.0 mg/l, concentration of phosphates from 0.016 to 3.801 mg/l, and the concentration of chlorides from 2.00 to 185.0 mg/l (Tables 1, 2). Most analysed parameters had non-normal distribution (Table 3), therefore, in such cases median values were used in data interpretation.

Table 1. Descriptive statistics of selected parameters in unfiltered (A) and filtered (B) water with the division into water taken from water works supply

Parameter	Unit	Mean	Median	Range (min. – max.)	Lower - upper quartile	Interval quartile	SD
Conductivity A	μS/cm	448.9	469.5	143.0 - 700.0	373.0 - 550.0	177.0	148.9
Conductivity B		283.9	273.5	52.00 - 534.0	181.0 - 364.0	183.0	134.4
Acidity A	pH	7.154	7.100	6.760 - 7.700	6.990 - 7.280	0.290	0.272
Acidity B		6.604	6.445	5.480 - 7.620	6.000 - 7.290	1.290	0.664
Hardness A	mg CaCO ₃ /l	240.8	251.76	90.00 - 339.9	219.9 - 290.0	249.9	73.16
Hardness B		87.76	79.01	11.60 - 247.9	36.20 - 123.0	236.4	59.50
Ca ²⁺ A	mg/l	77.75	82.96	20.04 - 113.8	67.33 - 92.99	25.66	26.36
Ca ²⁺ B		25.22	24.45	2.405 - 56.11	9.700 - 36.87	27.17	16.27
Mg ²⁺ A		11.37	11.43	0.970 - 25.28	8.265 - 13.90	5.635	5.009
Mg ²⁺ B		6.038	4.132	0.000 - 26.25	1.440 - 9.724	8.284	6.441
NO ₃ ⁻ A		1.890	2.201	0.505 - 4.982	0.864 - 2.230	1.366	1.226
NO ₃ ⁻ B		2.017	1.653	0.040 - 8.414	0.514 - 2.315	1.801	2.026
PO ₄ ³⁻ A		0.175	0.138	0.016 - 0.656	0.050 - 0.246	0.196	0.165
PO ₄ ³⁻ B		0.388	0.319	0.020 - 1.694	0.142 - 0.479	0.337	0.387
Cl ⁻ A		23.12	16.000	4.000 - 100.1	11.00 - 26.00	15.00	22.35
Cl ⁻ B		21.95	15.500	2.000 - 92.10	11.00 - 25.00	14.00	21.61

Table 2. Descriptive statistics of selected parameters in unfiltered (A) and filtered (B) water with the division into water taken from dug wells

Parameter	Unit	Mean	Median	Range (min. – max.)	Lower - upper quartile	Interval quartile	SD
Conductivity A	μS/cm	608.3	528.0	250.0 - 1305.0	444.0 - 703.0	259.0	308.3
Conductivity B		375.6	338.0	41.00 - 981.0	216.0 - 535.0	319.0	241.9
Acidity A	pH	7.177	7.250	6.450 - 8.290	6.750 - 7.390	0.640	0.486
Acidity B		6.065	5.810	4.710 - 7.700	5.650 - 6.550	0.900	0.811
Hardness A	mg CaCO ₃ /l	303.9	285.9	125.0 - 569.9	234.0 - 379.9	444.9	108.1
Hardness B		107.6	91.90	12.00 - 308.0	64.00 - 122.0	296.0	80.05
Ca ²⁺ A	mg/l	101.4	96.19	48.10 - 168.3	80.16 - 116.2	36.00	32.35
Ca ²⁺ B		29.20	24.85	4.810 - 73.75	17.64 - 44.08	26.44	18.59
Mg ²⁺ A		12.39	9.724	1.220 - 36.47	5.834 - 16.29	10.46	9.083
Mg ²⁺ B		8.438	5.340	0.000 - 30.14	2.100 - 11.18	9.080	8.965
NO ₃ ⁻ A		35.88	26.48	0.208 - 104.0	0.841 - 62.40	61.56	36.78
NO ₃ ⁻ B		16.08	6.100	0.159 - 78.78	2.750 - 22.05	19.30	21.32
PO ₄ ³⁻ A		0.770	0.430	0.063 - 3.639	0.279 - 1.039	0.760	0.922
PO ₄ ³⁻ B		1.074	0.772	0.150 - 3.801	0.459 - 1.204	0.744	0.918
Cl ⁻ A		41.01	20.00	8.000 - 185.0	15.00 - 37.00	22.00	46.19
Cl ⁻ B		33.38	18.00	6.000 - 118.0	14.00 - 37.50	23.50	33.16

Table 3. Statistical data on the normal distribution of measured water parameters

