FROM THE EDITOR

Dear readers

As I have already mentioned, in the year 2023 we will celebrate the centenary (1923-2023) of the Medical International Journal, therefore now is a good time to thank all the authors who contributed to our journal and also to all the members of the Editorial Committee of MIR , who with great dedication support the existence of MIR. This is difficult, because remaining Esperantist physicians very rarely contribute to MIR with Esperanto-language articles, which is why we mainly print articles written in other languages, especially in English.

On this occasion, I would like to mention an important event that can influence medical doctors to write and defend their doctoral theses in their native and Esperanto languages. On December 2, 2022, physiotherapist Jacek Piechowicz defended his doctoral thesis at the Faculty of Health Sciences of the Medical College of the Jagiellonian University in Kraków. While working at the UMEA Shinoda Medical Center in Krakow (1991-1999), Dr. Jacek Piechowicz had the opportunity to learn, through the Esperanto language, the hand therapy method developed by Master Masayki Saionji, which enabled him to complete an internship at the International Institute of Preventive Medicine. Practice in Tokyo (July 1991). Since then, he became interested in manual therapy, which resulted i.a. through a doctoral dissertation already defended. Thus, it can be said that thanks to the Esperanto language, Dr. Jacek Piechowicz initiated, to some extent, his education in the field of manual therapy, which he continues to this day. It can be mentioned that with the help of Esperanto it is possible to obtain a doctorate i.a. in medical fields. Scientific activity of future doctors can begin by publishing their specialized articles in our long-standing journal MIR. At this opportunity I am pleased to mention that Dr. Piechowicz was co-author of the articles published in MIR: Medicina Internacia Revuo 2013; 25(4), 101: 156-162; Medicina Internacia Revuo, 2013; 25(3) 100: 119-123; Medicina Internacia Revuo, 1995; 3(64): 145-147.

In closing, I am happy to mention the 22nd IMEK, which took place from 13 to 17 July 2022 in Hódmezővásárhely and at the Faculty of Pharmacy of the University of Szeged. Detailed information about the 22nd IMEK is freely available, because a conference book has been published and on this occasion we invite all authors to publish their lectures in MIR.

Professor Włodzimierz Opoka
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Vice-Editor-in-Chief

REDAKCIAJ VORTOJ

Karaj legantoj

Kiel mi jam menciis en la jaro 2023 ni festos jubileon de centjariĝo (1923-2023) de la Medicina Internacia Revuo, tial nun estas bona momento danki al ĉiuj aŭtoroj, kiuj kontribuis por nia revuo kaj ankaŭ al ĉiuj membroj de la Redakta Komitato de MIR, kiuj kun granda sindono subtenas ekziston de MIR. Tio estas malfacile, ĉar restintaj medicinistoj esperantistoj tre malofte kontribuas por MIR per esperantlingvaj artikoloj pro tio ni ĉefe presas artikolojn skribitajn en aliaj lingvoj, precipe en la angla lingvo.

Ĉi-okaze mi ŝatus mencii gravan eventon, kiu povas influi medicinistojn verki kaj defendi siajn doktorajn disertaĵojn en sia gepatra kaj Esperanto lingvoj. La 2-an de decembro 2022, fizioterapiisto Jacek Piechowicz defendis sian doktoran tezon ĉe la Fakultato de Sansciencoj de la Medicina Kolegio de la Jagelona Universitato en Krakovo. Laborante en UMEA Shinoda-Kuracejo en Krakovo (1991-1999), d-ro Jacek Piechowicz havis la ŝancon lerni per la Esperanto-lingvo la metodon de manterapio ellaborita de Majstro Masayki Saionji, kiu ebligis al li plenumi staĝon ĉe Internacia Instituto de Preventa Medicina Praktiko en Tokio (Julio 1991). Ekde tiam, li ekinteresiĝis pri mana terapio, kio rezultis i.a. per doktora disertaĵo jam defendita. Tiel, oni povas diri, ke dank' al la lingvo Esperanto d-ro Jacek Piechowicz iniciatis, iagrade, sian edukadon en la kampo de manterapio, kiun li daŭrigas ĝis hodiaŭ. Oni povas mencii, ke helpe de Esperanto estas ebleco doktoriĝi i.a. je medicinaj fakkampoj. Scienca aktiveco de estontaj doktoroj povas komenciĝi per publikigado de siaj fakaj artikolojn en nia multjara revuo MIR. Ĉe tiu ĉi ebleco kun plezuro mi mencias, ke d-ro Piechowicz estis kunaŭtoro de la artikoloj aperigitaj en MIR: Medicina Internacia Revuo 2013; 25(4), 101: 156-162; Medicina Internacia Revuo, 2013; 25(3) 100: 119-123; Medicina Internacia Revuo, 1995; 3(64): 145-147.

Finante mi kun ĝojo mencias pri la 22-a IMEK, kiu okazis de la 13-a ĝis la 17-a de julio 2022 en Hódmezővásárhely kaj ĉe Farmacia Fakultato de Universitato en Szeged. Detalaj informoj pri la 22-a IMEK estas libere riceveblaj, ĉar estas publikigita kongresa libro kaj ĉe tiu ĉi ebleco ni invitas ĉiujn aŭtorojn por apierigi siajn prelegojn en MIR.

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Editorial Board

MYCELIAL CULTURES AS A MODEL TO STUDY THE ACCUMULATION OF MEDICINAL COMPOUNDS – HISTORICAL PERSPECTIVE

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Abstract

Mycelial culture is a compilation of techniques used to maintain or grow mycelium under sterile conditions on a medium of known composition. This method is commonly used in fungal biotechnology. This review article describes the methodology of biotechnological processes using cultures of higher fungi, e.g. initialization of mycelial cultures, types of media used in in vitro cultures of higher fungi, types of mycelial cultures, and culture conditions. In addition, this paper describes selected biologically active compounds produced by mycelium obtained in vitro. Furthermore, the research on mycelial cultures conducted at the Department of Pharmaceutical Botany at the Jagiellonian University Collegium Medicum in Krakow is presented from a historical perspective.

Keywords: mushroom biotechnology, *in vitro* cultures, mushroom metabolites

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Introduction

Mushrooms have accompanied mankind since antiquity. In ancient Greece and Rome, they were used i.a. by warriors as food giving strength before battles. In the Far East, they constituted an important medicinal resource used in traditional medicine. Nowadays, mushrooms are of great interest in the scientific community, because of their potential usage in biotechnological processes utilized in such fields as medicine, cosmetology, pharmacy, agriculture, food industry and others [1]. Mycotechnology is an important direction of biotechnology in the broadest sense, using processes conducted with the participation of fungal organisms, of which the overriding task is to make the best possible use of the biochemical potential of mushrooms [2].

Important research objects in higher mushroom biotechnology are *in vitro* cultures, otherwise known as mycelial cultures, which are spawn cultivations conducted on synthetic medium under sterile conditions. The initiator of this method was the French mycologist Lucien Oddoux, who obtained mycelial cultures of more than 500 fungal species during many years of experimentation. For most of them, a species–pure cultures were obtained under laboratory conditions [3].

Mycelial cultures – general characteristics

Mycelial cultures can be initiated in several ways. The most common method is to isolate a fragment of the fruiting body from the stem or hymenial layer. The fruiting bodies, which are intended for the derivation of mycelial cultures, are cleaned (shriveled parts, soil residues and other impurities are removed) and sterilized [4]. Decontamination of the raw material is based on initial immersion of parts of the fruiting body in a decontaminating liquid, followed by several rinses in sterile redistilled water. The time needed for decontamination of each material should be determined experimentally The activity of disinfectants is usually surface based, so the source of culture infections are microorganisms residing inside the explants. A significant problem is latent bacteria, whose activity manifests itself only after a longer cultivation time. Therefore, it is required to add antibiotics to the culture medium [4]. Mycelial cultures can also be obtained by germinating sterilized spores obtained by exudation from the hymenial layer or by drenching the dried hymenium with water. Another option for initiating *in vitro* cultures is a transfer of commercially available strain onto agar medium [4].

The foundation of the effective growth of mycelial cultures is the selection of appropriate cultivation conditions. Factors affecting the growth of *in vitro* cultures can be divided into physical and chemical ones. A strategic factor affecting the development of mycelium *in vitro* is to supply essential nutrients. A source of carbon, nitrogen, macro– and micronutrients should be provided along with the medium. For a few species, the culture medium is additionally supplemented with vitamins and plant growth regulators [4,5].

The primary source of carbon are simple sugars, mainly glucose and fructose. Disaccharides – sucrose, maltose as maltose extract or polysaccharides (starch) are used less frequently. The most often used sources of nitrogen are organic compounds, such as amino acids, casein hydrolysate or peptone, which for many species can be used as a combined carbon and nitrogen source [5]. Some species prefer an inorganic nitrogen source such as ammonium chloride. Optimizing the carbon and nitrogen content in the medium has a huge impact on increasing biomass *in in vitro* cultures. With knowledge of the amount of carbon and nitrogen in specific compounds used for the cultivation, it is possible to determine their content in the medium. This establishes the ratio of total carbon to total nitrogen in the medium, known as the C/N ratio. This parameter

is particularly important in the growth of biomass cultured *in vitro* [5].

