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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

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ORIGINAL PAPER

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Chemical analysis of substitute drugs of abuse – "legal highs" from Lubuskie province, Poland

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ABSTRACT

Introduction. Interest in substitute drugs of abuse, commonly called "dopalacze" [literally "after-burns"] that is "legal highs", in Poland and abuse of such products, which could pose a significant life hazard, led to legislative action taken by the government. The decision by the Chief Sanitary Inspectorate made shops commonly called "smart shops" close down, while confiscated products were subjected to chemical analyses by national research institutes.

Aim. Determination of the chemical composition and possible presence of active substances contained in tested samples of substitute drugs of abuse.

Material and methods. The research material consisted of 171 samples taken for analysis at the end of 2010 from retailers in Lubuskie province. Samples of "legal highs" were tested in a specialized laboratory of the Institute of Rural Medicine, Lublin, by means of liquid chromatography with mass spectrometry (HPLC-MS).

Results. Laboratory analyses of "legal high" samples showed the presence of different psychoactive substances in 136 samples, representing 80% of the tested products. The compounds included psychoactive substances – MDPV (17%), 4-EMC (10%), AM-694 (10%), JWH-203 (7%), TFMPP (6%), as well as narcotics, such as mephedrone (5% samples), *Piper methysticum* (5%), JWH-250 (4%), JWH-200 (5%) and *Salvia divinorum* (2%). Chemical analyses showed that only 35 samples contained no substances that would affect the physiological and psychological condition of the human body.

Conclusions. Analyses of the chemical composition of "legal highs" showed that they contained a large group of different substances or their mixtures exhibiting psychoactive and narcotic activity that may pose a significant health and life hazard.

Keywords: legal high, substitute drugs of abuse, active substance, intoxicants.

Introduction

Recently there has been witnessed an increased interest in the use and abuse of the original end use (for collectors' only) of a new generation of psychoactive substances colloquially referred to as legal highs or uppers, dramatically spanning their markets share. That these products are gaining popularity with young people all the more calls for alert.

As regards their effects, legal highs may be characterised as products containing foreign substances capable of exerting psychoactive and/or narcotic effects on the human body [1]. They can stimulate the system and enhance the psychophysiological potential of the organism in a non-physiological manner. Although legal highs are not preparations for consumption by humans inter alia, the fact that they are used can be explained by a drive that may eventually bring the user to a state as close as possible to that resembling explicitly delegalised substances [2]. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) defined legal highs as psychoactive substances, produced illegally and altering the characteristics of a nar-

cotic so that they could be legally distributed, exhibiting narcotic effects upon use.

Production of such compounds involves mixtures of natural origin (*Piper methysticum, Salvia divinorum,* caffeine); however, these drugs are mostly based on newly designed and synthesised compounds whose chemical structure is similar to that of known psychoactive substances. Worth noticing is also the fact that the effect on the human body exerted by many components of legal highs has not been fully identified, especially once they have been blended in a single product. Moreover, their technology cannot be seen as controlled production and for that reason the contents of individual compounds in these drugs may vary considerably between different batches of the same product. A significant problem comes up from the fact that some of these substances are not listed on the product label. As a result it is practically impossible to predict any effect of their action. It also greatly hinders possible pharmacotherapy in the case of undesirable symptoms and disorders caused by consumed legal highs [3].

It also needs to be mentioned that until recently substitute drugs of abuse comprised a special group of "legal" narcotics, not covered by specific legal provisions or a ban on sales under the Anti Drug Abuse Enforcement Law or other provisions [4]. It was only amendments to the Anti Drug Abuse Enforcement Law that regulated the legal status of marketing as well as production of substances classified as substitute drugs of abuse. The first amendment of 2009 expanded the list of substances to be monitored by the State authorities to include substances which could have been components of legal highs [5]. A subsequent amendment passed in 2010 added up substances from the group of synthetic cannabinoids as well as a newly specified compound, mephedrone [6]. Furthermore, the same year other alternatives were implemented which provided that substances in legal highs unless subject to separate or appropriate general safety provisions, were to be governed by the provisions of the Anti Drug Abuse Enforcement Law [7]. Concurrently, the Law in force imposed a ban on production and marketing of substitute drugs of abuse, otherwise both being liable to a fine, while the Chief Sanitary Inspector was empowered to supervise enforcement [8].

Aim

As there was no data of scientific worthiness on marketable substitute drugs of abuse available, the objective of the study was shifted to analysis of chemical composition of and on determination of what substances that might have a potentially negative impact on human health and be ingredients of legal highs, on sale in Lubuskie province in 2010.

Materials and methods

The material for analyses comprised 171 samples of substitute drugs of abuse – legal highs, collected from points of sale in Lubuskie province at the end of 2010. The legality of psychoactive agents identified in the material was interpreted in compliance with the then legislation in force. Chemical analyses of legal high samples were performed by the laboratory of the Institute of Rural Medicine, Lublin, specialising in identification of hazardous substances. The test samples were subjected to ultrasound-assisted methanol extraction. The extracts were analysed in high performance liquid chromatography with mass spectrometry (HPLC-MS). A tandem quadrupole mass spectrometer – a time-of-flight analyser was used. A reversed phase system using a C18 column and the linear gradient of the mobile phase: 0.1% formic acid (A) and 0.1% formic acid in acetonitrile was applied to produce chromatographic separation. Compounds were ionised by means electrospray ionisation (ESI). Cations were collected in an MS scan mode within the range of 100–1000 m/z. The compounds were identified on the basis of a proprietary data base, drawn up specifically for the assays.

Results and discussion

The tested material of 171 substitute drugs of abuse revealed the presence of over 20 psychoactive and narcotic substances, with substances found most commonly in the products and the trade names of preparations containing them as shown in **Table 1**. These substances belonged to the group of cathinones, synthetic cannabinoids, piperazines and tryptamines. Moreover, analyses of legal highs detected the presence of structural analogues of these substances whose chemical structure was similar to that of the narcotic and psychotropic substances listed in appendices to the Anti Drug Abuse Enforcement Law. Apart from the above mentioned compounds, legal highs also contained pharmaceuticals, among which lidocaine was assayed most frequent. Moreover, synthetic derivatives of cocaine and legal substances, such as e.g. caffeine, were detected in the tested material. It has to be pointed out that most samples were of poor quality, while a considerable percentage (13%) contained large quantities of

Table 1. A list of psychoactive substances and intoxicants in the studied substitutes and trade names of "legal highs", in which these substances have been identified

Type of substances	Name of substances	Number and percent of detected psychoactive substances narcotic drugs	Commercial name of products
Identified the presence of psychoactive substances*	MDPV (methylenedioxy-pyrovalerone)	29 (17%)	*Ibiza, Speedway, Fresh and funky, L x2, Saddam x2, Kamikadze, Funky, Strong Men, Kokolino, Up up x3, Lord Koks, Ivory Speed, Diablo MDPV, Coco+, $, XXX, Matrix, Strong Man 2x, Nitro v2,0 x2, Funky Style, Speedooo x2*
	4-MEC and/or 4-EMC (4-Methylethcathinone)	17 (10%)	*Mefisto x3, L x2, Przerwa x2, Kokolino, Coco Jumbo x2, Lord Koks, Elektryczny Gisza, Charge+, Speedoo x2, Koko Cherry, Exotic Coco*
	AM-694 (1-(5-Fluoropentyl)-3-(2-iodobenzoyl)-indole)	17 (10%)	*Mr. Nice, Druits Fantasy, AK-47 x2, Bonzo, Spam x2, Baka x2, Nie ma lipy, Niezły wkręt, Spam, Baka, Black widow x2, Hammer, Smart Shiva*
	JWH-203 1-pentyl-3-(2-chlorophenylacetyl)indole)	12 (7%)	*Kosior, Smile, Mocarz x4, Ale urwał, Mr. Grzmot, Summer Mint, Wyrwidąb, Bobby Sense, Hammer, Smart Shiva*
	TFMPP (3-trifluoromethylphenyl-piperazine)	10 (6%)	*Super E, ABC, Kokolino, Loved Up x2, Shrooms x3, Lolly Pop, Super E,*
	Methylone	7 (4%)	*Lick x3, Kamikadze, Limit, Next Explosion, Ex-Extasy*
	Butylone	6 (3,5%)	*Fresh and Funky, Orange x2, Fresh x2, Ocean Snow*
	C11H15N (4-Phenylpiperidine)	8 (5%)	*Smiley, Shrek, Ice x2, Crazy Orange, $, Kolombo, Vanilla Sky*
	Caffeine	8 (5%)	*Diablo, Blue, Super E, Mitsu, Boom, LZD, Fungeez, Koks*
	Naphyrone	6 (3,5%)	*Lick x2, ABC, Kiss, Limit, XXX*
Identified the presence of narcotic drugs from the List*	Mephedrone	8 (5%)	*Vanilla Sky, Kolombo, Smileys, Shrek, Ice x2, Crazy orange, $*
	Piper methysticum	6 (3,5%)	*Beg, Smart shiva, Aztek, AK-47, Smart Shiva x2*
	JWH-250 (2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone	7 (4%)	*Czarny pirat, Aztek, Mr nice, Druits fantasy, Smile x2, Blach widow 2x*
	JWH-200 (1-(2-morpholin-4-ylethyl)indol-3-yl)-naphthalen-1-ylmethanone	8 (5%)	*Marshmallow, Black widow, Bonzo, Buszek, Mr. Grzmot x 2, Dj Feel, Wild beach*
	Salvia divinorum	4 (2%)	*Smart shiva x4*
	Properdin	3 (1,8%)	*Coco Jumbo x2, Koko Cherry*

** Act of 10 June 2010 amending the Anti Drug Abuse Enforcement Law (Journal of Laws Dziennik Ustaw of 2010 no. 143 item 962)*

chemical contaminants of no psychoactive effects (e.g. tributylamines, dihexylamines, diheptylamines and heptylamines).

Chemical analyses showed that the psychoactive compound identified most frequently in the tested material was methylenedioxypyrovalerone – MDPV (17% samples), a compound exhibiting, among others, stimulant properties. At lower doses it evokes effects comparable to those of methylphenidate, whereas in higher amounts it is similar to cocaine [9]. Stimulant properties observed following the use of MDPV entail an increase in energy, enhanced concentration, sexual arousal and mild empathogenic effects [9, 10]. Side-effects of this substance include fatigue, insomnia, trismus, fever, hyperhidrosis, cardiac arrhythmia, dilated pupils, headache, loss of appetite, kidney pain, numbness as well as respiratory problems. MDPV overdose causes long-term panic fits and anxiety [10].

Another psychoactive substance whose presence was detected in 8% of the tested samples, was an organic chemical compound, 4-methylonecathionine (4-EMC), a derivative of actinon whose chemical structure is similar to that of mephedrone. 4-EMC has a stimulatory effect on the central nervous system. It is a dopamine and noradrenaline reuptake inhibitor. It causes excitation and euphoria, attention problems, dilated pupils, flushing, hand trembling and tingling sensation. It also leads to elevated blood pressure and arrhythmia. At large doses of 200–500 mg administered e.g. intranasally euphoria is experienced, comparable to that after the use of mephedrone [11].

In this study in the analysed substitute drugs of abuse the synthetic psychoactive substance, AM-694, was identified in 8% samples. A combination of AM-694 with CB1 and CB2 receptors in the brain stimulates the feeling of pleasure and euphoric states [12]. Depending on the dose, this compound enhances the sensation of relaxation. Adverse effects include first of all ocular irritation and hyperaemia, fluctuating arterial blood pressure, dyskinesis, dizziness, vomiting and apathy. In extreme cases anxiety attacks, visual and auditory hallucinations may occur [11].

Analysis of the chemical composition of the tested material in 7% samples showed also the presence of the cannabinoid receptor agonist JWH-203 whose effect on the human body consists among others in inhibiting the neurotransmitter activity. This compound exhibits a strong affinity to CB1 and CB2 receptors [13, 14]. Dose-wise, JWH-203 causes deep relaxation, increased appetite, spatial disorientation, irritations and euphoric states. Adverse effects are connected mainly with ocular hyperaemia, fluctuations of arterial blood pressure, spatial disorientation, dryness of the mucosa and dizziness [13, 14].

A serotonin receptor agonist: 3-trifluoromethylphenylpiperazine (TFMPP), a psychoactive substance exhibiting stimulant properties and frequently combined with benzylpiperazine (BZP), was detected in 6% of the tested samples. It was observed that the effects of this mixture mimic those of 3,4-methylenedioxymetamphetamine (MDMA) [15, 16]. TFMPP together with BZP influence among others serotonin and noradrenalin levels, psychedelic effects and euphoric states, as well as stimulate hyperkinesia, the tingling sensation and the sensation of bliss. A single use effect of BZP mimics the those of amphetamine, while for TFMPP the effects resemble those of ecstasy (i.e. less than 30% MDMA activity) [17, 18, 19].

Chemical analyses of the tested material in 5% of the samples also showed the presence of methylone. Effects of its administration mimic those observed for ecstasy, although certain differences are observed between these substances [20, 21, 22]. According to Alexander Shulgin, who was the first to synthesise methylone, similarly as MDMA this agent exhibits antidepressant properties and influences the general feeling and enhances the sensation of pleasure [20]. Methylone also causes numerous side-effects, e.g. excessive sweating, dilated pupils, nausea, vomiting, abdominal pains, irritation, tachycardia and depression [21, 22].

Chemical analyses of tested substitute drugs of abuse showed that apart from the new substances not legally specified, samples of legal highs also contained substances controlled by the Anti Drug Abuse Enforcement Law in force since 2010, such as mephedrone, *Piper methysticum, Salvia divinorum* and synthetic cannabinoids (JWH-250, JWH-200).

In the case of the above mentioned narcotics the presence of mephedrone was detected in 5% of the tested samples. This agent exhibits effects mimicking those of MDMA, amphetamine, as well as cocaine [23]. It causes extreme euphoria, logorrhoea, increased libido and intellectual stimulation [24]. Adverse effects resulting from the administration of mephedrone are connected with palpitation, increased arterial blood pressure, intensive sweating, a cold wave sensation, as well as headaches and dizziness, gnashing teeth and trismus [25].

In 3.5% of the tested samples analyses detected *Piper methysticum*, a plant from the pepper family (*Piperaceae*), grown on islands in the western Pacific. Inhabitants of that regions use infusions from *Piper methysticum* for medicinal, sedative and relaxation purposes [26]. It contains compounds from the group of kavalactones, responsible for psychotropic and spasmolytic effects [27]. They cause a state resembling alcohol intoxication, as well as visual and auditory disorders. Worth noticing here is a study by Stickel et al., which indicated that *Piper methysticum* extracts and preparations may exhibit hepatotoxic effects [28].

Moreover, a chemical analgesic JWH-200, a synthetic cannabinoid receptor agonist, was also detected in the tested material (5% of the samples). JWH-200 shows affinity to the CB1 receptor and its action consists in the inhibition of neurotransmission [29]. Its psychoactive effect is stronger than that of tetrahydrocannabinol (THC) while a sedative action is several times weaker than that shown by THC [30]. Depending on the dose this compound brings about euphoric

states and a sensation of considerable relaxation, and stimulates olfactory and gustatory sensitivity. Adverse effects are connected with irritation and ocular hyperaemia, fluctuating arterial blood pressure, locomotor disorders and dizziness, while in extreme cases they include anxiety attacks, visual and auditory disorders, delusions and chronic mental diseases [11].

The chemical analysis of the tested material also showed (in 4% of the legal highs) the presence of a synthetic cannabinoid JWH-250, being an analgesic. It was synthesised by John Huffman as an analog and metabolite of THC. JWH-250 is a cannabinoid receptor agonist whose effect results from inhibition of neurotransmitters. This compound shows a strong affinity both to the CB1 and CB2 receptors [14]. Its binding with the above mentioned receptors in the brain causes an enhanced sensation of pleasure. The adverse effects of this substance are connected with ocular hyperaemia, fluctuating arterial blood pressure, locomotor disorders, dryness of the mucosa, dizziness and apathy. Anxiety fits, visual and auditory hallucinations, delusions, chronic mental diseases requiring hospitalisation are reported in extreme cases [11].

Analyses showed that 2% of the tested samples contained also one of the most potent natural hallucinogens, Diviner's sage (*Salvia divinorum*). In this plant the hallucinogenic effect is provided by salvinorin A, present in leaves; a compound identified in 1982 by Alfredo Ortega, and, independently, slightly later, by Leander Valdes [31, 32, 33]. Diviner's sage has a short, but strong hallucinogenic effect, accompanied by a lack of control over one's behaviour and serious locomotor impairment [33, 34]. A case of antidepressant effects was also documented [35]. The hallucinogenic effect of the preparation following oral administration is intense and lasts approximately for one to two hours [36, 37]. Individuals using such products declared confusion, as well as perception of sounds from the environment, colours and smells [37]. Side-effects of Diviner's sage have not been sufficiently identified, although users may be susceptible to injuries [38].

The body of scientific literature contains very few studies presenting analyses of chemical composition of commercially available substitute drugs of abuse, colloquially called legal highs. **Table 2** gives results of analyses verifying the presence of psychoactive substances in such products. Tests conducted by the National Institute of Drugs, Warsaw, showed that 15.9% of the examined samples contained narcotics banned by the legal regulations in force and illegally marketed active pharmaceutical agents. All tested samples contained psychoactive substances, mainly structural analogs of controlled substances. Overall approximately 90 substances were identified in analyses of these preparations, including over 57 exhibiting psychoactive properties (these included among others derivatives of cathinone, piperazine, tryptamine, phenylethylamine, synthetic cannabinoids and active pharmaceutical agents) [22]. With reference to the results presented in this paper the analyses performed by the National Institute of Drugs, Warsaw, showed that MDPV (22% vs. 17%) and butylon (11% vs. 3.5%) were found in a slightly higher percentage of examined substitute drugs of abuse, at a lower percentage for TFMPP (4% vs. 6%). High amounts of psychoactive agents in legal highs were also reported in studies conducted by the Research Institute of Sport, Warsaw, in which case the highest percentages of active substances were recorded for caffeine (44%), MDPV (22%), TFMPP (16%), JWH-81 (13%), MBZP (13%), ephedrine (9%), methylone (9%) and butylon (7%) [23]. In turn, results of tests conducted by the Institute of Forensic Research showed that the percentage shares of individual active substances in all tested samples amounted to 19% for caffeine, 14% for JWH-081, 12% for MDPV, 11% for RCS-4, 10% for butylon, 9% for JWH-122, 7% for lidocaine and 6% for TFMPP, respectively [23].

For this reason emphasis has to be made here that – similarly to studies conducted by the Lublin Institute of Rural Medicine, the Warsaw Research Institute of Sport, and the Warsaw Institute of Forensic Research – results

Table 2. Most common active substances in samples of legal highs tested by different research institutes

The active substances	Percent of detected active substances		
	National Medicine Institute	Institute of Sport	Institute of Forensic Research
MDPV	22	22	12
JWH-81	18	13	14
TFMPP	4	16	6
Butylone	11	7	10

of chemical analyses of legal high samples presented in this paper show that commercially available substitute drugs of abuse very often contained psychoactive agents, such as e.g. MDPV, JWH-081 and TFMPP. These data suggest that despite legislative action legal highs may still pose a real social threat and health hazard, as evidenced by an increased interest in this issue on the part of many institutions dealing with narcotic abuse issues, both on the national and European levels.

Conclusions

1. Substitute drugs of abuse, also called legal highs, contain both substances of natural origin obtained from plants and synthetic products.
2. Legal highs contain a group of diverse agents or their mixtures exhibiting narcotic or stimulant effects.
3. Analyses detected structural analogs of substances whose chemical structure is similar to that of narcotics and psychotropic substances listed in appendices to the Anti Drug Abuse Enforcement Law.
4. Analyses of legal high samples show that they are complex products, containing a number of active substances.
5. Required are further studies into the impact of active substances in legal highs on the human body, while educational campaigns need to expose harmfulness and hazards related to abuse of such products.

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ORIGINAL PAPER

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The influence of age and dosage on the pharmacodynamics of dexmedetomidine in rabbits

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ABSTRACT

Aim. This study aimed to examine the influence of maturation and dosage on the sedative and haemodynamic response observed in rabbits after the administration of dexmedetomidine.
Material and methods. The pharmacodynamics of dexmedetomidine was studied on 14 healthy New Zealand white rabbits at three periods of maturation; stage 1–1.5 months old, stage 2–2.5 months old and stage 3–6.5 months old ones. The administered dose of dexmedetomidine ranged from 25 μg/kg to 300 μg/kg of body weight. The pedal withdrawal reflex was used to measure the duration of anaesthesia. The heart rate and mean arterial pressure were measured at the third stage of the study to evaluate the haemodynamic response. A simple pharmacodynamic relationship between the dose and the duration of anesthesia was used to describe the data.
Results. We observed that young rabbits were less sensitive to dexmedetomidine than adult animals, as was reflected by the pedal withdrawal reflex, and we found that the haemodynamic response to dexmedetomidine depended on dosage of the drug. Dexmedetomidine decreased the mean blood pressure in a dosage-dependent manner with the highest decrease observed for the lowest dose. As the dose increased, the hypotensive effect of the drug was less noticeable. After the administration of dexmedetomidine the heart rate decreased to the same value regardless of the dose applied.

Keywords: dexmedetomidine, rabbits, pedal withdrawal reflex.

Introduction

Dexmedetomidine is an α2-agonist used for a short-term sedation of adults in the ICU. The central hypnotic mechanism is not associated with GABA, as in the case of benzodiazepines and propofol. The stimulation of an adrenergic system in the central nervous system at locus coeruleus elicits a conscious sedation whereas the analgesic properties are the effect of substance P suppression in the dorsal horn of the spinal column. Cardiovascular effects are bradycardia and hypotension. The lack of respiratory inhibition is one of the advantages of the drug [1]. Dexmedetomidine exerts cardioprotective, nephroprotective, hepatoprotective and neuroprotective activity, and the latter one seems to be of special importance to patients in the ICU, probably preventing the occurrence of delirium [2, 3].

Clinical studies on healthy volunteers have shown that dexmedetomidine pharmacokinetics is best described by a two-compartment model. The distribution phase is rapid with a half-life of 6 min. The drug

is highly protein bound. The volume of distribution at steady state equals 1.33 L/kg or 118 L. Metabolism of dexmedetomidine occurs mainly in the liver by cytochrome P450 (CYP) enzyme 2A6 and uridine diphosphate glucuronosyltransferase (UGTs) glucuronidation pathways, specifically UGT1A4 and UGT2B10, to inactive metabolites. Dexmedetomidine clearance equals 39 L/hr, and the elimination half-life of 2–2.5 hours [4].

Currently, dexmedetomidine in humans has been registered for sedation. There are still intensive scientific studies on possibilities of dexmedetomidine perioperative application in anaesthetized patients as premedication, adjuvant in neuraxial and general anaesthesia. The scientists test its narcotic-sparing effects, as well as effectiveness for awake fiberoptic intubation, and intraoperative sedation [2].

Dexmedetomidine, despite of the fact that it is registered only for adults, has been recently proposed for sedation in the pediatric ICU settings [5]. However, the problem which occurs in almost every drug administered in the PICU settings, is the lack of detailed pharmacokinetic and pharmacodynamic information on the drug in children population. Up to 70% of the drugs in pediatric intensive care and 90% of the drugs in neonatal intensive care, are prescribed in an off-label or unlicensed manner. Pediatric dosing regimens are usually empirically derived from adult regimens using linear extrapolations based on body weight. However, this calculation does not take into account all the physiological changes occurring during the maturation process. Child-ren differ from adults in their response to drugs, one of the reason may be a different metabolism which is faster in children than in adults. These differences cause changes in the pharmacokinetics (PK) and/or pharmacodynamics (PD) of drugs and they may also vary among children of different ages. This may lead to therapeutic failure and severe adverse effects or even death, like in the example of fatal complication after propofol infusion used for long-term sedation in neonates [6, 7]. Large interindividual differences in the pediatric population and also the need to broaden patients group to children for most of commonly used drugs show the problem which is occurring in PICU settings. The lack of PK and PD information on drugs in children has led to the European Regulation, which came into force in 2007. This law recommends to perform the studies in children in the early stages of the development of a new drug [6, 8, 9, 10]. Due to the ethical reason, it is difficult to eliminate the problem of interindividual variability and examine the influence of maturation process on pharmacodynamics of a given drug in one child in different stages of its ripeness. The study conducted in the animals seems to be a rational solution.

To examine pharmacodynamic response to given drug in the laboratory setting, scientists use various reflexes of different animals. One of them is the pedal withdrawal reflex. This reflex was proposed for assessing the sedative pharmacodynamic response to various drugs: propofol [11], midazolam [12], diazepam-ketamine-pentazocine [13] in rabbits. The aim of our study was to examine the influence of the maturation process on the sedative pharmacological response to dexmedetomidine in rabbits and to determine the influence of different doses of dexmedetomidine on rabbits' monitored hemodynamic parameters.

Materials and methods

All experiments with animals were conducted with approval by the local animal care committee and their care was in accordance with institutional and international guidelines.

Animals

The study was divided into 3 periods according to three stages of animals' growth. 14 healthy New Zealand white rabbits with the average weight of 1.2 ± 0.1 kg (mean ± SD), aged 42–54 days, were used in the first period. In the second period the average weight and age of the animals were 1.8 ± 0.2 kg and 75–85 days, respectively, whereas in the third period they were 3.2 ± 0.4 kg and 169–214 days, respectively. Two rabbits were not used in the second period due to the procedural reasons, whereas three new adult animals were added during the third period to examine the haemodynamic response to dexmedetomidine.

All the rabbits were housed individually in stainless steel cages under controlled environmental conditions. The room temperature and relative humidity were controlled at 20–22°C and 50–60%, respectively. The rabbits were provided with 125 g of commercial pelleted diet once a day between 08:00 and 12:00 h and they drank tap water ad libitum. Dexmedetomidine (Dexdomitor 0.5 mg/ml, Orion Corporation, Finland) was administered intravenously as a single bolus at different doses (25 µg/kg, 35 µg/kg, 50 µg/kg, 75 µg/kg, 100 µg/kg, 140 µg/kg, 150 µg/kg, 200 µg/kg, 250 µg/kg, 300 µg/kg of body weight). The experiment was conducted from November, 2013 till April, 2014.

Animals were fasted on the day of sedation. Just before infusion, the rabbits were weighed and placed into restraining cages. The hair over the auricular artery

and on the tail was removed and the skin cleaned with alcohol. A 22G catheter was inserted percutaneously into the central auricular artery and fixed with tape. Dexmedetomidine was administered to the marginal vein of the opposite ear. Warm fluids (38°C) were infused after each blood sampling. Rabbits from period 3 of examination additionally had arterial catheter attached to monitoring system Philips IntelliVue MP5 with Philips M1567A catheter and sedation included monitoring of cardiac and respiratory status.

The animals were oxygenated with 100% oxygen at 3 L/min via a facial mask; oxygen flow was continued until the animals recovered completely. Appropriate dose of dexmedetomidine was administered as a bolus injection. Rabbits from stage 3 of examination had additionally monitored heart rate and recorded from the curve of the arterial blood pressure during the first 30 minutes from administration. Blood oxygen saturation was monitored from the shaved tail by pulse-oximetry.

In order to monitor the level of sedation, two basic reflexes, i.e. pedal withdrawal reflex and corneal reflex, were tested. The reflex was tested in the following periods: initially, every 20, 40, 50 and 60 seconds, and every few minutes after administration, until full recovery. In this study, the recorded starting point and endpoint was loss and return of the pedal withdrawal reflex, whereas the corneal reflex was always retained during the experiments. The reflex is elicited by extending the limb and stimulating it to achieve its withdrawal.

Pharmacodynamics

The main parameter derived from pedal withdrawal reflex measurements is the duration of anesthesia (t_d). Assuming an IV infusion and one compartment disposition model the duration of anesthesia can be described by the following equation (1) [14]:

$$t_d = \frac{1}{k}(\ln D - \ln D_{\min}) \quad \longrightarrow \quad (1)$$

where k denotes elimination rate constant, D denotes the administered dose, and D_{min} denotes the minimum effective dose. For more complex PK the equation can also be used, as the terminal (the slowest) phase will mostly contribute to the time of responsiveness after administration of an anaesthethic. In such case, k will denote the slope of the terminal phase.

The statistical analysis was done in Matlab® Software version 7.0 (The MathWorks, Inc., Natick, MA, USA) using the Curve Fitting Tool.

Results

Table 1 shows comparison between administered dose of dexmedetomidine and rabbit response on the basis of loss and return of pedal withdrawal reflex. In all stages of our research, time required for loss and return of the reflex shortens and elongates, respectively, with given dose of dexmedetomidine. Small doses entail more time to cause anaesthesia and consequently shorten the duration of sedation. The higher doses of dexmedetomidine the longer is sedation and more time needed for pedal withdrawal reflex return.

The proposed model adequately described the experimentally determined durations of anesthesia (td) as shown in **Figure 1**. The linearity of anaesthesia duration with respect to logarithm of dose indicated that a linear PK can be expected within the studied range of doses. The following parameter estimates (coefficient of variation) were obtained for Stage 1: D_{min} = 20.8 (28.5%) mg/kg and k = 0.0369 (17%) min^{-1}; Stage 2: D_{min} = 11.4 (39.8%) mg/kg and k = 0.0411 (18.8%) min^{-1}; and Stage 3: D_{min}= 8.60 (83.0 %) mg/kg and k = 0.0367 (32.5%) min^{-1}. The obtained terminal elimination rate constants corresponded to half-lifes of 18.8 min (Stage 1), 16.9 min (Stage 2) and 18.9 min (Stage 3). No age related changes of terminal elimination rate constant were observed. However, a decrease of a minimal effective dose with rabbits age was evident. It suggested that young rabbits were less sensitive for dexmedetomidine than old ones.

Influence of the drug on blood pressure and heart rate was summarized in **Table 2** and **Figure 2**. The baseline MAP equaled 92 ± 8.4 mmHg. It decreased on average upon dexmedetomidine administration to the value of 76 ±9.6 mmHg**.** Interestingly, the highest decrease was observed for the smallest dose (35µg/kg). For larger doses the decrease in MAP was less apparent. The significant slope of 0.103 mmHg/(mg/kg) between mean MAP and dexmedetomidine dose was observed. This value indicates that the increase in the dexmedetomidine dose of 10 µg/kg is followed by the increase in mean MAP of 10.3 mmHg. This equation seems valid for the range of studied doses (35–250 µg/kg). The dexmedetomidine also decreased the heart rate from the initial (baseline) values of 207 ± 49.0 beats/min to 100 ± 20.8 beats/min noted during the anesthesia. The decrease in heart rate was constant, independent of the administered dose.