Parameter	Water works supply (n=18)		Dug wells (n=15)	
	<i>W</i>	<i>p</i>	<i>W</i>	<i>p</i>
Conductivity A	0.959	0.576	0.818	0.008
Conductivity B	0.973	0.855	0.938	0.359
Acidity A	0.937	0.257	0.952	0.554
Acidity B	0.933	0.222	0.876	0.041
Hardness A	0.889	0.037	0.932	0.290
Hardness B	0.918	0.122	0.859	0.024
Ca ²⁺ A	0.904	0.067	0.975	0.927
Ca ²⁺ B	0.942	0.314	0.906	0.116
Mg ²⁺ A	0.919	0.124	0.859	0.024
Mg ²⁺ B	0.798	0.001	0.831	0.009
NO ₃ ⁻ A	0.854	0.009	0.856	0.021
NO ₃ ⁻ B	0.806	0.001	0.743	< 0.001
PO ₄ ³⁻ A	0.830	0.004	0.672	< 0.001
PO ₄ ³⁻ B	0.758	< 0.001	0.786	0.002
Cl ⁻ A	0.699	< 0.001	0.664	< 0.001
Cl ⁻ B	0.738	< 0.001	0.733	< 0.001

Water samples from water works and from dug wells were similar in conductivity, pH, water hardness and the concentrations of magnesium and chlorides but differed markedly in the concentration of nitrates and phosphates. In the water from dug wells there was

12 times more nitrates and 3 times more phosphates than in the water from water works. Water from wells was a bit richer in calcium ions compared with samples from waterworks, yet the difference was statistically insignificant (Tables 1, 2 and 4).

Table 4. Analysed water parameters in relation to the type of water intake (comparison with *Mann-Whitney U* test)

Parameter	Sum of ranks - water supply	Sum of ranks - dug wells	<i>U</i>	<i>Z</i>	<i>p</i>
Conductivity A	274.0	287.0	103.0	-1.139	0.255
Conductivity B	278.0	283.0	107.0	-0.994	0.320
Acidity A	301.0	260.0	130.0	-0.163	0.871
Acidity B	370.0	191.0	71.00	2.296	0.022
Hardness B	267.0	294.0	96.00	-1.392	0.164
Hardness A	295.0	266.0	124.0	-0.380	0.704
Ca ²⁺ A	254.5	306.5	83.50	-1.844	0.065
Ca ²⁺ B	294.5	266.5	123.5	-0.398	0.691
Mg ²⁺ A	322.5	238.5	118.5	0.578	0.563
Mg ²⁺ B	291.0	270.0	120.0	-0.524	0.600
NO ₃ ⁻ A	237.0	324.0	66.00	-2.477	0.013
NO ₃ ⁻ B	228.0	333.0	57.00	-2.802	0.005
PO ₄ ³⁻ A	208.0	353.0	37.00	-3.525	< 0.001
PO ₄ ³⁻ B	216.0	345.0	45.00	-3.236	0.001
Cl ⁻ A	263.0	298.0	92.00	-1.536	0.124
Cl ⁻ B	269.5	291.5	98.50	-1.302	0.193

Significant correlations ($p < 0.05$, $n = 15$) between water hardness and the concentration of nitrates ($R_s = 0.650$) and chlorides ($R_s = 0.612$) were found in the water from dug wells. Similarly, meaningful correlations

were found between the concentrations of nitrates and calcium ($R_s = 0.650$) and between the concentration of chlorides and calcium ($R_s = 0.556$). No such correlations were noted for water from water works.

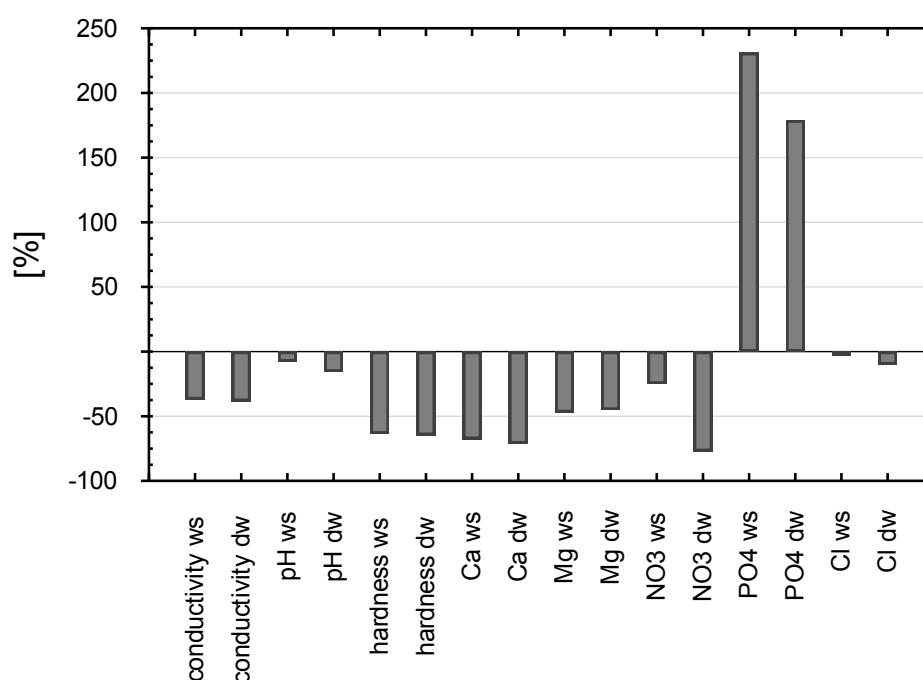


Figure 1. Percentage changes in values of chosen chemical parameters as a result of water filtration with household pitcher water filters (ws - water supply, dw - dug wells)

Table 5. Comparison of analysed water parameters in unfiltered (A) and filtered (B) water samples