A proper mycelium development is also determined by the presence of macronutrients such as phosphorus, magnesium, potassium, sodium, and calcium, which are supplied in the form of inorganic salts. Amongst the micronutrients, salts of zinc, fluorine, manganese, copper, molybdenum, and iron are used. The content of macro– and micronutrients ranges from micro– to milligrams per liter of nutrient solution. Due to the role in fungal metabolism played by enzyme cofactors, vitamins are added to the medium, primarily B vitamins: thiamine (B1), biotin (H), pyridoxine (B6) and inositol. The proper concentration of these compounds can significantly affect mycelial growth [6]. Substrates usually do not contain fat–soluble vitamins in their composition, while vitamin C is added as a reducing compound that does not directly affect mycelial growth [4].

It has been proven that the addition of plant growth regulators, e.g., cytokinins and gibberellins, to mycelial cultures has a positive effect on increasing mycelial weight. For example, such a relationship was proven for *Polyporus palustris* [6]. Also, in studies on the effect of auxins on the development of the mycelium of *Pellinus linteus*, an influence on mycelial growth was demonstrated [7,8]. However, for some species, the addition of plant hormones does not affect the development of mycelium and may even cause its inhibition. In studies conducted on the species *Pleurotus florida*, the addition of auxins and gibberellins did not affect mycelial growth [9,10].

Potato, yeast, or corn extracts are also added to the media, which can cover the needs of organic growth factors. In case of the mycorrhizal mushrooms, an extract from the roots of trees with which they exist in symbiosis (pine, spruce, oak) is added to the media. Final product precursors or substrates directly converted to the desired product without complex metabolic reactions are also used as components of substrates that act by modifying metabolism or biotransformation.

Precursors affect fast and efficient production, but also guarantee targeted synthesis of a single compound [4].

In biotechnology research, the often–used is medium according to Oddoux, MNM medium according to Merlin – Norkrans modified by Marx, medium according to Pachlewski, PDA medium (Potato – Dextrose Agar), medium according to Lubiński [3,11, 12] (Table1). During the preparation of the media, redistilled water and high purity reagents are used. Mediums are prepared by adding mineral salts (macro– and microelements as concentrated solutions) and then organic compounds. Further, the pH value of the obtained solution is measured and set at the desired level. Passaging of cultures is usually carried out every 4–6 weeks, due to the different rates of depletion of medium compounds, depending on the type of culture, the rate of mycelial growth and the temperature at which the culture is conducted [4].

Table 1. Composition of selected media used in mycelial cultures

Carbon source	Nitrogen source	Macronutrients	Micronutrients	Vitamins	Other
Medium by Oddoux					
Glucose: 10 g Maltose extract: 5 g	Casein hydrolysate: 1 g L–asparagine: 1 g Adenine: 0.12 g	NH_4Cl: 0.5 g KH_2PO_4: 0.5 g $MgSO_4 \times 7\ H_2O$: 0.15g $CaCl_2 \times 6\ H_2O$: 0.5 mL	$FeCl_3$: 1.5 mL $MnSO_4 \times H_2O$: 1.5 mL $ZnSO_4$: 1.5 mL	B_1: 50 mg B_6: 50 mg	Yeast extract: 30 mg

MNM medium by Merlin–Norkrans modified by Marx					
Glucose: 10 g Maltose extract: 3 g	Casein hydrolysate: 1g	KH_2PO_4: 0.5 g $MgSO_4 \times 7\ H_2O$: 0.15g NaCl: 0.025 g $CaCl_2$: 0.05 g $(NH_4)_2HPO_4$: 0.25g	$C_6H_5O_7Fe \times 5H_2O$: 1.2 mL	B_1: 50 mg	
Medium by Pachlewski					
Glucose: 20 g Maltose extract: 5 g	Casein hydrolysate: 1g	KH_2PO_4: 1 g $MgSO_4 \times 7\ H_2O$: 0.5g	$ZnSO_4 \times 7\ H_2O$: 0.5 mL $C_6H_5O_7Fe \times 5H_2O$: 0.5 mL $(NH_4)_2C_4H_4O_6$: 0.5 g	B_1: 50 mg	
Medium PDA (Potato – Dextrose Agar)					
Glucose: 10 g Potato extract: 200 g	Casein hydrolysate: 1g	KH_2PO_4: 0.5 g			
Medium by Lubiński					
Glucose: 5 g	Casein hydrolysate: 1g	KH_2PO_4: 0.5 g			Yeast extract: 1 g

Currently, cheaper alternatives for standard mediums are being sought. These include extracts of oat flakes, rice and wheat bran, tapioca, pea hulls and pineapple waste. In one of the conducted studies, it was documented, that a substrate composed of tapioca extract supplemented with additional aqueous peanut and rice bran extracts allows to achieve a significant increase in mycelia, characterized additionally by high protein content [13].

An important parameter conditioning the proper growth of mycelium *in vitro* is the pH of the medium. For Basidiomycota species, it is in the range of 5.0–6.0. In the case of arboreal mushrooms, the pH can be reduced even to a value of 4.0. It has been shown that the pH has a significant impact on the function of the cell membrane, the structure of the filaments, the processes of nutrient uptake and the production of metabolites [14].

Physical conditions, such as incubation temperature of the medium, shaking speed and aeration, are also important for mycelium growth. A suitable incubation temperature for mycelium is in the range of 20–30°C, while special attention should be paid to the often–differing optimal temperatures for mycelium growth and metabolite production. Higher temperatures are used during spore germination and biomass growth, while efficient synthesis of secondary metabolites may require lower temperatures [14]. The rate of shaking can affect the distribution of nutrients in the system. In the study on the impact of shaking method on biomass development, significant differences in cellular metabolism were observed within cultures with improper selection of process parameters [15]. Aeration is another important factor affecting the level of mycelium growth. In the case of mycelium of species of the Basidiomycota cluster, light – its length, intensity and photoperiod have a negligible effect on development [15].

Mycelial cultures are initiated as stationary–agar cultures and can be carried out on Petri dishes, in test tubes or conical flasks; they serve as starting material for experiments. In the initial phase, mycelium grows as a loose or compact homogeneous structure. Then, it is transferred as an inoculum into a liquid medium and continued as a shaken liquid culture (Figure 1). This is advantageous, as it ensures

mycelium growth occurring throughout the medium. At the same time, aeration of the culture occurs, resulting in an increase in the rate of biomass growth. Liquid cultures of mushrooms can also be carried out in bioreactors, devices for the cultivation of single–celled organisms such as bacteria or lower fungi, or multicellular organisms, built to maximally reduce or eliminate contamination. Furthermore, bioreactors offer the possibility of reducing the growth time of mycelium, consuming medium elements more efficiently, ensuring reproducible conditions, and markedly increased the efficiency of culture [16].

Development in mycelial culture involves two processes: the growth of hyphae and their propagation. Vegetative development consists in the proliferation of mycelial shreds with the formation of branched multicellular systems. Meanwhile the proliferative process, is the result of morphological and biochemical diversification of the mycelium after a period of dynamic growth [17].

In mycelial cultures usually there are obtained the same compounds that are products of secondary metabolism as in fungal fruiting bodies. However, with modified parameters, mycelia can produce new substances with specific biological properties not found in fruiting bodies. It is therefore important to compare the chemical composition of mycelia obtained *in vitro* with that of fruiting bodies obtained from natural sites. It is also necessary to compare the composition of the mycelium and the medium, in which the primary and secondary metabolites synthesized by the mycelium, are also deposited [18]. With appropriate manipulation of the composition of the medium and physical factors, it is also possible to influence the metabolism of the mycelium, causing an increase or decrease in the biosynthesis of chemical compounds and directing the mycelium to produce specific substances [18].