Discussion

During the study we examined the relationship between the pharmacological response to dexmedetomidine and

Table 1. Comparison of dose-dependent loss and return of pedal withdrawal reflex and duration of sedation in the subsequent stages of examination in healthy rabbits

Dose	Rabbit no.	Loss of pedal withdrawal reflex	Return of pedal withdrawal reflex	Duration of sedation
STAGE 1				
[μg/kg]		[min]	[min]	[min]
25	8	01:10	14:30	13:20
35	11	02:30	15:30	13:00
50	10	00:35	16:00	15:25
	13	00:40	09:00	08:20
	14	00:20	21:05	20:45
75	6	00:50	36:00	35:10
	9	00:40	35:00	34:20
100	4	00:35	60:00	59:25
140	3	00:40	59:30	58:50
150	5	00:20	56:00	55:40
200	1	00:40	27:40	27:00
	7	00:15	67:00	66:45
250	12	00:15	73:00	72:45
300	2	00:20	80:00	79:40
STAGE 2				
35	8	00:50	17:00	16:10
	11	00:36	19:00	18:24
50	10	00:20	23:30	23:10
	13	00:25	30:20	29:55
	14	00:35	45:00	44:25
75	6	00:55	57:00	56:05
	9	00:40	59:00	58:20
100	4	00:30	36:00	35:30
150	5	00:50	59:16	58:26
200	1	00:30	60:00	59:30
	7	00:26	72:00	71:34
250	2	00:35	80:00	79:25
STAGE 3				
35	8	01:25	27:00	25:35
	11	01:15	35:45	34:30
50	13	01:50	40:00	38:10
	14	00:50	46:00	45:10
75	6	01:50	55:00	53:10
100	2	01:00	105:00	104:00
	4	01:00	82:00	81:00
150	17	00:30	73:00	72:30
	18	00:50	95:30	94:40
200	1	00:45	62:16	61:31
	7	00:30	74:00	73:30
250	16	00:20	127:00	126:40
	19	00:50	53:00	52:10

the process of maturation in rabbits and the influence of the dosage of dexmedetomidine on the haemodynamic parameters. There have been reports on the use of dexmedetomidine in other animals, like rats [15], dogs [16], cats [17] or gerbils [18], but only adult ones. The administration of dexmedetomidine has also been tested on adult rabbits, mainly in the context of neuroprotection, ventilatory and haemodynamic properties [19, 20]. In our study we used the pedal withdrawal reflex to measure the duration of sedation after single intravenous administration. We found it useful for testing the level of sedation and anaesthesia both in adult

Table 4. The baseline and average mean arterial pressure and heart rate in rabbits after dexmedetomidine administration at the 3rd stage of the experiment

Dose	Rabbit no.	Body weight	Mean arterial pressure (MAP) [mmHg]			Heart rate (HR)bpm		
[μg/kg]		[kg]	Baseline	Mean	Baseline- Mean	Baseline	Mean	Baseline- Mean
35	8	3.2	105	68	37	230	128	102
	11	3.1	86	63	23	235	100	135
50	13	2,9	84	62	22	129	84	45
	14	2.8	94	64	30	190	159	31
75	6	3.3	95	70	25	214	91	123
	9	3.0	92	75	17	217	102	115
100	2	3.1	79	80	-1	183	81	102
	4	3.2	90	74	16	243	97	146
150	17	3.7	109	84	25	213	94	119
	18	3.9	91	77	14	306	106	200
200	1	3.2	86	95	-9	109	75	34
	7	2.8	102	85	17	158	83	75
250	16	4.0	82	81	1	243	91	152
	19	2.6	91	83	8	234	103	131

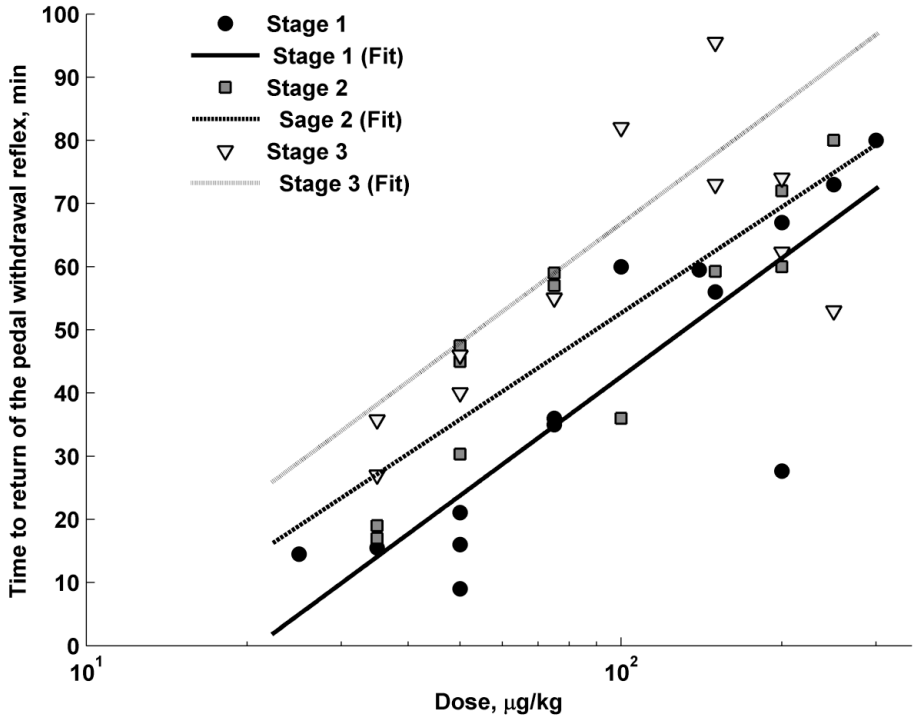

Figure 1. The relationship between the dexmedetomidine dose and the time to return of the pedal withdrawal reflex. The symbols denote the experimental data. The straight lines indicate the best fit of the Eq. 1 to the data for each stage

and young rabbits. There have been numerous studies that used this reflex to examine other drugs, such as propofol, midazolam, pentobarbital alone or in mixtures: diazepam-ketamine-pentazocine, katamine-xylazine, midazolam-xylazine-alfentanil [11, 12, 13, 21]. Our study was unique for the following reasons. First of all, we divided the study into 3 periods, taking into account the process of maturation of all animals individually. At stage 1 we chose young rabbits (42–54 days old), mostly because there is no available literature on intravenous administration of dexmedetomidine to this age group. We conducted the consecutive stages of the study with the same animals but at different ages to determine the influence of their maturation process on the pharma-

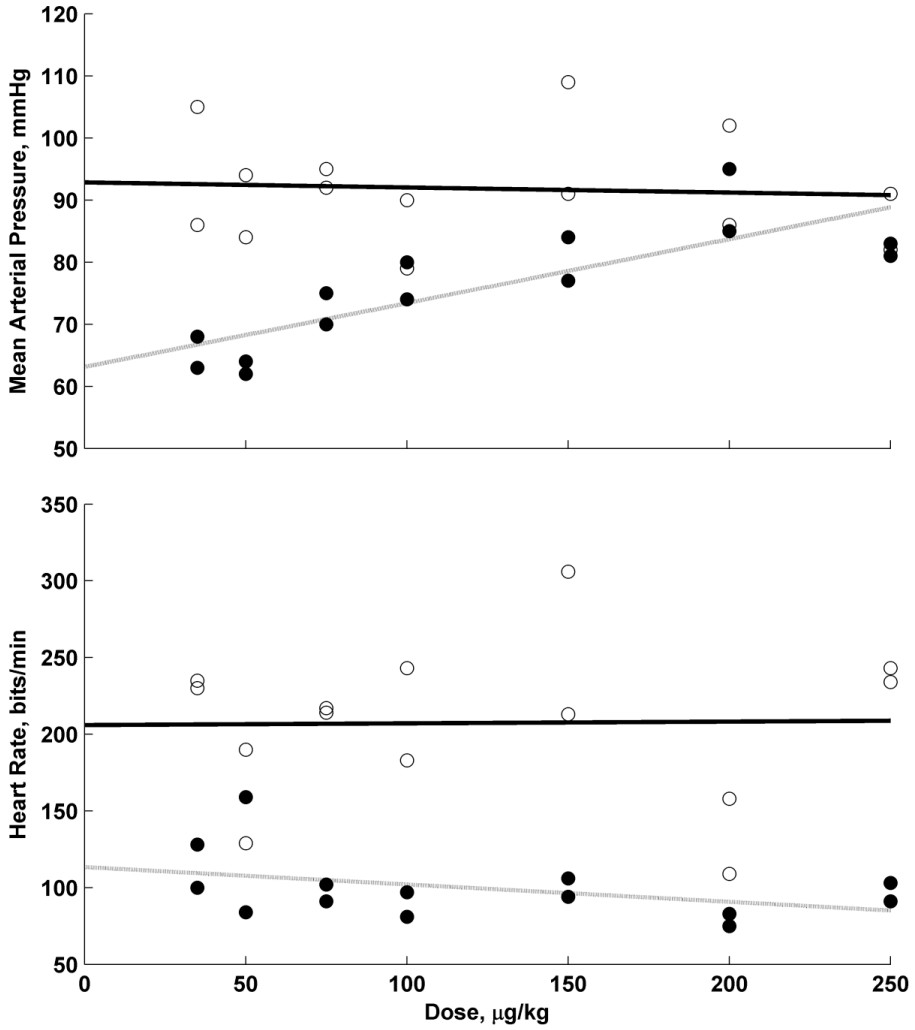

Figure 2. The relationship between the dexmedetomidine dose and mean arterial pressure and heart rate. The open symbols denote the baseline values and closed symbols the mean values noted during the anaesthesia for each rabbit. The straight lines indicate the trends in the data

codynamics of dexmedetomidine. Thus, we eliminated the problem of interindividual variability. We chose the rabbits whose weight was around 1 kg, as the titration of dexmedetomidine in smaller animals is more difficult and thus its application is limited [22]. For the first time we also examined the linearity of the pharmacodynamics of dexmedetomidine and its haemodynamic effects on rabbits at very narrow dosage intervals. The linearity of the pharmacokinetics of dexmedetomidine is of great clinical importance because there is only a limited number of data concerning the pharmacokinetics of this drug administered in prolonged (> 24 h) infusion at large doses (up to 2.5 μg/kg/h) and there are no such studies on children [23]. The results show the linear relationship between the logarithm of the administered dose of dexmedetomidine and the duration of sedation measured with the pedal withdrawal reflex. During our study we observed that as the rabbits grew older, they became more sensitive to the sedative effects of dexmedetomidine. We cannot transfer the results of our studies directly to humans, but we can conclude that children may require higher doses of dexmedetomidine than adults. However, it is necessary to make a pharmacokinetic analysis to explain this phenomenon fully and we are planning to do it in further parts of our project. Moreover, in the third period we examined the influence of different doses of the drug on the haemodynamic response. Thus, we confirmed the hypotonic effect of dexmedetomidine. As far as this effect is concerned, we noted that in spite of the fact that dexmedetomidine is known for its hypotensive effect, as doses of the drug increased, the blood pressure tended to increase, too. Potts et al. conducted a study on children after a cardiac surgery. The aim of their research was to determine the influence of the pharmacodynamics of dexmedetomidine on the haemodynamics of the circulatory system

in the children who had undergone cardiac surgeries and received a bolus dose of 1–4 μg/kg within 10 minutes. Large doses of dexmedetomidine were observed to cause the contraction of smooth muscles in blood vessels. This resulted in a short increase in the arterial pressure, whereas the application of small doses of the drug had the hypotensive effect [24]. Three doses of dexmedetomidine were previously applied to adult rabbits (20, 80 or 320 μg/kg) by Zornow who studied the haemodynamic effect of dexmedetomidine on rabbits. He noted that the administration of the three doses under study caused a decrease in the heart rate in a significant and dosage-related fashion, whereas the mean arterial blood pressure did not change significantly at any dose or time. Similarly to Zornow, we noted a significant decrease in the heart rate. However, contrary to his findings, we observed significant dosage-related changes in the MAP. In our study we observed that small doses of dexmedetomidne resulted in a significant drop in the blood pressure, but this effect was less noticeable with higher doses of the drug. The most important difference between these two studies consists in the fact that although our doses were the same as Zornow's, the intervals were more naoorw (25, 35, 50, 75, 100, 140, 150, 200, 250, 300 μg/kg) [20].

To conclude, young rabbits are less sensitive to the sedative pharmacodynamic response of dexmedetomidine than adult animals. The haemodynamic response to dexmedetomidine depends on dosage of the drug. There was a significant decrease in the blood pressure observed with small doses of the drug, but the effect was less noticeable with higher doses. After the administration of dexmedetomidine the heart rate decreased regardless of the dose applied.

Conflict of interest

The authors declare that there are no conflicts of interest.

Abbreviations

ICU – Intensive Care Unit
GABA – Gamma Aminobutyric Acid
PICU – Pediatric Intensive Care Unit
PK – Pharmacokinetics
PD – Pharmacodynamic
MAP – mean oriented blood pressure

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Prevention of cardiovascular disease and eating behavior in group of women and men aged 20 to 30 years

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ABSTRACT

Introduction. Proper nutrition and the use of preemptive care can prevent the development of cardiovascular diseases, which are the main cause of death in the world.
Aim. The aims of this study were to evaluate the nutritional habits, nutritional status, assessment of knowledge and use of nutritional prevention among group of women and men aged 20 to 30 years, living in Wielkopolska in the aspect of CVD risk.
Material and methods. In this study the method of 24-hour dietary recall to evaluate nutrition intake was used. To determine the nutritional status the anthropometric measurements (weight, height, waist) were taken, and BMI values were calculated. Assessment of knowledge and use of nutritional prevention in cardiovascular disease was performed by a questionnaire. The atherogenicity of the diets was evaluated using Keys score.
Results. The analysis of daily food rations showed differences between dietary guidelines and respondent diets, mainly in protein, fat, saturated fat and cholesterol intake. Tested prevention factors were well known in both groups. The exception involved the reduction of sodium intake in the diet. The use of preventive factors in daily routine differentiated treatment in groups. In the studied group men often exercised regularly, while women often limited intake of animal fats, cholesterol and simple sugars.
Conclusion. Inadequate nutrition (mainly incorrect saturated fatty acids (SFA), cholesterol and dietary fiber intake) and insufficient prevention care in the studied group may increase the risk of cardiovascular disease in the future.

Keywords: prevention of cardiovascular disease, nutrition intake, nutritional status, eating intake.

Introduction

Improper dietary habits can significantly affect the development of civilization diseases, including cardiovascular diseases (CVD), which are one of the most common causes of death in the world [1]. Improper dietary behavior in childhood and during youth have a significant impact on the development of many diseases in adulthood, among other can lead to the development of obesity, diabetes, cancer and cardiovascular disease [2, 3].

Aim

The aim of this study was to assess the knowledge on the prevention of cardiovascular disease and to evaluate of eating intake, nutritional status and dietary habits in group of men and women aged 20 to 30 years living in Wielkopolska.

Material and methods

The study was performed between February and June 2012 on randomly selected 195 women and 184 men aged 20 to 30 years, living in Wielkopolska. Applied methods were based on a questionnaire, which consisted of three parts: the first part concerned the anthropometric measurements, the second was associated with the knowledge about prevention in cardiovascular diseases, while the third was used to determine the daily

intake of energy ingredients, sodium, dietary fiber and cholesterol. The nutritional status of the study group was based on the Body Mass Index (BMI). The eating intake were evaluated by the method of 24-hour dietary recall. The atherogenicity of the diets was evaluated using Keys score calculated by the following formula [4]:

$$Keys\ score = 1.35 \times (2 \times SFA\ \% - PUFA\ \%) + 1.5 \times (\sqrt{}\ ((cholesterol\ [mg] / 1000\ kcal))$$

where: SFA – saturated fatty acids, PUFA – polyunsaturated fatty acids.

The daily energy intake and content of selected nutrients in the diets were compared to the "Human dietary standards" issued by M. Jarosz and B. Bulhak-Jachymczyk. The amount of consumed food products were determined by the "Album of photographs of food products and dishes" issued by the National Food And Nutrition Institute [5, 6]. Analysis of the results was performed using the application prepared in MS Access. The statistical analysis was performed using Statistica 10 software. Normality was tested using the Shapiro-Wilk statistical test. Diet effects were analyzed using a Mann-Whitney U test, and dietary habits were analyzed using a Fisher's exact test ($p \leqslant 0.05$).

Results

The BMI values in the study group indicated: a normal weight ($18.5 \leqslant BMI < 25$ kg/m^2), however the values were higher among group of men (23.3 kg/m^2) than in the group of women (20.6 kg/m^2). Overweight ($25 \leqslant BMI < 30$ kg/m^2) was observed in 37% of men and 9% of women, while obesity ($BMI \geqslant 30$ kg/m^2) in 4% of men and 1% of women. The BMI values (less than 18.5 kg/m^2), which indicate underweight were observed in 14% of all surveyed women and 1% of men.

The median waist circumference was in the normal range and was successively for men: 89 cm and for women: 71 cm (**Table 1**).

The daily food intake of the study subjects showed discrepancies compared to the dietary guidelines. They focused on the intake of fat, dietary fiber, saturated fatty acids and cholesterol, particularly among males (**Table 2**).

The median energy intake in the group of men was 2762 kcal/day, and in the group of women 2066 kcal/day, indicating a slight excess of energy in daily food rations in women (**Table 2**).

The protein intake in the studied group, regardless of gender was in the recommended range.

Median protein content in the analyzed diets was 108 g/day in group of men (15.8% of the dietary

Table 1. Anthropometric characteristic in the group of women and men

Parameter	Men Median (X ± SD)	Women Median (X ± SD)
Age (year)	24 (25 ± 5)	24 (25 ± 3)
Body weight (kg)	81 (81 ± 11)	57 (59 ± 10)
Height (cm)	181 (181 ± 7)	165 (167 ± 7)
Waist circumference (cm)	89 (89 ± 8)	71 (72 ± 9)
BMI (kg/m^2)	23.3 (24.6 ± 3.2)	20.6 (21.1 ± 2.9)

Table 2. Characteristic of daily food ratio in woman and man group

Parameter	Men			Women			p
	Median ($\bar{x}$ ± SD)	Q1	Q3	**Median** ($\bar{x}$ ± SD)	Q1	Q3	
Energy [kcal]	**2762** (2797 ± 890)	2142	3333	**2066** (2136 ± 618)	1669	2496	–
Carbohydrates [g]	**354** (372 ± 143)	260.8	466.1	**278** (293 ± 100)	223.7	350.5	–
Carbohydrates [% en.]	**54.1** (53.2 ± 10.4)	46.9	59.7	**55.0** (54.7 ± 9.3)	48.6	61.1	p = 0.248
Protein [g]	**108** (112 ± 37)	84	131	**81** (85 ± 27)	64	105	–
Protein [% en.]	**15.8** (16.5 ± 4.3)	13.0	18.9	**15.5** (16.4 ± 4.4)	13.4	18.9	p = 0.813
Fat [g]	**97** (101 ± 44)	67	130	**72** (77 ± 32)	54	94	–
Fat [% en.]	**31.9** (32.2 ± 9.2)	25.5	38.8	**30.8** (32.2 ± 8.7)	26.2	38.7	p = 0.982
SFA [% en.]	**11.7** (12.5 ± 4.7)	9.2	15.5	**11.5** (12.1 ± 4.5)	8.9	15.0	p = 0.416
MUFA [% en.]	**11.6** (11.9 ± 4.0)	9.3	14.4	**11.8** (11.9 ± 4.2)	8.6	14.2	p = 0.604
PUFA [% en.]	**4.2** (5.0 ± 3.1)	3.1	5.6	**4.3** (5.5 ± 3.3)	3.2	6.5	p = 0.110
Cholesterol [mg]	**378** (593 ± 540)	248.2	748.2	**291** (380 ± 274)	196.7	437.6	–
Dietary fiber [g]	**28** (28 ± 12)	19.2	35.2	**23** (25 ± 10)	18.0	29.0	–
Sodium [g]	**2.8** (3.0 ± 1.4)	2.1	3.8	**1.9** (2.1 ± 1.1)	1.3	2.7	–
Keys score	**44.0** (47.5 ± 16.9)	35.9	57.2	**43.9** (44.4 ± 16.3)	32.9	54.7	–

Q1 – lower quartile, Q3 – upper quartile, en – energy input
Mann-Whitney U test

energy) and 81 g/day (15.5% of the dietary energy) in group of women.

Carbohydrate intake in analyzed diets was 354 g/day (54.1% of energy) in men and 278 g/day (55%) in women, which indicates a slight deficiency of carbohydrates in the daily food rations in men.

The percentage of energy from fat in the diets in both studied groups was 30% which is slightly higher than the dietary guidelines.

Dietary saturated fatty acids (SFA) in both groups were higher than the recommended (up to 10% of energy intake). The percentage of energy from SFA in the diets was 11.7% in the men and 11.5% in the women. The percentage of energy from EFA in the diets was only 4.2% in men and 4.3% in woman.

The average daily intake of dietary cholesterol in food rations was statistically significant different between women and men ($p < 0.0001$). In the men group dietary cholesterol intake was 378 mg/day, but in the group of women 291 mg/day.

The dietary fiber intake with the diets was 28 g/day in men and 23 g/day in women.

The median sodium intake in the group of men was 2.8 g/day and in the women group was 1.9 g/day (**Table 2**).

The group of men consumed more liquids compared to the group of women. Respondents mainly consumed 1 to 1.5 liters of fluid per day in both groups (in group of men 34%, in group of women 45%) (**Table 3**).

Median of the Keys score in the respondents diets was similar to the values obtained in WOBASZ study conducted in 2005 (Multicentre Nationwide Study of the Polish population's Health) and was 44.0 for men and 43.9 for women.

Most of respondents assessed their eating habits as "good" (65% of women and 55% of men), only 3% of men and 2% of women assessed them as "bad" (**Table 3**).

Knowledge about preventive factors was similar in both groups (**Table 4**). Significant differences were observed in implementing preventive factors in daily life. The group of women often restricted animal fat, dietary cholesterol and monosaccharide intake in their diets. The group of studied men often exercised regularly, but for those with overweight it was still insufficient (**Table 4**).

Table 3. Characteristic group with regard to selected dietary habits – the percentage of total sample

	Men	Women
The number of meals consumed		
< 3	5%	3%
3	30%	19%
4	36%*	36%
5	24%	38%*
6	2%	4%
> 6	2%	0%
The regularity of meals consumed		
Always	18%	19%
Often	51% *	57%*
Rarely	19%	17%
Irregularly	12%	7%
The amount of liquid consumed		
< 1 liter	4%	11%
1–1.5 liter	34%*	45%*
1.5–2 liter	30%	36%
2–2.5 liter	20%	5%
> 2.5 liter	11%	3%
Assessment of their eating habits		
Very good	10%	5%
Good	55%*	65%*
Average	32%	29%
Bad	3%	2%

** The highest percentage in the study group*

Table 4. Knowledge of selected risk factors of cardiovascular disease in woman and man group – the percentage of total sample

Factor	Men			Women		
	1	2	3	1	2	3
Cessation or not smoking	2%	31%	67%*	4%	26%	70%*
Regular exercises	1%	35%	64%	2%	56%*	43%
Maintaining a healthy weight	4%	33%	63%*	2%	31%	67%*
Stress reduction	9%	53%*	38%	8%	53%*	38%
Reduction of sodium intake	43%*	29%	27%	27%	29%	44%*
Reduction of cholesterol intake	11%	52%*	37%	7%	30%	63%*
Reduction of animal fat intake	12%	61%*	28%	8%	37%	55%*
Reduction of sugar intake	20%	47%*	33%	8%	46%*	46%*

1 – do not know, 2 – know but do not apply, 3 – know and apply
** The highest percentage in the group*

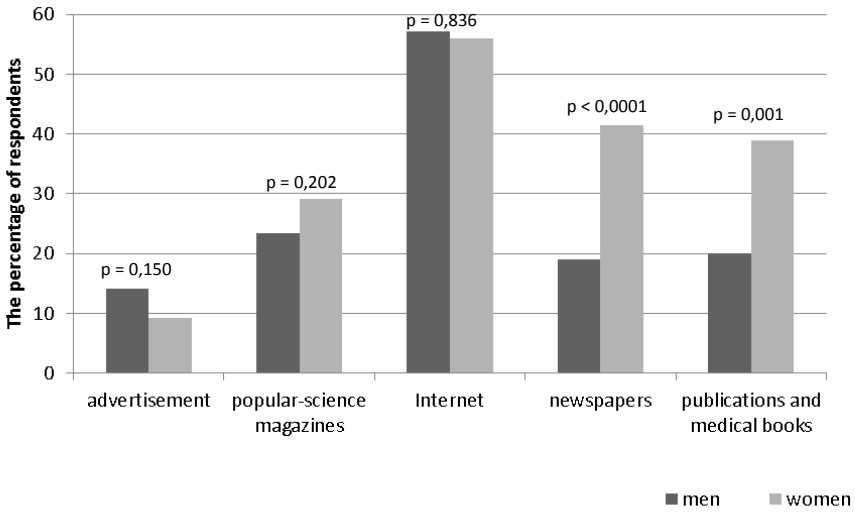

Figure 1. The main source of information on nutrition in woman and man group [%]. Fisher's exact test

The reduction of salt consumption was not known as a risk-reducing factor among respondents, 43% of men and 27% of women did not know that reducing sodium intake may reduce the risk of cardiovascular disease (**Table 4**).

The well-known preventive factor in both groups was maintaining a healthy weight, 63% of men and 67% of women strived to maintain a healthy weight. It was evident also in anthropometric measurements, mean BMI value and waist circumference conformed to the norms [5].

Cessation and not smoking were well-known preventive factors. Both groups, 67% of men and 70% of women did not smoke cigarettes and were aware that smoking increases the risk of heart diseases.

Main source of information about food and nutrition was the Internet (55.9% of women and 57.1% of men). The statistically significant difference was between the acquisition of nutritional knowledge from newspapers ($p < 0.0001$), and medical publications ($p = 0.001$). Women read medical publication and newspapers about nutrition more often than men (**Figure 1**).

Discussion

It is well known that main cause of civilization diseases is an unhealthy lifestyle. Lack of physical activity, excessive animal fat and sugar in the diet lead to increase in weight, increase body fat and contribute to occurrence of CVD.

These studies wanted to emphasize the important role of prevention against cardiovascular diseases.

The results of this study confirm earlier statements which documented an increase in the frequency of poor dietary habits in Polish young people.

The reported dietary intake and selected parameters of nutritional status studied correlate to preemptive care.

Analysis of BMI value, which is the most commonly used parameter to differentiate normal body weight from underweight, overweight and obesity [6] indicate the increased tendency to excess body weight in male and propensity to weight deficiency in women.

However, this has not been confirmed in waist circumference were the results were lower than the limit values. The correct values for this parameter should be less than 94 cm for men and less than 80 cm for women [7].

It is worth noting, that the higher values of this parameters can significantly contribute to increase the risk of coronary heart disease in the future [8]. In particular, in the group of men where the prevalence of overweight was significantly higher than in women.

Daily energy intake in the studied group of men and women, after adjustment by age, body weight and physical activity should be 2700 kcal/day for men and 1900 kcal/day for women [5]. In the study an excessive intake of energy was observed only in woman group although in the group of men prevalence of overweight and obesity was more common compared to the woman group. This could be due to the fact that women ate the meals more often and regularly during the day.

According to the current dietary guidelines, the proportion of energy components should be: 55–75% from carbohydrates, 10–15% from protein and 15–30% from fats [5].

The daily food intake of the study subjects showed discrepancies in dietary fiber, fat, saturated fatty acids and cholesterol intake, particularly among males.

The increase in the consumption of dietary fiber which was observed in woman group negatively correlated with the occurrence of diseases of the cardiovascular system. However, the recommended intake of dietary fiber in the daily food rations equals 20–40 g/day. In prevention of cardiovascular disease it is recommended to take in even 30–45 g/day dietary fiber [5, 8].

The higher fat and dietary cholesterol intake in the men group was associated with increased consumption of butter, cheese, chicken eggs and cream. In addition, increase the intake of SFA was due to increased consumption of sweets.

The recommended daily intake the discussed value should be no more than 10% of energy for SFA, about 8% of energy for PUFA and cholesterol should be lower than 300 mg/day [5, 8, 9].

In the prevention of cardiovascular disease it is important to reduce sodium intake in the diet, which should not exceed 5 g per day [8]. The average sodium intake in the group of men was correct. This element in the diet was within the normal range, although the dietary questionnaire did not include additional salting, which is the major source of sodium in the diet [10, 11]. The study analyzed only the sodium content of food.

Median of the Keys score in the respondents diets wae similar to values obtained in WOBASZ study conducted in 2005 where Keys score was respectively 41.8 for men and 41.1 for women [12].

Most of respondents assessed their eating habits as "good", mostly unaware of dietary mistakes.

An important factor in determining the correct nutritional dietary behavior is the appropriate level of nutritional knowledge. Increase in the level of knowledge about nutrition and prevention of lifestyle diseases in the population may help to reduce the incidence of these diseases. It is therefore important to promote knowledge, as well as to asses the level of knowledge in population [2].

To counteract the occurrence of cardiovascular disease it is necessary to include among others factors: maintaining a healthy weight, regular exercise, reducing the intake of animal fats, cholesterol, sugars and sodium, and cessation or not taking up smoking [8, 13, 14].

The preventive factors were well known in both study groups. Significant differences were observed in animal fat, dietary cholesterol and monosaccharide intake which was confirmed in their regular daily diet. For this reason, the proper emphasis should be put on proper nutrition among men.

Unawareness of the salt reduction in regular diet, which greatly contributes to reducing the risk of cardiovascular disease seems to be closely related to hypertension, which is one of the most common health problems in Polish population. In addition, the study revealed a positive trend for the reduction of smoking cigarettes in young people (aged 20 to 30 years).

Conclusions

Excessive content of total fat, SFA and cholesterol in the diets and insufficient intake of PUFA and dietary fiber may be the cause of increased risk of the CVDs in this group in the future.

The insufficient knowledge about nutrition in the studied group may cause increased risk of the CVDs in the future. It is important to promote national knowledge to raise social awareness of the dangers related to the lifestyle.

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General adiposity and adipose tissue distribution in young women from Warsaw

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ABSTRACT

Aim. The aim of this study was to determine the overall adiposity and adipose tissue distribution in young women from Warsaw.

Material and methods. The study covered 550 women from Warsaw. The following research methods were applied: bioelectric impedance, measurement of body weight, body height, waist and hip circumference, body mass index (BMI), adipose tissue distribution Waist-to-Hip Ratio (WHR), Waist-to-Height Ratio (WHtR); waist circumference was also analyzed based on the criterion of the International Diabetes Federation (IDF).

Results. The average value of body mass index pointed to the correct values within 18.5–24.9 kg/m^2 range, while the BMI distribution in the studied group covered a wide range, starting from values characteristic for the state of emaciation up to values indicating III° obesity. Analysis of adipose tissue distribution based on WHR indicator showed existence of central adiposity, or abdominal adiposity, in case of more than 60% of the surveyed women. However, based on WHtR indicator that takes into account waist circumference and body height, abdominal obesity was found in case of slightly more than 30% of women. Analysis of the waist circumference measurement showed that abdominal (visceral) adiposity was found in case of half of the surveyed women. Average overall adiposity was 18.5 kg. Adiposity on the left and right sides of the individual body segments did not differ significantly.

Conclusion. Overweight and obesity connected to the risk of developing cardiovascular disease exist among young women from Warsaw. Among the surveyed women a significant percentage of people with abdominal (central) type of adiposity was found, which creates the increased risk of cardiovascular disease. Prevention of cardiovascular disease should be based not only on the assessment of the overall adipose tissue and BMI but also on the assessment of its distribution in the body. Young women from Warsaw should be covered by preventive actions in order to prevent the development of cardiovascular disease through health education programs.

Keywords: distribution of adipose tissue, bioelectric impedance, women.

Introduction

Excessive adiposity is a well-documented risk factor for many diseases of civilization and in particular arterial hypertension, atherosclerosis, ischemic heart disease, hyperlipidemia and hypercholesterolemia, type 2 diabetes, as well as the majority of malignant neoplasms [1, 2].

Many epidemiological studies and experimental clinical trials demonstrated the existence of a strong causal link between obesity and cardiovascular disease [3, 4].

Adipose tissue is a storage of fat and serves as an organ of internal secretion, it is dispersed throughout the body, and its most important locations are abdominal visceral, subcutaneous abdominal and gluteal-femoral, which differ in structure, function, gene expression, metabolic and endocrine activity, and differently affect the functioning of liver and central nervous system (CNS) [5].

Abdominal obesity is the accumulation of adipose tissue in the abdomen, not proportional to the total

amount of fat in the body, which is an important and independent risk factor for many metabolic complications [6].

Abdominal obesity rating in epidemiological studies is mainly done indirectly based on anthropometric measurements and indicators. One of the most commonly used indicators is WHR (Waist-to-Hip Ratio), which is calculated based on the ratio of the circumference of the waist to that of the hips, whereas a division covers android (abdominal) and gynoidal (gluteal-femoral) obesity. The division criteria are based on the links between the WHR and the occurrence of the increased risk of developing cardiovascular disease [7].

The distribution and amount of the adipose tissue varies in terms of gender. The amount and size of adipocytes in the subcutaneous tissue are higher in case of females in comparison to men, and, therefore, the percentage of fat in relation to the total body mass is higher in case of women [8].

Aim

The aim of the study was to determine the overall adiposity and adipose tissue distribution in young women from Warsaw.

Material and methods

The study covered 550 women from Warsaw aged 22.1 ± 2.9 years (mean ± SD). The study was conducted in the period from January to April 2013 in the Laboratory of Clinical Trials of Higher School of Rehabilitation in Warsaw. The qualification for inclusion to the study was the women's written consent for voluntary participation in the project. All people joining the study were informed of the purposes and methods of research and the possibility of cancellation at any stage. Contraindications for measuring bioelectrical impedance (BIA): the early period of pregnancy, defibrillators and pacemakers, epilepsy, people with metal prostheses, prosthesis, implants were the criteria for exclusion from the srudy.

The research program, its goals, objectives and selected research methods obtained the certificate of conformity with the rules of ethics of the Senate Ethics Committee for Scientific Research at the Józef Piłsudzki University of Physical Education in Warsaw.

The following research methods were used:

- bioelectrical impedance analysis with the use of the TANITA BC 418 analyzer;
- anthropometric measurements of the following morphological characteristics: body weight (kg), body height (cm), waist circumference (cm) and hip circumference (cm);
- indicators: body mass index (BMI), adipose tissue distribution WHR (Waist-to-Hip Ratio), WHtR (Waist-to-Height Ratio); waist circumference was also analyzed based on the criterion of the International Diabetes Federation (IDF), where the abdominal obesity for women is when the waist circumference is: ⩾ 80 cm;
- statistical methods with the use of STATISTICA software.

Results and discussion

Table 1 presents the descriptive statistics of anthropometric measurements of the studied group. The average value of the body mass index indicated the correct values within the range of 18.5–24.9 kg/m^2, while the BMI distribution in the studied group was in a wide range, starting from the values characteristic for the state of emaciation up to the values indicating III^0 obesity. The average value of the WHR indicator pertaining to adipose tissue distribution indicates the type of visceral adiposity, otherwise as central, abdominal or androidal. The ratio of waist circumference to body height presented as the WHtR indicator reached the value below the criteria of the International Diabetes Federation, what indicates the abdominal obesity (**Table 1**).

BMI distribution analysis in the studied group of women (n = 550) showed that the largest percentage of women was characterized by a normal weight, while there was noted both the cases of emaciation and weight deficiency in a total of 6.7% of the surveyed women. Overweight was observed in 12.7% of women, whereas obesity, including III^0 obesity, in 6.6% (**Table 2**).

The data obtained are consistent with research of the second year students of the full-time Masters studies at the Faculty of Physical Education and the students of the second year of Masters studies at the Faculty of Human Nutrition and Consumer Agricultural University aged 22–28. Body mass index of most women was in the range of normal values, i.e., 18.5–25 [kg/m^2] (95% in the AWF, 87% in the WAU). Both groups noted values below normal (5% in both cases), and excessive body weight (BMI > 25 [kg/m^2]) in 7% of WAU students [9].

In the studies of Charkiewicz et al. anthropometric parameters of the tested female UM students in Bialystok showed that BMI in 75% people was in the normal range, while 23% were underweight, and only 2% – overweight [10].

Research conducted on students from the Universities of Poznan demonstrated higher scores, respectively: 38% were underweight and 6.7% were overweight [11].

In most studies of young women in Poland there is stated a significant proportion of normal nutritional status, however, there are still noted the increasing cases of women who are overweight and obese at such a young age.

These results are important mainly due to the fact that the BMI, as an index of nutritional status, is highly correlated with the total body fat content [12, 13].

However, we must remember that using only the analysis of trends of BMI changes, we cannot unequivocally demonstrate the negative health consequences of obesity. As this indicator does not distinguish the body content of the adipose tissue from muscle tissue, there is also no information concerning its distribution.

Therefore, it is considered as better to assess the distribution of body fat based on the WHtR index, regarded as an effective indicator of the body fat, particularly in the abdominal type. Given the waist circumference and body height, abdominal obesity was found in slightly more than 30% of the women (**Table 3**). This result is all the more important beasue WHtR is a recognized indicator of the risk of cardiovascular disease and metabolic syndrome [12].

Whereas the WHR indicator showed the presence of central adiposity (abdominal type) in more than 60% of the surveyed women. This may indicate that impaired fat distribution, consisting of the increased ratio of the visceral fat to the subcutaneous tissue. In humans, abdominal obesity is an independent risk factor of metabolic syndrome, type II diabetes, cardiovascular disease and certain types of cancer [13, 14, 15].

Whereas applying the analysis of the measurement only of the waist circumference according to the International Diabetes Federation criteria showd that abdominal fat type occurred in the half of the examined. In practice, due to the role of visceral fat in the development of complications of obesity, WC is increasingly gaining recognition, which is regarded, just like WHtR, as one of the most important indicators of body fat [16].