Water works supply (n=18)				
Parameter	<i>t</i>	<i>df</i>	<i>p</i>	Statistical test
Conductivity A & B	7.050	17	< 0.001	t- <i>Students</i>
Acidity A & B	4.394	17	< 0.001	
Ca ²⁺ A & B	8.333	17	< 0.001	
	<i>T</i>	<i>Z</i>	<i>p</i>	
Hardness A & B	0.00	3.725	< 0.001	<i>Wilcoxon</i>
Mg ²⁺ A & B	7.500	3.396	< 0.001	
NO ₃ ⁻ A & B	63.00	0.979	0.327	
PO ₄ ³⁻ A & B	27.00	2.548	0.011	
Cl ⁻ A & B	43.50	1.829	0.067	
Dug wells (n=15)				
Parameter	<i>T</i>	<i>Z</i>	<i>p</i>	Statistical test
Conductivity A & B	1.000	3.351	< 0.001	<i>Wilcoxon</i>
pH A & B	3.000	3.237	0.001	
Hardness A & B	1.00	3.351	< 0.001	
Mg ²⁺ A & B	6.000	3.067	0.002	
NO ₃ ⁻ A & B	7.000	3.010	0.002	
PO ₄ ³⁻ A & B	0.000	3.408	< 0.001	
Cl ⁻ A & B	28.00	1.817	0.069	
	<i>t</i>	<i>df</i>	<i>p</i>	
Ca ²⁺ A & B	12.75	14	< 0.001	t- <i>Students</i>

With the exception of phosphates, higher concentrations of the studied parameters were found in unfiltered than in filtered water. In water samples from both water works and dug wells, filtration process decreased electrolytic conductivity by more than 35% and calcium concentrations by about 70%. Concentrations of magnesium ions decreased by 45% in water from water works and by 64% in water from dug wells. Water pH decreased due to filtration, which was particularly visible in water from dug wells. In these water samples filtration decreased nitrate concentration by almost 80% and about 25% in samples from water works. In both types of water samples, filtration did not significantly affect chloride concentrations but markedly increased the concentration of phosphates (Figure 1, Tables 1, 2 and 5).

DISCUSSION

Values of physical and chemical parameters in samples of water from water works fell within the range given as a geochemical background for ground water in Poland [22]. In the samples of water from dug wells, however, the geochemical background values were exceeded in 80% of samples for nitrates and in 27% of samples for phosphates and electrolytic conductivity. These results suggest that water from dug wells is largely exposed to pollutant inflow and statistically significant correlations between water components indicate that the pollutants are of municipal origin or come from animal

breeding [10]. Noteworthy, water from individual intakes is not the subject to sanitary control in Poland and, in some cases, its quality may pose a threat to consumers' health, mainly because of high nitrate concentrations. Nitrates may cause methaemoglobinemia in infants and little children and diseases of alimentary tract and hypertension in adults. Long exposure to high nitrate concentrations may be carcinogenic [4, 8, 14]. The maximal allowable concentration of nitrates in drinking water in Poland is 50 mg/l [24]. The concentration of nitrates in ground water below 5 mg/l is natural and does not pose a risk for consumers' health. The process of filtration does not affect significantly the concentration of nitrate ions in water. Lack of significant changes in nitrate concentration after filtration through membrane filters was also noted by *Van der Bruggen et al.* [29]. Changes in the concentration of nitrates were, however, significant in water taken from wells. This water with its concentration of nitrates over ten times higher than in deep water intakes may be markedly improved after filtration.

Drinking water is an important source of calcium and magnesium in diet [5, 9]. The results of presented analyses of randomly collected water samples showed that filtration of water in households decreased water hardness and the concentrations of calcium and magnesium. It also led to water acidification. The revealed effects of filtration on water properties may have unfavourable consequences for the health of consumers.

Both calcium and magnesium participate in many physiological processes in human organism at subcellular, cellular and tissue level. Their deficit leads to hypocalcemia and hypomagnesemia [6, 17, 18, 19]. Chronic hypocalcemia may result in osteoporosis [17, 18]. Moreover, hypocalcemia increases the risk of cerebral stroke and leads to an increase of blood pressure [11]. Magnesium deficit in human organism contributes to diseases of blood circulation system, disturbs heartbeat rhythm, causes vertigo and muscle spasms [6, 25]. Larsson et al. [16] underlined that high magnesium intake may reduce the occurrence of colorectal cancer in women. In view of presented literature data one can hardly agree with a common belief that home filtration of water may improve its quality. Rubenowitz et al. [25] clearly indicate the correlation between the concentration of magnesium and the calcium to magnesium ratio in drinking water and health risk associated e.g. with heart diseases.

Filtration increased phosphate concentration in water. This was true for both water from water works and from dug wells. Systematic uptake of higher amounts of phosphates in drinking water may result in symptoms described e.g. by Kemi et al. [13] and Maziarska and Pasternak [17], including unfavourable effects on bone metabolism and disturbed calcium-phosphate equilibrium. Filtration of water markedly decreased its pH. Acidification of water after filtration is an effect of calcium and magnesium sorption on ion-exchanging resin and their replacement by hydrogen ions.