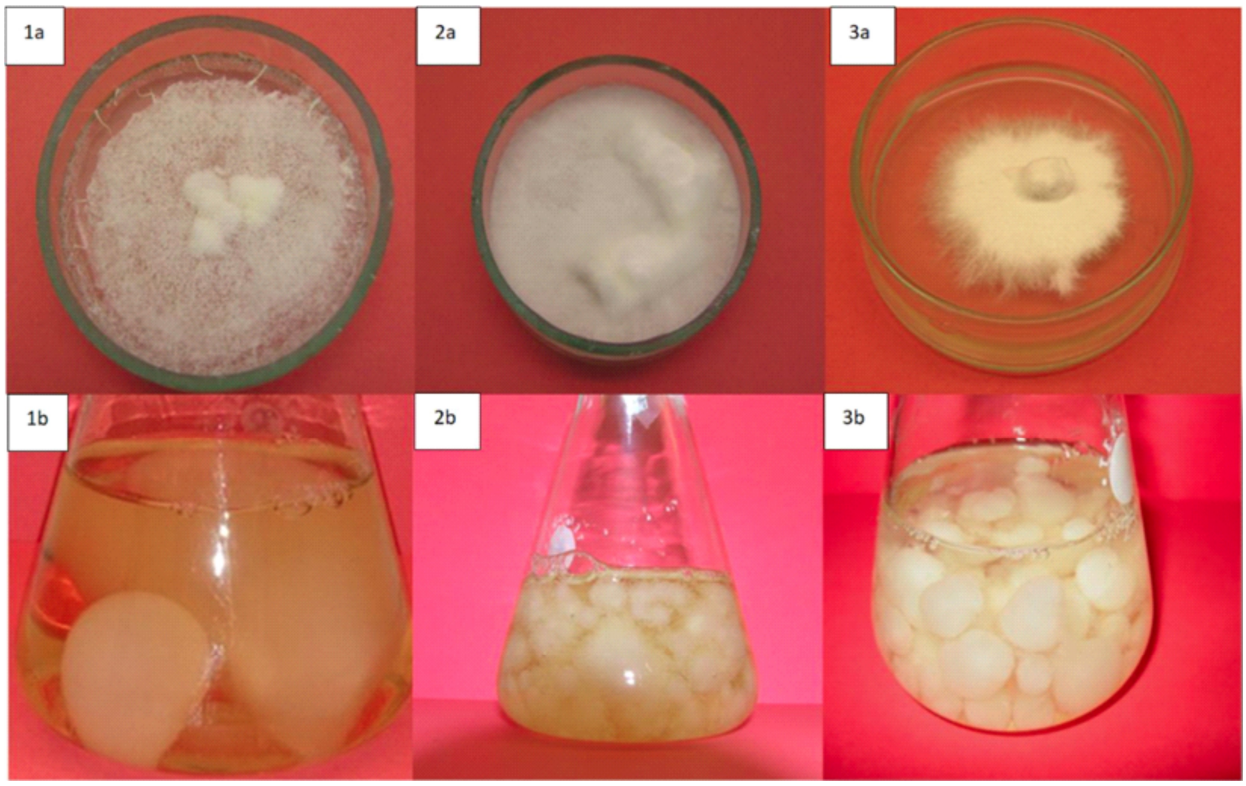

Figure 1. Mycelial cultures on agar medium and liquid medium – 1a, 1b *Laetiporus sulphureus*, 2a, 2b *Ganoderma applanatum*, 3a, 3b *Trametes versicolor* (Photo: K. Sułkowska–Ziaja).

Examples of compounds isolated from mycelial cultures – historical overview

The growing interest in mycology in recent years, has greatly enhanced the level of knowledge about mushrooms. It has been proven that they are species with a rich and diverse chemical composition, as well as high potential for application in medicine, pharmacy, and dietetics. A lot of research is currently underway to identify new compounds isolated from mycelial cultures and to determine their biological activities (Table 2).

One of the most thoroughly studied phenomenon is the ability of mycelial cultures to accumulate polysaccharides. The first studies were carried out in Japan, where were obtained polysaccharides such as lentinan, schizophyllan and krestin (PSK), which showed anticancer activity [19]. These compounds activate extracellular and intracellular apoptotic pathways by binding to so–called death receptors in the cell membrane [20]. Polysaccharides with anti–cancer effects also include β–glucan extracted from *Hericium erinaceus* cultures, which affects intracellular signaling pathways by increasing immune activity against mutant cells. Other polysaccharides isolated from this species also showed anti–tumor activity by arresting the cell cycle and inducing apoptosis [21]. From mycelial cultures there were obtained also other substances with anticancer effects. These include, for example, coriolin (sesquiterpene), a compound isolated from *Coriolus consors*, and calvatic acid from *Calvatia sp.* cultures, which inhibits the growth and development of *Yoshida sarcoma* [22,23]. Anticancer properties have been demonstrated also for cordycepin isolated from the genus *Cordyceps sp*. This compound acts by affecting AP–1, NF–κB and TNF–α signaling pathways, inhibiting the proliferation and migration of cancer cells. In addition, it has been shown that the substance can limit the process of angiogenesis. The properties of cordycepin have been studied i.a. on melanoma, colorectal cancer, and bladder cancer cells [24,25].

Substances with cytotoxic activity, such as ganodermic acid – a triterpene extracted from cultures of *Ganoderma lucidum*, nematolone – a sesquiterpene from cultures of the genus *Panus sp.* or ergosterol derivatives from cultures of *Trametes versicolor* can be obtained from mycelial cultures [26,27]. Cytotoxic as well as antioxidant activities are also characterized by p–terphenyl pigments – rickenyls A–E isolated from the mycelium *Hypoxylon rickii* [26,27].

Furthermore, mycelial cultures have become a source of other compounds characterized by bacteriostatic and fungistatic activity. Examples of such substances include mucidin, a polyketide with antifungal properties obtained from the mycelium of *Oudemansiella mucida*, and pleuromutilin, a sesquiterpene with fungistatic activity isolated from mycelial cultures of mushroom of the genus *Clitopilus* [28,29].

Strobilurins extracted from the mycelium of *Strobilurus tenacellus* have found use as fungicides due to their ability to inhibit the cellular respiration of filamentous fungi [30]. Oudemansin B found in cultures of *Xerula pudens also* exhibits a similar mechanism of action [31]. Among the numerous substances with antimicrobial activity produced by mycelial cultures, only some of them have been completely chemically characterized. These include, for example, pleurotin, a naphthoquinone extracted from mycelial cultures of *Hohenbuehelia grisea*, which is an inhibitor of Gram (+) bacterial growth, and lentinellic acid, a sesquiterpene–like compound extracted from cultures of *Lentinellus ursinus* [32,33]. Striatins from *Cyathus striatus* cultures have fungistatic and antimicrobial activity against Gram (+) and Gram (–) bacteria [34]. Polysaccharide compounds isolated from mycelial cultures of *Sarcodon imbricatus* have antimicrobial properties against Gram (+) bacterial strains such as *Enterococcus faecalis*, *Staphylococcus aureus* and Gram (–) bacteria – *Escherichia coli*

[35]. Polysacharides and triterpenoids found in the mycelium of *Ganoderma lucidum* show bacteriostatic activity i.a against *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli* and drug–resistant strains of *Staphylococcus aureus* [36].

Many substances from *mycelial cultures* are also characterized by antiviral activity. Such compounds include hispidin from *Inonotus hispidus* cultures, which *in vitro* causes inhibition of influenza A and B virus proliferation [37]. Compounds extracted from the mycelium of *Ganoderma lucidum* show potent antiviral activity against HIV–1, HSV–1 and HSV–2. Lucidenolactone and lucidenic acid O inhibit HIV–1 reverse transcriptase, while ganodermanontriol and ganoderiol F inhibit virus growth [38]. Both PSK and a polysaccharide–peptide complex extracted from *Trametes versicolor* cultures cause inhibition of HIV and cytomegalovirus replication [39]. On the other hand, a polysaccharide isolated from *Sarcodon imbricatus* mycelium, composed of glucose and fucose, has been shown to inhibit HSV–1 virus replication [35].

Compounds with antioxidant properties are also extracted from mycelial cultures. Among them, polyphenols play the most important role. Many species of Basidiomycota are characterized by a significant content of these substances. *Inonotus xeranticus, Phellinus linteus* or have significant antioxidant activity and free radical scavenging potential [40].

Cordyceps sinensis is another species possessing antioxidant properties. These activities are attributed to the presence of substances such as polysaccharides, cordycepin and mannitol. The obtained extracts showed the ability to inhibit linoleic acid peroxidation, chelate and reduce iron ions, and scavenge free radicals [25,41,42].

The previously mentioned cordycepin also has immunostimulatory effects by influencing humoral and cellular immunity. This compound shows the ability to activate the transcription factor NF–κB, resulting in an increase in the production of pro–inflammatory compounds by macrophages. In studies with rats, there was an increase in the concentration of T lymphocytes: CD4 and CD8, cytokines and interleukins, including IL–4, IL–10, and IL–12, as well as a decrease in the level of TGF–β and IL–2. Furthermore, cordycepin showed an effect increasing the activity of enzymes such as cyclooxygenase–2 (COX–2) and induced nitric oxide synthase (iNOS). Immunostimulatory effects may also be exhibited by certain polysaccharides. For example, polysaccharide compounds isolated from the *Cordyceps militaris* mycelium interacted with the immune system by affecting the NF–κB and MAPK transcriptional pathways and stimulating macrophages to produce pro–inflammatory factors [41,42].

Anti–inflammatory properties have also been demonstrated for numerous compounds isolated from mycelial cultures. Examples include ergosterol and cordycepin, which can inhibit the release of inflammatory mediators. The latter compound inhibits also the activity of NF–κB and MAPK factors, which can be used in the treatment of neurodegenerative diseases, reducing inflammatory processes within the nervous system. In addition, the compound exhibits significant antioxidant properties. In turn, studies with mice have shown an increase in GABA neurotransmission and a decrease in acetylcholinesterase (AChE) activity [24]. Neuroprotective effects were proven for diterpenoid derivatives of erinacine isolated from mycelial cultures of *Hericium erinaceus*. These compounds increased the activity of nerve growth factor (NGF) [43].

Mushrooms are also gaining popularity in supporting the treatment of civilization diseases. This is due to the presence in them of many substances that have positive effects on the circulatory system and organs such as the liver and pancreas, among others. Polysaccharides isolated from *Cordyceps militaris* show the ability to inhibit α–glucosidase, while cordycepin increases glucose absorption and uptake by cells, resulting in lower blood glucose levels.