The amount of general and segmental adipose tissue based on the analysis of body composition with the use of bioelectrical impedance (BIA) method showed a large variation in the group of examined women from Warsaw. The average overall adiposity was 18.5 kg, while the difference between the lowest and the greatest mass of adipose tissue was 48.3 kg. In case of the percentage of total adipose tissue similar trends were stated. Adiposity on the left and right sides of the individual body segments did not differ significantly (**Table 4**).

In people with central obesity there is accumulated both the visceral adipose tissue and the subcutaneous abdominal tissue. Several studies have shown that both deposits, regardless of themselves, condition the

Table 1. Somatic characteristics of studied women (average ± SD, minimum, maximum)

	Average ± SD	Minimum value	Maximum value
Body weight [kg]	63.2 ± 11.5	42.7	115
Body height [cm]	166.9 ± 5.3	155	186
Waist circumference [cm]	79.5 ± 9.0	58	116
Hips circumference [cm]	92.9 ± 6.6	68	115
BMI [kg/m²]	22.7 ± 3.8	16.7	42.2
WHR*	0.86 ± 0.07	0.68	1.13
WHtR**	0.48 ± 0.06	0.35	0.71

** waist-to-hip ratio, ** waist-to-height ratio*

Table 2. The distribution of Body Mass Index (BMI) in the studied group of women

Classification of nutritional status	BMI [kg/m²]	% of women (n = 550)
Emaciation	16.0–16.9	1%
Underweight	17.0–18.49	5.7%
Proper body weight	18.5–24.9	74%
Overweight	25.0–29.9	12.7%
Obesity I°	30.0–34.9	5.7%
II°	35.0–39.9	0.3%
III°	> 40.0	0.6%
Overall obesity	⩾ 30	6.6%

Table 3. Distribution of adipose tissue in the studied group of women (n = 550) based on WHR and WHtR indicators and waist circumference

Distribution of adipose tissue	Value of indicator	% of women (n=550)
WHR (waist-to-hip ratio)		
Androidal adiposity (abdominal)	⩾ 0.85	60.3 %
Gynoidal adiposity (gluteal-femoral)	⩽ 0.85	39.7 %
WHtR (waist-to-height ratio)		
Central adiposity (abdominal)	⩾ 0.5	30.3 %
Waist circumference		
Central adiposity (abdominal)	⩾ 80	50 %

Table 4. The amount of general and segmental adipose tissue based on the analysis of body composition with the use of bioelectrical impedance (BIA)

		Average ± SD	Minimum value	Maximum value
Overall amount of adipose tissue [kg]		18.5 ± 8.0	6.2	54.4
Percentage of adipose tissue [%]		27.7 ± 7.2	12.5	51.4
Segmental amount of adipose tissue [kg]	Right lower limb	3.7 ± 1.3	0.9	10.3
	Left lower limb	3.6 ± 1.3	1.1	10.1
	Right upper limb	0.9 ± 0.5	0.1	3.9
	Left upper limb	1.0 ± 0.6	0.2	4.3
	Trunk	8.9 ± 4.8	1.7	30.5
The percentage of segmental adipose tissue [%]	Right lower limb	30.8 ± 5.9	7.8	47.4
	Left leg lower limb	31.0 ± 5.8	9.4	47.9
	Right upper limb	27.7 ± 8.2	6.3	56.1
	Left upper limb	28.6 ± 8.0	7.6	56.9
	Trunk	24.7 ± 8.4	3.0	52.0

development of insulin resistance, and the particularly adverse significance is found in the visceral adipose tissue. Its excess favours the development of the metabolic syndrome and hypertension and coronary heart disease [17].

Depending on the applied indicator, the percentage of women with abdominal obesity in the group of 550 surveyed women ranged from 30% to as much as 60%. WHR > 0.8 indicates the accumulation of visceral adipose tissue in women. In own studies, more than half (60.3%) people had abdominal (visceral) adiposity. In comparison to the study of female students from Szczecin, where 29% exceeded the value of the indicator for abdominal obesity, the result of young women from Warsaw indicates the increased risk of disease strongly correlated with this type of adipose tissue distribution [18].

The WHtR indicator allows to determine the risk of diet-related disease that can be prevented or minimized by a proper lifestyle, especially thanks to the proper nutrition and physical activity. The average value of the WHtR indicator in the studied group of women did not indicate the risk of developing the above mentioned diseases, including cardiovascular disease, similarly to the results of female students of the University of Szczecin being in the same age [18].

Abdominal obesity, called visceral, central or android obesity is characteristic for the "apple" body type. According to the medical sources, from the health point of view, among all types of obesity, abdominal obesity is highly unfavourable because most often it leads to the metabolic disorders, lipid metabolism disorders, diabetes, hypertension, which create the metabolic syndrome. Such adipose tissue distribution in connection with hypertension or diabetes significantly increases the risk of complications of cardiovascular diseases, leads to heart attack and stroke. In case of individuals with general or gluteal-femoral adiposity, the metabolic profile is more advantageous than in the central obesity [19, 20, 21].

High consumption of alcohol, improper diet (high-fat diet, fast foods), stress, smoking and many other factors associated with unhygienic lifestyle may result in the development of the abdominal obesity [22, 23, 24].

On the other hand, the increased physical activity, as indicated by the guidelines of preventive health care, reduces the risk of its occurrence [25].

Due to the differences in fat content, depending on the application of particular indicators, the discussed measurement methods may not be used interchangeably. While it is important to undertake research towards the risk assessment of the scale-dependent diseases of central fat distribution and increased total body fat among young women in order to develop effective programs for prevention of obesity and related health complications.

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ORIGINAL PAPER

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Brain segmentation unmasks association between body composition and central nervous system structures

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ABSTRACT

Introduction. Excess body fat is currently the major health problem. We suggest that marker of fatness like BMI or percent of body fat are associated with gray matter volume (GMV) or brain areas responsible for cognitive functions.
Material and methods. Study was performed in 89 healthy individuals (mean age 58 yrs, 51 women).
For brain segmentation and GMV we used whole-brain, high-resolution 3D T1-weighted images. For assessment of the fat content as a proportion of total body mass a bio-impedance analyzer was used.
Result. GMV was correlated, significantly and negatively with age but was not associated either with BMI nor body fat content. Body fat content was significantly and negatively associated with hippocampus and thalamus.
Conclusion. We demonstrate that brain segmentation was able to unmask the association between body fat content and brain structures particularly involved in cognitive function.

Keywords: body fat, body mass index, gray matter volume, hippocampus, thalamus, brain segmentation.

Introduction

Excess body fat is currently a major health problem. Rising body weight in concert with hypertension and diabetes contributes to the development of cardiovascular complications such as myocardial infarction, heart failure or stroke [1, 2, 3]. It has also been shown that fat distribution between different compartments may affect brain structure and function [4]. Moreover, current research has suggested that high body mass index (BMI) may represent a risk factor for the development of cognitive decline or dementia [5, 6, 7]. In the present study we address the question whether a marker of obesity such as BMI or the percentage of body fat are associated with gray matter volume (GMV) or brain areas responsible for cognitive functions.

Material and methods

The study was performed on 89 healthy individuals (mean age 58 years, 51 women). None of the subjects were taking any medication. The Poznan University Ethics Committee approved the study protocol and a written informed consent was obtained from all participants.

Magnetic Resonance Imaging of the brain

Magnetic Resonance Imaging (MRI) was performed using a 1.5-T magnet with a head 12-channel coil (Magnetom Avanto System, Erlangen Germany). We used axial and sagittal acquisition of T1-weighted, T2-weighted, PD, FLAIR and Diffusion Weighted Images (DWI). T1 and T2-weighted images were scanned

with 4 millimeter slices, covering the whole brain. We used 230x230 field of view (FOV) and 256x256 scan matrix.

Whole-brain, high-resolution 3D T1-weighted images (Magnetization Prepared Rapid Gradient-Echo) were acquired for morphological analysis. The acquisition parameters were as follows: bandwidth = 190 Hz/pixel, flip angle = 7°, TR/TE/TI = 2.73s/3.44 ms/1s. This sequence was obtained to calculate grey matter volume (GMV) and brain segmentation.

Morphometric analysis of brain structure was completed with MRIcro Version 1.35 software (www.sph.s.edu/comb/rorden/mricro.html).

For detailed brain structure segmentation into volumes and surfaces, with the use of the state-of-the-art algorithms, the FreeSurfer Version 5.1 (Laboratory for Computational Neuroimaging, Athinoula A. Martinos Center for Biomedical Imaging) software package was used (http://surfer.nmr.mgh.harvard.edu), (**Figure 1**).

Body fatness assessment

For the assessment of the fat content as the proportion of a total body mass, a bio-impedance analyser (MC180MA, Tanita Corp USA) was used. Bio-impedance analysis was performed using multifrequency technology.

Statistical analysis

The results are expressed as mean ± SEM, and Pearson's correlation coefficient was calculated. The statistical analyses were performed using MedCalc (MedCalc Software, Ostend, Belgium), with statistical significance set at $P < 0.05$.

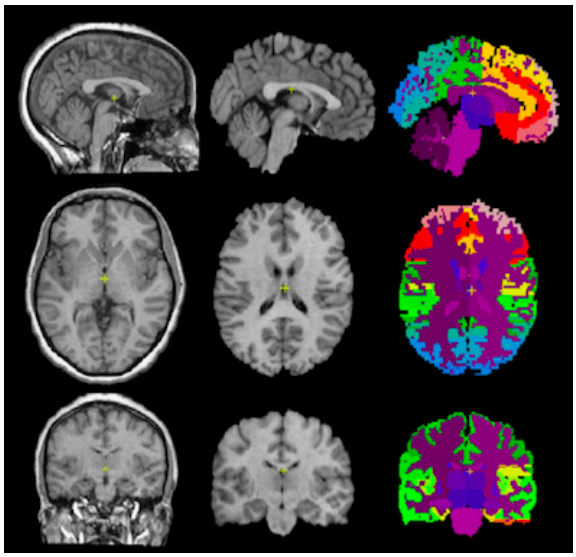

Figure 1. Representative maps of the white and gray brain matter in T1-weighted images. Segmentation of the brain

Results

Patients characteristics are presented in **Table 1**.

Table 1. Clinical characteristics of the study participants

Characteristic	
Age (years)	58 ± 1
M/F	48/51
BMI (kg/m^2)	26 ± 0.4
Body fat (%)	27 ± 1
Cholesterol (mg/dl)	210 ± 4
SBP (mmHg)	122 ± 1
DBP (mmHg)	74 ± 1

M – male, F – female, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure

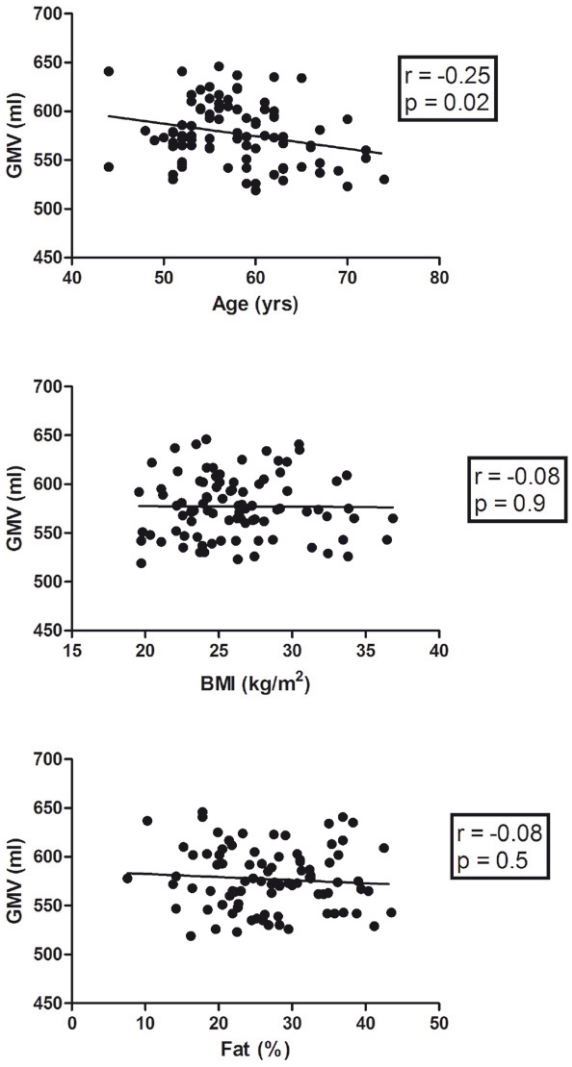

Figure 2. The association between gray matter volume, age, body mass index and percentage of body fat. GMV – gray matter volume, BMI – body mass index

Association between gray matter volume, measures of fatness and age.

In **Figure 2**, the correlation between gray matter volume, BMI and body fat content is presented. GMV was correlated, significantly and negatively with age ($r = -0.25$, $p = 0.02$) but was not associated either with BMI ($r=-0.08$, $p = 0.9$) or body fat content ($r = -0.08$, $p = 0.5$).

Interaction between brain structures extracted by segmentation and measures of fatness

The hippocampus and the thalamus were extracted with the use of FreeSurfer software. Body mass index correlated significantly and positively with body fat percentage ($r = 0.5$, $p < 0.0001$), nevertheless a high degree of correlation does not mean that these measures could be regarded as interchangeable. As shown in **Figure 3**, both the hippocampus and the thalamus correlated significantly and negatively with age ($r = -0.37$, $p = 0.0005$; $r = -0.29$, $p = 0.009$, respectively). The hippocampus and the thalamus were not correlated with BMI ($r = 0.02$, $p = 0.8$; $r = -0.12$, $p = 0.2$, respectively). However, body fat content was significantly and negatively associated with both structures, namely the hippocampus and the thalamus ($r = -0.22$, $p = 0.04$; $r = -0.24$, $p = 0.03$, respectively).

Discussion

Apart from being an important risk factor for the development of diabetes, heart failure or stroke, obesity is thought to play a major role in cognitive decline and Alzheimer's disease. It has been demonstrated that Alzheimer's disease and obesity are both associated with brain volume decrease [7, 8]. Gustafson et al. [9] examined body mass index (BMI) in relationship to cerebral atrophy in a representative sample of women followed up from 1968 to 1992 as part of the population study. The authors were particularly interested in changes observed in temporal lobe since this area is highly susceptible to the effects of ischemia and other vascular insults to the brain. Moreover, it appears to be an early hallmark of Alzheimer's disease. Women with atrophy of the temporal lobe were, on average, by 1.1 to 1.5 kg/m^2 higher in BMI. Moreover, multivariate analyses showed that age and BMI were the only significant predictors of temporal atrophy. It is also important that there were no associations between BMI and atrophy measured at three other brain locations. Taki et al. [10] obtained brain MR images from 690 men and 738 women. Volumetric analysis revealed a significant negative correlation between BMI and the gray matter ratio (which represents the percentage of gray matter volume in the intracranial volume) in men (adjusting for age, lifetime alcohol intake, history of hypertension and diabetes mellitus), but not in women. Brain segmentation revealed that, in men, the regional gray matter volume of the bilateral medial temporal lobes, anterior lobe of the cerebellum, occipital lobe, frontal lobe, precuneus and midbrain showed significant negative correlations with BMI, while those of the bilateral inferior frontal gyri, posterior lobe of the cerebellum, frontal lobes, temporal lobes, thalami and caudate heads showed significant positive correlations with BMI. Thus, even these two articles demonstrated that body fat content estimated by BMI is to some extent associated with brain structure alterations. Moreover, it seems that in order to observe this correlation a large sample of subjects is required to increase the strengths of a statistical test applied to estimate such association. It needs to be noted that BMI is a measure of excess weight relative to height but not necessarily the excess adiposity. It is stressed by the fact that BMI in our study correlated only to some extent ($r = 0.5$) with body fat composition estimated by bioimpedance. We were also unable to observe any correlation between BMI and gray matter volume or white matter volume (data not shown). However, when we performed brain structure segmentation with the delineation of the thalamus and the hippocampus, both of these structures demonstrated association with body fat content but not with BMI. It is thought that the thalamus is involved in cognitive and perceptual function, while the hippocampus is involved in minute-to-minute cognitive processing including spatial information processing, temporal sequencing and formulating the relationships between objects in the environment [11, 12]. There are several reports with data indicating that body fatness may be associated with cognitive function. Yoon et al. [13] observed 250 subjects (mean age 60 years) to investigate a direct association between visceral adiposity and cognitive performance. It was concluded that high adiposity, particularly visceral adiposity, was associated with poor cognitive functioning in younger elderly persons. Interesting data indicate that under real-world stress higher body fat percentage may be associated with endocrine stress vulnerability, with consequences for deleterious cognitive performance [14].

Our study, although it involved a relatively small population, thanks to state-of-the-art brain segmentation used was able to unmask the association between

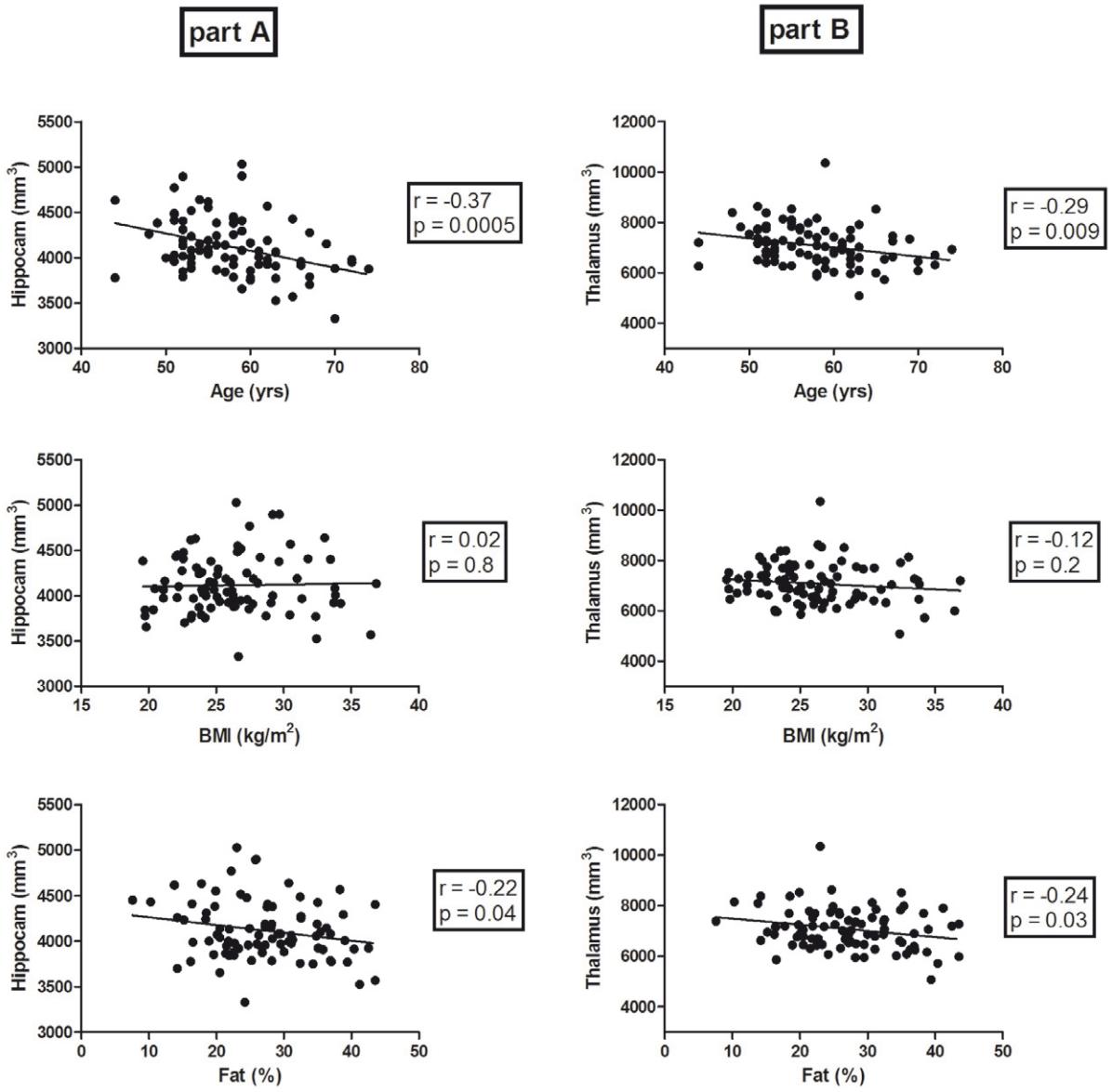

Figure 3. The association between volume of the hippocampus or the thalamus and age, body mass index or body fat. GMV – gray matter volume, BMI – body mass index

body fat content and brain structures particularly involved in cognitive function. The results of the study suggest that the improvement in the technique of MRI interpretation and delineation of structures of particular interest allow better insight into the pathophysiological link between body fat composition and brain arrangement.

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REVIEW PAPER

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Monogenic diabetes – an unappreciated problem among physicians

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ABSTRACT

Monogenic diabetes results from one or more mutations in a single gene. It is a relatively rare genetic condition, therefore, it was frequently unappreciated among clinicians. Consequently, monogenic diabetes is misdiagnosed as type 1 diabetes or type 2 diabetes. Such misclassification leads to an inappropriate treatment, often inconvenient for the patients, such as insulin injections with their permanent glycemic control. The correct diagnosis may completely change previous methods of treatment. Patients diagnosed with GCK mutations may be completely treated with adequate diet. HNF1A/HNF4A affected patients are extremely sensitive to low dose sulphonylureas. Moreover, the exact diagnosis has an impact on patients' relatives. Mostly, misdiagnosing of monogenic diabetes is caused by its rare occurrence and insufficient training in this area among physicians. According to different studies it may comprise 1–4% of all cases of diabetes. The aim of this article is to emphasize that despite the fact that monogenic diabetes is an uncommon disease, it should always be considered in cases of diabetes with unusual course.

Keywords: monogenic diabetes, prevalence, diagnosis, treatment.

Introduction

Proper function of insulin-producing pancreatic beta-cells is crucial for the regulation of glucose homeostasis. Pancreatic beta-cells secrete insulin in amounts appropriate to the respective blood glucose concentration. In brief, glucose is taken up by the beta-cells via glucose transporter 2. It is then phosphorylated to glucose-6-phosphate by an islet specific glucokinase, resulting in an increase in adenosine triphosphate (ATP) concentration [1]. Rising ATP levels close ATP-sensitive potassium channel (KATP channel, intramembrane cellular channel controlling K^+ efflux depending on ATP concentration) and lead to cell membrane depolarization. The beta-cell KATP channel is a complex of 2 subunits: *Kir6.2* (inwardly rectifying potassium-channel subunit) and *SUR1* (regulatory sulfonylurea-receptor subunit) and both are needed for the correct function of the channel [2].

Thereby beta cells react with opening calcium channel, enabling Ca++ influx and together with calmodulin allowing the release of secretory granules containing previously synthesized insulin. In addition, other factors such as hepatocyte nuclear factors (HNF) 4-alpha, 1-alpha, and 1-beta play a role in the regulation of glucose homeostasis and affect transcription of many genes in beta-cells. Defects in HNF lead to abnormal glucose level. The exact mechanism of hyperglycemia is not clear, but it has been connected with reduced insulin secretory response to glucose, suggesting primary genetic defect in insulin secretion [1, 3, 4].

Single genes at any stage of these pathways lead to monogenic diabetes (MGD) also known as MODY (maturity-onset diabetes of the young). MGD is a heterogeneous group of rare forms of diabetes. The mutation might occur de novo or might be inherited dominantly or recessively [4, 5, 6].

MGD can be detected in infancy, as well as in adolescence or in adulthood. The one present in infancy is called neonatal diabetes mellitus (NDM). NDM is defined as diabetes, which occurs in the first 6 months of life [2] and has an estimated incidence of around 1 in 200,000 live births [7, 8]. It is divided into two clinical subtypes: transient neonatal diabetes mellitus (TNDM), which can resolve after a few weeks or months and permanent neonatal diabetes mellitus (PNDM), which requires treatment following diagnosis [7]. According to different studies 50% of cases of PNDM and 20% of TNDM are caused by mutations of the KATP channel genes (*KCNJ11* or *ABCC8*) encoding the *Kir6.2* and *SUR1* subunits respectively [6, 9, 10]. Patients diagnosed with *KCNJ11* or *ABCC8* mutations are able to switch from insulin injection to orally administered sulphonylurea drugs. In addition, such a transfer allows lowering the level of glycated hemoglobin (HbA1c) and reduces microvascular complications [11]. On the other hand, insulin therapy is required for patients with other non-KATP channels forms of NDM.

Typically, the diagnosis of MGD in young adolescents is based on clinical characteristics including: family history of diabetes with an autosomal dominant mode of inheritance and non-ketotic diabetes with the age of onset before 25 years of age [12]. There are at least eight well known genes involved in these MGD (**Table 1**). Approximately 70% cases are caused by mutation in *GCK, HNF1α or HNF4α* [13, 14]. It is important to conduct the genetic diagnostics because MGD is commonly misdiagnosed. 90% of genetically confirmed monogenic diabetes is initially treated as type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) [15, 16, 17]. Appropriate diagnosis has profound impact not only on predicting clinical course of the disease and explaining other associated clinical features, but mostly on introducing proper treatment. Moreover, the exact diagnosis will also carry implications for the patient's relatives [18].

Finally, it is worth mentioning that there are a few genetic disorders associated with diabetes mellitus, including: Wolfram syndrome, also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness) or Alström syndrome.

Wolfram syndrome is caused by the mutation in at least two different genes: *WFS1* and *ZCD2*. The reason of diabetes mellitus in Wolfram syndrome is impaired homeostasis of beta cells (increased apoptosis and/or failure of regenerative processes), however some clinical symptoms as ketosis prone juvenile diabetes and absence of obesity are similar to T1DM [19].

Alström syndrome is a rare genetic condition caused by mutations in the *ALMS1* gene. It is characterized by multiorgan dysfunction, including endocrinologic features such as hyperinsulinemia and early-onset type 2 diabetes [20].

Is monogenic diabetes undiagnosed and untreated?

Recent data from American Diabetes Association (ADA) annual meeting sheds light on monogenic diabetes, especially on the lack of appropriate diagnosis and its consequences. It was emphasized that almost no training has taken place in this area. Commonly, for clinicians, there exist only two types of diabetes: type 1 and type 2. In everyday practice other types of diabetes are forgotten [21]. It results mostly from the fact that the exact prevalence of MGD is unknown, but based on different studies it may comprise 1–4% of all cases of diabetes [13, 14, 22–26]. Moreover, early symptoms might be mistaken and MGD is frequently diagnosed as T1DM [15, 16, 17]. Patients with no evidence of pancreatic autoantibodies are classified as having antibody-negative T1DM or idiopathic diabetes [27]. Lack of autoimmune markers might be useful as an indicator for further genetic testing, especially if measured at diagnosis. Most patients with T1DM will have detectable islet cell autoantibodies and less than 10% will need a combined antibody testing, including glutamic acid decarboxylase autoantibodies (GADab), protein tyrosine phosphatase-like protein autoantibodies (IA2-ab), insulin antibodies (IAA) [28, 29]. The autoimmunity detection rate will rise up to 98%, if the measurement of zinc transporter-8 autoantibodies (ZnT8A) will be added [30]. Assessment of autoantibodies level at the diabetes onset plays the key role in differential diagnosis, because islet cell autoantibodies may disap-

Table 1. Genes in which mutations cause MGD characteristic for neonates, young children and adolescents or adults

Affected gene	HFN4A	GCK	HFN1A	IPF 1	HFN1B	NeuroD1	KCNJ11	ABCC8
Symbol	MODY 1	MODY 2	MODY 3	MODY 4	MODY 5	MODY 6	PNDM	TNDM
Chromosome locus	20q13	7p13	12q24	13q12	17q12	2q31		
Function	Transcription factor	Glycolytic enzyme	Transcription factor	Transcription factor	Transcription factor	Transcription factor	coding Kir6.2	coding SUR1

pear over time after disease onset in previously proven autoimmune diabetes [31]. The prevalence of GADab or/and IA-2ab is less than 1% in patients diagnosed with *GCK*, *HNF1α* /*HNF4α* and *HNF1B* mutations. Therefore, positive results of islet autoantibodies make monogenic diabetes very unlikely [32].

Very interesting research was conducted by the Search for Diabetes in Youth Study Group (SEARCH) in the United States. This group screened for three most common MGD subtypes among SEARCH participants who were diabetic autoantibodies (DDA) negative with fasting C-peptide ⩾ 0.8 ng/ml. The frequency of having one of three commonest mutations was 8% and the frequency of mutation carriers was 4.4% for *HNF1α*, 1.2% for *HFN4α* and 2.4% for *GCK*. Only 6% of MGD positive individuals received proper diagnosis and adequate treatment. Most MGD(+) participants were misdiagnosed and classified as T1DM (36%) or T2DM (51%). According to this study the estimated prevalence of these three MGD subtypes is at least 1.2% in the US population of pediatric diabetes [33]. Similar trends can also be noticed in European countries. The first prevalence study of MGD in a nationwide population of children with diabetes was conducted in Norway and showed that the minimum prevalence of MGD in 2,756 children with newly diagnosed diabetes was 1.1% [32]. German/Austrian study of 40,757 children and adolescents revealed the prevalence of clinical and genetic MGD 0.83% and 0.65%, respectively [14]. A nationwide genetic screening campaign among Polish diabetic children found the prevalence of MGD between 4.1% in 2005 and 3.2% in 2011. *GCK* mutations were the most often detected form of MGD and they account for 83%. The frequency of other types was 7% for neonatal diabetes, 4% for *HNF1α/HNF4α* mutations, 2% for *HNF1B* mutations and diabetes in Wolfram and Alström syndromes 2% and 1%, respectively. This study showed that MGD is more common in pediatric population with low frequency of obesity rather than T2DM or cystic fibrosis-related diabetes (CFRD) [26].

When to suspect that the diagnosis of diabetes type may not be accurate?

Available epidemiological data indicates that despite MGD is a rare genetic condition, it should be considered in unusual diabetic cases. Uncommon course of diabetes may suggest to the physician that this is different than T1DM or T2DM and some genetic test should be considered. There are a few clinical features which may be useful in making diagnosis. Based on International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines MGD should be pondered if diabetes was diagnosed within the first 6 month of life, family history indicates diabetes with a parent affected or mild fasting hyperglycemia (5.5–8.5 mmol/l) accompanied by other extra pancreatic features occurs. Furthermore, in case of previously recognized T1DM, with prolonged "honeymoon" phase after 3 years of diabetes onset, with still maintained significant endogenous insulin production, should raise doubts about proper diagnosis. Mainly, doctor should be suspicious, if there was not any evidence of autoimmunity process at the beginning of the diabetes. On the other hand, lack of acanthosis nigricans and other features of insulin resistance, including fasting C peptide within the normal range, low C-reactive protein level and ethnic backgrounds with a low prevalence of T2DM may suggest a possibility of monogenic diabetes in patients previously diagnosed as T2DM [35]. The similarities and differences between MGD, T1DM and T2DM are shown in **Table 2** [36].

Furthermore, the clinical features should be considered together rather than separately. Moreover, the patient with a diagnosis of MGD should present the features of a specific genetic subtype of MGD (**Table 3**) [35].

Why to diagnose monogenic diabetes?

An accurate diagnosis of MGD is not meaningless. First of all, it allows understanding the patient's origin of diabetes and explaining other associated clinical fea-

Table 2. The similarities and differences between MGD, T1DM and T2DM, in modification

Features	T1DM	T2DM	MGD
Non-insulin dependent	NO	YES/NO	YES
Parents affected	0–1	0–2	1
Age of onset < 25 yr	YES	YES	YES
Obesity	Uncommon	Common	Uncommon
Acanthosis nigricans	Uncommon	Common	Uncommon
Racial groups (Type 2 prevelance)	Low	High	Low
C-Reactive Protein	Normal	High	Low
Presence of B-cell antibodies	> 90%	Rare	Rare

Table 3. The most characteristics of common forms of monogenic diabetes

	Inheritance	Typical age of presentation in pediatric clinic (range)	Typical glucose presentation (range) mmol/l	Other clinical features
HNF1A	Dominant	14 (4–18)	17 (11–26)	Large increase of glucose level in an OGTT
				Low renal threshold
				Progresive hyperglycemia with age
				Sensitive to sulphonylureas
HNF4A	Dominant	17 (5–18)	15 (9–20)	Similar to HNF1A, but renal threshold normal
				Very common macrosomia
				20% prolonged neonatal hypoglycemia
GCK	Dominant	10 (0–18)	11 (5.5–16)	Usually incidential finding at diagnosis
				Fasting glucose in range 5.5–8 mmol/l
				Little deterioration in glycemia

tures. Secondly, it gives the physician an opportunity to predict the clinical course of the disease. Finally, it is paramount to introduce appropriate treatment. Such patients might not need any treatment or might be able to switch from insulin injections to tablets such as sulfonylurea [11, 37]. As a final point, it is worth mentioning that an exact diagnosis also has implications for other family members often modifying their diagnosis and treatment as well as allowing proper genetic counselling.

Typically, individuals with glucokinase mutation present the fasting hyperglycemia, however their blood glucose level is regulated at a higher set point [38]. Commonly, such patients are diagnosed incidentally on routine physical examination or during pregnancy in case of women. Usually, deterioration in glycaemia control is not observed and they do not develop any diabetic chronic complications even when they have not received any treatment throughout life [39]. Therefore there is a clear conclusion: these patients do not need treatment, especially in pediatric age. Confirmed genetic diagnosis allows avoiding repeated, unnecessary diagnostic tests and explains disturbances of glucose metabolism in case of additional diseases or pregnancy, which can exacerbate clinical manifestation of diabetes mellitus.

On the other hand, diabetes caused by mutations of the *HNF-1α* gene and the *HNF-4α* gene requires pharmacological treatment. Patients with *HNF-1α* gene mutation, typically are young, slim adults with symptomatic diabetes, glucose range between 300–500 mg/dl and can present extrapancreatic features such as renal cysts and liver adenomatosis. They demonstrate progressive deterioration in glycemic control throughout life and they are at risk of developing diabetes-related chronic complications [38, 40]. An important point is that these patients are extremely sensitive to low dose sulphonylureas [41, 42]. Moreover, patients receiving sulphonylureas achieved better glycemic control than those on insulin treatment [43].