The results of presented analyses of randomly collected ground water samples showed that using filters decreased the concentrations of calcium and magnesium, substantially decreased pH value and increased phosphate concentrations. Water from deep intakes contains natural concentrations of nitrate ions and is the subject of control by sanitary services. Improving its quality by filtration is fruitless. Filtration of water may only decrease the risk of exposure to high nitrate concentrations in the case of water from dug wells polluted by these substances.

CONCLUSIONS

1. The improvement of water quality with home methods when the water is supplied by water works and analysed by sanitary services seems pointless.
2. In the case of dug wells, using water purification devices decreases health risk subsequent to the intake of nitrates with water.
3. Due to the lowered calcium and magnesium concentrations in water treated with pitcher water filters, it is advisable to supplement diet with these elements.

Conflict of interest

The authors declare no conflict of interest.

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BODY COMPOSITION AND FATTY TISSUE DISTRIBUTION IN WOMEN WITH VARIOUS MENSTRUAL STATUS

Agnieszka Dmitruk¹, Jan Czezelewski², Ewa Czezelewska³, Jolanta Golach³, Urszula Parnicka⁴

¹Department of Biological Development of Man, Faculty of Physical Education and Sport in Biała Podlaska, J. Piłsudski's Academy of Physical Education in Warsaw, Poland

²Chair of Human Nutrition, Faculty of Physical Education and Sport in Biała Podlaska, J. Piłsudski's Academy of Physical Education in Warsaw, Poland

³Department of Nursing, Faculty of Health Sciences, Collegium Mazovia Innovative Higher School in Siedlce, Poland

⁴Chair of Recreation, Faculty of Tourism and Health in Biała Podlaska, J. Piłsudski's Academy of Physical Education, Warsaw, Poland

ABSTRACT

Background. Menopause, also referred to as climacterium, is a period of multiple changes in the structure and functions of a woman organism.

Objective. Determination of differences in body composition and fatty tissue distribution in women from groups discriminated based on their menstrual status.

Material and Methods. The survey covered 312 women aged 38-75 years. Menstrual status of the surveyed women was established according to WHO guidelines based on answers to a questionnaire, and three groups were discriminated: women in the premenopausal period (group 1), in the perimenopausal period (group 2), and in the postmenopausal period (group 3). The following anthropomological measurements were taken: body height, body mass, waist and hip circumference, and thickness of 6 skinfolds. Their results enabled evaluating the somatic built of women in the separated groups. Fatty tissue distribution was determined based on TER distribution index calculated as a ratio of the sum of trunk skinfolds (TSS) to the sum of extremity skinfolds (ESS). Body composition of the women, including percentage of body fat, lean body mass, soft tissue mass, and total body water, was assessed using an IOI 353 analyzer by JAWON MEDICAL. In addition, percentages of women with underweight, normal content of fatty tissue, and these with overweight and obesity were calculated. The WHR index was computed in the case of obese women.

Results. The highest values of body mass, hip circumference and most of the skinfolds were determined in the perimenopausal group, whereas the postmenopausal women were characterized by the highest percentage of body fat (PBF) and by the lowest contents of lean tissue, soft tissue, and total water content in the body. The highest percentage of obese women was found in the postmenopausal group, including 40% of them having visceral type obesity. The occurrence of the menopause contributed to changes in fatty tissue distribution, causing its shift from extremities toward the trunk.

Conclusions. The study showed differences in the somatic built and body composition in groups of women distinguished based on their menstrual status.

Key words: *menstrual status, body composition, women*

STRESZCZENIE

Wprowadzenie. Menopauza, zwana inaczej przekwitaniem, to okres licznych zmian w budowie i funkcjonowaniu organizmu kobiety.

Cel. Określenie wielkości różnic w składzie ciała i rozmieszczeniu tkanki tłuszczowej u kobiet w grupach wydzielonych na podstawie statusu menstruacyjnego.

Material i metody. Badaniom poddano 312 kobiet w wieku 38-75 lat. Na podstawie odpowiedzi udzielonych na pytania ankiety określono status menstruacyjny badanych kobiet zgodnie z zaleceniami WHO. Wydzielono trzy grupy kobiet będących w okresie premenopauzalnym (grupa 1- 69 kobiet), perimenopauzalnym (grupa 2 – 45 kobiet) i postmenopauzalnym (grupa 3- 198 kobiet). Wykonano pomiary antropologiczne: wysokości ciała, masy ciała, obwodu pasa i bioder oraz grubości 6 fałdów skórno-tłuszczowych w celu oceny budowy somatycznej kobiet w wydzielonych grupach. W celu określenia dystrybucji tkanki tłuszczowej obliczono wskaźnik dystrybucji TER, będący stosunkiem

Corresponding author: Agnieszka Dmitruk, J. Piłsudski's Academy of Physical Education in Warsaw, Faculty of Physical Education and Sport, Department of Biological Development of Man, Akademicka Street 2, 21-500 Biała Podlaska, Poland, Tel. +48 83 342 87 38, e-mail: agnieszka.dmitruk@awf-bp.edu.pl

sumy fałdów skórno-tłuszczowych na tułowie (TSS) do sumy fałdów skórno-tłuszczowych na kończynach (ESS). Skład ciała oceniono przy pomocy analizatora składu ciała IOI 353 z oprogramowaniem JAWON MEDICAL. Pozwoliło to na określenie m.in. procentowej zawartości tkanki tłuszczowej, beztłuszczowej masy ciała, masy tkanek miękkich oraz całkowitej zawartości wody. Obliczono również odsetek osób z niedowagą, prawidłową zawartością tkanki tłuszczowej oraz z nadwagą i otyłością. U otyłych kobiet obliczono wskaźnik WHR.