The compound has potential for use in the treatment of hyperlipidemia, by lowering LDL, VLDL, triglyceride and cholesterol levels. *In vitro* studies have also indicated an anti–aggregative effect of cordycepin [41,42]. Polysaccharides isolated from *Hericium erinaceus* cultures may be potential anti–ulcer agents due to their ability to stimulate anti–inflammatory cytokines and inhibit the release of pro–inflammatory cytokines. Recent studies also indicate their possible action against *Helicobacter pylori* [44]. In addition, polysaccharides induce a hepatoprotective effect related to their antioxidant activity and the reduction of triglycerides, cholesterol, and low– and medium–density lipoproteins [44]. Hypolipemic properties are also possessed by such compounds as eritadenine, which is a nucleotide derivative extracted from *Lenlinula edodes*, or ganodermic acids isolated from the mycelium of *Ganoderma lucidum* [45,46]. In turn, the triterpenoids of *Antrodia cinnamomea*, show hepatoprotective, hypotensive, and antidiabetic effects [31].

Mushrooms produce many secondary metabolites that are involved in important biological processes, such as transcription. A significant number of them function as drugs, for example as antibiotics or immunosuppressants. For instance, the species *Penicillum chrysogenum* was used to discover the first antibiotic – *penicillin*, which was a breakthrough in the treatment of bacterial infections. Cyclosporin A extracted from *Tolypocladium inflatum* found use in transplantology as an immunosuppressive agent to protect against a transplant rejection by the recipient's body [47]. In turn, lovastatin isolated from *Aspergillus terreus* cultures, has become a key drug used in hyperlipidemia that inhibits HMG–CoA reductase, an enzyme involved in cholesterol synthesis [47,48]. Other examples include ergotamine produced by the genus *Claviceps* used as an anti–migraine drug, as well as gibberellins – growth hormone–like compounds produced by Fusarium fujikuroi [47,48].

Table 2. Overview of compounds isolated from mycelial cultures and their biological activities

Biological activity	Chemical compounds	Species of mushrooms
Antineoplastic	β–glucan	*Hericium erinaceus*
	coriolin	*Coriolus consors*
	calvatic acid	*Calvatia* sp.
	cordycepin	*Cordyceps* sp.
Cytostatic	ganoderic acid	*Ganoderma lucidum*
	nematolin	*Panus* sp.
	ergosterol derivatives	*Trametes versicolor*
	p–terphenyl pigments	*Hypoxylon rickii*
Fungistatic	mucidin	*Oudemansiella mucida*
	lenthionine	*Lentinula edodes*
	pleuromutilin	*Clitopilus* sp.
	strobilurins	*Strobilurus tenacellus*
	oudemansin B	*Xerula pudens*
Antibacterial	pleurotine	*Pleurotus griseus*
	lentinic acid	*Lentinellus ursinus*
	striatins	*Cyathus striatus*
	ganomycin	*Ganoderma lucidum*
	polysaccharides	*Sarcodon imbricatus*

Antiviral	**ergosterol peroxide**	***Inonotus hispidus***
	lucidenolactone, lucidenic acid, ganodermanontriol, ganoderiol F	*Ganoderma lucidum*
	polysaccharide–peptide complex	*Trametes versicolor*
	polysaccharides	*Sarcodon imbricatus*
	β–glucan	*Inonotus obliquus*
Antioxidant	polyphenols	*Inonotus xeranticus* *Phellinus linteus* *Anthrodia camphorata*
	polysaccharides, cordycepin, mannitol	*Cordyceps sinensis*
	ergothioneine	*Lentinula edodes*
Immunostimulant	cordycepin, polysaccharide	*Cordyceps militaris*
Anti–inflammatory	cordycepin, ergosterol	*Cordyceps militaris*
Neuroprotective	erinacine	*Hericium erinaceus*
Hypoglycemic	polysaccharides, cordycepin	*Cordyceps militaris*
Hypolipemic	cordycepin	*Cordyceps militaris*
	polysaccharides	*Hericium erinaceus*
	eritadenine	*Lentinula edodes*
	ganoderic acid	*Ganoderma lucidum*
Anti–ulcer	polysaccharides	*Hericium erinaceus*
Hepatoprotective	polysaccharides	*Hericium erinaceus*

Metabolic processes and the accumulation of medicinal compounds

Metabolism, which is defined as the totality of chemical reactions and related energy transformations taking place in the cells of organisms – is the foundation of all biological processes. It can be divided into two types: primary metabolism and secondary metabolism. Primary metabolism, as a universal one that includes all species, concerns substances that allow to carry out the vital functions: carbohydrates, fats, proteins, nucleic acids – compounds that make cell division, growth, respiration, and reproduction possible. On the other hand, the consequence of secondary metabolism is the formation of substances that enable the survival under harsh environmental conditions such as increased UV radiation [48]. The production of secondary metabolites is under the control of the relevant genes, which form aggregations – clusters (groups of genes). Clusters contain a transcription factor whose activity is specific to particular set of genes. It has been confirmed that this activity can also affect genes located elsewhere in the genome. An example is the transcription factor aflR, which is not only responsible for regulating genes related to aflatoxin production in *Aspergillus flavus* and *Aspergillus parasiticus* as well as sterigmatocystine in *Aspergillus nidulans*, but it also additionally affects 3 other genes located elsewhere. The production of secondary metabolites is also influenced by global transcription factors encoded by genes that are not associated with gene clusters [49].

Research on mycelial cultures in the Department of Pharmaceutical Botany at the Jagiellonian University Collegium Medicum in Kraków

In the 1970s, an innovative direction of research on mycelial cultures of mushrooms belonging to the taxa Basidiomycota and Ascomycota was initiated in the Department of Pharmaceutical Botany at Jagiellonian University Collegium Medicum in Cracow.

At that time, research was mainly focused on the isolation and analysis of polysaccharides produced by mycelial cultures of species such as

Aleuria aurantia (Ascomycota), *Saccharomyces cerevisiae* (Ascomycota), *Trametes hirsuta* [50,51]. In addition, a mycelial culture of *Tylopilus felleus* species was established, which became a source of a glucan with similar, but not the same structure and activity as tylopilan – a glucan isolated from the fruiting bodies of this species [52].

The next compounds analyzed in mycelial cultures were lectins – metabolites with probable anticancer activity. The research focused not only on trying to isolate lectins from biomass, but also on analyzing their biological properties. The occurrence of lectins in mycelial cultures of *Laetiporus sulphureus* and the effect of culture conditions on the degree of their accumulation were proven. Moreover, it was confirmed that lectins are deposited in the culture medium [53,54].

In the 1980s, there was begun research on the content of non–hallucinogenic indole–derived compounds in the fruiting bodies and mycelial cultures of native Basidiomycota and Ascomycota species [55]. These studies were with the aim of identifying pharmacologically heterogeneous compounds that can affect the toxicity of edible species. In biomass from mycelial cultures of *Lepista nuda*, 3–indoleacetic acid and a small amount of L–tryptophan were determined. Both compounds were also present in fruiting bodies where tryptophan derivatives kynurenine and 3–hydroxykynurenine were additionally determined [55]. *In vitro* culture studies on *Calocera viscosa* species compared the content of indole compounds in biomass obtained from different types of mycelial cultures. In cultures that were carried out on liquid medium, L–tryptophan, 5–hydroxytryptophan and scatole were detected, while scatole was identified in cultures from solid medium. With chemical analysis of the ether extract, β–carotene and its oxidation product 5,6–epoxide were identified in both fruiting bodies and *in vitro* cultures [56]. In mycelium from *in vitro* cultures of *Aleuria aurantia* (Ascomycota) there were obtained i.a. L–tryptophan and 3–indoloacetonitrile. These substances were also contained in the comparatively tested fruiting bodies [55].

In further research, numerous compounds having the chemical structure of indole were found in mycelial cultures of the edible species *Tricholoma equestre* and *Xerocomus badius*. In the mycelium of *Tricholoma equestre*, compounds with an indole structure were isolated: L–tryptophan, 5–hydroxytryptophan, serotonin and melatonin; of which L–tryptophan was quantitatively the most abundant [57]. Products derived from the degradation of tryptophan – kynurenic acid, kynurenine sulfate and tryptamine – were found in the mycelium of *Imleria badia* [58].

Recently, the object of the study constituted species from the genus *Pleurotus sp.* and *Cordyceps militaris, Fomitopsis betulina, Hypsizygus marmoreus* in which the presence of substances such as cordycepin, ergothioneine, lovastatin, indole compounds sterols, terpenes and55 phenolic compounds were detected [59–62].

Conclusions

Mushroom fruiting bodies have been used as food and a valuable medicinal resource for centuries. Mycelial cultures may be a promising alternative for extracting substances with approved medicinal effects. Submerged mycelial cultures are a rapid process that yields a high–quality mycelium, against crops that take much longer to produce fruiting bodies. The presence of bioactive compounds in the mycelium makes it an attractive compound that is now being used as a dietary supplement or nutraceutical.

Resumo

Micelia kulturo estas kompilo de teknikoj uzitaj por konservi aŭ kreskigi micelion sub sterilaj kondiĉoj sur medio de konata kunmetaĵo. Ĉi tiu metodo estas ofte uzata en funga bioteknologio. Ĉi tiu reviziartikolo priskribas la metodaron de bioteknolo-

giaj procezoj uzantaj kulturojn de makrofungoj, ekz. inicialigo de micelaj kulturoj, specoj de amaskomunikilaro uzitaj en in vitro kulturoj de pli altaj fungoj, specoj de micelaj kulturoj, kaj kulturkondiĉoj. Krome, ĉi tiu artikolo priskribas elektitajn biologie aktivajn komponaĵojn produktitajn per micelio akirita en in vitro. Krome, la esplorado pri micelaj kulturoj farita ĉe Katedro pri Farmacia Botaniko ĉe la Jagelona Universitato Collegium Medicum en Krakovo estas prezentita el historia perspektivo.