Diabetes caused by mutations of the *HNF-4α* is less common than the mutation of the *HNF-1* α and should be considered when the mutation of *HNF-1α* gene is not detected and the clinical features strongly suggest the mutation of *HNF-1α* gene [44]. These patients are also sensitive to sulphonylureas [45].

Conclusions

Despite the fact that the MGD is a relatively rare genetic condition and there is lack of studies which would allow estimating the true prevalence of these diseases it should be always considered in the case of unusual course of diabetes. According to the latest ADA meeting's opinions, in some cases the possibility that a patient has other types of diabetes than type 1 or type 2 should be taken into consideration. MGD is a challenge for physicians due to its rare occurrence. As a consequence patients are misdiagnosed and receive incorrect treatment. Patients for genetic testing should be selected carefully. Prior to making a decision to perform genetic tests the clinician should analyze the family history for diabetes. It is crucial to determine the exact age of diabetes onset, especially if diagnosis was made earlier than 6 months after birth. In addition, assessment of autoantibodies level at the time of diabetes diagnosis is one of the most important aspects influencing appropriate diagnosis. Preferably combined antibody testing should be performed. The website www.diabetesgenes.org provides information for patients and professionals on research and clinical care in genetic types of diabetes (Probability Calculator for patients diagnosed < 35 years based on clinical criteria). Determining the cause of monogenic diabetes may

have implications for treatment, prognosis and genetic counselling; not only for a patient, but for other family members as well.

Key points

- The most frequent forms of monogenic diabetes are GCK, HNF1α andHNF4α MODY.
- Patients with absence of islet autoantibodies, especially when measured at diabetes diagnosis should be evaluated for either GCK or transcription factors MGD.
- In MGD characteristic for young adolescents the genetic subtype determines clinical picture and treatment response.
- Identification of a patient with MGD may allow switching from insulin injection to tablets such as sulphonylurea.
- Individuals with *GCK* mutations rarely need pharmacological treatment and the majority can be managed with diet alone.

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REVIEW PAPER

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Current directions in searching for tuberculostatic substances

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ABSTRACT

Yearly, 8 milion people advance to active tuberculosis (TB) and nearly 2 milion victims die of their infection. Long drug regimen is blamed for the emergence of drug resistant TB. Moreover, 20% of TB isolates are already resistant to the first line antituberculosis drugs. This situation has required to develop new, more active anti-TB substances. Several novel drug candidates from different groups of chemical compounds undergo clinical trials. Others, also promising agents, have been obtained recently. They are the basis for further modifications heading for improvement of their physicochemical, biological and toxicological parameters.

Keywords: tuberculosis, tuberculostatic agents, clinical trials.

It is a very complex process to develop and introduce new drugs, requiring years of hard work and large expenses. It often takes 12–15 years from qualifying a substance to clinical tests to their successful completion. What is also important is that only 10% of substances qualified to clinical tests reach the end of the study with positive notes. There are a few new tuberculostatic drugs of various chemical composition being currently in different clinical trial phases [1, 2, 3]. Virtually no new effective tuberculostatic drugs appeared on the pharmaceutical market since rifampicin has been introduced (1970). In the meantime, new multi-drug-resistant bacterial strains have evolved, making the antituberculotic treatment even harder. That is why it is necessary to search for new substances with different antituberculotic effects. In the next few years, there should be at least a few new and effective drugs of that kind. The tested drugs include: two bicyclic nitroimidazole derivatives: PA-824 and OPC-67683 [4], a pyrrol derivative LL- 3858 and some quinoline derivatives. From the beginning of the 21st century, a lot of new substances were identified that stand chances to become some of leading tuberculostatic drugs. Some of them are already being tested, and others, equally promising, have been discovered recently and are still subject to further research to improve their physicochemical, biological and toxicological properties.

This study will try to present the most interesting and promising tuberculostatic substances that have been reported in scientific news in the last twelve years.

At present, one of the diarylquinoline-type substances named TMC-207 (R-207910) has successfully undergone the second trial phase. This is one of the most promising tuberculostatic drugs. It will probably become a part of multi-drug therapy against multi-drug-resistant *M. tuberculosis* strains. There are twenty chemical substances in the diarylquinoline group, with MIC values below 0.5 μg/ml in *in vitro* studies on *M. tuberculosis* H37Rv. Antituberculotic effect was confirmed *in vivo* for three diarylquinoline combinations [5]. The one with the most advantageous characteristics was chosen, named TMC-207 and had undergone a number of further tests. This compound turned out to be an *in vitro M. tuberculosis* growth inhibitor for strains susceptible to the most common drugs as well as drug-resistant strains [6]. Its activity in *in vivo* tests was stronger than the activity of rifampicin and isoniazide and, furthermore, it was effective against latent bacterial forms. The antituberculotic effect increased when rifampicin, isoniazide and pyrazynamide were replaced by diarylquinoline TMC-207 (R-207910), leading to total bacilli elimination within two months of treatment. Moreover, this compound has a new

mechanism of action – it is a proton pump inhibitor of a $F_0F_1H^+$ ATP-synthase, which leads to a damage of the ATP structure and acid-base balance disorders in bacterial cells.

The tuberculostatic effect was also observed in 4-amine-7-chloroquinoline derivatives [7]. The strategy for obtaining these compounds was based on a substitution of chlorine atom in C-4 position in a 4,7-dichloroquinoline molecule by ethylenediamine chain, which is an ethambutol pharmacophor, and subsequently by its homologues: propylene- and butylenediamine and so on. The objective of further modifications was to determine the Structure-Activity Relationships (SAR) for the obtained products. It was observed in *in vitro* tests that in a group of 4-amine-7-chloroquinoline derivative compounds with different substituents in terminal carbon atom of alkyl chain, the most active compound was the one with a chlorine atom (MIC = 12.5 μg/ml). The MIC value changed adversely after substitution of halogens with amine group – NH_2 or azide group – N_3. The effect of the alkyl substituent length on antituberculotic effect was also tested. It turned out that lateral chain elongation up to ten carbon atoms leads to an increased tuberculostatic effect (**Figure 1**).

Another prospective antituberculotic drug is a substance identified as SQ-109. This compound was singled out of a group of 63,000 diamines in a search for antituberculotic substances [8–11]. This substance's mechanism of action inhibits the cell wall formation in tubercle bacilli. In the *in vitro* conditions, SQ-109 has even 35 times stronger tuberculostatic effect compared to another diamine group member, ethambutol. This substance is also effective against multidrug-resistant microbes. Adding SQ-109 to rifampicin and isoniazid in antituberculous therapy makes the latter ones more effective. Different tests assessing efficacy of SQ-109 and other antituberculotic drugs combinations as well as SQ-109 monotherapy treatment are currently being conducted. Apart from antituberculotic effect, this substance is effective also against *Helicobacter pylori*. This effect is now being tested and is in the second phase of clinical trials [12].

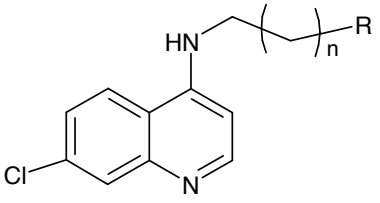
Further search for new, better antituberculous drugs yielded a discovery of the group of β-sulphonylacetamide compounds. One compound with the highest activity was selected out of this group and marked FAS-20013. It has been proved that this substance is a mycolic acid synthase inhibitor and that it disturbs energy production in bacterial cells, causing their systematic dying. FAS-20013 is also effective in the case of drug resistance. Moreover, this drug was observed to cause no serious adverse effects [13, 14] typical of other similar drug classes.

Sutezolid, initially marked as PNU-100480, is an oxazolidinone antibiotic currently in the second phase of clinical trials. Sutezolid is three times more active against MDR-Tb strains than structurally similar linezolid, both *in vivo* and *in vitro*. Compared to linezolid, it is much less toxic and has better pharmacokinetic characteristics resulting from drug absorption. Mechanism of action for both oxazolidinones consists in binding the 50S subunit of the ribosome, and thus inhibiting one of the earliest stages of essential bacterial proteins synthesis [15].

It was observed in another study that some of pyrrole derivatives have a tuberculostatic effect on *M. tuberculosis* in i*n vitro* conditions. The most active compound – LL-3858 has been selected out of this group and is characterized by being more effective in monotherapy than isoniazide *in vivo*. The mean MIC value is 0.19 μg/ml, also for drug-resistant strains [16]. Its mechanism of action has so far remained unknown.

Chemical structures of the described compounds are displayed in **Figure 2**.

Pleuromutiline belongs to the class of semisynthetic antibiotics, produced from *Pleurotus mutilis* strains. Two members of that class, tiamulin and valnemulin, are veterinary antibiotics acting against avian myco-



R= -H, -NH_2, -OH, halogen, -N_3, etc.

R= -NH_2

n=6 MIC=25.0 μg/ml

n=8 MIC=6.25 μg/ml

n=10 MIC=3.12 μg/ml

Figure 1. The MIC values for quinoline derivatives against M. tuberculosis

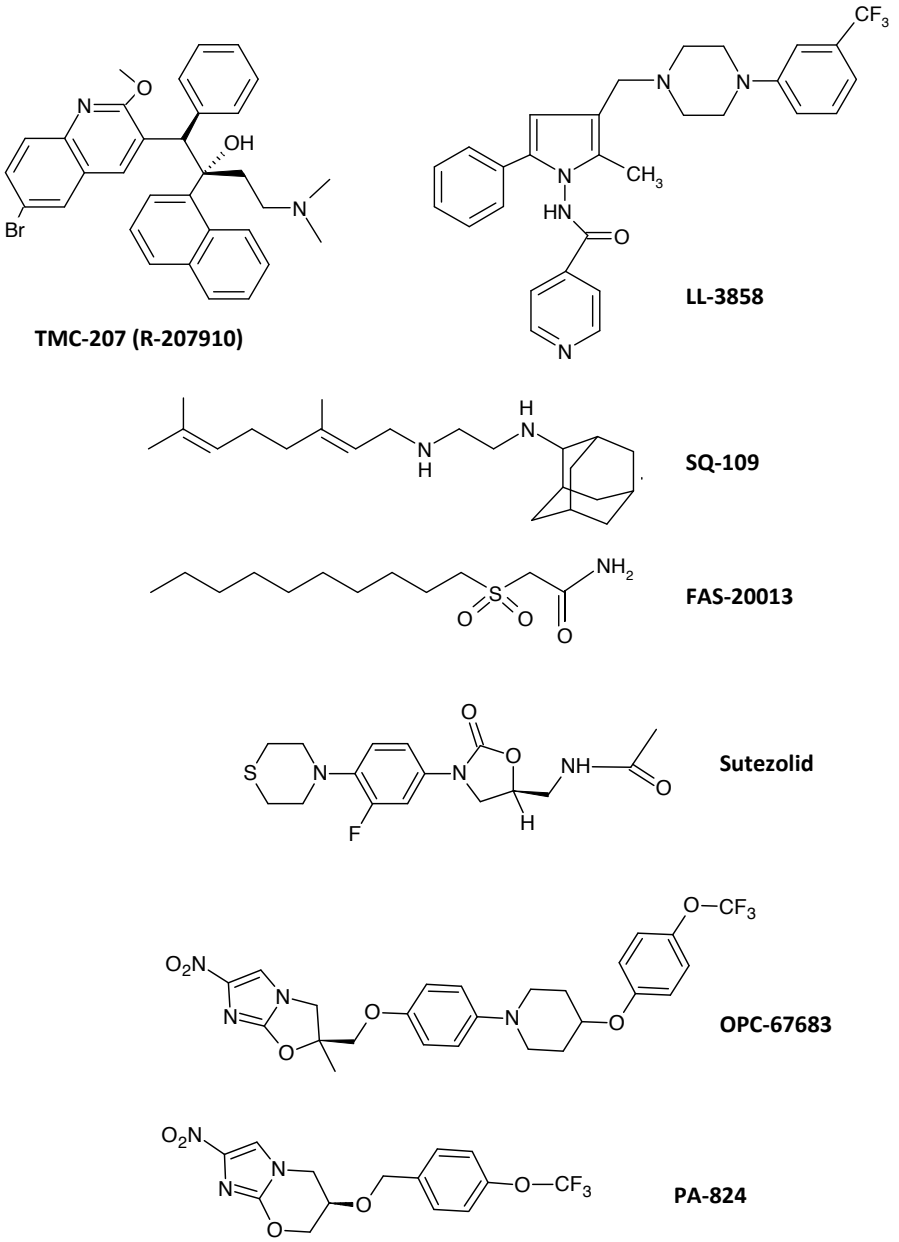


Figure 2. The structures of choosen tuberculostatic agents currently undergoing clinical trials

plasmosis. Pleuromutilines inhibit bacterial protein synthesis by binding the 50S subunit of the bacterial rRNA and thus inhibiting formation of peptidic bonds [17]. This mechanism of action suggested a possibility of using these antibiotics in antituberculotic treatment. Further studies proved these antibiotics to have antituberculotic effects, also against drug-resistant strains. Chemical structures of these substances are shown in **Figure 3.**

While searching for new antituberculotic drugs, previously known antibiotic classes were re-analyzed. Two fluoroquinolones were found that way: gatifloxacin (GAT) and moxifloxacin (MXF) [18]. Both drugs are now in the third phase of clinical trials. Gatifloxacin has a tuberculostatic effect *in vitro* as well as *in vivo*. In *in vitro* tests, the greatest antituberculotic effect is observed during the first two days of testing [19]. Similar results were obtained when gatifloxacin was used in combination with isoniazide (INH) or rifampicin (RIF) [19]. The other tuberculostatic drug in the fluoroquinolone class, moxifloxacin, is more promising. In *in vitro* conditions, it destroys populations of tubercle bacilli which developed resistance to rifampicin. This distinguishes moxifloxacin among other tuberculostatic fluoroquinolones of prior generations: ciprofloxacin and ofloxacin [18]. It is likely that moxifloxacin influences protein synthesis in slow metabolizing bacteria. This mechanism significantly differs from rifampicin's

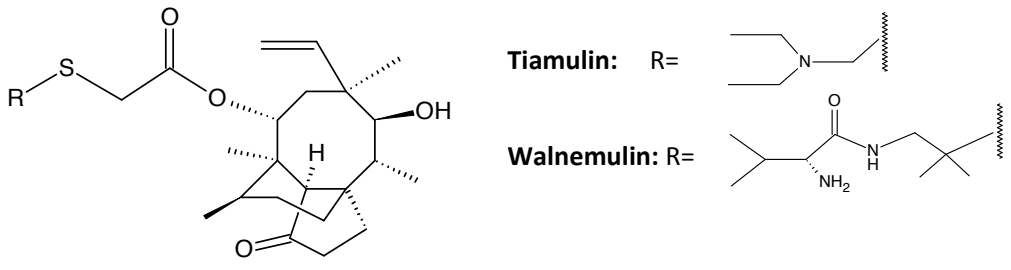
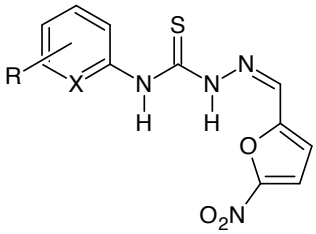


Figure 3. The structures of choosen pleuromutilins



R	X	MIC (μg/ml)
3-Br	CH	0.54
2,4-$(NO_2)_2$	CH	0.52
3,5-Br_2	N	0.22
H	N	12.1

Figure 4. N'-substituted semicarbazonic derivatives of 5-nitrofurfural with tuberculostatic activity

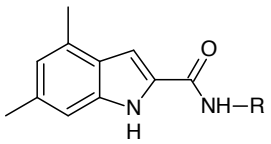
mechanism of action. In *in vivo* trials, MXF's activity is comparable to that of isoniazide. Furthermore, combination of MXF and pyrazynamide is more effective than the combination of isoniazide, rifampicin and pyrazynamide [20].

Other authors have proved antituberculotic effect of N'-substituents of 5-nitrofurfural semicarbazone derivatives [21] (**Figure 4**).

The highest efficacy was related to the presence of electron accepting substituents such as chlorine atom or nitro group in the aryl fragment. In some cases, MIC values were lower compared to their equivalents of isoniazide, rifampicin and ciprofloxacin. Pyridil thiosemicarbazone derivatives proved to be more effective than their phenyl equivalents. These compounds eradicate slowly growing mycobacteria which could reduce therapy duration.

A number of scientific reports discuss tuberculostatic effect of monosubstituted bicyclic amides of heterocyclic compounds derivatives, both with one and two nitrogen atoms. One of the papers [22] presents a number of amide substituted indole derivatives (**Figure 5**).

Derivatives 3–5 with expanded cyclic systems from the amide group had the highest tuberculostatic effect. Their MIC values were lower compared to the MIC of isoniazide, TMC- 207 and PA- 824 [22]. Further-



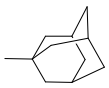
Compound	R	MIC (μM)
1		0.93
2		0.055
3		0.013
4		0.012
5		0.012

Figure 5. The MIC values for obtained heterocyclic compounds

more, compounds 1, 2 and 3 had a significant effect against multi-drug-resistant XDR-Tb strains. What is extremely positive about this group of drugs is that the obtained substances are not toxic. These findings make the amide indole derivatives one of the most promising groups antituberculotic compounds.

Other authors [23] indicate a significant tuberculostatic effect of amide imidazo-[1,2-*a*]-pyridine derivatives (**Figure 6**).

The compounds having 4-(4-halogenohenoxy-substituted)benzyl substituent at amide nitrogene atom (7–9) had the lowest MIC values amounting to ⩽ 0.006 μM. It has also been observed that the tuberculostatic effect of these compounds against drug resistant XDR-Tb strains in *in vitro* tests was ten times higher than that of PA-824 [23].

The obtained results of biological trials determined the following relationships between the structure of the study compounds and their tuberculostatic effect:

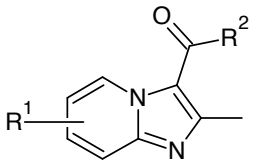
- the most advantageous factor is the presence of amide group in the second position of the bicyclic configuration;
- an expanded, three-dimensional lipophilic substituent is preferred by the amide N atom;
- N-1 atom in a heterocyclic system without any substituent and single substituted amide group N atom determine the manifestation of antituberculotic effect;
- presence of methyl groups in C-4 and C-6 positions of indole and C-6 and C-7 positions of imidazopyridine increases activity of these compounds.

Yokokawa et al. have recently described a number of compounds having another bicyclic system of pyrazol[1,5-*a*]tetrahydropyrimidine in their structure [24]. A substance with the most profitable physicochemical properties and the best biological activity has been selected based on antituberculotic test results (**Figure 7**).

That is why it is very likely to become one of the tuberculostatic drugs in the nearest future.

Chemical synthesis in laboratory is not the only way of creating new antituberculotic drugs. Some of them are naturally found in the environment. For example, Caribbean Sea corals of *Pseudopterogorgia elisabethae* genus produce a group of pseudopterosins and pseudopteroxazoles. McCulloch et al. [25] submitted these natural substances to some chemical modifications and obtained a number of semisynthetic tuberculostatic substances with MIC values comparable to these of ethambutol, kanamycin, capreomycin and cycloserine. The derivative with a methyleneimidazol substituent had the best parameters, with its MIC value at 34 μM. Its molecular structure is shown in **Figure 7**.

The clofamizine derivatives are another group of very promising compounds – clofamizine belongs to the riminophenazine group, used until now in leprosy treatment [26]. Its efficacy against drug-resistant *M. tuberculosis* strains has been known since the 1960s. Unfortunately, the possibilities of putting clofamizine to common use in antituberculotic treatment are largely limited due to its lipophilicity and the common side effect consisting in skin pigmentation disorders. That



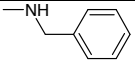
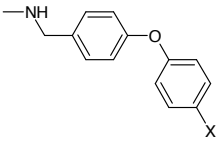
Compound	R^1	R^2	X
6	6-CH_3		-
7	7-CH_3		-Cl
8	6-CH_3		-F
9	7-CH_3		-F

Figure 6. The structures of some imidazo[1,2-a]pyridine derivatives

is why clofamizine derivatives with C-2 pyridilamine substituent were synthesized, which were less lipophilic and had no adverse effect on the skin, while retaining the desired tuberculostatic effect (**Figure 8**) [26].

Summary

Despite the constantly developing prophylaxis and increase of public awareness, tuberculosis remains a huge health problem, both worldwide and in Poland. According to WHO data for 2011 [27], tuberculosis was the second (directly after AIDS) most common cause of death all over the world.

An increased number of cases of multidrug resistant tuberculosis (MDR-Tb) resistant to isoniazide and rifampicin is now being observed [1]. Cases of XDR-Tb, i.e. multidrug resistant tuberculosis with additional resistance to other important tuberculostatic drugs: fluoroquinolones, amikacin, kanamycin and capreomycin, have presented in many countries [28].

Each year, a number of researcher groups working at different research institutes and industrial laboratories make successful attempts at synthesizing new antituberculotic substances. Hopefully, among these new chemical combinations there will be compounds that will become modern antituberculotic drugs.

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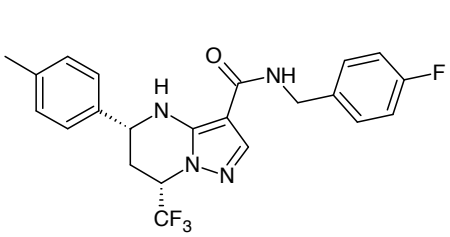
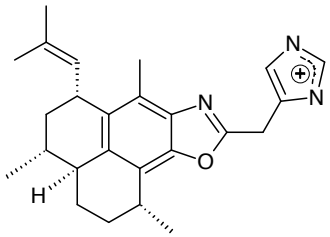
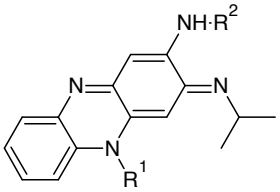


Pyrazol[1,5-*a*]tetrahydropirimidine



Pseudopteroxazole with methyleneimidazol substituent

Figure 7. The structural formula of pirazol[1,5-a]tetrahydropyrimidine and pseudopteroxazole with imidazomethylene substituent



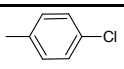
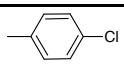
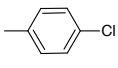
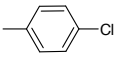
Compound	R^1	R^2	ClogP	MIC (μM)
clofazimine			7.137	0.25
10			5.322	0.57
11			3.507	1.23
12			5.322	0.07

Figure 8. The structural formula of clofazimine and their active derivatives

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REVIEW PAPER

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The role of the orthodontist in the early simulating plate rehabilitation of children with Down syndrome

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ABSTRACT

Introduction. Abnormalities in the oro-facial complex in children with Down syndrome can be corrected through oro-facial therapy which makes use of a palatal plate.
Aim. The aim of this study was to present the therapeutic effects of orthodontic palatal plate therapy in children with Down syndrome.
Material and methods. The study was conducted on 50 children with Down syndrome between the ages of 3 months and 2 years who were treated by means of stimulating plate therapy a period of two years.
Results. The study found that in 50% children with Down syndrome who underwent palatal plate oro-facial rehabilitation there was a visual improvement of the mimetic muscles, tongue retraction as well as lip closure.
Conclusions. The care by orthodontist and early palatal plate therapy ought to constitute an integral part of the comprehensive multidisciplinary rehabilitation of children with Down syndrome.

Keywords: Down syndrome, oro-facial rehabilitation, stimulating palatal plate.

The first description of a patient with Down syndrome was presented by Jean-Étienne Dominique Esquiriol in 1838. In 1866 John Langon Down published an article in which he delineated the characteristic features of this disorder, hence the condition being named after him. Down syndrome is a disorder conditioned by the presence of an extra autosomal chromosome. The mutation involves the presence of an extra copy of chromosome 21 in a pair of autosoms. There are three types of Down syndrome. The most common type is simple trisomy 21, which accounts for 90–95% of all Down syndrome cases. Translocation trisomy is much less frequent and occurs in 5–6% of cases. The rarest form is mosaic trisomy. In people with a mosaic karyotype the characteristic features of Down syndrome are less pronounced [1, 2]. The phenotypic features of Down syndrome occur when there is triple the number of genes characteristic of chromosome pair 21, regardless of which karyotype contains an extra copy. The incidence of Down syndrome in Poland is estimated at 1 in 604 live births. Publications on the subject identify the age of the pregnant woman as one of the risk factors of this disorder [3].

Typical phenotypic features of Down's syndrome which permit clinical diagnosis after birth include hypotonia; brachycephaly; extra skin folds on the neck;

midfacial hypoplasia (flat face); flat facial profile; widely spaced upslanting palpebral fissures; a flat nasal bridge; small misshapen ears; small wide hands with simian creases across the palms; and short stature [4]. The features which occur in the mouth include a slack, fissured and seemingly enlarged tongue; decreased tone of the orbicularis oris and buccinator muscles; and a disturbed balance of forces in the mouth with the tongue muscles being dominant. Incorrect tongue position can cause open bite, which is often diagnosed in children with Down syndrome. Because of the habitually open mouth and mouth breathing, such patients frequently develop malocclusion as well as disorders related to breathing, swallowing and articulation [5, 6, 7]. Additionally, the development of the mid-face region is inhibited [8]. In a study conducted in 1990, German researchers proved that the most severe symptom in children with Down syndrome is facial muscle hypotonia. The abnormalities and dysfunctions observed include varying degrees of mouth opening, the tongue protruding beyond the dental arches, inadequate chewing, and impaired articulation [9]. As a result of the phenomenon of neuroplasticity, which refers to the ability of the nervous system to change in response to external stimuli, manual rehabilitation exercises are typically introduced in early infancy. Such exercises help to harmonise the movements of the mandible and the tongue during sucking and swallowing, which promotes the correct development of chewing and speech functions [10]. The position of the tongue is evaluated when the child is lying down, sitting, playing, eating, drinking and speaking. A palatal plate with a stimulation element ought to be an integral part of such therapy. The aim of this study was to present a visual assessment of the facial features of children with Down syndrome made by the parents/guardians and orthodontists in order to obtain information regarding the effectiveness of oral rehabilitation through the use of a stimulating palatal plate, documented in the form of medical photographs as well as entries in examination reports and answers to questionnaire questions at two-month intervals.

Material and methods

From among the patients who received treatment as part of the programme of orthodontic care for children with congenital craniofacial defects, a group of 50 small children with Down syndrome were selected. The children were undergoing rehabilitation through the use of a palatal plate with a stimulating element for changing tongue position. The group comprised 28 girls and 22 boys aged between 3 months and 2 years. There were 22 only children among these children with Down syndrome, 21 had a brother or sister, and the remaining 7 came from large families. Twenty seven of the children were first-borns, 18 were second children, and 5 were later in the order of births, including 2 who were born as fifth children. In addition to anamnestic and clinical examinations of the children, the research project also included the parents, who were asked to closely monitor their children's progress and provide answers to a questionnaire conducted by an orthodontist during each of the bimonthly check-ups. The questions related to observing the facial appearance and tongue position of the children with Down syndrome; the frequency and duration of palatal plate usage; the child's willingness to perform exercises; as well as other observations made by the parents/guardians at home such as, for example, excessive salivation or the duration of lip closure without the plate. There were also questions relating to the observations made by an orthodontist during the regular appointments. Palatal plates, together with the stimulation element, were replaced every 6 months so as not to inhibit the development of the jaw. The study compared the effects of using palatal plates with two types of stimulation elements.

A statistical analysis of the data collected made it possible to answer the following questions:

- Does using plates with different stimulation elements in early infancy in children with Down syndrome produce better visual treatment results than using them at an older age?
- Which type of stimulation element produces better visual results according to the parents and orthodontists?
- What is the effectiveness of a stimulating plate in children with Down syndrome when it is used irregularly and when cooperation with the parents/guardians is inadequate?

Statistical analyses were performed using the Statistica 10.0 (StatSoft Inc., USA) program. The compatibility of the analysed quantitative variables with a normal distribution was tested by means of the Shapiro-Wilk test. For inter-group comparisons the Kruskal-Wallis test was used. A correlation analysis of the variables on the qualitative scales was performed using a chi-square test of independence. The assumed level of significance was $\alpha = 0.05$. The results were considered statistically significant at $p < 0.05$.

The research was approved by the Bioethics Committee at the Poznan University of Medical Sciences (decision No 97/11).

Results

The position of the tongue was assessed in an initial examination by an orthodontist and by the parents/guardians on a four-point scale (**Table 1**). In the initial assessment by the orthodontist and the parents the tongue was positioned between the lips in the majority of the children. No differences were observed in the tongue position in children of different genders ($p = 0.861$). Both in boys and in girls in the rest position the tongue was lying between the lips or between the alveolar margins (**Table 2**).

In 44 children the first palatal plate had a cylinder with a "roller", and 6 children had a plate with a movable bead (**Table 3**). Examples of plates and their application in a patient's mouth are presented in **Figures 1** and **2**.

An analysis of the photographic documentation collected throughout the course of the study revealed that in 86% of children with Down syndrome who received stimulating plate therapy there was a significant improvement as regards tongue position and lip closure and a resultant improvement in facial expression. The parents of 3 children temporarily withdrew from the treatment programme after using the first plate due to the need for other medical treatment, but

Table 1. Assessment of tongue position in children with Down syndrome by an orthodontist and by a parent/guardian

Tongue position	Orthodontist	Parent/Guardian
Inside the mouth	2	6
Between the alveolar margins	14	12
Between the lips	23	23
Protruding from the mouth	11	9

Table 2. Tongue position assessed by an orthodontist in children with Down syndrome according to gender

Tongue position	Gender		
	Boy	Girl	Total
Inside the mouth	1	1	2
Between the alveolar margins	6	8	14
Between the lips	9	14	23
Protruding from the mouth	6	5	11
Total	22	28	50

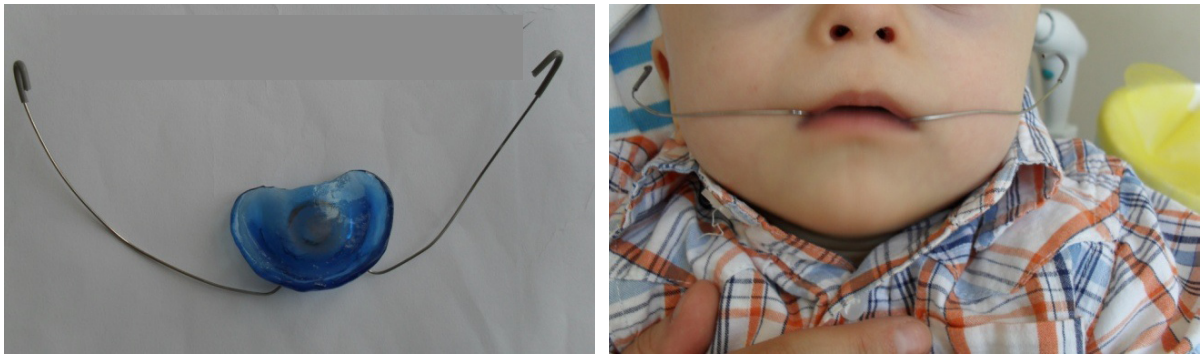

Figure 1. A palatal plate (left image) with a stimulator in the form of a cylinder with a "roller" and wire "whiskers" – patient G.S. (right image)

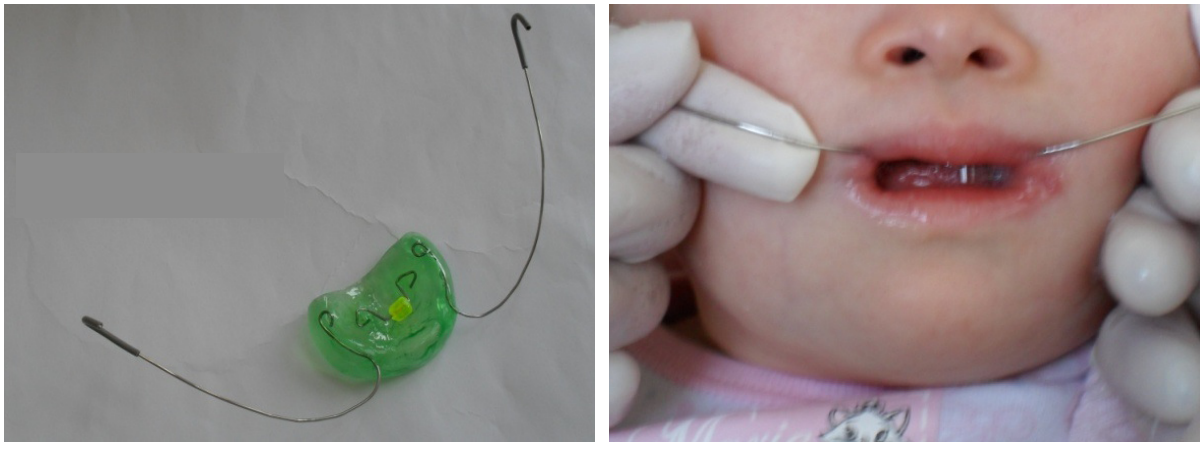

Figure 2. A palatal plate (left image) with a stimulator in the form of a movable bead and wire "whiskers" – patient B.M. (right image)

they expressed an interest in continuing rehabilitation at a later date. After using the first plate 10 patients discontinued treatment. In the opinions of both the parents and orthodontists, in the case of 7 patients there was no marked improvement in the tongue position and mimetic muscles (**Table 5**).

The assessments of the type of stimulator used made by the parents/guardians and the orthodontist were mostly identical. It was observed that using a plate having a cylinder with a "roller" produced the best results as regards lip closure in 33 patients, whereas in 10 patients a movable bead was more effective. In 28 patients tongue retraction was more pronounced after using a movable bead, whereas a cylinder with a "roller" worked better in 9 children. In the case of 6 patients both stimulation elements were rated equally. Based on an analysis of photographic documentation and the opinions of the parents of the children with Down syndrome, in 40 patients a cylinder with a "roller" produced a significant visual improvement in the mimetic muscles. The study also analysed the correlation between the age of the children and the adaptation of a palatal plate. The Kruskal-Wallis test rendered a statistically significant result ($p = 0.003$). The Dunn's multiple comparison test indicated that there were statistically significant differences in age between the children with good plate adaptation and those who quickly became discouraged from exercises ($p = 0.002$). The children in whom plate adaptation was good were approximately one year old (**Table 6**). In the analysis of correlation between plate adaptation and the exercise routine the chi-square test rendered a statistically significant result ($p = 0.027$). It was demonstrated that there is a correlation between plate adaptation and the exercise routine, as well as between the duration of exercises with a palatal plate and plate adaptation ($p = 0.004$). Longer and more frequent exercises resulted in a better assessment of the results by both the parents/guardians of the children with Down syndrome and the orthodontists.