Wyniki. Najwyższe wartości masy ciała, obwodu bioder i większości fałdów skórno-tłuszczowych wystąpiły w grupie perimenopauzalnej, a w grupie postmenopauzalnej – najwyższe wartości całkowitej zawartości tkanki tłuszczowej (PBF), przy równoczesnym najniższym poziomie tkanki beztłuszczowej, tkanek miękkich i całkowitej zawartości wody w organizmie. Najwyższy odsetek otyłych kobiet wystąpił w grupie postmenopauzalnej, przy czym u 40% badanych z tej grupy była to otyłość wisceralna. Wystąpienie menopauzy przyczyniło się do zmian w rozmieszczeniu tkanki tłuszczowej, powodując przesunięcie jej z kończyn w kierunku tułowia.

Wnioski. Stwierdzono występowanie różnic w budowie somatycznej i składzie ciała kobiet w grupach wydzielonych na podstawie statusu menstruacyjnego.

Słowa kluczowe: *status menstruacyjny, skład ciała, kobiety*

INTRODUCTION

Menopause is a very important period in every woman's life. First symptoms of reproductive functions ceasing appear on average 5 year beforehand. This period is called the perimenopausal period and is characterized by, e.g., decreased concentrations of estrogens, ovarian androgens, progesterone, and growth hormone [11]. These hormonal changes result in irregular menstrual cycles – initially shortened and then extended with excessive profuse or scanty menstruation [14]. In about 85% of the women being in that period suffer from the so-called climacteric syndrome, symptoms of which include, e.g., heats, night sweats, palpitation, as well as vertigos and headaches. Some psychical disorders like the feeling of fatigue, mood swings, trouble sleeping etc., are likely to appear as well [1, 6, 10].

Lack of menstruation for 12 months is the basic criterion in menopause diagnosis. A study by Kaczmarek [8] indicates the mean age at the natural menopause in Poland determined with the *Kaplan-Meier* method to be 49.5 years. In turn, menopausal age determined with the probit method is at 51.74 years [17]. The age at which the menopause appears is significant for the health of women. The loss of the hormonal activity of ovaries induces many metabolic disorders that may contribute to increased incidence of multiple diseases hazardous to both their health and life. Postmenopausal women face a higher risk of development of osteoporosis, cardiovascular diseases, visceral obesity, and type II diabetes [5, 19].

Changes are also observed in body composition. Hormonal fluctuations in the period of menopause cause an increase in fatty tissue content, induce changes in the ratio between body fat percentage and lean body mass – to the disadvantage of the latter, and contribute to water body loss [13, 17].

This study was aimed at determining differences in body composition and fatty tissue distribution in women from groups discriminated based on their menstrual status.

MATERIAL AND METHODS

Material

The survey included 323 women aged 38-75 years from the Bialski District (Lubelskie Province) attending the Third Age University and from the Siedlecki District (Mazovia Province) attending the so-called bridge studies at the Collegium Mazovia Innovative Higher School in Siedlce, 224 of whom (69.3%) lived in urban areas and the other 99 (30.6%) in rural areas. Participation in the survey was voluntary. Analyzed were results gathered for 312 women. The lower final number of women was due to the appearance of artificial menopause in 11 women (3.52%) as a result of radiotherapy or hysterectomy and/or ovariectomy.

Methods

To collect necessary data, a questionnaire was constructed that comprised two parts. The first part included questions related to, e.g., date of birth and place of residence, whereas the second part included such questions as “do you still menstruate?” or “are your menstrual cycles regular?”. The menostatic women were asked to state the date of their last menstruation. Based on the collected answers, the menstrual status of the women was determined following WHO guidelines [22] and respective groups of women were discriminated. The first group included regularly menstruating women (premenopausal women). The second group (perimenopausal period) included women with irregular menstruation and the time from the last menstruation till the day of the survey shorter than 12

months. Women in the case of whom the menostasis was longer than 12 months were classified to the third group (postmenopausal women). Numbers and age of the women were provided in Table 1.

Table 1. Number and age of the surveyed women in groups discriminated based on their menstrual status

Group	Number of women		Age of women		
	n	%	\bar{x}	SD	$\pm 95\%$ CI
Group 1	69	22.12	44.48	2.22	40.94 \pm 46.01
Group 2	45	14.42	48.47	1.75	47.94 \pm 48.99
Group 3	198	63.46	66.59	6.69	65.65 \pm 67.53

In addition, the women were subjected to measurements of the basic anthropological features, including: body height (BH), body mass (BM), waist circumference (WC), hip circumference (HC), and to measurements of the thickness of left-sided skinfolds: at biceps (BIC), at triceps (TRC), under the scapula (SSC), over the iliac crest (SIC), at the abdomen (ABD), and at the calf (CLF). Body composition including, e.g. percentage of body fat (PBF), lean body mass (LBM), soft tissue mass (SLM), and total body water (TBW), was evaluated using an IOI 353 analyzer by JAWON MEDICAL.