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POSSIBILITIES OF USING CRANIOSACRAL OSTEOPATHY AFTER HEAD INJURIES IN KICKBOXING PRACTITIONERS

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Abstract

Osteopathic medicine constitutes a new branch of medicine, dealing with a man as indivisible organism. The human body is treated as a whole and has a mechanism of self-regulation and also the structures and functions that are highly dependent on each other, creating a balanced mechanism for mutual support. Described in the article craniosacral approach used in neurotherapy is especially important, mainly because of the small number of contraindications and ability to use even in the case of patient in coma. The paper presents the research and selected examples of techniques and osteopathic therapy, so that this medicinal form was brought closer to the reader. It was also emphasized that only an experienced osteopath, constantly supplementing related to this area knowledge, is able to efficiently and correctly follow the form of treatment. Kickboxing is associated with frequent and repeated micro-injuries of the head, which give a variety of ailments in the treatment of which cranio-sacral therapy turns out to be effective.

Keywords: osteopathy, craniosacral technique, head injury, kickboxing

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Introduction

The increase in global interest and the dynamic development of martial arts increase the number of kickboxing practitioners, as well as related injuries [1]. Important and relatively underestimated are head injuries area serious health problem, especially since they tend to increase. Mostly it is said about injuries to the sideburns, knees and muscle syndromes. And rarely about head injuries. In kickboxing, and in particular competitive kicking, the head is exposed to strong blows and kicks that cause micro-brain injuries. The accumulation of minor and major head injuries becomes disastrous. Compared to other martial arts in kickboxing, among both amateurs and professionals, the head is particularly vulnerable. P. Anderson in his publication estimates that every year between 54 and 60 million people in the world suffer head injuries[2]. The most common causes of these injuries are: traffic accidents, falls from heights, beatings or the effects of loss of consciousness. Unfortunately, many victims are under the influence of alcohol or drugs at the time of the accident [3]. A similar head injury can be suffered as a result of a blow and kick in the head. Trauma depends on many factors, but affects all stages and ages [4]. In this situation, a beneficial factor is the developing medicine, enabling an increasingly higher level of patient care and giving a chance to survive and improve its functions.

Osteopathic medicine in the craniosacral approach can be an effective complement in the case of head injuries due to its holistic and individual approach to each patient, including the possibility of conducting therapy with people with very diverse ailments.

History and main assumptions of osteopathy based on manual therapy. Manual therapy in osteopathy includes manual treatment of disorders, in particular of the spinal joints, in which there was no clear damage to their morphological structure - that is, functional disorders, reversible. This therapy aims to restore the joints of the spine and peripheral joints to the anatomical position, that is, the right one, in order to eliminate pain by removing pressure on the nerves. The methods used in manual therapy derive from osteopathy and chiropractic (this is scientifically and medically documented chiropractic), which can be integrated with massage and corrective exercises.

Osteopathic Medicine was born at the end of the twenty-first century in the United States. The creator of this method is considered to be Andrew Taylor Still, who was a surgeon by education, but as a result of disappointment with classical medicine, he decided to create a new branch of it, which was to be closer to its roots, i.e. put man and the laws of nature in the foreground [5,6,7].

For Still, the musculoskeletal system, and above all the spine, played a major role. He noted that many diseases and health dysfunctions are accompanied by a limitation of spinal movement.

His merit for medicine is the creation of 4 basic principles on which Osteopathy is based to this day:

1. the human body is a whole,
2. the human body has self-regulation mechanisms,
3. the structure and functions in the human body are interdependent,
4. rational treatment is based on the above principles.

In osteopathy, we can distinguish several main therapeutic approaches:

1. craniosacralne,
2. wisceral,
3. fascial,
4. muscle energy,
5. structural (manipulative).

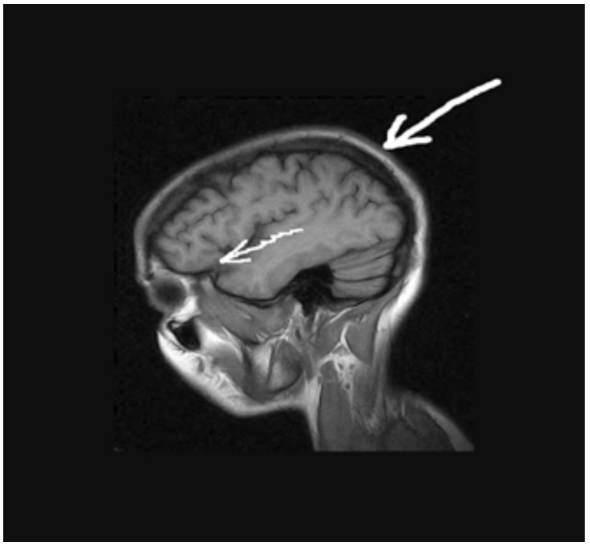
Osteopathy is often placed within the fields of alternative treatment, such as bioenergy the-

rapy, homeopathy, acupuncture, reflexotherapy. This is due to the fact that many people involved in alternative medicine use osteopathic techniques incorrectly. There are many institutions offering courses of selected techniques for masseurs, psychologists, often also laymen who, in order to increase the attractiveness of the courses offered, add the adjective "osteopathic" to the name of their services.

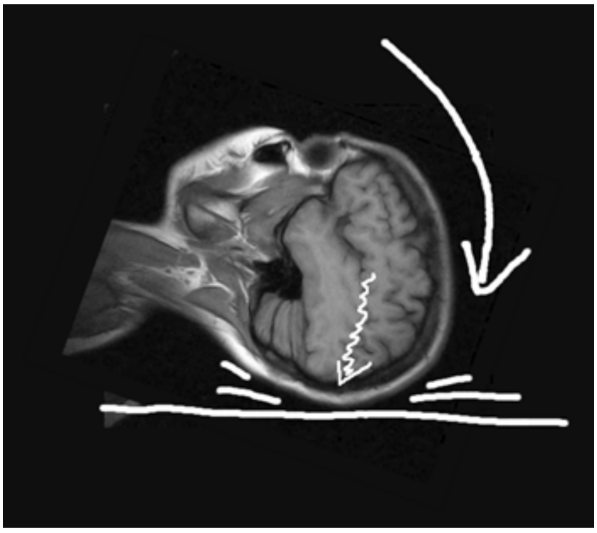
It should be emphasized, however, that almost all manual therapy techniques used in physiotherapy are based on osteopathic concepts, usually being their significant simplification, but they are most often found within academic medicine.

Application and role of osteopathy in the treatment of patients after head injuries: It should be realized that in the case of patients after craniocerebral injuries, osteopathy plays a complementary role for other therapies such as: physiotherapy, occupational therapy, speech therapy. Even a small head contributes to the development of multiple structural, functional and neuropsychological disorders. This event causes a particular variety of symptoms regarding motor impairment, as well as disorders related to memory, concentration, sleep, headaches, noise and many others, which within the framework of traditional physiotherapy can not always be regulated.

Serious craniocerebral injuries are the most common cause of death in the group of traumatic patients. These injuries most often occur as a result of direct brain damage with skull bone fragments and rapidly acting acceleration or delay forces (Fig. 1,2), which damage the nervous tissue both at the site of the injury and on the opposite side, the so-called contrecoup phenomenon (Fig. 3), which is usually caused by the result of shock wave displacement in conjunction with inertia. The brain that arises in this case occurs as a result of the action of various accelerations:



acceleration injury



deceleration injury

Fig.1. Linear accelerations (Own elaboration)

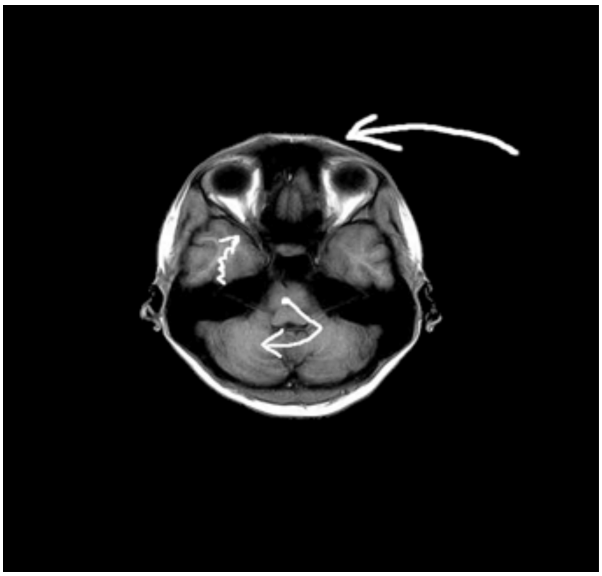


Fig. 2. Rotational acceleration (Own elaboration)