Discussion

In children with Down syndrome it is necessary to carefully observe the stomatognathic system in order to determine the best time for starting therapy, monitor its progress and assess the results. Muscular abnormalities in the oro-facial complex become apparent at birth and their manifestations include the baby's difficulty in sucking, swallowing and closing its mouth as well as tongue protrusion. The first motor responses of the oral muscles in a newborn baby are reflex responses

Table 3. Type of stimulation element in 1st, 2nd and 3rd stimulating plate

Stimulation element	1st plate	2nd plate	3rd plate
Cylinder with a "roller"	44	16	2
Movable bead	6	24	7

Table 4. Position of stimulation element in 1st, 2nd and 3rd stimulating plate

Position	1st plate	2nd plate	3rd plate
Close to the front, immediately behind alveolar margin/teeth	8	1	0
In the centre of the plate	30	10	1
Close to the rear of the plate	12	29	8

Table 5. Improvement in tongue position and mimetic muscles in children with Down syndrome

Has there been an improvement in tongue position and mimetic muscles?	Number
Yes	43
No	7

Table 6. Correlation between a patient's age and palatal plate adaptation in children with Down syndrome over the entire course of therapy (2 years)

Plate adaptation	Average age	SD	p
Good plate adaptation	Below 1 year	0.8	0.003
Increased salivation	1.5 years	1.3	
Rather quick discouragement of the child	Over 2 years	0.7	

to tactile stimuli. Irritation of the mouth area produces the rooting reflex, turning the head towards the stimulus and opening the mouth. Stimulating the gums produces automatic jaw movements, the so-called bite reflex [11, 12]. It is recommended that the Castillo-Morales oro-facial regulation therapy should be implemented immediately after birth. The therapy ought to be conducted under the supervision of an experienced therapist because it is a significant component of neurophysiological therapy. The treatment includes massaging by touching, stroking and stretching, as well as using the most common vibration technique [10, 13, 14]. A child who starts to receive this therapy from the earliest stages of life can develop motor patterns which are close to the norm. This principle is the basis of functional maxillofacial orthopaedics and the effects clearly confirm the validity of such an assumption [15].

In addition to the manual stimulation of certain areas of the face, another method of oro-facial rehabilitation is a therapy which makes use of individual stimulating palatal plates, vestibular plates or other orthodontic appliances. Such forms of treatment promote an increase in the activity of the muscles responsible for swallowing, chewing and articulation. The rehabilitation treatment is adjusted to the individual needs of each patient, depending on the severity of the defect and any concurrent medical conditions. The therapy can include two components (massage and an appliance) or only one. Out of the patients analysed in the present study 33 children with Down syndrome underwent therapy consisting of both components, and those children achieved better results in the visual assessment of facial appearance over the period of two years [12, 16].

At every stage of therapy it was essential to cooperate closely with the children's parents and to motivate them to become involved and to carefully monitor the effects of treatment, as the ultimate outcome of the therapy largely depended on them, which has also been stressed by other researchers [17]. The parents of these children with Down syndrome were aged between 25 and 49, and the majority were between 31 and 40 years old. The average age of the mother when giving birth to a baby with Down syndrome was 35 years; and this was slightly lower than the age of the father which was 36 years. This data is consistent with the reports by Klotzka and Trojnarska, in whose study the age of the parents of children with Down syndrome confirmed the assertion that the probability of this anomaly is greater in the case of older mothers [18]. Hennequin et al. showed that the risk of Down syndrome is higher in the first pregnancy than in subsequent pregnancies, but also in later ones when there is a long interval between pregnancies [5].

Two important elements in the visual assessment of a child with Down syndrome are tongue position and lip muscle tone, which affect the facial features and play a considerable part in how the child is perceived by other people, which has been described by numerous researchers [9, 11, 19]. In this study only two children with Down syndrome were able to fully retract the tongue into the oral cavity, which confirms that it is the parents of children with an incorrect tongue position that bring their children for treatment. This coincides with the findings obtained by Fischer-Brandies et al. in 1988, as well as Hohoff and Ehmer in 1999. Those researchers observed a correct tongue position in about 5% of children with Down syndrome between the ages of 1 month and 6 years. This study did not discover significant differences in tongue position in the children with Down syndrome depending on gender when comparing its position during the initial examination, as well as during and after rehabilitation on the basis of the photographic documentation produced for every visit. An improvement in the mimetic muscles and tongue position was achieved in 43 out of the 50 analysed patients with Down syndrome. This result is higher than that reported by Hohoff et al., where improvement in tongue position was observed in 65% of patients; and by Schuster et al., where an improvement was recorded in 55% of patients treated with a stimulating plate [19, 20, 21]. The findings are similar to the findings of earlier studies conducted in Poland by Radwańska and Żmuda-Stawowiak, who reported an improvement in 45 out of the 55 patients who had received treatment [22]. Improvement was also recorded in 68 children in a study by Zavaglia et al., as well as in 42 children with Down syndrome aged between 6 and 21 months in a study by Bäckman et al., where there was significant improvement in respect of almost all the parameters in comparison to the initial condition [23, 24].

In almost all the children, inserting a palatal plate triggered the reaction described by Fisher-Brandies and Avalle, Limbrock et al., Nęcka et al., Matthews-Brzozowska et al., which involved retracting the tongue into the mouth and raising it towards the stimulation element located at a specific place on the palatal plate, which resulted in bringing the lips closer together and making it possible to close the mouth. The children who had the best plate adaptation in this study were the youngest group, around 12 months old [12, 25,

26]. Good plate adaptation in the youngest children was observed earlier by Schuster and Giese, thus it can be stated that introducing systematic rehabilitation of children at the youngest possible age can increase the chances of good palatal plate adaptation [19]. The parents reported that their children became discouraged from exercises when their teeth started to erupt, which was also observed by Bäckman et al. [27]. They also declared that the improved muscle tone continued after the plate had been removed, which was confirmed by Krombacher et al., who conducted long-term observations of children with Down syndrome who had undergone early oro-facial therapy through the use of a palatal plate [14].

Good plate adaptation was usually connected with performing the exercises correctly and systematically, which required a considerable involvement of the parents/guardians. However, it has to be borne in mind that though the parents or legal guardians may become fully involved in the therapy and accept it, they also have the right to discontinue rehabilitation. Thus it is extremely important to clearly explain to them what the treatment involves and what its results are likely to be. If the expectations of the parents/guardians are different, their involvement may turn out to be inadequate [28]. It was observed that among the patients who were doing the palatal plate exercises correctly and systematically, 19 children with Down syndrome (65%) were also undergoing manual therapy according to the Castillo-Morales method, thus the involvement of the parents/guardians in the rehabilitation of their children was evident.

This study also analysed the different elements of a stimulating palatal plate and their influence on tongue position and lip closure, as well as a visual improvement in the mimetic muscles. The assessments of the type of stimulator used made by the parents/guardians and the orthodontist were mostly identical. It was observed that using a plate with a cylinder with a "roller" produced the best results as regards lip closure in 33 patients. Tongue retraction was more pronounced in 28 patients with Down syndrome after using a movable bead. Based on an analysis of photographic documentation and the observations of the parents of the children with Down syndrome, in 40 patients a cylinder with a "roller" produced a significant visual improvement in the mimetic muscles. Thus it can be stated that a stimulator in the form of a movable bead, used mostly in the 2nd and 3rd plate, had the greatest impact on tongue position and retraction; whereas a cylinder with a "roller" had a greater influence as regards lip closure and a visual improvement in the appearance of the mimetic muscles. In part (63%), these findings are consistent with the data published by Castillo-Morales in 1992.

To recapitulate, oro-facial therapy has a significant impact as regards tongue position and lip closure, as well as a visual improvement in the mimetic muscles. Children with Down syndrome who began stimulating plate therapy in infancy in a considerable number of cases achieved a visual improvement in the mimetic muscles.

Conclusion

The results from oro-facial stimulating plate therapy obtained in this study clearly demonstrate that this method, together with the substantial involvement of an orthodontist, plays a crucial role in the rehabilitation of children with Down syndrome.

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REVIEW PAPER

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The role of viruses in the cancerogenesis

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ABSTRACT

It is estimated that seven key viruses such as Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human T-lymphotropic virus (HTLV), Human papilloma viruses (HPV), Kaposi's sarcoma-associated herpes-virus (KSHV), Epstein-Barr virus (EBV) and Merkel cell polyomavirus (MCV), are responsible for about 11% of cancers all over the world. Viruses however are not only associated with cancerogenesis process. Scientific researches from recent years emphasize the possible use of the microorganisms as antitumor therapy. Oncoviruses, also defined as tumor viruses cause cancers whereas oncolytic viruses infect the host's cancer cells leading to destruction of tumor and due to that they are described as cancer killing viruses. It offers the potential application of viral infections to the cancer therapy.

Keywords: cancers, cancerogensis, viruses.

Early days

A number of findings published within the past few years have confirmed pathogens' involvement in the aetiology of a significant share of human malignant cancers. According to 2008 data, 2 million out of 12.7 million newly diagnosed carcinomas worldwide are estimated to be associated with infectious factors, of which 1.9 million are assumed to be caused by four pathogens: Hepatitis B and Hepatitis C viruses, Human Papilloma Virus (HPV), and *Helicobacter pylori* [1]. The role of viruses and bacteria in carcinogenesis is becoming more and more apparent and can be the basis for actions aimed at limiting cancer prevalence, having eliminated carcinogenic pathogens by vaccination or eradication. This type of phenomena has been observed after Hepatitis B immunization was applied population-wide [2]. Effective primary prophylaxis was made possible after over 150 years of combined efforts of biologists, chemists, and physicians who have been searching for evidence in laboratory and epidemiological studies. A lecture by Domenico Antonio Rigoni-Sterna, held in 1824 in Verona, was the first documented suggestion of viral origins of the cancer. In his medical practice, Rigoni-Sterna learned that endometrial cancer was significantly more common in married women than in nuns. However, there were no methods available at that time to differentiate the results into cervical cancer and endometrial cancer, which makes it difficult to objectively evaluate these findings [3].

In 1882, Charles Chamberland, a French biologist, and Louis Pasteur developed a porcelain filter that could be used to remove micro-organisms from a solution. Many believe the year 1882 was the birth date of virology. The Chamberland-Pasteur filter could be used to obtain a filtrate which apparently contained no living microorganisms [3].

However, a decade later, Dmitry Ivanovsky, a Russian scientist, studied a plant disease called mosaic disease in tobacco. The results indicated that the filtrate contained some invisible, unquantifiable pathogenic microorganisms, which the scientist believed were toxins produced by bacteria [4]. Ivanovsky did not dissem-

inate his findings as, apparently, he was oblivious to their true significance.

Another scientist, Martinus Beijerinck, also investigated the mosaic disease and repeated the experiment in 1898 without having any knowledge of Ivanovsky's work. He named the infectious microbes, *contagium vivum fluidum*, and discovered that, unlike *contagium fixum,* they could not be extracted from water with any filters at hand. Beijerinck was the first to discover that *contagium vivum fluidum* can only be formed in the presence of living cells and became the first person in modern era biology to use the word 'virus' [4].

Virions – the virus particles

In early 20th century, with significant technical progress and improvements made to the filters, more and more sophisticated methods were developed to isolate the still elusive particles – virions. First experiments on animals were performed after a much clearer filtrate was successfully obtained. Two veterinary physicians, Vilhelm Ellerman and Olaf Bang, working at the University of Copenhagen, demonstrated the way leukaemia, or more specifically erythroblastosis, could be transmitted to healthy chickens by injecting them with a cell-free filtrate obtained from tissue. These experiments paved way for Peyton Rous to perform more research. In his work, Peyton Rous focused on investigating the biology of solid tumours in animals. He believed environmental factors to be the key to their origin. In his break-through publication entitled *Transmission of a Malignant New Growth by Means of a Cell-Free Filtrate* published in 1912 in *The Journal of Experimental Medicine*, Rous demonstrated that, when using sub-cellular filters, chicken sarcoma could be similarly transmitted [5]. Rous' studies were initially considered interesting, though entirely unreliable. Nevertheless, the scientist was awarded the Nobel Prize in 1966. A record time of 55 years had to pass from the first discovery until the results were fully recognised by the scientific community, proving the ground-breaking nature of the experiments performed, pointing to the viral aetiology of cancers. Despite much scepticism, some new papers on the alleged infectious origin of cancers were published at the end of the first decade of the 20th century [6].

Rous resumed his studies on chicken sarcoma in the 1920s and confirmed the initial results. First studies on mammals were conducted in the 1930s. American virologist, Richard Edwin Shope, who later identified the influenza virus and created the first effective vaccine, was the pioneer of studies on mammals. He discovered that rodents infected with the papilloma virus, now known as the Shope papilloma virus, developed a specific type of cancer, a keratinous tumour growing exophytically on the animal's head [7]. The theory of "particle-free pathogens" was abandoned after the mosaic disease virus was first crystallized in the 1930s by an American chemist, Wendell Meredith Stanley, working at the University of California, Berkley. Stanley, who later won the Nobel Prize, discovered that viruses were composed of nucleic acids and thus proved them-to be made up of particles [6, 7].

Isolation of the murine leukaemia virus (MuLV) and polyomavirus, both evidently associated with carcinogenesis, was another important contribution to virology in the early 20th century. Both discoveries were made by Ludwik Gross, a Cracow-born virologist working in the USA [8].

Viral carcinogenesis in humans

Works by Michael Anthony Epstein and his assistant, Yvonne Barr, marked the most important step towards investigating the role of viruses in carcinogenesis. In collaboration with Bert Achong, expert in the field of newly invented electron microscopy, the scientists analysed preparations from Uganda sent by Denis Burkitt, a surgeon. By combining epidemiological data and endogenous cancer sites, thesis was formulated and evidence of viral carcinogenesis provided [9]. During his work at the American Health Institute in the 1960s, Baruch Samuel Blumberg isolated viruses causing hepatitis in humans. These were Hepatitis A and Hepatitis B viruses. Despite numerous indications of their role in carcinogenesis, epidemiological evidence was brought up only 20 years later. In the1980s, Bernard Poiesz and Francis Ruscetti studied retroviruses at the Robert Gallo Laboratory and discovered the Human T-lymphotropic virus, etiologically related to T-cell leukemia/lymphoma [10]. The isolation of human papilloma virus, strains 16 and 18, still remains one of the most important discoveries in clinical terms. It was accomplished by a team led by Harald zur Hausen, who won the Nobel Prize in 2008. Human papilloma virus strains 16 and 18 cause 70% of all cases of cervical cancer, which can be prevented by effective immunization. In many interviews, professor Harald zur Hausen argued that the vaccine could have been developed as early as in the 1980s, however, no pharmaceutical companies decided to take up production since the financial forecasts were unsatisfactory [11]. In 1987, Michael Houghton and David W. Bradley independently identified a virus which was formerly known as the Non-A, Non-B Hepatitis virus.

The Hepatitis C virus is one of the most common infectious factors which leads to the development of hepatic cancer. In the 1990s, a married couple of scientists, Patrick Moore and Yuan Chang used the Representational Difference Analysis (RDA) to isolate Kaposi's sarcoma-associated herpesvirus (KSHV), classified as Human Herpes Virus 8 (HHV-8). In the first years of the 21st century, they developed a new virus isolation method, the so-called Digital Transcriptome Subtraction (DTS). It was used to prove that the Merkel cell carcinoma was associated with the polyomavirus [13].

In recent years, the relationship between viral infections and carcinoma has been discussed in the context of eliminating cancer cells by infecting them with viruses. For example, infection with human cytomegalovirus may affect cancer in a process called oncomodulation, which favours the growth of more malignant cell clones [14]. Oncomodulation has been investigated by several research centres, and the results obtained can reasonably be expected to contribute to the development of new therapeutic interventions [15, 16].

Oncolytic viruses open up new possibilities in developing new cancer treatment options [17]. It was demonstrated that some viruses preferentially infect and kill cancer cells. Therapies based on this type of viruses could be used if the cancer fails to respond to traditional chemotherapeutics [18]. Oncolytic viruses were in the spotlight after cases of cancer regression following natural infection or immunization have been observed. Oncolytic viruses preferentially attack cancer cells, penetrate them using cell receptors, use molecular pathomechanisms associated with malignant transformation, and block interferon-mediated anti-viral response. Viral replication in the cancer cell leads to its destruction and regression. In light of these observations, cancer cell infections with oncolytic viruses are now examined for their potential use in cancer treatment [19].

Summary

It is estimated that the seven key viruses: Hepatitis B and Hepatitis C viruses, Human T-lymphotropic Virus, Human Papilloma Virus (HPV), Kaposi's sarcoma-associated herpesvirus (KSHV), Epstein-Barr Virus, and Polyomavirus associated with Merkel cell carcinoma [20] are responsible for 11% of all cases of cancer worldwide. In recent years, potential opportunities to use viral infections in the development of new cancer treatment options were investigated [18]. Oncovirus, synonymously called a 'tumour virus', is a virus that can cause cancer, whereas an oncolytic virus preferentially infects the host's cancer cells and lyses them, causing tumour destruction, and is thus referred to as a 'cancer killing virus'. Viruses which have been associated with cancer so far,may become a promising therapeutic tool as the mechanisms of their functioning in the host's cells are more closely investigated.

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Contemporary rehabilitation at cancer centres

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ABSTRACT

Aim. Cancer rehabilitation is an important, but often underutilized treatment in the comprehensive care of the cancer patient. The lack of appropriate referral by physicians unfamiliar with the concept of rehabilitation was identified as primary barriers to optimal delivery of rehabilitation care. Therefore, the aim of this paper is to describe the current situation in the world of science of cancer rehabilitation and to describe availability of professional resources in the selected countries in the world.

Material and methods. This paper is a review article to describe rehabilitation models in cancer services for patients in selected cases in the world.

Results. Rehabilitation should be applied in various settings, depending on the level of disability, extent of disease, medical acuity level of the patient, and available services. However, the rehabilitation systems in the world differ depending on the various social security and health-care systems, but they are largely based on a similar, multidimensional and multidisciplinary understanding of cancer rehabilitation.

Conclusions. On the basis of description of the bio-psycho-social models, it can be seen that rehabilitation must be an integral and continuous part of all cancer care. There is strong evidence that rehabilitation is a well-tolerated and safe adjunct therapy that can mitigate several common treatment-related side effects among cancer patients.

Keywords: oncology, rehabilitation medicine, physical activity, physiotherapy, occupational therapy.

Introduction

The concept of rehabilitation in cancer care is part of the new situation characterized by a stable rise in incidence of cancer overall in most countries, concurring with a rise in the number of cancer survivors [1]. The lack of identification of patient problems and of appropriate referral by physicians unfamiliar with the concept of rehabilitation were identified as primary barriers to optimal delivery of rehabilitation care.

The World Health Organization (WHO) has defined rehabilitation as "the use of all means aimed at reducing the impact of disabling and handicapping conditions and at enabling people with disabilities to achieve optimal social integration" [2]. The Model of Functional Health as established in the WHO's International Classification of Functioning, Disability and Health (ICF) is considered to provide a theoretical framework of rehabilitation [3, 4]. The ICF complements the International Classification of Diseases (ICD) and provides a conclusive conceptual framework which incorporates the biological, as well as the individual and the social aspects of health conditions [5, 6].

Based on the bio-psycho-social model of the WHO and a holistic approach of rehabilitation, the cancer rehabilitation comprises multidisciplinary efforts including medical, psychological and physiotherapeutic treatment, as well as occupational therapy and functional therapy, depending on the patient's functional status. Recommendations have been made for finding better methods for identifying and managing the broader effects of cancer and its treatment and for integrating a more holistic interdisciplinary approach during and after the treatment of patients with cancer. The role of physical therapy is well established in some areas (e.g., post-mastectomy exercises, lymphedema management), and it is exciting to see the programmes that are emerging across the countries and the breadth of involvement of exercises discipline across cancer types and through the continuum of cancer care.

Aim

The aims of this paper are to provide a description of the current situation in cancer rehabilitation, to give an overview of the state of science of cancer rehabilitation and to describe availability of professional resources within the general health-care systems in selected countries in the world.

Material and methods

This paper is a review article to describe professional resources and rehabilitation models in cancer services for patients in selected countries of the world. This article presents rehabilitation programms in the United States, Canada, and Germany as well as an overview of ongoing studies.

Prior to treatment

The limited available data suggest that short-term, presurgical exercise training is feasible, well-tolerated, and potentially associated with significant improvements in aerobic capacity [6, 7].

During cancer therapy

The vast majority of studies were conducted in patients with early stage disease, predominantly women with breast cancer, receiving conventional cytotoxic therapies such as chemotherapy, radiotherapy, or androgen deprivation therapy (in the case of prostate cancer patients). Results of these studies indicated that supervised exercise training following traditional guidelines [i.e. aerobic training (3 d/wk, for 30–45 min per session, at 50–75% of age-predicted heart rate maximum or baseline aerobic capacity for 12–15 weeks) or resistance training (3 d/wk, 10 exercises, at 60–70% of one-repetition maximum)] was associated with improvements in terms of aerobic capacity, upper and lower extremity muscle strength, functional QOL, and several other psychological outcomes. Overall, the current literature base indicates that supervised exercise therapy is safe and feasible and associated with significant improvements in selected outcomes in patients with early-stage disease receiving conventional cytotoxic adjuvant therapy [1, 6, 7, 8].

Past adjuvant therapy

Speck et al. [9] reported that 60% of all studies in cancer rehabilitation were conducted in the post-adjuvant therapy setting. Results of the presented studies indicated that supervised exercise training following traditional guidelines was associated with improvements in measures of aerobic capacity, upper and lower extremity muscle strength, overall QOL, fatigue, and several other psychological outcomes (e.g. mood disturbance) [5–8].

Discussion

Rehabilitation needs vary greatly across tumour groups and individuals, as the medical effects depend on both the cancer type and the treatment regimen. Cancer can cause multiple impairments and the bio-psycho-social model as a core concept of modern definitions of rehabilitation supports the interdisciplinary team approach to cancer rehabilitation (**Table 1**). Depending on the cancer disease, patients may suffer from various functional symptoms such as, loss of motor control, cognitive and speech problems, swallowing problems, and sensory loss. Thus, evaluation studies related to cancer rehabilitation cover a wide variety of interventions and programs, ranging from specific treatments such as urinary incontinence training for prostate cancer patients to multidimensional rehabilitation programs covering several interventions from physical exercise to relaxation training and psycho-educational interventions [8, 10]. In general, the existent body of research indicates that rehabilitative interventions reduce symptom distress in cancer patients and increase quality of life, functioning and general well-being. However, the evidence levels for rehabilitative interventions range from good (e.g. for exercises or relaxation training and psychosocial counseling) to low (lymph drainage and art therapy). Multiple studies of cancer patients receiving interdisciplinary rehabilitation in inpatient settings have shown functional gains equal to or better than in control patients, who exhibited similar impairments without having received a cancer diagnosis [10]. Other studies of exercise interventions for cancer patients, both during and after treatments, have demonstrated positive outcomes in several realms, and not just increased exercise tolerance. DePompolo [11] described successful rehabilitation outcomes from an interdisciplinary inpatient consultation team. Hospice based rehabilitation interventions and outcomes have also been documented [12]. An acute inpatient cancer rehabilitation unit within a comprehensive cancer center, using a comprehensive interdisciplinary team, may be most appropriate. Patients referred for inpatient rehabilitation tend to be those with multiple impairments and multiple co-morbidities, with the likelihood of higher rates of complication after discharge.

Outpatient rehabilitation programs have been successful in reducing symptoms and in improving physi-

Table 1. Rehabilitation team interventions for cancer patients

Physical Medicine and Rehabilitation Physician – physiatrist	Inpatient consultation in cancer centre, coordination inpatient and outpatient cancer rehabilitation (prescribes treatments performed by professionals from other disciplines, such as physical, occupational, and speech therapy et al.), pharmacologic treatments in pain, spasticity, bowel and bladder dysfunction, mood stabilization, decreased initiation, and other symptoms and adverse effects, joint injections, trigger point injections, or botulinum toxin injections for symptom control
Physical therapist	Strengthening, range of motion exercises, endurance activities, and mobility training (e.g. transfers, gait, stair climbing)
Occupational therapist	Training in activities of daily living such as bathing, grooming, dressing, toileting, meal preparation, and homemaking. In addition, occupational therapists evaluate home environments for potential modification, provide instruction in driving with adaptive devices, and implement interventions to promote upper extremity ROM, strength, endurance, and coordination
Speech therapist	Cognitive assessment and training and swallowing evaluation and treatment communication deficits, dysphagia, and train patients in use of alternative means of speech and communication, including adaptive communication devices, laryngeal speech, oesophageal speech, and use of a prosthetic larynx. Treatment of patients who have oral defects or experience aphasia also falls within the purview of the speech therapist
Psychologist	Providing assessment and treatment to assist in management of cancer-related psychological distress. As a member of the rehabilitation team, the psychologist also assists other team members when psychological issues, either in patients or family members, complicate efforts to provide effective therapy. The goal of consultation of the psychologist with other team members is to maximize the benefit derived by the patient during the rehabilitation process
Social worker	Counselling services to patients and families regarding emotional support, community resources, finances, lifestyle changes, and treatment participation. In some settings, social workers often serve as leaders for support groups and also may provide active assistance in discharge planning activities, such as arranging home care services and transfer to other health care settings
Dietitian	Teaching patients and family members about the importance of appropriate diet in successful rehabilitation

cal and psychosocial functioning for patients during and after oncological treatment. Investigators have also found positive effects of outpatient exercise training, not only in terms of aerobic capacity, strength, and flexibility but also significant gains in multiple domains of quality-of-life (positive affect, decreased distress, enhanced wellbeing, and improved function) [13]. The American College of Sports Medicine (ACSM) recommends rehabilitation programs for patients with cancer diagnosis, on the grounds that these meet the goal of maintaining cardiovascular endurance, muscular strength, and function. The benefits include: decreased nausea, decreased fatigue, increased endurance, and improved quality of life. The 2008 US Department of Health and Human Services (US DHHS) Physical Activity Guidelines for Americans recommends weekly aerobic activity of 150 minutes of moderate intensity exercise or 75 minutes of vigorous-intensity exercise or an equivalent combination. As well as 2–3 weekly sessions of strength training for major muscle groups and stretching of major muscle groups on days which other exercises are performed [7]. The National Comprehensive Cancer Network (NCCN) recommends 30 minutes per day, 5 days per week as a goal for exercise for patients with cancer.

The rehabilitation programs in the United States, Canada, and Germany

The first model of cancer rehabilitation was written in 1940's by dr Howard Rusk with dr Taylor in *New Hope for the Handicapped* in the United States (US). Cancer as a "special rehabilitation" problem was described in the chapter on rehabilitation of surgical patients [14]. First volume by Howard Rusk *Rehabilitation Medicine* contained a full chapter on cancer rehabilitation in the initial 1958 edition [15].

The next stage of development of this program took place in 1971 when the National Cancer Act was passed, and funds became more readily available for the development of training, demonstration, and research projects in rehabilitation and were administered through the Division of Cancer Control and Rehabilitation, National Cancer Institute (NCI) of the National Institute of Health (NIH) [16, 17].

The cancer rehabilitation history certainly would not be complete without the pioneer rehabilitation programs. Two early programs were conducted at the University of Texas MD Anderson Cancer Center and in New York [18].

At present, at MD Anderson Cancer Center in Texas, in the US, the inpatient cancer rehabilitation interdisciplinary team includes a physiatrist, a nurse practitioner, a physical therapist, an occupational therapist, a speech therapist, a rehabilitation nursing specialist, a nutritionist, a pharmacist, a case manager, working together to achieve the goal of safe patient discharge [19]. This team works closely with primary medical and surgical oncologists to coordinate care for ongoing chemotherapy, complex surgical wounds, adverse effects

from treatment, and the effects of disease progression. Ready access to medical and surgical specialists, internists, and intensive care specialists is required to manage the complex acute issues which these patients can present. An exception to this rule is a subset of patients who have developed significant deconditioning after systemic chemotherapy, and the rehabilitation goal is to improve their function back up to the performance level at which they would be eligible for their next round of systemic chemotherapy. These patients' planned discharge disposition is to go back to their oncology team for further inpatient treatment [20, 21, 22].

In Canada, in 1997, the Ottawa Regional Cancer Centre began to offer an Oncology Rehabilitation Program to patients with cancer. The primary goal of this program was to improve the quality of life, functional performance and psychosocial adjustment of patients with cancer who were undergoing active therapy. An indoor walking and jogging track, a group exercise area, free weights and an array of strength and aerobic training machines are available [23]. If the patient is eligible for participation, an exercise specialist (an exercise physiologist with a relevant degree) completes a compulsory physical fitness assessment and an optional nutritional assessment and determines baseline values for health-related quality of life.

In Germany, in turn, rehabilitation is an integral part of a comprehensive social security system which roots date back to the 19th century. The slogan "rehabilitation before retirement" captures the idea of rehabilitation as a prevention of early retirement in a nutshell. Based on the historical background of the German social security system, the German rehabilitation system evolved as a specific and independent system which is unique and distinct from the system in many other European countries where rehabilitation measures are part of primary health care [24]. Nowadays, based on the social laws, rehabilitation measures are mainly carried out as inpatient programs in specialized rehabilitation clinics, which are staffed with multidisciplinary rehabilitation teams.

The drawback issues of cancer rehabilitation

More rehabilitation professionals (including physiatrists – physical medicine and rehabilitation physican, physical therapists, occupational therapists, and speech and language pathologists, social workers) need to have specific training in the field of oncology rehabilitation. The American Academy of Physical Medicine and Rehabilitation has developed a Medical Rehabilitation Council that specifically contains a Cancer Rehabilitation Medical Subspecialty Group to help physiatrists share information, network, and foster the development of oncology rehabilitation [25]. Oncologists and other physicians need education on appropriate screening for and referral to rehabilitation for cancer patients. This cross-disciplinary interaction is an important part of developing and fostering oncology rehabilitation services. Interdisciplinary care for oncology patients and cancer survivors needs to become part of the standard of treatment. Rehabilitation and oncology professionals need to find ways to work together to provide optimal cancer rehabilitation services to the many patients who need them. There is clearly a significant deficit in cancer care when rehabilitation is not offered to those who will likely benefit from it. Bridging the gap between these two disciplines can be challenging but is an important goal to provide the best possible care for cancer survivors. By improving physical functioning, we can also positively influence the survivor's social and emotional functioning [26]. In some cases, a patient-centred approach with an individualized comprehensive treatment plan may need to be developed for the survivor. This is best accomplished by means of an interdisciplinary rehabilitation team that also includes expertise in, for example, occupational therapy, nutrition, speech–language pathology and exercise physiology.

Weaknesses of the study

The paper does not take into account the poor accessibility and financing the cancer rehabilitation services of the holistic approach in cancer care despite their obvious benefits.

Conclusion

Cancer rehabilitation can occur in various settings, depending on the level of disability, extent of disease, medical acuity level of the patient, and available services. Rehabilitation has substantial effects on the patients' physical, psychological, social and existential well-being. There is strong evidence that rehabilitation is a well-tolerated and safe adjunct therapy that can mitigate several common treatment-related side effects among cancer patients.

Conflict interest

The authors indicated no potential conflicts of interest.

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The impact of the glycemic index and glycemic load of food products on human health

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ABSTRACT

The supply of carbohydrates, contained in food products and meals, to the organism causes an increase of glucose in blood, which is referred as "glycemic effect". The concentration of glucose in blood increases after eating each meal and usually reaches its maximum after 20–30 min after food consumption, and then is gradually reduced. It returns to the level of fasting within 1–2 hours. However this pattern shows some individual variation.
The increase of interest of the glycemic index and glycemic load of food products came through the interconnection of these indicators of noncommunicable chronic diseases like diabetes, cardiovascular disease, cancer, overweight and obesity.

Keywords: glycemic index, glycemic load, carbohydrates, nutritionally dependent illnesses.

Providing the organism with carbohydrates products and meals causes an increase of blood glucose, which is referred as "glycemic effect". After 20–30 minutes of food consumption the glucose concentration reaches its maximum, then decreases gradually and is returning to the level of fasting after 2 hours. Human insulin hormone protects against excessive glucose concentration in blood, it is stimulating the conversion of excess glucose into glycogen and its storage in the liver. The indicator that is used for classification of the carbohydrate food products in terms of increase in the concentration of blood glucose after the ingestion is the glycemic index (GI). GI is an average increase of blood glucose concentration after ingestion, by a statistically representative group of people (10–12 persons) a portion of product which contains 50 grams of digestible carbohydrates compared to the increase in blood sugar level after the ingestion of the reference glucose solution. The increase of blood sugar concentration after ingestion of 50 g glucose is the base of scale (IG = 100) [1].

Easily digestible carbohydrate from products with a high GI are rapidly digested and absorbed from the gastrointestinal tract. They cause a rapid increase of blood glucose and then a rapid decrease of glycemia after a meal. Products with a low GI cause a low and slow increase of glucose concentration in blood. The lowering of the blood glucose is also slower after 60 minutes [2]. The value of glycemic index depends on the physico-chemical factors of food and the individual ability to digest carbohydrates, so GI should not be the only criterion for assessing the suitability of products in patients nutrition [3]. The glycemic index defines only the type of carbohydrates which can increase blood glucose, and does not indicate the quantity of carbohydrates consumed. It is an important complement of the glycemic load (GL). It is the product of the glycemic index and the content of the digestible carbohydrates in the given product or meal. The value of the glicemic load has a practical use, because it is expressed per portion. This facilitates to compare products taking the usual consumption into account and calculate the total load of dishes by summing the values of the glicemical load of its particular components [1]. The usefulness of the glycemic load of products in the diet planning is based on the assumption that high GI foods, consumed in small quantities, have the same impact on the secretion of insulin into the blood as low-GI products consumed in large quantities [4].

The first studies on the GI values for different products and their role in human nutrition have been taken 31 years ago and are continued to the present. The glycemic index is used throughout the world as a physiological indicator differentiating carbohydrate-containing food products, depending on the degree of increase in postprandial blood glucose [5, 6]. This indicator was then popularized as a way to select foods to decrease risk of obesity, diabetes and cardiovascular disease. Knowing the GI values of foods also allows to select food products which are stimulating the secretion of insulin and resignation from products which can contribute to the resistance of cells to this hormone [7, 8].