In groups distinguished based on the menstrual status, we also calculated the percentage of women with underweight, with normal body fat percentage, with overweight and obesity. The underweight women were those whose PBF was below 20% acc. to reference values of the IOI 353 analyzer, the group with normal body fat content included women whose PBF ranged from 20 to 30%, whereas the group of overweight and obese women included those whose PBF was: 30-35% and over 35%, respectively. The WHR value being a ratio of waist to hip circumference was additionally calculated for the obese women. It allowed calculating the percentage of women with WHR>0.85, namely these with android obesity, in each analyzed group distinguished based on the menstrual status.

Adipose tissue distribution in extremities and at the trunk was determined by calculating the TER index being a ratio of the sum of skinfolds at the trunk (TSS) and the sum of skinfolds in extremities (ESS).

Differences observed between groups discriminated based on the menstrual status in mean values of somatic traits (BH, BM, WC, HC, BIC, TRC, SSC, SIC, ABD, CLF), percentage of body fat (PBF), lean body mass (LBM), soft tissue mass (SLM), total body water (TBW), fatty tissue distribution index in limbs and trunk (TER), and the sum of skinfolds (TSS and ESS) were evaluated using one-way analysis of variance (ANOVA) and *Newman-Keuls* post-hoc test. The frequency of occurrence of women with various

percentage of body fat and obese women with high WHR values (>0.85) in groups discriminated based on the menstrual status was determined with the *Chi²* test. The significance of tests was adopted at $p \leq 0.05$ or higher. Statistical analyses were carried out using Statistica v. 12 PL software.

The study was approved by the Commission of Ethics of Scientific Research at the Academy of Physical Education in Warsaw and conducted followed guidelines of the Helsinki Declaration.

RESULTS

The somatic characteristics of women in groups discriminated based on their menstrual status was presented in Table 2. Data provided in the table show significant differences in body height of the women, i.e. the highest BH value was reached by women in the premenopausal period, and the lowest one by postmenopausal women. Waist circumferences was observed to increase successively, starting from the premenopausal to the postmenopausal group (from 83.58 cm to 88.29 cm), but statistically significant differences were observed only between groups 1 and 3. The analysis of values of body mass, hip circumferences and thickness of skinfolds at: triceps and biceps, under scapula, at the abdomen, and over the iliac crest showed no significant differences between the groups of women. In most cases, the highest values of these traits were found in the perimenopausal women (group 2). An exception was skinfold thickness at the calf: the mean value of which was the highest in women from group 1, and the lowest in these from group 3 (statistically significant differences between groups 1 and 3 as well as 2 and 3). The analysis of the sum of thickness of three skinfolds at the trunk (TSS) and of the sum of thickness of skinfolds at extremities (ESS), and TER distribution index (TSS/ESS) revealed the highest value of TSS in group 2 and of ESS in group 1, as well as successively increasing value of TER in subsequent groups of women discriminated based on their menstrual status.

The anthropometric measurements were completed body composition analysis with the bioelectric impedance method. The analysis of fatty tissue content demonstrated significant differences in body adiposity between women from the studied groups. The highest percentage of body fat (PBF) was determined in the group of postmenopausal women who were additionally characterized by the lowest lean body mass (LBM) and soft tissue mass (SLM). These women had also the lowest content of total body water (TBW). The analysis of variance ANOVA showed the differences observed between the discriminated groups to be statistically significant (Table 3).

Table 2. Somatic characteristics of women in groups discriminated based on their menstrual status

Variables	Group 1			Group 2			Group 3			ANOVA	Newman-Keuls test
	\bar{x}	SD	$\pm 95\%$ CI	\bar{x}	SD	$\pm 95\%$ CI	\bar{x}	SD	$\pm 95\%$ CI	F	
BH (cm)	161.71	5.95	160.28 \pm 163.14	161.23	5.05	159.71 \pm 162.75	157.21	5.82	156.40 \pm 158.03	20.409**	1-3; 2-3
BM (kg)	72.35	13.58	69.09 \pm 75.61	76.50	22.02	69.89 \pm 83.12	72.82	12.21	71.11 \pm 74.53	1.389	
WC (cm)	83.58	11.97	80.70 \pm 86.45	85.50	14.46	81.15 \pm 89.84	88.29	11.20	86.72 \pm 89.86	4.362*	1-3
HC (cm)	102.93	9.98	100.53 \pm 105.33	105.64	13.32	101.64 \pm 109.65	104.89	8.22	103.74 \pm 106.04	1.429	
BIC (mm)	11.85	4.64	10.74 \pm 12.97	12.02	5.13	10.48 \pm 13.56	10.76	3.87	10.21 \pm 11.30	2.719	
TRC (mm)	20.48	7.14	18.77 \pm 22.20	19.32	5.35	17.71 \pm 20.92	19.74	6.28	18.85 \pm 20.62	0.530	
CLF (mm)	15.39	5.19	14.14 \pm 16.63	14.51	3.32	13.54 \pm 15.48	12.46	4.03	11.90 \pm 13.03	14.100**	1-3; 2-3
SSC (mm)	21.43	8.72	19.33 \pm 23.52	21.91	8.53	19.53 \pm 24.47	22.09	7.66	21.01 \pm 23.16	0.174	
ABD (mm)	26.77	8.03	24.84 \pm 28.70	27.36	7.95	24.97 \pm 29.75	26.17	8.02	25.05 \pm 27.30	0.462	
SIC (mm)	21.76	8.63	19.69 \pm 23.83	21.91	8.83	19.25 \pm 24.56	20.30	7.27	19.29 \pm 21.32	1.358	
TSS (mm)	69.95	23.70	64.26 \pm 75.65	71.18	11.34	64.09 \pm 78.27	68.57	20.48	65.69 \pm 71.44	0.313	
ESS (mm)	47.71	14.00	44.35 \pm 51.08	45.84	11.34	42.44 \pm 49.25	42.95	10.69	41.46 \pm 44.45	4.718**	1-3
TER (mm)	1.48	0.38	1.39 \pm 1.57	1.54	0.31	1.44 \pm 1.63	1.63	0.45	1.57 \pm 1.69	3.519*	1-3