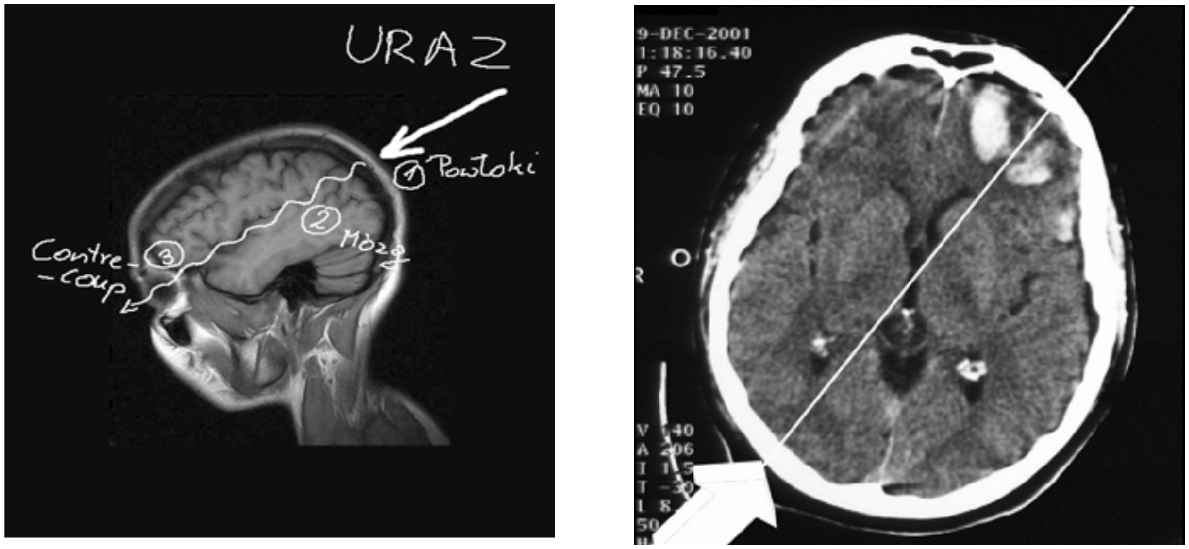


Fig. 3. Countrecoup effect (Own elaboration)

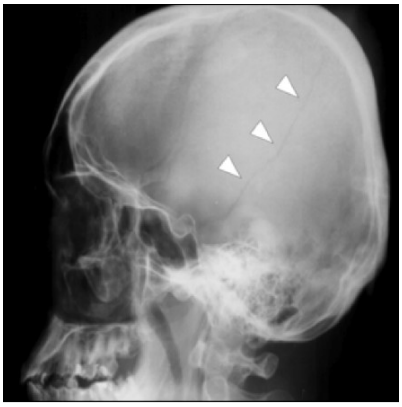


Fig.4. Fracture of the skull bone (Own elaboration)

Table 1. Examples of the use of craniosacral therapy

Examples of the use of craniosacral therapy	
General medical	Myofascial pain syndrome Increasing chronic systemic failure Palliative neoplasm treatment phase
Psychological	Anxiety, depression Disorders, post-traumatic stress disorder Anxiety syndromes Anxiety accompanied by mitral valve prolapse
Development	Growth delays Learning difficulties Hyperactivity deficit disorder (ADHD) Infantile colic
Other	Headache Temporomandibular joint dysfunction Whiplash injury to the neck Hemiparesis after a stroke Hemiparalysis after brain injury Allergic rhinitis Chronic otitis media Direct cranial trauma without fracture

Source: Own elaboration based on Leon Chitow, Cranial manipulations on bone and soft tissues. Theory and practice Leon Chitow, DB Publisching 2010

Given these effects, it should be noted that for the treatment of patients after brain injuries s a craniosacral approach is particularly useful. This therapy has a very small number of sermon counters, and at the same time it does not require logical accounts with the patient, which is why it is often used in infants with developmental disorders [8]. Therefore, it is also suitable for use in patients with mild brain damage and even during coma and other periods. during which contact is difficult. This therapy is also used in many other dysfunctions (Tab 1).

It should be remembered that craniosacral therapy is an element of osteopathic medicine and is based on 4 principles developed by Still, therefore the above list cannot be treated as a list of indications for therapy. The primary indication is Cranial Rhytmic Impulse (RIC). The professional approach of the therapist is also based on the knowledge of contraindications to the techniques performed [9].

Contraindications (medically or structurally unstable conditions):

1. Developing stroke,
2. Suspicion of subarachnoid hematoma,
3. Suspicion of acute cranial or cervical fracture,
4. Suspicion of cancer not yet diagnosed or in the process of diagnosis,
5. The possibility of metastasis, if treatment is still sought,
6. Acute encephalopathy or meningitis,
7. Prolapse of the intervertebral disc,
8. Dizziness, loss of consciousness, visual disturbance with neck rotation/side flexion,
9. Local infection, dermatitis or abscess,
10. Untreated fracturede,

A thorough interview and examination before starting therapy allows to determine whether a given person has contraindications, but the patient should also be asked if his health condition has changed.

Very good results are brought by the use of craniosacral therapy according to J. Upledger, derived - like most manual techniques - from osteopathy. In the 30s of the twentieth century, William Garden Sutherland put forward the thesis that the bones of the skull retain mobility. These are minimal movements that take place in the cranial sutures. He conducted many experiments using a specially constructed helmet that compressed and immobilized specific bones of the skull. In this way, depending on the type of brain structures located under the immobilized bone, he managed to cause headaches, dysfunctions of the organs of vision, hearing and balance, and emotional changes. On this basis, he developed a system of subtle hand positions and therapeutic grips, improving the movements of individual skull bones, thus removing the aforementioned symptoms. Scientific research conducted in the 70s of the twentieth century confirmed Sutherland's theories. One of the continuators was the American osteopath J. Upledger, who devoted himself to working with children with developmental defects and cerebral palsy, achieving excellent results.

The cranio-sacral system consists of a set of dura meninges, inside which the brain and spinal cord are located. The whole structure is filled with cerebrospinal fluid, whose natural production rhythm runs at a frequency of 6 to 10 cycles per minute. This mechanism is innate and completely independent of the will and consciousness of man. Any disturbance of this mechanism can cause disease, as it affects the nervous system and the homeostasis system in the body. Craniosacral therapy releases tensions in both the central and peripheral nervous systems. A study related to this approach was conducted in a large psychiatric hospital, as a result of which its effect on brain activity was determined. The author reports that it showed a specific change in the amplitude of alpha and theta brain waves in the back of the brain. It has been suggested that this has a positive effect on sleep disorders, general fatigue, susceptibility to drug addiction, and stress tolerance by "silencing" the

brain [10,11]. The craniosacral approach is also indicated for people overloaded with mental work, having a feeling of a heavy head, helps with depressive states and neuroses. It has a beneficial effect on strengthening the immune system, protects against infections and allergies. An interesting study was also conducted by D. Lopez, which analyzed the impact of osteopathic therapy, which placed particular emphasis on the craniosacral approach and its impact on the structures of balance control and postural stability in older people. The publication presents evidence of improved patient balance after therapy [12].

Osteopathy is based on thebody's self-healing abilities, therefore the therapist's goal is to stimulate these slingshots. Craniosacral therapy does not cure stroke, its task will be to eliminate dysfunctions that slow down self-healing processes or completely stop them.

Academic medicine tries to determine the tissue, joint, structure responsiblefor the patient's complaints. In the case of, for example, knee pain, the patient will be performed ultrasound which will show changes in the patellofemoral joint, leczewill not rely on the administration of cartilage rebuilding preparations, physiotherapeutic procedures toimprove tissue trophic and strengthen or stretch forspecific muscle groups. The whole therapy will be focused around the knee joint and its immediate surroundings.

Osteopathy is based on a holistic approach and probably the osteopath in the case of the above-described patient will devote very little time in his therapy to the knee joint itself, treating cartilage disorders in the patellofemoral joint as an expression of overload, not a disease in itself.

To clarify this issue, two possible approaches to knee therapy in the osteopathic concept are presented. Thereare endless such approaches, but it should illustrate why in the case of osteopathic therapies we do not rely on indications based on damage or abnormalities in a given tissue, but instead we look for disorders and chains of connections that may be associated with a given problem .

Knee therapy: Example 1

During the examination, the osteopath finds impaired mobility within the lower costal arches, impaired mobility of the stomach, reduced rotation in the thoracolumbar passage, and increasedheel in the iliopsoas muscle. Impaired gastric mobility introduces diaphragm dysfunction, which secondarily leads to blockage in the thoracolumbar passage of the spine (TH-L), and secondary tension of the iliac-lumbar muscle, which in turn causes the femur to be positioned in internal rotation, which changes the path of movement of the patella, leading to overload changes. Alignment of the entire chain, consisting in visceral manipulations of the stomach, manipulation of the thoracolumbar transition, relaxation of the iliopsoas muscle, leading to the correct positioning of the hip bone, and thus, to the restoration of the natural path of the patella and the elimination of the barrier that disturbed the self-healing process.

Knee therapy: example 2

A year ago, the patient suffered a torsional injury of the ankle joint, as a result of which there was a blockagein the upper ankle joint and limitation of the movement of rotation of the tibia relative to the talus during the movement of the dorsal flexion, which consequently also changes the path of movement of the patella. Osteopathic treatment in this case will be aimed at restoring normal mobility of the talus bone, which disturbed the automatic rotation in the knee joint.

Bothhigher in the ultrasound image and the clinical picture will be identical and this will be referred to as degeneration in the Velcro-femoral joint, while from an osteopathic point of view there will betwo completely different cases treated in a different way.