It was assumed that the glycemic index of food products less than 55 is low, the average is in the range of 56 to 69, and the high GI above 70.

The value of glycemic index depends on many factors such as:

- ratio between amylose and amylopectin fractions in starch. Amylopectin is characterized by the branched molecules, as a result of which it is more susceptible to digestion and the release of glucose. Food containing more amylopectin has a higher GI than products containing more amylose, such as: white rice compared to basmati rice. Amylose is composed of strongly interconnected glucose molecules in the straight chain and thus access for digestive enzymes is restricted;
- physical structure of the product – the GI value of a product increases with increasing fragmentation (e.g., mashed potatoes have a higher GI than conventionally cooked potatoes);
- content of protein and fat in product – if the product contains more of these nutrients, then it contains less of carbohydrates. In addition, fat slows down the digestion rate of starch (such as potato chips have a lower GI than boiled potatoes). The higher content of fat and protein in the product reduces the GI due to increased glucose utilization, by increase of insulin secretion;
- fructose content – fructose belongs to the group of products with a low GI, thus the value of GI decreases with the increase of fructose participation in the product / dish;
- degree of ripeness of fruits and vegetables – along with an increase in the degree of ripeness the amount of starch decreases which increases the content of free carbohydrates, which promotes the growth of products GI;
- content of organic acids in food – these acids slow down the digestion of ingested food. An example would be a bread made from flour subjected to a fermentation process with yeast. This kind of bread contains higher amounts of the organic acids and thus has a lower GI value than white bread;
- dietary fiber content – whole grain cereal products containing more fibers have lower GI, and thereby delay the absorption of glucose into the bloodstream;
- thermal processing – it increases the GI of products, through intensification of swelling of the starch molecules, their rupture and consequently easier access of digestive enzymes to the glucose molecules of the starch (such as: pasta al dente has lower IG value than soft-boiled pasta);
- processing of food – starch from refined and highly processed food is more easily digested and has a higher GI (eg, white rice has a higher GI than brown rice);
- diversifying of the meal composition lowers the glycemic index (eg pasta has a GI of about 50, while serving the pasta with meat sauce reduces the IG to 20) [8–12].

The increased interest in the glycemic index and load followed as a result of connecting these indicators with the noninfectious chronic diseases, including diabetes, cardiovascular disease, cancer, overweight and obesity [13].

There were suggestions indicating that food with a high glycemic index is harmful to health and that consumers should be informed about the values of GI products that have a negative effect on the metabolism of glucose [14].

The disease closely associated with disorder of carbohydrate metabolism is diabetes (Diabetes mellitus), characterized by a reduction or inhibition of insulin secretion from β cells of Langerhans islets of the pancreas. This leads to metabolic disorder and abnormal use of carbohydrates in the body [15].

The concentration of glucose in blood and increased synthesis and secretion of insulin are dependent, inter alia, from the GI and origin of food products. Glycemic response to carbohydrates contained in the nutritional ration allowes to predict the changes in concentration of insulin in the blood. The synthesis and secretion of the insulin, in addition to glucose, can also induce amino acides. Hyperinsulinemia promotes the development of insulin resistance and overstimulation of the β-cell to produce insulin, which is the reason for development of type 2 diabetes and increases the risk of coronary heart disease [2, 16, 17].

The Pavlicek's research [18] showed that a diet taking into account the GI of food products by the

patients with type 2 (insulin-dependent) diabetes has an impact improving the glycemic. The experiment was held for 24 weeks, involving 210 people aged approximately 60 years. The author proved that a diet based on low GI exerts a positive impact on the cardiovascular health among patients with type 2 diabetes. Moreover, there was a reduction in glycated hemoglobin in the blood and an increased concentration of HDL cholesterol in the blood. Barakatun (2009) conducted a study involving patients with type 2 diabetes. The aim of the study was to compare the severity of postprandial glycemia and insulin response among subjects who received meals with the same energy and macronutrient supply, and differed only in the value of the glycemic index. In subjects who ate meals with low GI values lower glycemic response and a decrease in insulin levels in the blood was observed. It was found that the meals having a low GI glucose decreased glycemia after their ingestion, as well as insulin response among patients with type 2 diabetes. It was recommended to replace food with high GI by products with a low GI [19].

Gestational diabetes mellitus (GDM), is a disorder characterised by impaired carbohydrate tolerance with a very high probability of progressing to diabetes diagnosed during pregnancy [20]. This kind of diabetes increases the risk of complications of pregnancy and childbirth, fetal development and newborn condition if it is not recognized, diagnosed too late or improperly treated [21]. In the Moses'es study [22], conducted among 63 pregnant women with GDM has shown that eating low GI meals from the first trimester has a positive effect on pregnancy. Women with GDM were divided into two groups. Among 31 women consuming meals with a low GI – 9 required insulin treatment during pregnancy, while a group of 32 women who were eating high GI foods, up to 19 met the criteria predisposing to start insulin therapy. Nine from nineteen women managed to avoid the use of insulin by replacing the food rations of products with a high GI by products with a low GI. Application of a low GI diet, taking into account the meals, by women with GDM significantly reduced the necessity of insulin treatment and had no adverse effects on pregnancy.

Improper diet increases the risk of cardiovascular disease by increasing levels of serum cholesterol, especially LDL cholesterol, lower HDL cholesterol, increasing serum triglycerides, increasing blood pressure and impact on the development of abdominal obesity, as well as disability of glucose tolerance [23].

Based on the Frost's research [24], performed between 1986 to 1987 in England with the participation of 2,200 people aged 16–64 years, with an average BMI of 25.0 kg/m^2, an inverse correlation between GI diet and concentrations of serum HDL cholesterol was found. It was also found that body mass index (BMI), smoking cigarettes and GI diets are potential, modifiable factors that affect the concentration of HDL cholesterol serum. This suggests that a diet based on low GI may reduce the risk of developing atherosclerosis and coronary heart disease. In the studies of Liu [25], which were conducted with the participation of 75,521 women, aged 38–63 years, without any prior diagnosis of diabetes, myocardial infarction, stroke and other cardiovascular diseases, an inverse relationship was detected between glycemic index and prevalence of myocardial infarction. On the basis of information regarding the subjects – medical history, lifestyle, consumption of food products, containing mainly carbohydrates, it was found that the intake of foods with a high GI was strongly associated with risk of coronary heart disease. This applied especially to women with a BMI > 23.0 kg/m^2.

Hyperglycemia and resistance to insulin are also risk factors for cardiovascular diseases. Large variations in postprandial glycemia by patients with overweight have negative impact on structure and function of blood vessels [26]. Studies have shown that eating food with a low index and low glycemic load helps to improve the health status of patients with cardiovascular disorders, regardless of the existing risk factors of these diseases, such as: older age, smoking, intake of high energy value of food rations. This leads to a reduction of triglyceride levels and increased HDL cholesterol concentrations in blood [9, 25].

There is also evidence that use of a diet based on low glycemic index food products for five weeks, among healthy men, contributed to the improvement of the lipid profile of blood plasma and consequently led to a reduction in total body fat mass and increase in fat free body mass [27]. In the years 2003–2004 a study was conducted, involving 290 patients with coronary heart disease and a control group consisting of 290 healthy individuals. Based on collected information regarding their dietary habits, glycemic loads of individual meals for each person were calculated. The total average of the glycemic load of patients meals was significantly higher compared to the control group. These studies have shown that high glycemic load diet is an independent risk factor for coronary heart diseases. Therefore, it is recommended to consume meals containing vegetables and fruit with low GI [28].

People with a high Body Mass Index (BMI) and impaired energy balance are more often diagnosed with metabolic diseases, mainly obesity. Obesity, according to the WHO assumed to be a global epidemic. It is a disease characterized by excessive accumulation of fat in the human body [23, 29].

In recent years, in the prevention and treatment of obesity the limitation of fat consumption was widely recommended. However, scientists started to wonder about the alternative recommendations. Hence the interest in the role of diet and products with a low GI in the treatment of this disease. Food with low values of this ratio satisfy the hunger for longer and reduce appetite more effectively. These products do not result in rapid weight gain. Upon delivery to the body of high GI meals blood sugar increases rapidly. It is a natural consequence of the rapid digestion and absorption of carbohydrates contained in these products. Products with low GI may be more useful for effective weight loss than moderate carbohydrate restriction in food ration [30].

In 2004, a study on rats was conducted to evaluate the effects of diets with different IG values. The experiment lasted 18 weeks. Animals were randomly assigned in two groups. The first group consisted of 11 animals receiving feed with a high GI, and the second group of 10 rats received a feed with a low GI. The feed of all rats contained 69% carbohydrate, 20% protein and 11% of fats and both, the first and second group, included starch in amount of 542 g/1 kg of feed. The group which consumed high GI food received 100% of amylopectin, and participation of the various starch fractions in the second group was: 60% amylose and 40% amylopectin. The other feed ingredients were: gelatin, casein, sucrose, soybean oil, wheat bran, methionine and a mixture of vitamins and minerals. It has been found that a diet with a high GI caused an increase of fat cover in rodents. Despite similar average body weight in rats fed a diet rich in carbohydrates compared to rats fed with a low GI feed, there was a 40% higher increase in body fat mass (higher susceptibility to obesity). These rats were characterized by furthermore 45% lower physical activity and resistance to insulin. Already in the 7^{th} week of the experiment almost three times higher concentration of triglycerides in the blood of rats fed feed with a high GI was observed. In this group of laboratory animals, the glucose concentration was already significantly higher at the 5^{th} week and remained such to the end of the experiment. A much higher percentage of pancreatic islet was clearly abnormal, with the disorganized structure and extensive fibrosis [31].

Thomas et al. (2007) assessed the impact of low GI diets on body mass of people with overweight. The study involved 202 volonteers. In humans who applied the low GI diet, higher loss of body mass (1.1 kg), fat mass (1.1 kg) and BMI (1.3) were observed. Moreover a higher reduction of total cholesterol and its LDL fraction was found. (respectively 0.22 mmol/l and 0.24 mmol/l) [32].

Studies also indicate a high correlation between diet based on a high glycemic index and the risk of colorectal cancer (colon and rectum). To such conclusions have led, among others, researches conducted in the years 1992–1996, with participation of 1,125 men and 828 women with confirmed colorectal cancer. Age of the subjects ranged from 19 to 74 years. GI, GL and dietary fiber content in food portions were calculated using data obtained from the questionnaire concerning the amount and type of consumed food. It was observed that dietary GI and GL of patients was positively correlated with the consumption of breads, sweets, sugar and energy value of food ration, and negatively correlated with the consumption of fruit and vegetables. Thus, studies have proven negative meaning of refined carbohydrates in the etiology of the disease [33]. Similar conclusions have been reported in Augustin's research [34] conducted in 1991–1994, involving 2,569 women with confirmed breast cancer. The collection of information on the consumption took place in the same way as in the previous study. It was noted that the risk of breast cancer was associated with consumption of high GI products, particularly in the postmenopausal period. It is necessary to carry out further studies to understand the mechanisms of adverse effects of refined carbohydrates diet on the human organism.

Postprandial hyperglycemia significantly influences the growth and poliferation of neoplastic cells. It encourages several metabolic changes on cellular level as well as on tissue level. The results of the epidemical research, connected with involvement of general risk of cancerous disease development or particular type of cancer, are inhomogeneous. Most of them indicate an increase of this risk together with rising GL of diet. Researches have also proven that reducing digestible carbohydrate in the diet has possitive impact on the stability of the disease and an increased tolerance for radio- and chemotherapy [35].

Consumption of food products / meals with low GI and GL cannot only be beneficial for reduction of

postprandial glucose and insulin response, contributing to the preservation and improvement of health, but also for lipid metabolism and regulation of the energy changes in our organism. Application of a diet based on low GI and GL is an alternative to the current recommendations, often requiring restrictive limitations of food energy intake. This increases, among others, probability of long-term sustainable results of weight reduction.

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REVIEW PAPER

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The effects of vitamins and trace minerals on chronic autoimmune thyroiditis

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ABSTRACT

Hashimoto's thyroiditis (HT), also known as chronic lymphocytic thyroiditis is one of the most frequent types of inflammation of the thyroid gland. The prevalence of the overt HT is about 2% but it is believed that Hashimoto thyroiditis is more frequent than expected. Hashimoto's thyroiditis is characterized by dysfunction of the immune system, which leads to impaired tolerance of own tissues and increased production of autoantibodies against the thyroid cells. Thyroid peroxidase antibodies (anti-TPO), thyroglobulin antibodies (anti-Tg) and/or TSH receptors antibodies are the principal markers of the disease. The essential element of the treatment of HT is the supplementation of L-thyroxine. In Hashimoto's disease, like in many other autoimmune diseases, researchers attempted to support pharmacological treatment by adequate nutrition. The aim of this paper was to review the existing literature on the levels of antioxidants (vitamin A, C, E, selenium, zinc) and vitamin D in patients with HT, as well as the influence of the nutritional supplementation of the above mentioned elements on the metabolism of the thyroid gland hormones and the level of anti-thyroid peroxidase (anti-TPO) antibodies.

Keywords: Hashimoto's thyroiditis, antioxidants, vitamin D, selenium, vitamins and trace minerals supplementation.

Introduction

Hashimoto's thyroiditis also know as chronic lymphocytic thyroiditis is the most frequent type of inflammation of the thyroid gland. Although the disease has been described more than 100 years ago, it is still not fully understood.

Hashimoto's thyroiditis is characterized by dysfunction of immune system, which leads to impaired tolerance of own tissues and the increased production of autoantibodies against thyroid cells. Thyroid peroxidase antibodies (anti-TPO), thyroglobulin antibodies (anti-Tg) and/or TSH receptors antibodies are the principal markers of the disease. In addition HT revealed lymphocytic infiltration which destroys thyroid follicular cells. The concentration of free thyroxine (FT4) and free triiodothyronine (FT3) in patients with Hashimoto's disease is reduced. HT is the most frequent type of hypothyroidism. The prevalence of the overt HT is about 2% but it is believed that Hashimoto's thyroiditis is more frequent than expected [1]. The incidence rate of HT is estimated at 0.3–1.5/1,000 cases per year. Chronic autoimmune thyroiditis may occur in all age groups, also in children. The peak incidence is between 45 and 65 years of age. Women suffer from HT 10–20 times more frequently than men, which confirms the participation of estrogen in the development of the disease [2]. HT develops slowly, starting without any symptoms. In the initial phase of the disease may mild, temporary hyperthyroidism occur, which after a short period of *euthyroid state* changes into permanent hypothyroidism. There are many causes recognized in the etiology of the disease such as: environmental factors (nicotine, iodine and selenium intake, drugs, pollution), physiological state of the organism (pregnancy, menopause, emotional and physical stress), genetic factors (specific HLA allele, polymorphisms of *PTPN22* and thyroglobulin gene), bacterial and viral infections, female sex, and age [2, 3, 4]. Treatment of Hashimoto disease involves

L-thyroxine supplementation (1–2 mcg/kg/day) in order to normalize TSH and FT4 concentrations. In Hashimoto, like in many other autoimmune diseases, researchers attempted to support pharmacological treatment by adequate nutrition. Existing publications focused on vitamins and trace minerals supplementation in patients with Hashimoto disease. The aim of this paper was to review the literature on antioxidants (vitamin A, C, E, selenium, zinc) and vitamin D supplementation in HT and to assess its immunomodulatory effects and influence on metabolism of the thyroid gland hormones.

Antioxidant vitamins and trace minerals in Hashimoto's disease

Autoimmune Hashimoto's thyroiditis is associated with impaired antioxidant status and detrimental action of oxidants and free radicals. Enzymatic and non-enzymatic mechanisms play an important role in counteracting the harmful effects of the reactive oxygen species. The results of studies evaluating the effect of hypothyroidism on the body's antioxidant status are poor and inconsistent. Study conducted by Erdamar [5] the effects of hypo- and hyperthyroidism and treatment of those conditions on the antioxidant status markers revealed an increased generation of reactive oxygen species and impairment of the antioxidant system in patients with hyper- and hypothyroidism. This was seen particularly in patients with hypothyroidism. Similarly, Reddy's study [6] conducted on 72 patients with subclinical or overt hypothyroidism showed, that the malondialdehyde (MDA) and glutathione peroxidase (GPx) values were elevated, while glutathione (GSH), ferric reducing ability of plasma (FRAP) and superoxide dismutase (SOD) were decreased in both patient groups compared with controls. No change in activities of catalase (CAT) and glutathione reductase (GR) were observed in both patient groups. One of the recent studies [7] in this field indicated significant differences in the levels of glutathione (GSH), glutathione peroxidase (GPx) in patients with newly diagnosed HT as compared to the control group ($P < 0.001$). Since the activity of some antioxidant enzymes depends on the availability of copper, zinc (SOD) and selenium (GPx) it should come to mind, that changes in the concentration of these trace minerals (resulting from the pathophysiology of the disease) may affect the ability to defend against reactive oxygen species.

Zinc is an essential trace element for the conversion of thyroxine to triiodothyronine. Previous study [8] suggested that zinc deficiency leads to a reduction in the level of FT4 and FT3 and development of hypothyroidism symptoms. Existing studies did not examine the relationship between the occurrence of zinc deficiency and the risk of Hashimoto's disease. The study conducted by Erdal et al. [9] to evaluate the serum copper, zinc, magnesium, and selenium in patients with subclinical hypothyroidism did not confirm association between thyroid function and zinc level. Similarly, Przybylik-Mazurek et al. found no differences in copper, zinc, and Zn/Cu ratio between Hashimoto patients and control group [10]. Furthermore, the results of study [11] on the effects of zinc supplementation and thyroid hormone concentrations were not clear. A study conducted by Pathak [12] which evaluated the influence of zinc supplementation on the abnormal serum levels of triiodothyronine (FT3), thyroxine (FT4) (decreased) and thyroid-stimulating hormone (TSH) (increased) in male Wistar rats revealed, that 8 week administration of zinc regulated the T3, T4 and TSH concentrations. However, clinical research did not confirm that zinc supplementation in humans without substantial zinc deficiencies leads to regulation of thyroid hormone metabolism [13].

In addition to the enzymatic mechanisms, an important role in antioxidant defense is played by exogenous antioxidants like vitamin A, C, and E, which are delivered to the body with food. L-tocopherol, the most biologically active and most widespread vitamin E form in the body has a protective effect on cell membranes. As an antioxidant, vitamin E acts as a free radical scavenger, it inhibits lipid peroxidation and causes the extinction of singlet oxygen. Vitamin C (L-ascorbic acid) is involved in the reduction of tocopherol, hydroxyl radicals and reactions with superoxide anion. Animal study [14] which assessed the effects of antioxidants against methimazole (MMI) induced hypothyroidism in male Wistar rats showed benefits of supplementation with vitamins C and E for the thyroid gland. The rats who received vitamins C and E along with MMI showed statistically significant reduced weight (38–55% less) of the thyroid glands ($P < 0.01$) and less suppressed FT4 and FT3 levels (2–6% and 7–35% respectively) as compared to the controls. Other experimental study [15] indicated the influence of vitamin E supplementation on the reduction of malondialdehyde level in the group of male Sprague Dawley rats with propylthiouracil-induced hypothyroidism. Additionaly, vitamin E supplementation significantly increased liver and kidney reduced glutathione levels.

A different result was obtained in the study conducted by Adali et al. [16], who found no beneficial effects of vitamin E supplementation on the antioxidant status in patients with hypothyroidism.

Vitamin A has the ability to directly react with free radicals, scavenging lipid peroxides and singlet oxygen. Moreover, it plays an important role in the regulation of thyroid hormone metabolism and the inhibition of TSH secretion. So far, only a few studies evaluated the effect of vitamin A supplementation on thyroid function and treatment of Hashimoto's disease. A 4-month randomized, double blind controlled trial conducted by Farthang et al. [17], on 84 premenopausal women (56 women with obesity) showed, that vitamin A supplementation (25,000 IU/d retinyl palmitate) significantly reduced the serum TSH concentrations ($p = 0.004$), therefore it might reduce the risk of subclinical hypothyroidism in premenopausal women. Another study [18] demonstrated that the supply of vitamin A alone or in combination with iodine had a positive effect on the functioning and size of the thyroid gland.

It was previously suggested, that high levels of anti-TPO are associated with deficiency of antioxidant vitamins (E, A, C), trace mineral (Se) as well as iron and copper, which are important antioxidant enzyme cofactors. This thesis was not confirmed by Dellal et al. in their study [19], in which no relationship was observed between the levels of vitamin E, A, D, folate, Fe, Cu, Se and the occurrence of Hashimoto's disease. The study demonstrated the existence of a negative correlation between the concentration of vitamin B12 and the anti-TPO level (despite the absence of vitamin B12 deficiency). This generates the necessity to screen patients with HT for atrophic gastritis. Earlier studies [20, 21] on the relationship between thyroid dysfunction and levels of vitamin B12 showed that 7–12% of patients with HT suffer from pernicious anemia, therefore the concentration of cobalamin should be evaluated every 3 to 5 years. The study [22] on a large population (1,401 subjects) with mild and severe thyroid dysfunction indicated no correlation between the levels of zinc, selenium, vitamin C and the markers of thyroid gland function although, Moncayo et al. found, that the percentage of patients whose levels of vitamin C, zinc and selenium were below the reference values amounted to, respectively 8.7%, 7.8% and 20.3%.

Selenium is a necessary trace mineral for humans because of its antioxidant and anti-inflammatory properties. It is present in specific selenoproteins such as glutathione peroxidase (GSH), iodothyronine deiodinase and thioredoxin reductases (TRs). Selenium plays a major role in the thyroid hormone metabolism by conversion of triiodothyronine (T3) to tetraiodothyronine (FT4). The recommended daily intake of selenium varies from 55–70 µg depending on the geographic region (55 µg /day in the USA, 60–70 µg in England, 1 µg/ kg of body weight per day in France). Exceeding the dosage of 400 µg per day is toxic and leads to selenosis. The best food sources of selenium are meat, fish, shellfish, offal, eggs, cereals. Even a mild zinc deficiency can result in an increased risk of the development and progression of autoimmune thyroid disease.

Several previous studies confirmed that serum selenium level in patients with HT was lower than in the healthy control group. According to the Polish research [23] the average content of Se in serum of patients with Hashimoto disease (63.03 ± 17.31 µg/L) was significantly lower ($p < 0.0007$) in comparison with the control group (75.16 ± 19.92 µg/L). Similarly, Erdal et al. [24] revealed lower levels of selenium in serum of patients with Hashimoto's thyroiditis (67.7 ± 10.4 mg/l) as compared to the control group (83.7 ± 17.3 mg/l). It appears that in the publications demonstrating the benefits of selenium supplementation in autoimmune diseases, many of the studies focused on examining the effect of selenium supplementation on the course of HT, in particular the normalization of thyroid gland hormone levels and the reduction of anti-TPO concentrations. These results were not conclusive [25, 26]. One of the earliest studies [27] in this field revealed, that in patients with Hashimoto's disease selenium supplementation decreased anti-thyroid antibody levels and improved the ultrasound structure of the thyroid gland.

Several clinical studies in patients with autoimmune thyroid disease demonstrated, that the 6 month long Se supplementation (200 micrograms) caused an increase in serum selenium level from 70–75 mg/l to 86–125 mg/l, which can cause better functioning of the thyroid gland [23].

Similarly, study conducted by Zhu et al. [28] in 2012 on autoimmune thyroiditis patients with different thyroid functional status, revealed that selenium supplementation with 200 mg for 6 months resulted in the reduction of anti-TPO concentration (12.6% in subclinical and 20.4% in the overt form of the disease). Five other studies [29–33] on the effect of selenium supplementation on HT confirmed, that the selenium intake in a dose of 100 to 200 mg/day for 3–12 months decreased the anti-TPO but did not cure the underlying autoimmune thyroid disease. In a blinded, placebo-controlled trial, Gärtner et al. [34] observed, that in the group of HT females receiving 200 micrograms (2.53 micromol) sodium selenite per day, orally for 3 months, the mean anti-TPO concentration decreased significantly (63.6% vs. 88% in the placebo group).

Moreover, patients with anti-TPO greater than 1200 IU/ml revealed a mean 40% reduction in anti-TPO concentrations, as compared with a 10% increase in anti-TPO in the placebo group. The mean TSH, FT4, and FT3 levels were unchanged in both groups. According to Comps et al. [35], supply of 200 micrograms of selenomethionine per day for 28 months caused no clinically significant changes in thyroid hormone concentrations. Similar results were presented by Rayman et al. [36], who supplemented 501 elderly HT patients with selenium (100, 200 and 300 mg per day) for a period of six months. The study found no significant changes in thyroid function (TSH, FT4, FT3) in selenium-treated subjects. In addition, the average concentration of selenium in serum of patients with HT measured before the test were normal and remained at 91 mg/l. Olivieri et al. [37] showed a significant decrease in the FT4 level in selenium-treated (100µg/day) elderly subjects as compared to the control group. In view of these ambiguous results, routine supplementation of selenium in the prevention and treatment of hypothyroidism with respect to each population is not recommended. Moreover, many reports drew attention to the danger of administration of uncontrolled excessive doses of selenium.

Vitamin D and Hashimoto's disease

Vitamin D is responsible for the regulation of calcium-phosphate homeostasis. It also affects cell proliferation and differentiation, insulin secretion and cardiac contractility. Furthermore, it controls the function of the immune system by decreasing the activity of T-cells and production of pro-inflammatory cytokines [8]. Vitamin D deficiency defined as serum 25(OH)D3 below 10 ng/ml is associated with the development of a variety of autoimmune diseases including HT. The case-control study [39] that included 161 patients with HT and 162 healthy controls showed that vitamin D deficiency in HT patients was significantly higher (148 of 161, 92%) than in the healthy controls (102 of 162, 63%, p< 0.0001). Among patients with HT, the occurrence of vitamin D deficiency showed a higher trend in overt hypothyroidism (47 of 50, 94%) or subclinical hypothyroidism (44 of 45, 98%) than in euthyroidism (57 of 66, 86%), but the differences were not significant (p = 0.083). Kivity et al. [40] demonstrated, that the prevalence of vitamin D deficiency (level above 10 ng/ml) was significantly higher in patients with autoimmune thyroid diseases as compared with healthy individuals (72% vs. 30.6%; p < 0.001), as well as in patients with HT compared to patients with non-AITDs (79% vs. 52%; p < 0.05). Furthermore, vitamin D deficiency correlated width the presence of antithyroid antibodies (p = 0.01) and abnormal thyroid function tests (*P* = 0.059).

Camurdan et al. [41] in 2012 published a study evaluating the problem of vitamin D deficiency in children with autoimmune thyroiditis. As in the case of the adult population, deficiency of 25 (OH)D3 was more frequent in children with HT as compared to the control group (73.1% vs. 17.6%, p < 0.0001). In the group of children with Hashimoto's disease, mean 25(OH)D levels were significantly lower as compared with the control group (31.2 +/-11.5 vs. 57.9 +/-19.7 nmol/L, p < 0.001) and were inversely correlated with the anti-thyroid peroxidase (anti-TPO) levels (r = -0.30, p = 0.007). This research suggested that vitamin D deficiency may play a role in the development of autoimmune response in Hashimoto's thyroiditis. Recent report [42] confirmed the results of the previous studies. In a group of patients suffering from HT in a euthyroid state, who were on a stable dose of L-thyroxine (the average level of vitamin D was 11.4 ± 5.2 ng/mL and it was lower as compared to the control group (15.4 ± 6.8 ng/mL, p < 0.001). Serum 25(OH)D levels directly correlated with thyroid volume (r = 0.145, p < 0.001) and inversely correlated with anti-TPO (r = -0.361, p < 0.001) and anti-TG levels (r = -0.335, p < 0.001). The serum 25(OH)D levels (10.3 ± 4.58 ng/mL) were significantly lower in female chronic HT patients as compared to male control subjects (19.3 ± 5.9 ng/mL, p < 0.001). From the presented data, it can be assumed, that vitamin D supplementation could reduce the incidence of HT and alleviate the disease. However, there is a need for further research on this topic.

Conclusions

Many previous studies confirmed the involvement of impaired antioxidant status and vitamin D deficiency in development of autoimmune diseases. Nevertheless, there is no sufficient proof confirming benefits of supplementation with vitamins and trace minerals for the treatment of Hashimoto's disease.

Because of the potential impact of vitamins A, C, E, selenium and zinc reducing oxidative stress as well as the effects of vitamin D in reducing the serum anti-TPO level, it is necessary to pay more attention to nutritional education of patients suffering from HT.

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REVIEW PAPER

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Osteopontin – a multifunctional protein and its impact on an insulin resistance development

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ABSTRACT

Osteopontin (OPN) is one of the many physiological elements creating human musculoskeletal system. It is suspected that this protein is one of the most important mediators responsible for osseous tissue mass resorption, regulated by parthormon. The origin of its name comes from one of its physiological action – rebuilding of the bone mass structure (*osteo* – bone, *pontin* – bridge). Osteopontin fulfils many different actions being secreted by many different types of cells, including macrophages, lymphocytes, epithelial cells, vascular smooth muscle cells, and osteoblasts. OPN plays an important part in inflammatory process. It provokes macrophages and dendritic cells to movement into the destination where inflammatory process takes place. It also stimulates macrophages to interleukin 12 (IL12) and interferon γ (IFN γ) secretion. Increased OPN concentration in blood stream might be regarded as a novel, independent indicator of coronary artery disease. Osteopontin plays an important role in macrophage infiltration of the adipose tissue and at the same time contributes to insulin resistance. Obesity induces chronic, low-grade tissue inflammation. Positive correlation was observed between body mass index (BMI) and number of macrophages accumulated in the fat tissue. Once aroused monocytes infiltrate the adipose tissue, which leads to persisting chronic inflammation. At the same time the excreted by them cytokines may be connected with the mechanisms of obesity-induced insulin resistance.

Keywords: osteopontin, coronary artery disease, insulin resistance.

At the early beginnings of eighties in last century, osteopontin (OPN) was thought to be mainly associated with osseous tissue metabolism, particularly with its reconstruction. Later it started to be connected with neoplasms, probably because its chemotactic abilities and its elevated concentration in cancerous diseases. In 21st century we are certainly sure that OPN is also involved in the development of obesity related diseases, such as type 2 diabetes, insulin resistance, and cardiovascular disorders.

For the first time in history osteopontin was described in 1979 as a fosfoprotein secreted by cancerous epithelial cells [1]. In scientific literature it occurs under different names, such as: Eta – 1 (early T lymphocyte activation protein 1) [2]; Spp1 (secreted phosphoprotein – I) [3]; 2ar [4] or uropontin [5].

The origin of its name comes from one of its physiological action – rebuilding of the bone mass structure (*osteo* – bone, *pontin* – bridge) [6]. OPN is one of the many physiological elements creating human musculoskeletal system. Its total amount constitutes 0.2% of whole bone mass [7]. OPN synthesis in skeletal system is controlled by calcitriol stimulation. It is suspected that this protein is one of the most important mediators responsible for osseous tissue mass resorption, regulated by parthormon (PTH) [8].

Osteopontin fulfils many different actions and it is why is a secreted by many different types of cells, including macrophages, lymphocytes, epithelial cells, vascular smooth muscle cells, and osteoblasts [9]. It has been proved that OPN is even involved in each part of phosphate urolith development [10]. OPN shows the capacity to adhere to the surface of target cells, what stimulates them to further migration. This protein was also classified as a T helper type 1 (Th1) stimulator [11].

OPN concentration is significantly higher in the cancer cells than in the healthy tissues, also its level grows proportionally to the malignancy and clinical stage of the neoplasm [12].

Osteopontin plays an important part in inflammatory process. It provokes macrophages and dendritic cells to movement into the destination where inflammatory process take place. It also stimulates macrophages to interleukin 12 (IL12) and interferon γ (IFN γ) secretion [13]. OPN is being regarded as a proinflammatory protein. Others cytokines involved in inflammatory process such as: tumor necrosis factor – α (TNF – α), transforming growth factor – β (TGF – β) and interleukin 1b (IL – 1b) increase its synthesis [14]. At the same time osteopontin secretion might be stimulated by insufficient concentration of oxygen and hyperglycemia [15].

Simultaneously other authors proved that OPN also shows anti-inflammatory activity, by induction of nitric oxide synthase isoform (iNOS) to synthesis of nitric oxide (NO) in macrophages [16].

Nevertheless, vast majority of scientists regards OPN as an inflammation stimulating agent. To inflammation-associated diseases with elevated plasma level of osteopontin we can include: autoimmune diseases, Crohn disease, atherosclerosis, and of course obesity [17].

Nowadays, studies are being conducted on an osteopontin inhibitor. This potential molecule might be an agelastatin A. The performed *in vivo* trials on o group of patients with skin, breast and bladder cancer revealed promising effects. Osteopontin inhibitor slows down cancerous cell adhesion, invasion, and colony formation [18].

Beside traditional cardiovascular risk factors, increased OPN concentration in blood stream might be regarded as a novel, independent indicator of coronary artery disease [19]. Fitzpatrick et al showed high osteopontin concentration inside atherogenically changed blood vessel walls [20]. The total content of OPN in plasma may predict sustained VT/VF (ventricular tachycardia/ventricular fibrillation) in HF (heart failure) patients at high risk for SCD (sudden cardiac death). The combination of elevated OPN levels and high hsCRP levels (> 3 mg/l) were significantly associated with increased risk of all-cause mortality, re-infarction and heart failure [21].

Experimental trials performed on rodents revealed elevated OPN levels in experimental diabetes mellitus [22]. There are also many other evidences suggesting OPN involvement in glucose homeostasis and keeping energy balance on the stable level. One of them is the fact that osteopontin deficiency in mice with obesity developed by overfeeding was associated with a 50% reduction of macrophage infiltration in adipose tissue and improved insulin sensitivity [23].

Kiefer et al conducted study on C57BL/6J mice. The rodents were served a high-fat diet which leads to obesity. At the same time animals were receiving intravenous OPN-neutralizing antibodies. This method of treatment allowed to improve insulin sensitivity and decreased inflammatory process both in adipose tissue and liver. Anti-OPN therapy decreased expression of inflammatory gene by intensive macrophage apoptosis and reduction c-Jun NH2-terminal kinase activation. The influence of OPN neutralizing antibody leads to major improvement in insulin sensitivity in obese mice. The authors also observed that OPN is an important factor which regulates activation of hepatic signal transducer and is an activator of transcription 3 (STAT3). Blocked action of OPN induced decrease inflammation in adipose tissue. OPN concentration may be regarded as a novel link for obesity-related metabolic disorders treatment [24].