*statistically significant difference at $p \leq 0.05$ **statistically significant difference at $p \leq 0.01$

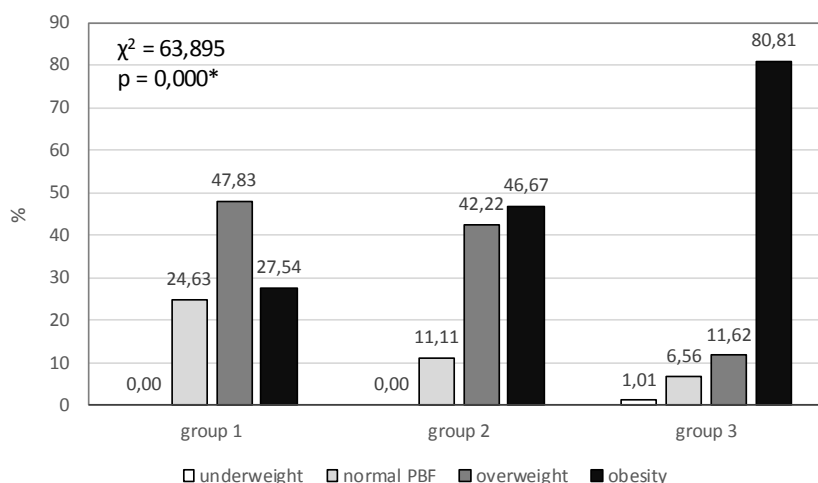
Table 3. Analysis of body composition of the surveyed women in groups discriminated based on their menstrual status

Variable	Group 1			Group 2			Group 3			ANOVA	Newman-Keuls test
	\bar{x}	SD	$\pm 95\%$ CI	\bar{x}	SD	$\pm 95\%$ CI	\bar{x}	SD	$\pm 95\%$ CI	F	
PBF (%)	33.83	5.70	32.46 \pm 35.20	35.53	4.47	34.19 \pm 36.87	37.79	4.96	37.09 \pm 38.48	16.763**	1-2; 1-3; 2-3
LBM (kg)	47.26	5.83	45.86 \pm 48.66	48.51	10.26	45.43 \pm 51.59	44.96	6.36	44.07 \pm 45.85	6.269**	1-3; 2-3
SLM (kg)	43.17	5.15	41.93 \pm 44.41	44.20	9.06	41.48 \pm 46.92	40.87	5.78	40.06 \pm 41.68	7.193**	1-3; 2-3
TBW (%)	34.02	4.20	33.01 \pm 35.03	34.91	7.38	32.71 \pm 37.15	32.38	4.59	31.73 \pm 33.02	6.248**	1-3; 2-3

** statistically significant difference at $p \leq 0.01$

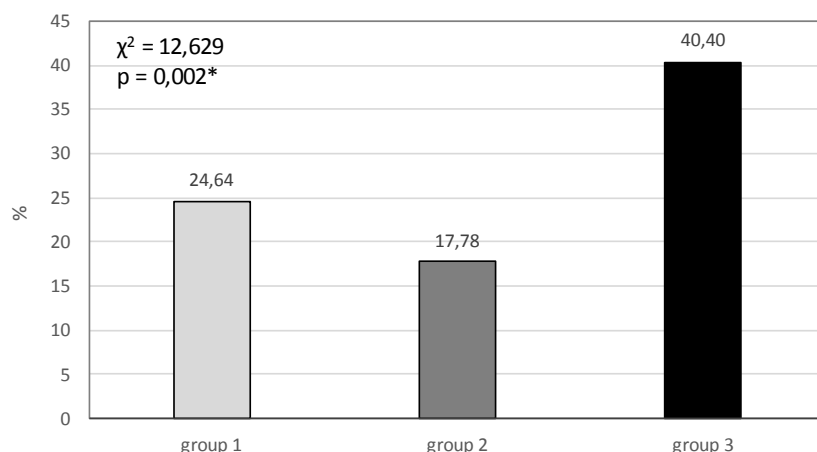
The boundary PBF values determined based on reference values of the IOI 353 body analyzer allowed discriminating three groups of women: with underweight, with normal PBF values, and with overweight and obesity. The highest percentage of premenopausal women were overweight (47.83% of the surveyed women in this group), whereas in groups

2 and 3 the highest percentage of women were obese, however the percentage of women with obesity in group 3 was significantly higher (by 34.14%) than in group 2 (Figure 1). In each of the discriminated group, the WHR index - being a ratio of waist to hip circumference - was calculated for obese women. The highest percentage of women with $\text{WHR} > 0.85$ was observed in group 3 and the lowest one in group 2 (Figure 2).