Similarly, it is not possible to determine the

exact indications for specific techniques associated with a given cranial injury. Appropriate therapy will be developed on the basis of the studied RIC disorders.

Cranio-sacral therapy

The creator of craniosacral therapy is Still's student, Dr. William Garner Sutherland (1837-1954). Itis based on the hypothesisof the mechanistic forces that move the skull, including fluctuations in cerebrospinal fluid and RIC [10,11]. This concept was created on the basis of many years of research by W.G. Sutherland in the field of skull anatomy, clinical observations of cranial mobility in asymptomatic patients, abnormal cranial mobility in patients with various symptoms, as well as the study of the impact of pressure on individual parts of the skull using a specially constructed device [10,11]. The author based his concept on five key elements, which are:

1. Internal motility of the brain and spinal cord,
2. Fluctuations in cerebrospinal fluid,
3. Mobility of intracranial and spinal meninges,
4. Mobility of the skull bones,
5. Involuntary mobility of the sacrum between the iliac bones.

The above elements presuppose the existence of a Cranial Rhythmic Pulse, which is possible to palpate. In this system of therapy, RIC is the most controversial element of osteopathic medicine, in which osteopathy is very often treated on a par with bioenergy therapy or other alternative methodsof treatment. There are many studies denying its existence and the possibility of palpationa [13]. However, more and more objective studies provide about thestate of summer, which may confirm the existence of RIC and the possibility of influencing the structures of the skull through cranio-sacral techniques.

In 1999, a study using X-rays andmagnetic resonance frames showed changes of about 0.38 mm inside the skull during alternating sagittal and frontal dilation [14]. D.C. Kostopoulos and G. Keramidas in 1992 proved a change in the length of the sickle brain by 1.44 mm under the influence of craniosacral techniques . In addition, with the help of objective research tools, rhythmic movement within the skull was observed at a frequency of 6-9 rhythms per minute. In the light of objective scientific evidence, the existence of RIC is less and less controversial, but the question of what actually causes Rytmic Impuls Cremains unanswered [15]. Looking for answers, L. Chitow proposes several different models:

1. Internal mobility of the brain and nervous system,
2. Movement of cerebrospinal fluid ("pressure" hypothesis),
3. Muscle no and movement force,
4. Limfatyczna pump,
5. Venous mobility and/or change in vessel caliber,
6. Tissue pressure,
7. Oscylacja Grape Herring Meyer,
8. Dostrajaniand [9]

Particularly interesting and probably closest to the truth is the theory of fine-tuning, because it connects Trabue-Hering-Mayer waves to RIC. Itimplies that fine-tuning is the integration or harmonization of oscillations. J. M. McPartland and E. A. Mein identifies the way in which the different rhythms and pulsations that make up the RIC with the process observed in physics and nature, in which patterns and cycles tend to align over time [16].

Craniosacral therapy methodology

In craniosacral therapy, the therapist strives to equalize tensions within the skull of both bone structures, membranes and connections of individual bones, and to normalize RIC.

The therapist begins with palpation, in which he tries to identify the following elements:

1. stopof asymmetry,
2. impaired movement potential,
3. abnormal tissue structure,
4. sensitivity to light pressure,
5. RIC test,
6. careful testing of freedom of movement at the level of the seams and between specific bones,

Then he selects the appropriate technique for cranial therapy (Table 2).

Table 2. Types of cranial manipulations

Types of cranial manipulations	
Direct techniques	Technique performed against tissue resistance. The therapist tries to "stretch" the tissue/structure that is limited or dysfunctional. Cranial therapy uses "tilting" rather than movement, as is the case in classic joint manipulations
Indirect techniques	Technique performed in the direction of tissue resistance. The therapist tries to aggravate the existing dysfunction / limitation, then maintains the above-mentioned state expecting tissue relaxation.
Separation or withdrawal technique	The technique is most often used in the case of limitations within the seams. The therapist moves the limited joint apart with little force.

Source: Own elaboration based on: Cranial manipulations on bone and soft tissues. Theory and Practice" Leon Chitow", DB Publisching 2010

Thanks to the variety of techniques, osteopathy gives the opportunity to choose the right therapy tailored to a specific patient.

Examples of craniosacral therapy techniques

It is not possible to learn to perform these techniques just by reading. Osteopathic therapy is a holistic concept and is not based on performing a series of manual movements according to a strictly defined scheme. The therapeutic procedure is created for each patient individually and is based on a logical chain of dysfunctions resulting from anatomical or functional connections.

Mobilization of the frontal bone

When performing this form of therapy, the patient is in a lying position on his back, the therapist sits on the side of the patient's head, he puts his hands on the patient's frontal bone as illustrated in Figure 5 stofeel the RIC and any disorders.

Figure 5. Mobilization of the frontal bone (Own elaboration)

The osteopath, after finding dysfunction, performing very gentle pressure with a force of about 10 grams coming out of the elbow joints, carries out the so-called "spred"

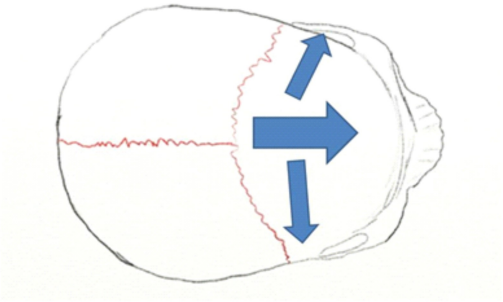


Figure 6. "spred" of the frontal bone

Then the therapist maintains the introduced tension to the momentu in which there will be complete relaxation and obtaining the so-called "liquid tension"2, after which he moves on to the second phase of this lifting technique (Fig. 7). The osteopath performs a pulling movement with a force of about 10 grams, again waiting for full relaxation of thefrontal bone. Pabout its achievement, ceases to introduce any forces and again b a da RIC, which should be normalized.

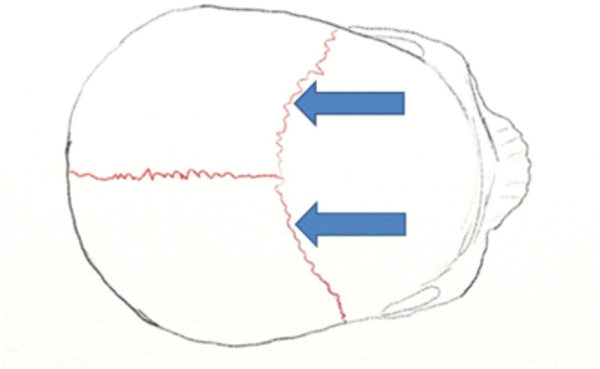


Figure 7. Lifting the frontal bone

If the RIC asymmetry is still felt, it may be due to a blockage at the level of one of the stitches. The technique of mobilization of the frontoparietal suture should then be performed (Fig. 8). The patient lies on his back with his head slightly rotated. The therapistplaces the second and third fingers of one hand on the frontal bone and the other on the parietal bone.

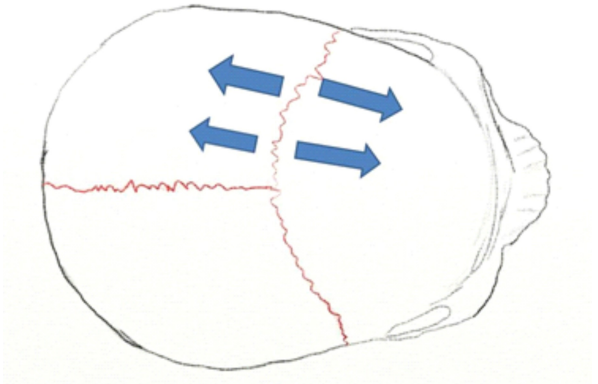


Figure 8. Mobilization of the frontoparietal suture (Own elaboration)

Then wpdistributes tension within the seam, also with a force of about 10g. trying to "stretch" it. It maintains tension until full relaxation occurs, after reaching it, it tests the RIC again.

Clinical case

The clinical case of the therapy shows that osteopathic medicine can be practically used in athletes who have been training kicboxing for years. In clinical practice, structural, visceral and, above all, cranio-sacral therapy was used, which turned out to be therapeutically important. The treated patient, 49 years old, is a long-time practitioner and kickboxing competitor. The main symptoms that did not disappear after various forms of therapy were: headaches combined with a feeling of heaviness and various neuropsychological disorders.

The rehabilitation programme was based on the use of osteopathic elements. Sessions were conducted individually and lasted appro-

ximately 30 minutes. As part of the treatment, visceral, craniosacral and structural techniques were used.

The therapy included the following treatments:

1. Strain/counterstrain technique and reflex point treatment, also known as ischaemic compression,
2. Normalisation of mesenteric root,
3. Barall's technique - 3D liver,
4. Technique for diaphragm normalisation (domes and cruses),
5. Craniosacral technique balancing dura maters,
6. AO technique for occiput balancing dura maters and vagus nerve.

During osteopathic therapy, such ailments as stomach ache or nausea occurred. Both during the procedure and post-operatively, reactions were sporadic. The duration of the procedure was, on average, below 30 minutes, in which it also always contained interview elements to ensure safety and effectiveness of therapy. Hand position, pressure and amplitude were selected on an on-going basis. All of these treatment parameters were individually adjusted to the patient and were always done in the facilitated direction. All presented techniques were performed during 1 session. Sessions were performed regularly 2-3 times a week for 4 weeks.