For obesity it is characteristic that massive macrophage infiltration is present in the fat tissue. Adipocytes – fat tissue cells produce excessive number of different kinds of cytokines, which is directly associated with insulin resistance and type 2 diabetes. Osteopontin plays an important role in macrophage infiltration of the adipose tissue and at the same time contributes to insulin resistance. Therefore it seems so important to understand the regulation of osteopontin expression by adipocytes. Well known, confirmed underlying mechanisms have not been yet established. There are multiple factors, including TLR4 (Toll-like receptor 4) activation induced by lipopolysaccharide (LPS) or palmitic acid, act in concert to up-regulate osteopontin expression by mononuclear cells through an IL-6-mediated mechanism.

Obesity induces chronic, low-grade tissue inflammation. Except of stimulated monocytes, and macrophages also adipocytes are actively involved in the secretion of proinflammatory cytokines. In case of obese individuals the levels of inflammatory markers such as high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor receptor (TNF – α) or interleukin 6 (IL-6) are consistently increased [25]. Those elements correlate positively with the risk of development cardiovascular diseases, and type 2 diabetes and insulin resistance [26]. Positive correlation was observed between body mass index (BMI) and number of macrophages accumulated in the fat tissue [16]. Once aroused monocytes infiltrate the adipose tissue, which leads to retaining chron-

ic inflammation. At the same time secreted by them cytokines may be connected with the mechanisms of obesity-induced insulin resistance [27]. Increased OPN serum blood concentrations were found in the group of obese patients as compared with those with an appropriate weight [28].

As mentioned earlier OPN reveals great proinflammatory potential. It activates macrophages and dendritic cells. OPN also stimulates them to secretion of IL12, IFN γ and many other chemotactically active substances. They subsequently are able to increase insulin resistance. All these confirm the hypothesis that osteopontin might be the missing link between glucose intolerance and an obesity (as a low-grade chronic inflammation). Potential resolution of existing correlation between obesity and insulin intolerance might require knocking-down OPN genes or introducing into blood system its inhibitors. This will not only improve insulin sensitivity and help lose weight but it also could slow down tumor metastasis, cancerous cell adhesion and, prolong human live. Further investigations conducted in the future will bring us the correct answers.

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Cerebrovascular mediated subclinical brain injury – interaction between cardiovascular function, brain structure and cognitive function – study rationale, design and principal methods

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ABSTRACT

Project entitled "Cerebrovascular mediated subclinical brain injury – interaction between cardiovascular function, brain structure and cognitive function- study rationale, design and principal methods" is a cross-sectional study in a group of older individuals with no apparent symptoms of stroke, transient ischemic attacks or cognitive impairment. The primary aim of the projects is further our understanding of the patomechanism of cerebrovascular disease associate brain injury as well as vascular cognitive impairment and give a better insight into the interplay between arterial wall, ventriculo-vascular coupling, wave reflection and pulsatility of the flow and how these interplays are mirrored by MRI neuroimaging. With our novel, interdisciplinary approach, we will define the methodology and build tools for those who want to follow in this challenging and non-standard direction.

Keywords: brain structure, cognitive function, arterial stiffness, arterio-vascular coupling, gray matter volume, white matter lesions.

Introduction

A population study performed with the magnetic resonance imaging (MRI) demonstrated high prevalence of salient changes in central nervous system [1, 2]. Among 3301 participants (mean age 65 years) who underwent MRI scanning and denied a history of stroke or transient ischemic attack only 4.4% had scans which were assigned a white matter grade of 0, indicating that they were free of any abnormal signal in the white matter. Thus, most of this population showed degenerative changes, which may not be considered benign because they were associated with impaired cognitive and lower extremity function [2]. *A large part of this problem is enhanced by vascular disease contributing to subclinical brain injury, silent brain infarction or clinically apparent stroke.* The clinical spectrum of vascular contribution to cognitive impairment and dementia ranges from subclinical forms, being in fact risk factors for the development of clinically overt syndrome to overt stroke. Recently a broader terminology was introduced namely "vascular cognitive impairment" (VCI) in order to capture the entire spectrum of cognitive disorders linked to all forms of cerebral vascular brain injury [3]. Thus, VCI is regarded as syndrome with evidence of stroke or subclinical vascular brain injury based on clinical as well as neuroradiological imaging. Moreover, these changes are linked to impairment of ⩾ 1 cognitive domain.

Cerebrovascular disease brain injury (CVBI) ranges from overt stroke to microinfarction, brain athrophy or

white matter lesions (WMLs). WMLs are highly correlated with white matter ischemic injury while gray matter volume changes are associated with both CVBI and Alzheimer [4]. In populations study WMLs as well as silent cerebral infarction are very common [5]. Nevertheless, several studies demonstrated the association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality [6]. The risk factors for the development of CVBI include hypertension, atherosclerosis, tobacco, diabetes and atrial fibrillation [7].

Non-traditional risk factors for vascular brain injury

Recently considerable interest was focused on non-traditional risk factors for the development of vascular cognitive impairment. Increased arterial stiffness or arteriosclerosis, represents a physiological part of aging that leads to diminished elastic properties of the media and increased arterial stiffness. This process may be further accelerated by hypertension, diabetes, renal insufficiency and other risk factors [8]. There are several methods, such as pulse wave velocity, digital volume pulse and pulse wave analysis or ultrasonographic indices of local arterial distensibility that allow the assessment of general, segmental or local arterial stiffness as well as central hemodynamics or pressure wave reflection. These measures as well as derived indices served as indicators of increased risk of cardiovascular complications independently of other well known risk factors [9, 10, 11].

Henskens et al. [12] demonstrated that increased arterial stiffness measured by aortic pulse wave velocity (PWV) is associated with silent cerebral small-vessel disease in hypertensive patients. Kearney-Schwartz et al. [13] in a prospective study performed in 198 elderly (mean age 69 years) hypertensive patients showed that increased PWV, brachial endothelial function as well as increased intima-media thickness in carotid artery are associated with white matter hyperintensities and cognitive performance. There are several possible explanations for the pathophysiological link between increased arterial stiffness and microvascular brain injury. First, increased arterial stiffness leads to increased pulsatility in the ascending aorta which has a direct transfer to brain vasculature. Second, arterial stiffness is linked to endothelial dysfunction and oxidative stress both of which are associated with microvessel injury. Third, arterial stiffness leads to left ventricular hypertrophy, a risk factor for subclinical brain damage and dementia.

Cardiac diseases are associated with increased risk of cognitive impairment or dementia. It is obvious that chronic cerebral hypoperfusion due to severe carotid stenosis caused by atherosclerosis or significantly reduced cardiac output caused by left ventricular dysfunction may lead to cognitive impairment. Therefore, the majority of research concerning relationship between cardiac function and cerebral structure and cognitive performance was done in the population of subjects with chronic heart failure [14]. Recently, in preliminary studies we demonstrated that grey matter volume is correlated with some metrics of central hemodynamics as well as with arterial and left ventricular stiffness in a population of healthy subjects. Moreover, subclinical white matter lesions were present in those with higher arterial stiffness and increased values of aortic excess pressure [15, 16].

Study hypothesis

It is established that indices of subclinical atherosclerosis, wave reflection, arterial stiffness and cardiac dysfunction predict cardiovascular complications. Several investigators demonstrated that the characteristics of vascular beds obtained by these techniques are not synonymous and indicate different aspects of vascular status. Currently it is not known what exact contributions to cerebrovascular disease associated brain injury and/or vascular cognitive impairment may be attributed to each cardiovascular metric. We hypothesized that complementary assessment of vascular wall properties as well as cardiac structure and function combined with MRI evaluation of brain structure and cognitive testing allow to quantify the contribution of different cardio-arterial properties to subclinical brain injury and cognitive decline.

Aim

To perform complimentary assessment of arterial structure and function, left ventricular structure and function, peripheral and central hemodynamics in conjunction with brain magnetic resonance imaging. Additionally cognitive assessment will allow to performe analysis assessing interaction between cardiovascular and brain morphology metrics and cognitive performance.

The novelty input and influence of obtained results

The novelty of our basic research is comprehensive evaluation of arterial and cardiac structure and function, together with central hemodynamics in the frame of neuroimaging and neuropsychologic evaluation of study subject. We hope that the obtained results will help to determine the optimal cardiovascular and brain

structure metrics for testing in a future research performed in risk factors groups as well as subjects with established diagnosis of brain injury in order to prevent further deterioration or to monitor targeted therapy.

Method and experimental plan

Study group

The study group will include 230 volunteers, age > 60 years with the perception of normal health, without apparent history of stroke, transient ischemic attack or cognitive decline. The exclusion criteria are: neoplasm, chronic kidney disease requiring hemodialysis, status post kidney or heart transplantation, atrial fibrillation, active infection, hyperthyreosis, implanted artificial device preventing MRI assessment (pacemakers, hip replacement etc.).

Brain magnetic resonance imaging

All brain examination will be performed on 1,5-Tesla MRI (Avanto Simens Medical System, Germany) using standard, circular, polarized volume head coil. MR images will be done in standard sequence. For the automatic separation of brain and nonbrain matter as well as the evaluation of white matter and gray matter brain volumes the FSL (University of Oxford) package as well as the SPM (Wellcome Trust Centre for Neuroimaging, London) and VBM (Structural Brain Mapping Group, University of Jena) Matlab toolboxes will be used. Additionally, use will be made of the MRICRON software to further analyze the regions of interest in the transformed images.

MR angiography of brain arteries will be made with Time of Flight sequence (TOF) without contrast injection.

For detailed brain structure segmentation into volumes and surfaces with the use of state-of-the-art algorithms the FreeSurfer (Laboratory for Computational Neuroimaging, Athinoula A. Martinos Center for Biomedical Imaging) software package will be used. White matter hyperintensities will be assessed with the use of the LST (Structural Brain Mapping Group, University of Jena) Matlab toolbox.

Measurements

All patients will be evaluated in a temperature controlled laboratory. Anthropometric measurements (height, weight, Waist-to-Hip Ratio [WHR]) will all be made according to standard guidelines. Brachial blood pressure will be obtained by an oscillometric method (M-6, Omron Healthcare Co, Ltd, Kyoto, Japan) in the supine position, after 10-minutes rest. Plasma glucose, cholesterol and creatinine levels will be obtained from subjects after an overnight fast. Six minute walk test will be performed.

Cognitive function testing

Psychological assessment

To account for psychological outcomes related to cardiovascular and brain changes we will measure subjective health status (Nottingham Health Profile) and cognitive function (neuropsychological battery) with reliable and validated measures. These assessments will be assigned, coded, and interpreted by trained psychologists. This approach will provide a comprehensive examination of outcomes resulting from identified medical conditions and enhance its unique interdisciplinary application. Findings will be particularly relevant to the discipline of psychocardiology and neuropsychology.

Neuropsychological battery

A comprehensive battery of neuropsychological assessments, including standard clinical neuropsychological instruments with established reliability and validity will be completed by all participants. We will evaluate attention-executive function with the Trail making B time to completion test, Digits Forward and Backwards, and Controlled Oral Word Association Test. Memory will be assessed with California Verbal Learning Test-II immediate recall and delayed recall. A total cognitive composite score will also be calculated. We will account for the premorbid functioning with WAIS-R vocabulary subtest. Composite measures will be computed for each test by converting raw scores to standardized z-scores and averaging them across the tests in each composite [17–22].

Amount of body fat

A bio-impedance analyzer (Bodystat 1500, Bodystat Ltd, UK) will be use to measure the fat content as a proportion of total body mass. Bio-impedance analysis (BIA) will be performed with a single frequency (50 kHz) device.

Digital volume pulse analysis

Measurement of the digital volume pulse (DVP) waveform will be performed by a photoplethysmographic method (Pulse Trace 2000, MicroMedical, UK). The Stiffness Index of the DVP (SI_{DVP}) will obtained from the subject's body height (h) divided by the time between the systolic and diastolic peaks of the DVP. The SI_{DVP} is

an estimate of pulse wave velocity in large arteries and is regarded as a measure of large artery stiffness.

Peripheral and central pressure waves assessed by pulse wave analysis (PWA) for measurement of wave reflection and central hemodynamics

The SphygmocorMx validated system (AtCor Medical, software version 7.0) will be used for measurement of the wave reflection indices and central hemodynamics. Non-invasive beat-to-beat finger arterial blood pressure will be recorded continuously with the use of a volume-clamp photoplethysmograph (Portapres 2, FMS, The Netherlands) with the sensor on the middle finger of the right hand. Calculations of mean blood pressure (MBP), heart rate (HR), total peripheral resistance (TPR) will be made using the Modelflow algorithm.

Carotid Intima-Media Thickness and local carotid stiffness evaluation

All patients will undergo common carotid artery (CCA) measurements with a novel system (ArtLab, Esaote, Italy) based on high-resolution echotracking technology (WallTrack system), including the use of a 128 RF line multiarray. This novel technology gives access to all major mechanical parameters. The system employs dedicated software (RF-data technology involving RF Quality Intima-media Thickness (RFQIMT) and RF Quality Arterial Stiffness (RFQAS); Esaote Medical Systems).

Echocardiography

Patients will be imaged using a commercially available system (Vivid 9, General Electric-Vingmed). Data acquisition will be performed using a 3.5 MHz transducer, at a depth of 16 cm in the parasternal and apical views. Standard M-mode and 2D images will be obtained and analysis will be performed off-line. Peak systolic longitudinal strain and strain rate will be assessed on apical two-chamber, four-chamber, and long-axis views using speckle tracking analysis. Left ventricular end systolic stiffness, effective arterial elastance and ventricular-arterial coupling will be estimated with the single beat technique. Total arterial compliance (TAC) is an additional measure of arterial pulsatile load on the heart and it will be estimated by dividing SV by the pulse pressure (PP).

Conclusion and dissemination of the results

It is expected that the work proposed will further our understanding of the patomechanism of cerebrovascular disease associated brain injury as well as vascular cognitive impairment and give a better insight into the interplay between arterial wall, ventriculo-vascular coupling, wave reflection and pulsatility of the flow and how these interplays are mirrored by MRI neuroimaging. Also, with our novel, interdisciplinary approach, we will define the methodology and build tools for those who want to follow in this challenging and non-standard direction.

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The project "understAID" – a platform that helps informal caregivers to understand and aid their demented relatives

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ABSTRACT

"UnderstAID" is a platform that helps informal caregivers to understand and aid their demented relatives. It is an international project initiated by Denmark, Poland and Spain.
The aim of the project is to design, and implement the multimedia platform "understAID" to support informal caregivers of dementia patients. The project was launched in April 2013 and is expected to end 36 months later. The project is divided into five tasks concerning the final aim. The aim of task 1 is the management of the project, as well as the exploitation and dissemination of gathered information. Task 2 is meant to define the contents and solutions of the CarePlatform based on the knowledge gained from real-case studies. Demented elderlies from each country (n = 40) suffering from different degrees of dementia were evaluated by formal caregivers and dementia professionals. The aim of task 3 is the development of the social learning interface. Task 4 focuses on the CarePlatform development and system integration. Finally, task 5 assumes testing and validation of the platform. The platform is devised to be available in two versions, namely the light one for mobile appliance and the premium version. Also different activities leading to the popularization of the platform are planned.

Keywords: dementia, caregivers, software, help, platform.

General information

The project "understAID" – a platform that helps informal caregivers to understand and aid their demented relatives was awarded in the 5th contest of the Ambient Assisted Living Joint Programme (AAL).

The members of the international Consortium (listed below) signed administrative bilateral Agreements on 11 April 2013 (effective date):

- Danish Agency for Science (Technology and Innovation) and Sekoia Assisted Living ApS;
- Danish Agency for Science (Technology and Innovation) and Faculty of Health Science, VIA University College;
- Danish Agency for Science (Technology and Innovation) and Danish Alzheimer Association;
- Danish Agency for Science (Technology and Innovation) and Skanderborg Municipality;
- National Institute of Health Carlos III – Instituto de Salud Carlos III and The Centre of Supercomputing of Galicia (CESGA);

- Ministry of Industry, Energy and Tourism/Ministerio de Industria, Energía y Turismo (MINETUR) and Balidea Consulting and Programming;
- Ministry of Industry, Energy and Tourism/Ministerio de Industria, Energía y Turismo (MINETUR) and Provincial Association of Pensioners and Retired People (UDP) from A Coruña/Asociación Provincial de Pensionistas y Jubilados de A Coruña (UDP A Coruña);
- Centre for Research and Development/Narodowe Centrum Badań i Rozwoju, Poland and Poznan University of Medical Sciences (PUMS); Centre for Research and Development/Narodowe Centrum Badań i Rozwoju, Poland and Wiktor Dega's Orthopaedic and Rehabilitation Clinical Hospital Poznan University of Medical Sciences (ORSK).

Management

The project management structure is designed in several bodies, the main are: Steering Committee and Consortium Management.

Steering Committees of the above partners are represented by: PUMS (Ewa Mojs, Włodzimierz Samborski, Michał Musielak); ORSK (Przemysław Lisiński, Agata Bednarek); VIA (Lars Kjeldsen); Sekoia (Morten Mathiesen); Cesga (Maria Malmierca); Balidea (Angel Otero); DAA (Nis Nissen); UDP (Ana Maseda); Skan (Lisbeth Hyldegaard).

Consortium Management Coordinator is Lars Peter Bech Kjeldsen as Coordinator supported by the Technical Manager Angel Piñeiro Otero from Balidea and the Exploitation and Dissemination Manager Morten Mathiesen from Sekoia.

Ethics

Bioethical Committee at Poznan University of Medical Sciences on 8th November, 2012 accepted all the project's protocols and forms (number 990/12).

Finance

The total value of the granted funds in Polish Zlotys is 479,983.80 for PUMS and 165,841.21 for ORSK. The total value of the project for all participants was 1526217 Euro. Funds were used to cover personal costs of the project's participants and the costs of promoting the project. Additionally, new equipment, tools and software were purchased.

Project objective and schedule

The aim of the project is to design, and implement the multimedia platform "understAID" to support informal caregivers of dementia patients. The project is divided into five tasks concerning the final aim (**Figure 1**).

Task 1. Project management, Exploitation and Dissemination

Activity type: management
Leader: Sekoia
Duration: 1–36 months
The aim of this task is to collect information from a variety of sources and convert it into a form which allows translating them into real applications and content of understAID. In order to be tested by its end-users in each participating country, this task also aims at translating the contents into Danish, Spanish and Polish.

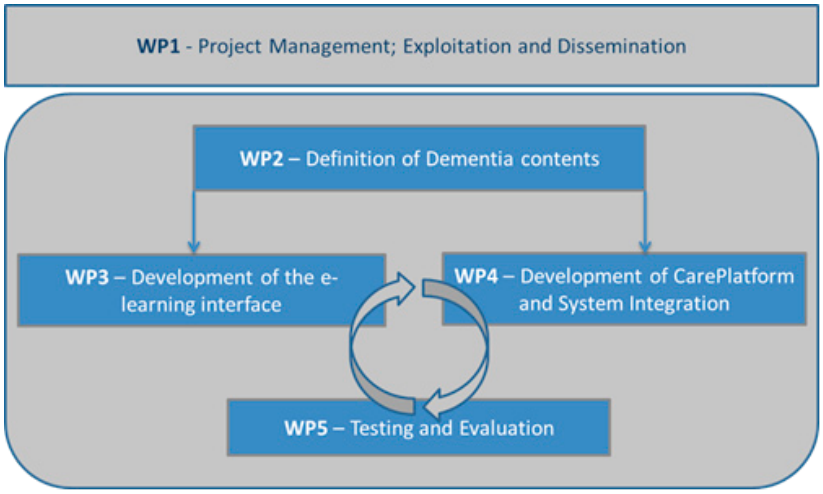

Figure 1. Project tasks

Detailed objectives:

1. Ensure effective communication and management of the project.
2. Enable robust transfer of expertise and knowledge to each of the Consortium members.
3. Ensure timely and correct delivery of all milestones and deliverables.
4. Provide objective mediation of conflicts.
5. Ensure that all project results are formulated and compiled into a protectable form.
6. Dissemination of technology and knowledge beyond the Consortium to technological and market stakeholders.
7. Enable exploitation through knowledge sharing and documentation.
8. Engaging a market access provider to partnership with the Consortium for taking products to market in the post-project period.
9. Use socioeconomic impact to influence end-users' investment decisions and to create awareness around the novel CarePlatform.

Task 2. Definition of contents and solutions for the CarePlatform

Activity type: research

The objective of this work package is to define the contents, the data and the solutions for the CarePlatform in order to support informal caregivers in the most efficient way. The knowledge about what the informal caregiver needs in different situations has to be extracted from a variety of sources, including published scientific literature, "gray literature", knowledge possessed by individuals and organizations that work with informal caregivers and by professional health staff who work with dementia patients in practice. The aim of this task is therefore to collect this information from a variety of sources and convert it into a form that the other tasks can translate into real applications in the CarePlatform.

Task 3. Development of the social learning interface

The objective of this task is to define specifications of the platform, preparation of the state-of-the-art review for e-learning interfaces and to define the e-learning model.

Task 4. Development of the CarePlatform and System Integration

The aim of this tasks is to apply specifications, design and programming. Subsequently, the development of the online CarePlatform server solution and integration of the system is also planned.

Task 5. Testing and validation

The main objective of task 5 is to test the CarePlatform in all its aspects and to evaluate its ongoing development. This work package will be an iterative process to constantly evaluate, validate and improve the platform in order to fulfil end-users' needs.

Detailed description of the tasks

Task 1

Task 1.1. Overall management and risk contingency planning

The Coordinator is responsible for the continuous verification of consistency with the project tasks and deliverables before transmitting them to the AAL Association. Any minor deviations from the project plan is reported to the Steering Committee (SC) which considers problems and, where appropriate, makes recommendations for implementing the contingency plan(s). Where alternative contingency plans are needed, the Coordinator together with the Technical Manager and other relevant persons drafts them. The Coordinator ensures that conclusions are communicated to all members and included in the project plan.

Task 1.2. Formal responsibilities of the Coordinator

The Coordinator directs the technical progress during the project ensuring that the schedule is met. This means reviewing all reports and project progress, solving potential partnership problems and mediating in case of conflict, planning and organizing the regular meetings.

Task 1.3. Formal administration

The Coordinator's administrative tasks include: collation of deliverables, milestone reports, final report; administration of the financial funding and distribution of shares to partners; submission and organization of cost statements and Consortium agreements; keeping records and financial accounts; resolution of any administrative or contractual issues.

Task 1.4. Protection of project results

The appointed Exploitation and Dissemination Manager (EDM) is responsible for identifying and assessing the project results and reporting his findings to the Coordinator and the Steering Committee (SC). The

EDM will discuss with the partners and make recommendations concerning the need for protection of each output and the SC will decide on the appropriate protection strategy.

Task 1.5. Dissemination of project results

The Coordinator will establish, in dialogue with all Consortium members, how best to disseminate project results. The EDM will prepare a comprehensive dissemination plan with the input from all partners and subjected to the Consortium approval. Dissemination activities will include attending conferences and meetings, presentations, fair trades, and active engagement in contacts with customers, investors, academia and regulators/policy-makers.

Task 1.6. Exploitation of project results

The EDM in cooperation with the Coordinator will deliver a plan detailing how to exploit the project in terms of production, distribution and sales. In particular, they will survey and select, together with all partners, a strong market player in medical instrumentation/devices that can enable market access for post-project commercialization of the products developed.

Task 2

Task 2.1.

The aim of this task is to perform a strong and comprehensive report of the literature review which can be used by the experts responsible for building the learning models in task 3. In addition, a review paper in a peer-reviewed scientific journal based on the results is demanded to be published.

Task 2.2. Real-cases study

The aim of this task is to collect knowledge from real-case studies. Demented elderlies from each country (n = 40) suffering from different degrees of dementia will be analysed by formal caregivers and dementia professionals. The primary end-users, i.e. informal caregivers (mainly relatives) in charge will, in interviews, provide input on their day-to-day situation, describing the routines and behaviour of the elderly, the most stressful situations, the solutions most commonly employed and respective results. Questionnaires will also be sent online to other informal caregivers through the end-users' networks. Moreover, the burden of the caregivers will be measured using the Zarit Burden Scale for comparison at the end of the project to assess the impact of understAID.

Task 2.3. Definition and building of contents

The aim of this task is to find and define the most important problems as well as the possible solutions on the basis of the previously gathered information and then to build a database (text, audio and video contents).

The situations, behaviours and corresponding solutions will be ranked according to their importance and regularity as well as the predicted point in the progression of dementia.

A great amount of relevant situations is considered, e.g. leaving a building without being adequately dressed or at unsuitable times, getting lost, feeling confused and anxious, verbal or physical aggression, improper sexual behaviour, etc.

Task 2.4. Translation of contents into Danish, Spanish and Polish

The aim of this task is to translate the contents of the database into Danish, Spanish and Polish in order to allow effective testing and the accessibility of the understAID solutions to caregivers. The second aim is to peer-review these solutions by specialists in dementia, namely to test them in user trials within task 5.

Task 3

Task 3.1. Platform specifications

Two versions for the platform: "light" and "premium" are planned. The decision regarding the final content of each version will be defined considering the users' needs.

The "light" version is an application on multiple operating systems/platforms Android, iOS, Windows, Windows Mobile, Linux, etc. The functionality of the "light" version is thought to provide relevant content for the end-user, but limited when compared to the "premium" version.

The test of the "premium" version is planned to go ahead on Sekoia's web platform. The premium version will include a complete questionnaire to provide the most relevant content for each situation, communication channels with informal and formal caregivers (forums, chats, etc.), video content, audio content (chatterbot), etc. The application from the "light" version is to be included in the "premium" solution, with additional functionality:

- easy upload of recorded instructional videos to the web platform,
- access to calendar,
- access to instructional videos,

– scanning of QR codes located in-house for access to instructions,
– receiving reminders.

The web platform is thought to supply an administration area with the following functionality:

– chat/forums,
– administration of medicine reminders,
– "drag and drop" calendar where everyday activities of daily life can be organized and visually presented,
– module for creating tutorial "video recipes" for the demented elderly on "how to do...". This could be a "how to make coffee" video that the informal caregiver records in own surroundings and leaves to "play at a touch" for the care receiving person to use when in need. Elements/ tutorials can be also used in the "drag and drop" calendar in relation to activities,
– administration of QR codes for easy in-house access to instructions,
– access to the virtual dementia coach including access to videos.

Defining the light and premium versions is fundamental at this stage in order to establish the e-learning models for both versions. Despite the fact that a similar approach will be used for both versions, the premium version will be more complex, requiring an extensive and advanced questionnaire, a much more innovative and advanced e-learning interface, etc.

Task 3.2. Analyse current state-of-the-art e-learning for support of informal caregivers

This task aims at reviewing and identifying the most adequate existing state-of-the-art e-learning interfaces (such as LMS, CMS, SLI, MOOCs, Groupware, etc.) that can be used by adult individuals from all educational and cultural backgrounds to support informal learning and to build a learning network, as well as identifying their weaknesses. The aim is to find a model that helps adult caregivers learn collaboratively, connect to other learners in their network and obtain information needed quickly, while keeping the online interface as user-friendly as possible.

Task 3.3. Definition of e-learning model

This task is aimed at providing the best environment for informal caregivers to learn, find answers, and communicate with other caregivers, therapists and health care professionals. In order to do so, this task is considered to determine the best e-learning model to be used, based on user needs analysis results. As a basis, some e-learning methodologies seem more adequate, such as connectivism, blended learning, user-centered design, all of them focused on the individual needs of an individual caregiver in combination with research-based knowledge rather than presenting large amounts of information and learning material to be browsed through and selected from. A precondition analysis component should provide online guidance and support to relatives/informal carers to help them make qualified choices in relation to choosing relevant e-learning materials, peers or deciding whether it would be more appropriate to seek guidance and support, in the virtual kin networks or from health care professionals respectively. Online communication tools should facilitate all sorts of connections and sharing among users, both asynchronous and synchronic, through text, audio and video resources. User entries into the application should allow the application to make the necessary analysis and selection of e-learning resources. Based on entries and user choices, the application will collect data in order to both improve future services targeted at the specific user and develop the application's content elements. This function could be described as a kind of Web 3.0 solution.

The model is thought to be designed to function both as a "Virtual Dementia Coach", a "First Aid Kit" and a "Social forum for knowledge and experience sharing" for relatives/informal carers. The "Virtual Dementia Coach" should support the development of the learner's knowledge and skills by presenting factual, validated knowledge about dementia and information on how the learner can optimally support the demented person. It should also introduce e-learning programs that focus on supporting caregivers and develop their knowledge, skills and attitudes in relation to the specific challenges they are facing. If a caregiver has previously received formal instruction, then the application can work further to develop this knowledge and these skills by interacting with the content of the" Virtual Dementia Coach".

The "First Aid Kit" can act as a "here and now" resource that can present on-the-spot suggestions for the resolution of some of the problems that caregivers usually meet in their daily dealings with the demented person.

Task 4

Task 4.1. E-learning application specifications

An extensive survey among end-users in the participating member-states will be performed to fully understand their needs and the expected output of the

applications. Requirements and specifications will be defined, namely the design, contents organization and structure, touch technology, etc.

When defining the e-learning application, state-of-the-art technologies and approaches will be taken into consideration and they will include:

- mobile learning, ubiquitous e-learning,
- Web 3.0, augmented intelligence,
- connectivity,
- usability and accessibility standards,
- open source technologies,
- cloud computing (SaaS).

Task 4.2. Design and programming of the e-learning application

The application is thought to be built on an open source heterogeneous software service in order to allow a smooth and error-free integration into the final platform (e.g. using CORBA, Web 3.0, ICE, or related technologies).

Task 4.3. Development of CarePlatform

The platform is meant to be based on cloud technologies in order to provide easy deployment of new contents, to have access from several devices (end-users will log in into the server and will have direct access to their profiles from any smartphone, tablet or computer), allow input and communication from end-users, etc. This demands open source heterogeneous software service that can be accessed by multiple platforms (Android, Linux, iOS, Windows, C, C++, Java, C#) e.g. using CORBA, W3C Web services, ICE, or related technologies.

Task 4.4. Integration of the system

This task focuses on the integration of the e-learning interface and dementia contents with the CarePlatform in the final version. This integration requires extensive hardware and software implementation tasks. The CarePlatform should be integrated in an iterative fashion, producing several prototypes: on principals in actions, on wireframes, on graphical user interface, on cloud based services, on individual application – before testing and implementing the final system version 1.0.

Task 5

Task 5.1. Testing the CarePlatform

The work should be carried out according to the implementation goals and objectives defined at the beginning of the project. The iterative process makes sure that testing goes on in close relation to implementation ideals, making testing the first part of commercialisation.

Task 5.2. Evaluation and validation

Evaluation is key to establishing a feasible market model that needs little customization in relation to the following marketing and commercialisation.

End-users assist in the evaluation process in relation to testing activities to follow the intention of developing closely in relation to end-user, buyer and other stakeholder needs.

Task 5.3. Documentation of how well the final CarePlatform product assists caregivers

In order to provide a sound business case, it is necessary to demonstrate that the final product has a positive effect on stress realized on caregivers and that caregivers in fact will continue to use the CarePlatform over longer periods.

The final validation consists of three parts. A quantitative study with the aim to address whether there is a perceived measurable effect on depression and perceived life quality. Additionally, qualitative interviews about how the CarePlatform has been used in everyday life is planned. Finally, a focus group of professional caregivers and representatives from the Alzheimer Association should present feedback on the platform as the final version.

The protocol for the quantitative and the qualitative study is inspired by the results found in the DAISY study. Hence, the inclusion criteria include patient diagnosis, time since the diagnosis was provided, age and gender of the caregiver, relation between the caregiver and patient, caregiver educational background, etc.

The quantitative data collection consists of a questionnaire that can be filled out online by the caregiver with or without support by the trained staff from VIA or PUMS. The questionnaire addresses questions about depression and perceived life quality, so the results are comparable with the DAISY study.

Expected results

Apart from building the final "understAID" platform in two versions, different activities popularizing the platform are planned. The aim is not only to circulate the knowledge but also to sell the software to the wide international audience.

Additionally, a review publication of end-users psychological condition, a review paper based on the results of the report of task 2 and a research study of

dementia patients and their caregivers are planned. Also, the results of these papers, as well as dementia patients and their caregivers' needs will be promoted at some conferences or meetings with specialists and politicians.

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THOUSAND WORDS

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A thousand words about microparticles in cardiology

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ABSTRACT

Microparticles (MPs) are membrane vesicles of 0.1–1 μm in diameter produced mainly by platelets, vascular endothelium and blood cells in response to cell activation and stress factors. MPs can be also released during malignant transformation or apoptosis. The essential step in MP formation is the loss of the cell membrane asymmetric phospholipid distribution as response to the increased intracellular calcium levels. MPs contain, proteins and genetic material (DNA, miRNA, mRNA) which enables them to interact and influence target cell. MPs are considered to be markers of ongoing pathophysiological processes in cardiovascular system, due to their role in inflammation and coagulation.

Keywords: laboratory diagnostics, extracellular microvesicles, endothelial dysfunction, platelet activation.

Microparticles (MPs) are membrane vesicles of 0.1–1 μm in diameter shed from vascular endothelium and blood cells (e.g.: lymphocytes T and B, macrophages) or platelets in response to cell activation, various forms of stress (e.g. hypoxia, mechanical trauma or inflammation). MPs can be also released during malignant transformation or apoptosis [1]. The mechanism of MPs formation can be studied by stimulating cultured cells *in vitro* with numerous cytokines and apoptotic stimuli [2]. The role of MPs in various diseases can be explored by their isolation from peripheral blood [3]. So far the endothelial MPs and platelet (PMPs) microparticles have been studied extensively in cancer, atherosclerosis, sepsis, diabetes and cardiovascular diseases [1, 3].