* statistically significant differences

Figure 1. Percentage of body fat (PBF) in the surveyed women from groups discriminated based on their menstrual status



* statistically significant differences

Figure 2. Percentage of obese women with high WHR values (>0.85) in groups discriminated based on their menstrual status

DISCUSSION

A man's body undergoes successive stages of the physiological development in a strictly defined, irreversible order. Some of the traits of somatic built develop in a progressive way, but once they reach the maximum value they are subject to successive regression. One of such traits is body height which begins to decline at the age of around 40 [7]. This thesis was confirmed in our study. Body height of the women from the oldest age group (postmenopausal group) was on average by 4.5 cm lesser compared to the women from the premenopausal group. A significant regression in body height of women and men over 50 years of life was also observed by *Zajac-Gawlak* and *Groffik* [23]. The size of spine intervertebra cartilage decreases as well as tension of abdomen and chest muscles declines with age, which aggravates spinal curvatures and, hence, contributes to body height decrease.

An opposite trend is observed in the case of the fatty tissue, the percentage of which increases successively in the subsequent stages of individual development, and whose rapid increment is observed in women in the peri- and postmenopausal period [17, 20]. Before the menopause, the adipose tissue is deposited most of all subcutaneously, usually in the thigh and gluteal region. In this period, adipocytes may reach significant sizes, while lipogenesis and lipolysis are strongly enhanced [3, 21]. Accumulation of the fatty tissue in the thigh and, gluteal region results from increased secretion of estrogens and the effect of progesterone. These hormones regulate lipolysis and lipogenesis, have a modulating effect upon expression of transcription factors and proliferation of adipocytes, as well as regulate the production of adipokines – leptin, angiotensin, resistin, and adiponectin [16].

In the perimenopausal period, distribution of fatty tissue increases and the tissue begins to accumulate mainly in the trunk and abdominal region. This is due

to decreasing concentration of estrogen as a result of declining functionality of the ovaries [12, 19]. It is usually concurrent with increased secretion of androgens [2]. Our study demonstrated the highest percentage of body fat (PBF) in bodies of women classified to the postmenopausal group, and the lowest one in bodies of women who were still regularly menstruating. In turn, the analysis of the percentage of subcutaneous fatty tissue, evaluated based on the thickness of selected skinfolds, revealed the highest content of this tissue in perimenopausal women, except for the skinfolds on triceps and calf, the greatest thickness of which were found in the premenopausal women. In addition, we observed an increased value of TER index in the discriminated groups of women, which was indicative of fatty tissue distribution from extremities towards the trunk. This may confirm the above-described observations related to the preponderance of the subcutaneous fatty tissue in the women before the appearance of menopause symptoms and its migration to the abdominal region (visceral adiposity). Different results were reported by *Ho et al.* [4] in a longitudinal survey carried out among women in China. In the successive periods distinguished based on the menstrual status of the women, they demonstrated no changes in adiposity of extremities, but showed increased adiposity of the trunk already in the perimenopausal period and the highest one in the postmenopausal period, which caused a successive increase in the value of the TER index in the subsequent groups of the women.

Distribution of fatty tissue around waist and on hips is described by the waist-to-heap ratio (WHR). Its high value may be indicative of visceral type obesity being typical of the androgenic type of adiposity. In our survey, over 40% of the obese women from the postmenopausal group were characterized by this type of adiposity. In the two other groups, the percentage of women with $WHR > 0.85$ was significantly lower. Investigations conducted by *Rębacz et al.* [15] demonstrated positive

correlations between WHR value and age of the surveyed women being over 50. In turn, Skrzypczak et al. [17] showed statistically significant differences in WHR values between premenopausal and postmenopausal women. These differences were significant regardless of the calendar age of the women. Similar observations were reported by Kanaley et al. [9].

CONCLUSIONS

1. The study demonstrated differences in the somatic built and body composition of women from groups discriminated based on their menstrual status. The highest values of body mass, hip circumference, and thickness of most of the analyzed skinfolds were demonstrated in the perimenopausal group. In turn, the postmenopausal women had the highest percentage of body fat (PBF) and the lowest lean body mass, soft tissue percentage, and total body water.
2. The highest percentage of obese women was found in the postmenopausal group, and 40% of these women had visceral type obesity.
3. The appearance of menopause contributed to changes in distribution of fatty tissue, causing its shift from extremities toward the trunk.

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Conflict of interest

The authors declare no conflict of interest.

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Legislative acts:

6. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off J EU L* 364, 20.12.2006.

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7. The Rapid Alert System for Food and Feed (RASFF) Portal. Available <https://webgate.ec.europa.eu/rasff-window/portal> (accessed 18.10.2010)

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