Additionally to MPs, exosomes (Ex) and apoptotic bodies are sometimes considered members of „microparticles family". However, MPs have different size, biological properties release mechanism [4, 5]. Apoptotic bodies are cell fragments formed in the terminal phase of apoptosis during cell fragmentation, they are larger than MPs and, like exosomes, exhibit lower clotting capacity comparing to MPs. While Ex are produced in the endocytic-lysosomal system and released from cell by the fusion of multivesicular bodies (MVB) with plasma membrane, MPs are formed by cell membrane shedding [6, 7] (**Figure 1**). The essential feature of this process is the loss of the cell membrane asymmetric phospholipid distribution in response to the increased intracellular calcium levels. This in turn regulates membrane flippase, floppase and scramblase activity, leading to phosphatidylserine (PS) and phosphatidylethanolamine (PE) exposure on the outer membrane leaflet, and the activation of contractile proteins (**Figure 2**). PS is a negatively charged phospholipid and, in the presence of calcium ions, it assembles the prothrombinase complex and activates coagulation *in vivo* [3].

PMPs are the most abundant circulating MP subtype [1]. They are released from platelets after their activation by thrombin, ADP plus collagen, calcium ionophore A23187 and high shear stress. Endothelial cells, monocytes and vascular smooth cells can release MPs after activation by bacterial lipopolysaccharide, inflammatory cytokines (e.g. interleukin-1α, IL-1α), complement complex C5b-9 or reactive oxygen species, hyperhomocysteinemia, hyperglycemia, hypoxia and tumor necrosis factor α [8, 9, 10]. MPs

contain enzymes and genetic material which enables them to interact and influence target cell (**Figure 3**) [7]. Endothelial MPs transfer cell adhesion protein VE-cadherin, T-cadherin, E-selectin and sialomucin (CD34). Endothelial MPs are also the carrier of many active enzymes, including matrix metalloproteinases and their inhibitors acid sphingomyelinase, nitric oxide synthases (eNOS), NADPH oxidase. These microparticles also regulate intercellular lipid transfer (PS, PA, sphingomyelin, arachidonic acid). Additionally, MPs contain DNA, mRNA, microRNA- and, possibly, other non-coding nucleic acid chains, indicating that MPs may play a role in the cell-to-cell transfer of genetic contents [11].

MPs play role in inflammation, coagulation and vascular function and are considered to be markers of ongoing pathophysiological processes. All of these processes contribute to cardiovascular risk factors (e.g.: diabetes and hypertension) or disorders (e.g.: atherosclerosis, coronary artery disease, stroke, cardiomyopathy or thromboembolism) [2, 3].

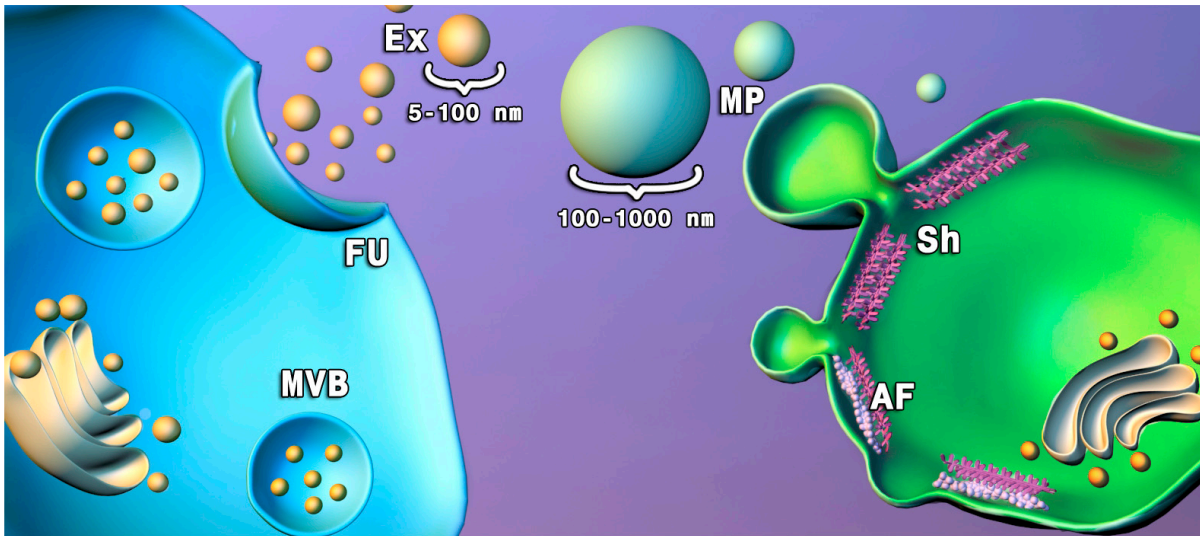

Figure 1. The comparison of exosomes (Ex) and microparticles (MPs). AF – actin filaments, FU – fusion of cell and vesicle membrane, MVB – multivesicular body, Sh – membrane shedding

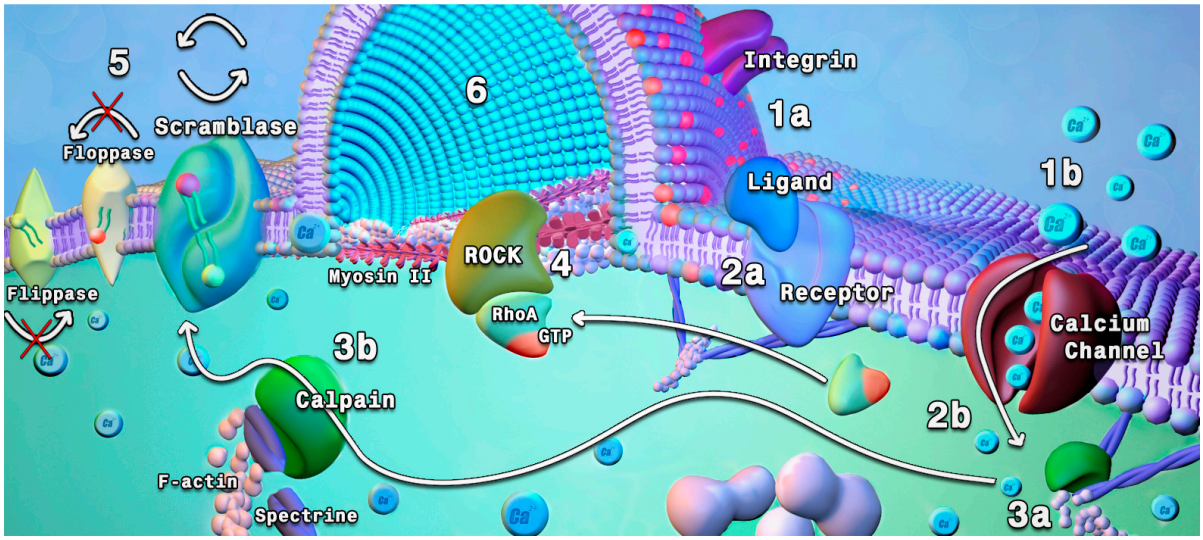

Figure 2. The regulation and physiology of cell membrane budding and microparticle formation. This model describes the stages of cell activation leading to membrane phospholipid asymmetry. The first step is cell stimulation via ligand-to-receptor binding (1a) or calcium channel activation (1b). A receptor transmits a signal towards the cell (2a) and/or calcium influx occurs (2b). Increased calcium activates calpain to brake F-actin and release spectrin from submembrane compartments (3a, 3b) or evokes RhoA phosphorylation (4). Membrane lipid asymmetry is regulated by the cooperative activities of three transporters: (flippase) the ATP-dependent aminophospholipid-specific translocase, which rapidly transports PS and PE from the cell's outer-to-inner leaflet; the ATP-dependent nonspecific lipid floppase, which slowly transports lipids from the cell's inner-to-outer leaflet; and the Ca2+-dependent nonspecific lipid scramblase, which allows lipids to move randomly between both leaflets (5). Finally, a microvessicle is formed and myosin II contraction controls its release (6)

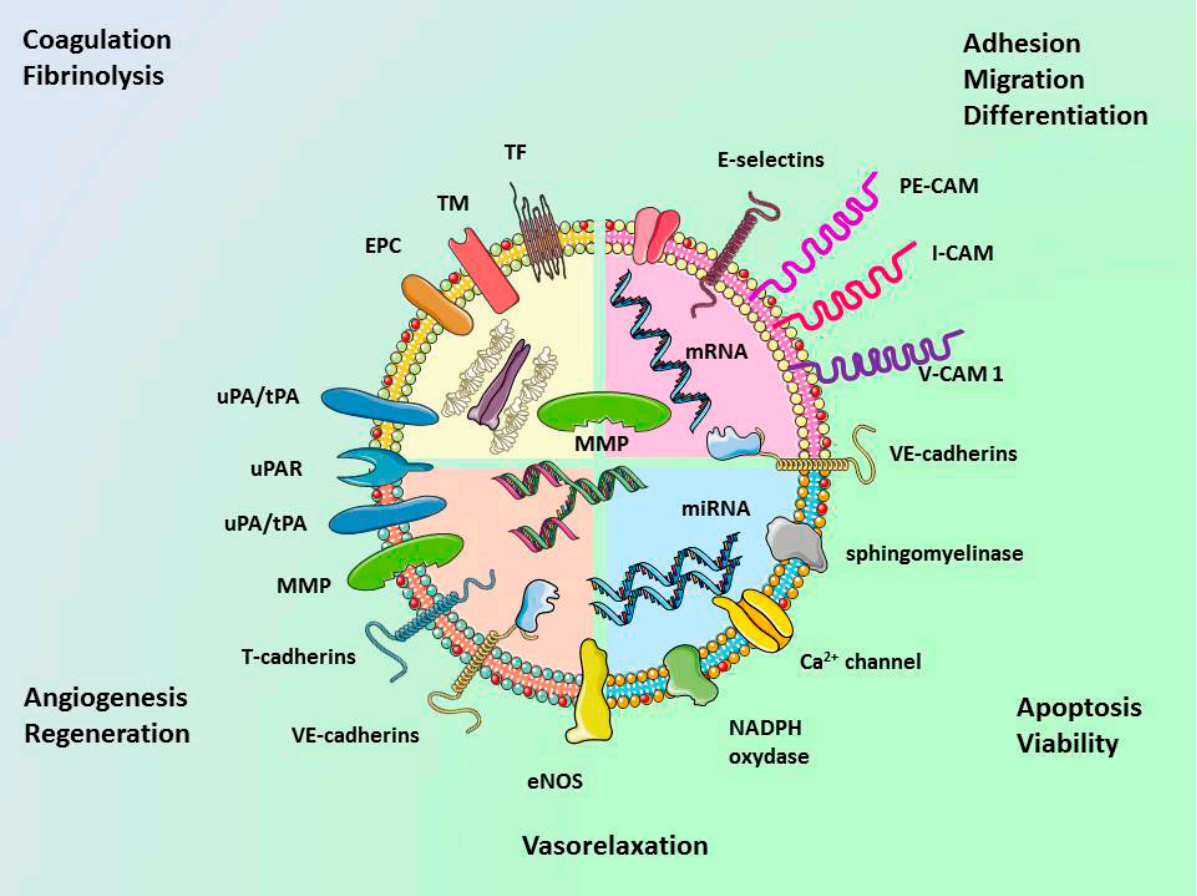

Figure 3. The scheme of typical EMP content. CAM – cell adhesion molecules, eNOS – endothelial nitric oxide synthase, EPC – endothelial protein C, MMP – matrix metalloproteinase, TF – tissue factor, TM – thrombomodulin, uPA/tPA – urokinase/tissue plasminogen activator, uPAR – urokinase plasminogen activator receptor. Based on Stępień and Targosz-Korecka 2013 [7], with agreement and modified

Atherosclerosis is an inflammatory process caused largely by transendothelial migration and accumulation of macrophages and neutrophils in the arterial wall [12]. The endothelial MPs stimulate endothelial cells to secrete cytokines responsible for activation and chemotaction of leukocytes [13]. Platelet MPs, by delivering arachidonic acid to endothelial cells, increase adhesion of monocytes to human umbilical vein endothelial cells (HUVEC), following upregulation of cellular adhesion molecules (ICAM-1) on endothelium and CD11a/CD18 and CD11b/CD18 on monocytes [14]. Leukocyte to leukocyte adhesion is also mediated by platelet MPs thorough the up-regulation of CD11b on cell surface. After the arterial wall is infiltrated with leukocytes, they start to secrete cytokines and growth factors that activate the proliferation of vascular smooth muscle cells, and initiates formation of atherosclerotic plaque [15].

Coagulation (thrombogenesis) is an important part of hemostasis. Cardiovascular patients are at high risk of thrombosis and they are predisposed to develop serious adverse cardiovascular events such as myocardial infarction, stroke, and acute lower limb ischemia. MPs have a large impact on coagulation mainly from the exposure of negatively charged PS on the outer membrane leaflet [5]. In the presence of calcium ions PS forms the prothrombinase complex assembly and increases thrombogenesis. Moreover, activated factor V (a clotting activator) can be found at the MPs surface. In the presence of calcium and phospholipids, an activated factor V merges with an activated factor X to produce thrombin (protrombinase activity). In turn, to catalyze factor X conversion to its active form, the complex of tissue factor (TF) with factor VIIa is necessary. That again leads to MPs because TF is present in the surface of platelet-derived microvesicles [16]. In patients with myocardial infarction the higher coagulation plasma potential due to MPs can be assumed (**Figure 4**).

Boulanger et al. (2001) showed the MPs impact on the vascular function [17]. Authors exposed rat aortic rings (with endothelium) for 24 hours to circulating MPs isolated from 7 non-ischemic patients and 19 patients with acute myocardial infarction. They found

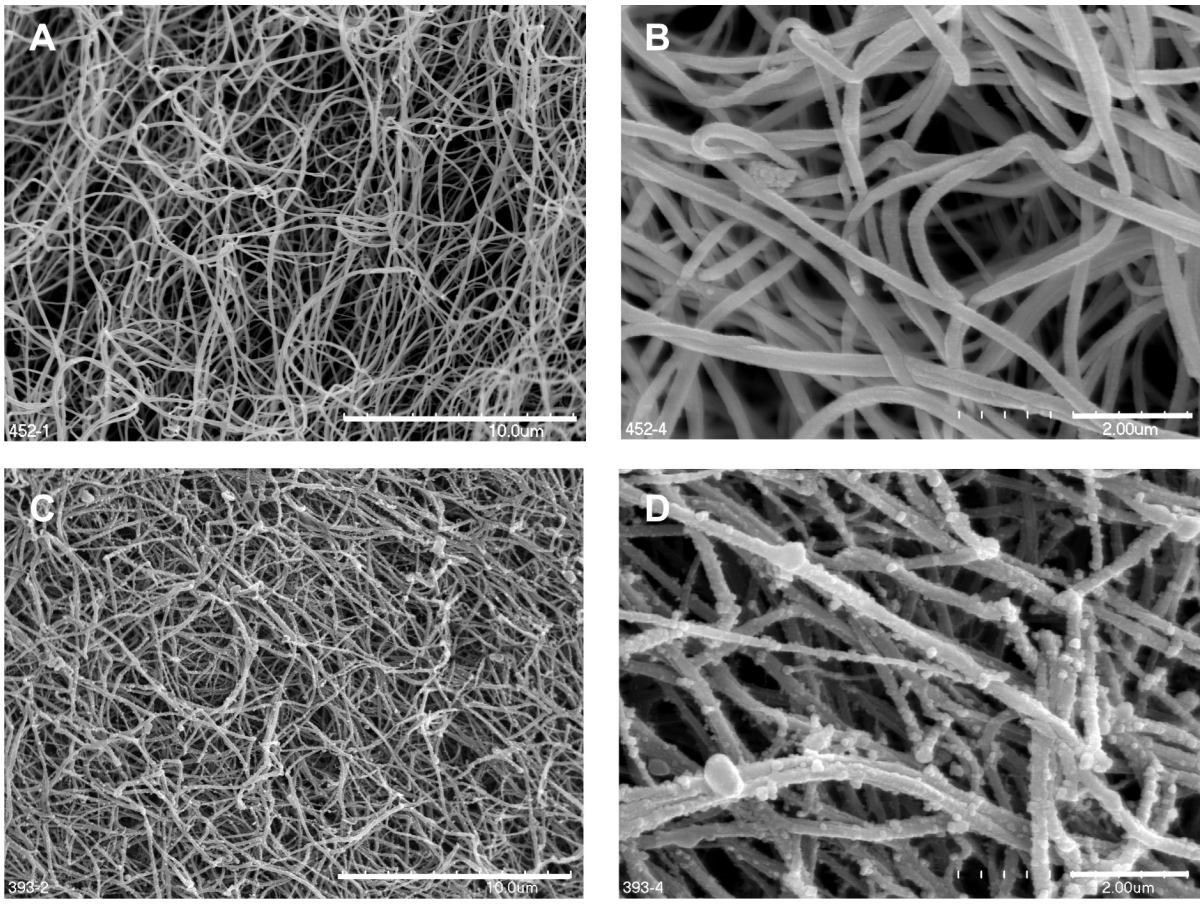

Figure 4. Microphotographs of fibrin clots obtained from acute cardiovascular patient plasma, incrusted with MPs emerging from fibrin fibers. Micrographs were taken using scanning electron microscopy (SEM) at 5000 (A, C) and 20000 (B, D) times magnification in collaboration with Mrs. Jadwiga Faber from Department of Cell Biology and Imaging, Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland. Bar represents 10 μm (A, C) and 1 μm (B, D)

that endothelium-dependent relaxations to acetylcholine were reduced in samples exposed to MPs from patients with myocardial infarction but not from non-ischemic patient [17].

Many in vivo studies regarding MPs in cardiovascular diseases, concerning their overall amount and composition, have been published in recent years, e.g.: endothelial MP levels were elevated comparing to control in deep vein thrombosis or pulmonary embolism [18], in acute coronary syndrome [19], in acute ischemic stroke [20], in diabetes [21, 22] or in the severe hypertension with systolic pressure correlation [23]. Very interesting study was carried out in 2005 by Koga et al. who proved that elevated endothelial MP levels were predictive for the presence of coronary artery lesions in diabetic patients referred for coronary angiography these microparticles were the strongest risk factor for CAD irrespective of other risk factors such as length of diabetic disease, lipid concentration, and hypertension. The elevated EMP were particularly useful in identifying patients with angiographically confirmed coronary artery disease and without typical angina symptoms [24].

In summary, MPs are diverse biological objects with various structure and function. Their impact on coagulation, inflammation and vascular function is important for endothelial function [25]. Precise knowledge of their release and activity *in vivo* may help to identify patients with increased cardiovascular risk in the future and perhaps apply appropriate therapies before acute complications occur.

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THOUSAND WORDS

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A thousand words about cardiac mobile health

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ABSTRACT

Cardiac mHealth (mobile health) is an innovative method of integrating technological and medical advances to provide healthcare in a convenient and cost effective manner in cardiology. While still considered an experimental and upcoming technology, its potential use in cardiology is feasible and may soon replace some standard medical practices. From basic encouragement of lifestyle modification to chronic disease self-management, mHealth can be a personal "pocket-doc". It can provide personal health benefits and immediate life-saving interventions to those who are unable to access medical care. mHealth's potential has much to offer to both physicians and patients.

Keywords: mobile health, cardiology, smart devices, smart applications, telemonitoring.

Mobile devices, which are like small computers, affect every aspect of our lives, including medicine and healthcare, and mobile health (mHealth) becomes a new and independent entity [1–6].

In this mini-review we describe cardiac mHealth. The term mHealth depicts *medical and public health practice supported by mobile devices, such as mobile phones, patient monitoring devices, personal digital assistants, and other wireless devices* [4]. This definition will certainly extend as new solutions and devices are developed, tested and introduced to the health market. Some additional devices not mentioned above include wearable patches that can be attached to a patient's skin for continuous monitoring 24 hours/day (e.g., ZephyrLife, Zephyr™, USA) and implantable devices (e.g., InSYNC pacemakers from Medtronic, cardioverters-defibrillators (ICD) or cardiac resynchronization therapy (CRT) devices), which transfer clinical data to monitoring centers via mobile phones [7–10]. A list of different devices with cardiac mHealth potential is presented in **Table 1** with some examples in **Table 2** [7, 8, 11–15]. A schematic mHealth data flow to and from a patient is pictured in **Figure 1**.

Potential indications for cardiac mHealth

An increasing number of cardiac mHealth solutions are used to solve different problems for a variety of users including patients, their families, healthy people, medical professionals, health care providers, and insurance services [1–6, 16]. The data and information flow in cardiac mHealth is shown in **Figure 2** whereas a list of potential indications and applications of cardiac mHealth is presented in **Table 3**.

Most commonly, cardiac mHealth is applied as a source of information and education. Patients find information on different health-related topics: a diet for weight reduction, smoking cessation, non-pharmacological control of blood pressure or how to safely perform physical exercise [1–4, 6, 16, 17]. Medical professionals search different resources for information on specific drugs or normal laboratory values.

Dozens of smart phone applications (apps), usually free of charge, measure pulse rate directly on mobile devices – an instant measurement of the rate of capillary pulsation is started right after placing a patient's finger on the smartphone's camera. The obtained signal of pulse rate can be further analyzed for computing heart rate variability to describe the autonomic influences on the heart [18].

The use of mHealth as a medical alert the next possible indication, by reminding individuals to take medication or measure glucose level, or by informing the patient about potential progression or aggravation of clinical condition (e.g., extremely high blood pressure or increased lung congestion) [10, 16].

Table 1. A variety of devices and solutions supporting cardiac mHealth. Abbreviations: CRT – cardiac resynchronization, ECG – electrocardiography, ICD – implantable cardioverter-defibrillator

Devices & solutions with cardiac mHealth potential
Medical devices directly transferring measured vital signals or parameters via built-in communication mode: *ECG machine, blood pressure monitor, cardiac impedance monitor*
Medical devices transferring measured vital signals/parameters via other mobile device: *blood pressure monitor*
Medical accessory measuring vital signals/parameters connected to or communicating with a mobile device: *ECG electrodes, heart rate belt*
Medical application installed in a mobile device: *smart applications for instant heart rate*
Medical patch attached to skin measuring vital signals/parameters communicating with a mobile device: *patches measuring ECG, respiration, temperature, activity, position etc.*
Implanted medical device/sensor used for the treatment or diagnosis communicating with a mobile device: *pacemaker, ICD, CRT, implantable loop recorder*

Table 2. List of sample devices and solutions with mHealth potential [11–15]. **Abbreviations: CPR – cardiopulmonary resuscitation, ECG – electrocardiography,** EEG – electroencephalography, ICD – implantable cardioverter-defibrillator

Examples of cardiac mHealth devices & solutions	
Implantable cardioverter defibrillator	A modern ICD is able to remotely communicate with the monitoring center after any intervention or report the current status of the device and electrodes
PocketCPR	This device offers instructions for bystanders to provide effective life-saving chest compressions in case of cardiac arrest
Zypher BioHarness 3 belt	This device performs accurate physiologic monitoring of numerous parameters including heart rate, blood pressure, and respiratory rate among others in a variety of real world conditions
AliveCor Heart Monitor	This smartphone accessory takes electrical impulses from patient's skin to record ECG at any time and anywhere and transmits data to most mobile devices
Flexible Skin-Worn Patch Monitor	These electronic patches can accurately monitor ECG and EEG under a variety of real world conditions
LIFENET 5.0	This mobile application offers physicians the ability to perform rapid consults, provide management decisions, notify appropriate healthcare services of patient condition and arrival, and provide monitoring of patient status all from a mobile device wherever they may be

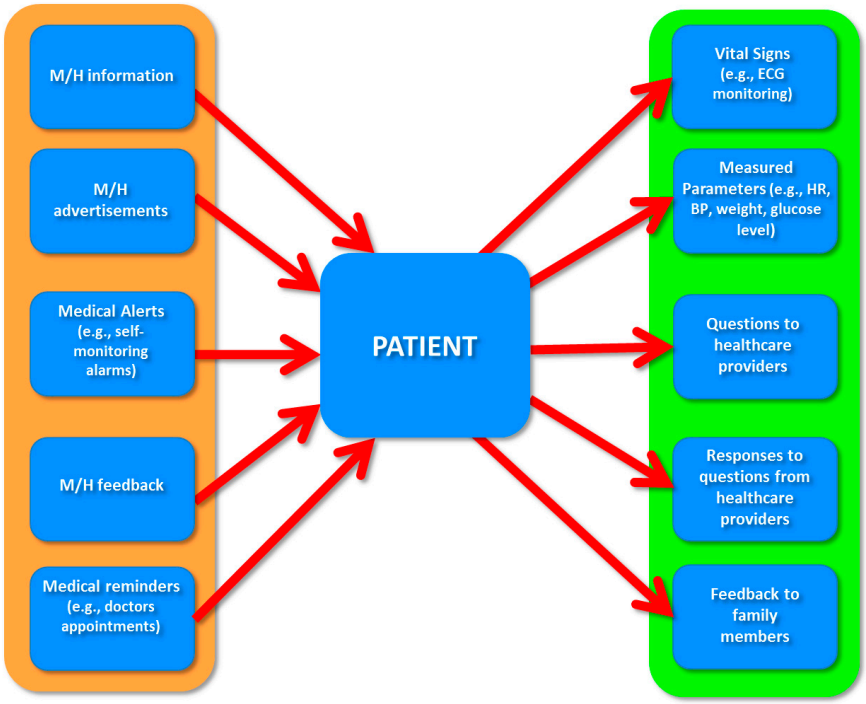

Figure 1. Type of mHealth data generated by or sent to patients. Abbreviations: BP – blood pressure, ECG – electrocardiography, HR – heart rate, M/H: Medical/Healthcare

Another excellent example of mHealth is a system for remote rapid consults in critical conditions (e.g., acute coronary syndromes) and fast clinical decisions (Lifenet 5.0 package, Physiocontrol, USA) [15]. This application can automatically activate special protocols helping hospital teams to prepare in advance for an incoming patient with a heart attack.

The cardiovascular risk assessment is an additional cardiac mHealth application to determine risk profile and the necessity of prescribing a statin. This can be done by using the SCORE (Systematic Coronary Risk Evaluation) Risk Chart available on a mobile app [19] or by the atherosclerotic cardiovascular disease (ASCVD) estimator [20].

The application of mHealth for diagnostic purposes is still in the preliminary phase and its use is mainly restricted to medical doctors. AliveCor is an accessory connected to a mobile phone which takes patient's ECG – it may help to diagnose myocardial infarction or ischemia [13].

The next instance for mHealth is the monitoring of the effectiveness of medical therapy. This can be done in several ways by professional medical devices like blood pressure monitor, glucometer or body composition analyzer, which control specific parameters and send readings to monitoring centers. One very specific mHealth solution that can be applied by anyone, including bystanders, is PocketCPR either installed on a smartphone (ZOLL Medical Corporation, USA) for free or purchased as a separate small device (BIO-DETEK Inc, USA) [11]. In both cases, the smartphone or device is held in both hands of a person performing cardiopulmonary resuscitation while it provides a real-time feedback and guidance through the entire resuscitation, improving the effectiveness and success rate.

Some other examples of mHealth are advanced solutions that allow monitoring and transmitting short ECGs with arrhythmias after activation by a patient,

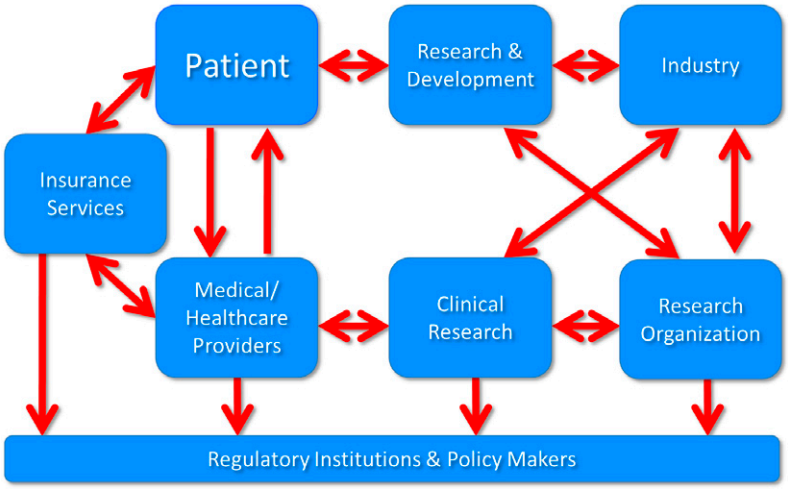

Figure 2. Data and information flow in mHealth

Table 3. Potential indications and applications of cardiac mHealth. Abbreviations: CRT – cardiac resynchronization, ECG – electrocardiography, ICD – implantable cardioverter-defibrillator, ILR – implantable loop recorder, INR- international normalized ratio

Potential indications & applications of cardiac mHealth
FOR PATIENTS
Assessment of the risk of various cardiovascular diseases and their complications
Assessment of the risk of various cardiovascular invasive procedures
Monitoring of vital signals and selected parameters in high-risk patients (*e.g. with recurrent arrhythmia or syncope*)
Education of patients and their family members on cardiovascular risk, prevention, and algorithms of action in case of life emergency
Regular reporting on various cardiovascular measures (*e.g. blood pressure, heart rate, INR*)
Remote monitoring of implanted devices, long-term skin patches with measuring vital signals
FOR MEDICAL PROFESSIONALS
Remote control of the effectiveness of therapy and progress of cardiovascular disease (*e.g. heart failure*)
Remote assistance in cardiac rehabilitation (*post-infarction, heart failure, post cardiac surgery*)
Remote control of implanted therapeutic (*pacemaker, ICD, CRT*) and diagnostic (*ILR*) devices
Remote reading and monitoring of selected vital signals and parameters in high-risk patients
The need of regular and repeated education of patients and their family members

family member or witness. The ECG tracings can also be automatically sent via an implanted device (e.g., ICD) directly to monitoring centers [7–9, 12, 15].

Potential benefits of using mHealth

It is rather a matter of a short time when cardiac mHealth will be routinely used and prescribed – a small medical application can easily turn a standard smartphone into a professional medical device.

There are many potential benefits for the application of cardiac mHealth (**Table 4**) like acquiring real-time monitoring of vital signals, collecting clinical data on a large scale, reduction in costs of healthcare, which is particularly important in low-income and developing countries, and improved personalization of the medical treatment [1, 3, 4–8, 10, 16, 17, 18, 21, 22, 23].

Potential mHealth related risks and limitations

The capability of cardiac mHealth is anticipated to be one of the fastest growing areas in medicine and has also prompted research for its use in other areas (engineering, programming etc.) [1–4, 6, 7, 8, 23, 24]. However, the application of mHealth can be accompanied by risk (**Table 5**) some of which may convey an increased cardiovascular morbidity and mortality [2, 3, 4, 21]. The responsibility of using and prescrib-

Table 4. Potential benefits of cardiac mHealth for the general population, patients, medical practitioners, and healthcare providers

Benefits of using cardiac mHealth
Collecting community and clinical data at low cost and real time
Continuous delivery of updated healthcare information and services to practitioners, researchers, and patients at any time
Real-time monitoring of patient's vital signs
Provision of healthcare in a convenient and cost effective manner
Portability of mHealth devices – a "pocket-doc"
Encouragement of health and self-surveillance for patients
Supporting mHealth contemporary solutions in developing nations
Beneficial interventions and programs on health in both high- and low-income countries
Accessibility from home and any place to mHealth with a reduction in number of office visits patients need to make
Easier and faster communication with medical personnel
Automatic data collection that are sent directly to medical personnel, monitoring centers, researchers and developers, clinical scientists, and insurance system
Increasing patient's mobility and ability to travel even for those who have chronic cardiovascular diseases
Easier communication with patients and their families
Ability to review patient data and determine course of management from anywhere
Ability to communicate course of management to patient and their families
Reduction of costs of diagnosis, monitoring, prophylaxis, risk assessment and treatment in selected diseases.
Transfer of some medical procedures closer to patient's natural environment
More personalized approach to health and medical care

Table 5. Potential risks of cardiac mHealth for general population, patients, medical practitioners and healthcare providers

Risks of using cardiac mHealth
Not established standards for medical and sensitive data transfer between different users of mHealth
Dependence on the availability of access to communication network
Not standardized protocols, algorithms, sensors with a variety of mobile devices which are not primarily medical devices
Lack of personal responsibility for taking decisions
Lack of legal regulations for using mHealth
Lack of reimbursement from insurance systems
Troubles with using advanced mHealth solutions by substanial number of people not familiar with modern internet & communication technologies
Lack of data from prospective, multicenter and randomized trials
Lack of recommendations of using mHealth in specific cardiovascular problems or prophylaxis
Lack of controlling institutions ready to certify mHealth solutions
Massive development of unverified medical and health-related smart apps by people with no medical background, expertise and skills
No certificates for majority of mHealth solutions
No assistance in choosing right mHealth solution for specific cardiovascular problems
Lack of face-to-face contact between patients and medical/health care professionals
Misunderstanding of instructions, comments, questions and answers from medical/health care professionals

ing mHealth is undetermined and it is unclear whether insurance companies will reimburse mHealth. As of right now, there are no legal regulations on mHealth, and the first documents are either in very preliminary forms or are under construction [2, 3, 4, 22].

Nevertheless, the development of cardiac mHealth seems unstoppable – the first mHealth devices and applications have gotten approval from the Food and Drug Administration in USA for the use by medical professionals while many others are being introduced to the market by medical industry without approval from any regulatory institution [2–5].

While mHealth provides a sort of "pocket-doc", there is no replacement for the physical and emotional support or a live personal physician. As with any innovative technological advancement, mHealth comes with its limitations. It may offer advantages in specific aspects of healthcare but its vast reach is not finite. There are neither big, prospective, multicenter and randomized clinical studies exploring the application of mHealth to cardiac patients nor guidelines on the use of cardiac mHealth from any of the largest cardiac societies [3, 4, 5, 17].

Summary

Users adopt the cardiac mHealth tools nearly as quickly as they are developed for different purposes. With the rising popularity of mobile devices, cardiac mHealth has the potential to help modifying lifestyle, aid in chronic cardiovascular disease (e.g., heart failure, hypertension, arrhythmias), self-management and surveillance [1, 3–8, 10, 16, 17, 18, 22, 23, 25, 26]. Cardiac mHealth may improve physician, researcher, and patient understanding of cardiovascular diseases and risk factors, reduce needs for healthcare visits, help to collect community and clinical health data and vital signals, and provide immediate, personalized medical interventions. Cardiac mHealth can bring ease for people to take care of their own health and promote lifestyle changes, and supply immediate access to medical information whenever and wherever they require it.

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Standard journal articles

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Personal author(s)

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