After the therapy containing osteopathic techniques, in particular sacral-cranio, it brought effects in the form of a significant reduction in headache symptoms and neuropsychological ailments.

Resume

Cranio-sacral therapy arose from clinical experience, which shows that health is something active and dynamic, and not just the absence of diseases. This form of therapy, as well as other types of manual treatment, maintain a favorable state of health and the mechanisms that regulate it. It also helps to increase physical vitality and well-being not only through its impact on structural changes, but also through broader emotional and spiritual implications. Training and the impact of kickboxing has been controversial for years. Compared to various sports, it turns out that kickboxing is not such an injured sport. Compared to breaststroke swimming, there are fewer lumbar pain syndromes in people who train kickboxing [17]. Kickboxing practitioners are exposed to head injuries. These injuries give various complications and especially in those who have gone through them many times. There are patients who have the greatest disturbance of the homeostasis of the nervous system from the point of view ofcraniosacral osteopathy. Working with this type of patient is a challenge for thetherapist, for whom the craniosacral approach is a good therapeutic tool. Significantly complementary to other neurotherapy methods [18]. It can also be a tool for treatingailments that other methods have not always been able to cope with. The most exposed to chronic ailments requiring treatment are those who have suffered loss of consciousness. Neurotherapy in various forms is now widely used, and the use of biofeedback in sport and the treatment of sports injuries is becoming increasingly popular [19]. Martial arts elements are an attractive element of therapy, which is why tai-chi used in health care units is becoming more and more popular [20]. Looking for an effective method of treating various ailments, cranio-sacral therapy is part of the increasingly widely used Osteopathic Medicine, which already works as a method of work and can be combined with other forms of therapy [21]. Preventing the effects of head injuries requires education and awareness, as well as appropriate cooperation between athletes, coaches and medical professionals.

Resumo

Osteopatia medicino konsistigas novan branĉon de medicino, traktante homon kiel nedivideblan orga-

nismon. La homa korpo estas traktata kiel tutaĵo kaj havas mekanismon de memreguligo kaj ankaŭ la strukturojn kaj funkciojn, kiuj estas tre dependaj unu de la alia, kreante ekvilibran mekanismon por reciproka subteno. Priskribita en la artikolo kraniosakra aliro uzata en neŭroterapio estas speciale grava, ĉefe pro la malgranda nombro da kontraŭindikoj kaj la ebleco uzi ĝin eĉ en kazo de paciento en komato. La artikolo prezentas la esploradon kaj elektitajn ekzemplojn de teknikoj kaj osteopatian terapion, por ke ĉi tiu kuraca formo alproksimiĝu al la leganto. Oni ankaŭ emfazis, ke nur sperta osteopato, konstante kompletiganta scion rilatan al ĉi tiu areo , kapablas efike kaj ĝuste sekvi la formon de kuracado. Kickboxing estas asociita kun oftaj kaj ripetaj mikro-vundoj de la kapo, kiuj donas diversajn malsanojn en kies traktado kranio-sakra terapio montras sin efika.

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INTERRELATIONS OF BODY COMPOSITION AND QUALITY OF LIFE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Abstract

Background: Interrelations of body composition and quality of life in chronic obstructive pulmonary disease (COPD) are important, since low body mass index (BMI) and muscle atrophy are basic factors of low exercise capacity and have reliable predictive value of the progressing disease. The aim of our study was to assess how body composition affects the quality of life, exercise capacity and respiratory function of COPD patients

Methods: We performed body composition measurements on 120 COPD patients of the National Koranyi Institute for Pulmonology in Budapest between February 1, 2019 and February 1, 2020, using OMRON Healthcare BF511 body composition analyser. The disease-specific COPD Assessment Test (CAT) questionnaire measured the quality of life; respiratory function and anthropometric data were extracted from the electronic health record system.

Results: Underweight patients (10.8%) were less work loading (6MWD (m) 250 vs. 320; p=0.098) and had significantly lower quality of life (CAT: 32 (29-36) vs. 28 (23.5-30) vs. 24 (16-30); p=0.004), than normal or overweight patients. Those with higher body fat percentage (women: 36-42%, men: 25-30%) had better lung function (FEV1) and significantly better quality of life (CAT). Muscle percentage correlated also significantly with 6-minute walking distance (6MWD: p=0.514; p <0.001) and quality of life (CAT: p=-0.344; p <0.001).

Conclusions: Our results suggest that measurement and assessment of body composition is useful in managing COPD patients and should routinely be performed during therapy, thus we propose to add body composition assessment to the COPD severity assessment (BODE index).

Keywords: chronic obstructive pulmonary disease (COPD); body composition; quality of life; COPD Assessment Test (CAT); BODE index

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Introduction

COPD (Chronic Obstructive Pulmonary Disease) is insidious and irreversible that progresses and worsens at different rates depending on specific circumstances [1]. There are many factors affecting the quality of life and life expectancy of COPD patients, such as breathing capacity, frequency of acute exacerbations, timely and effective treatment, good lifestyle habits such as regular exercise, adequate diet quality and quantity, smoking cessation and quality of air [2].

Body composition, dietetics, and adequate quantity and quality of nutrition are also becoming increasingly prominent of COPD patients' quality of life and treatment, since inadequate body composition is leading to a decrease in muscle volume and increases the fat content, with adverse consequences in pathological cases as sarcopenia or sarcopenic obesity, resulting in malaise, frailty and poorer life outcomes [3].

Previous studies highlighted that early detection and initiation of nutritional therapy may improve significantly the respiratory function, exercise tolerance and quality of life, as well as reducing morbidity and mortality of COPD patients [4-6]. Actually, body composition analysis is not routinely performed in COPD management, although it should be, because restricted diet, low physical activity and the production of inflammatory cytokines change metabolism to catabolism with increasing incidence of infections, development of nutritional abnormalities thus reducing the quality of life [7]. The aim of our study was to assess how body composition affects the quality of life of COPD patients.

Materials and methods

Study Design and Population

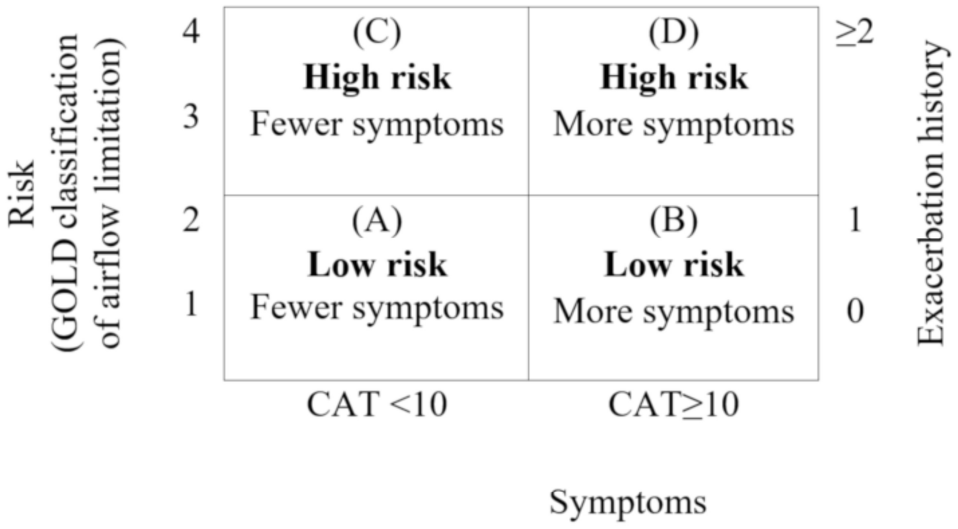
Our cross-sectional study included 120 COPD patients in the National Koranyi Institute of Pulmonology. The study approved the local TUKEB Ethical Committee Licence Number: TUKEB 44402-2 / 2018 / EKU - approval date 28 August 2018 - and complied with the Helsinki Declaration. Inclusion criteria were: age ≥40 years and diagnosed with COPD (post-bronchodilation of FEV1/FVC <70%) [8]. Exclusion criteria: implanted pacemaker, pregnancy, severe dehydration or oedema, further chronic diseases (e.g. cancer, endocrine system) with major impact on outcomes of instrumental measurements of nutritional status.

Patient and public involvement

Inclusion of patients was based on the institution's electronic database. All patients learned oral and written medical information about the study and signed a statement of consent. Participation was voluntary. Patients and the public were not directly involved in the design of the research or the objectives of the study. However, at patient group meetings, the results of the research and the views expressed by the patients were also made public to the working group and the patients themselves.

Examination of respiratory function

All patients underwent a baseline respiratory function test by automated computerized spirometer for assessing respiratory function. Dynamic lung volumes were defined as the amount of air expelled in the first second [(FEV1 (ref%)], vital capacity [(FVC (ref%)], the degree of airway obstruction (FEV1/FVC), inspiratory capacity in litres and percent [(IVC (L), IVC (ref%)], with GLI-defined (Global Lung Function Initiative) normal spirometry (z-score) [8]. Patients were classified in GOLD A-D stages according to the current and future risk parameters, as spirometric values, relevant symptoms and exacerbation rate (Figure 1) [8].



GOLD=Global Initiative for Chronic Obstructive Lung Disease; CAT=COPD Assessment Test; mMRC= The modified Medical Research Council scale;

Figure 1: GOLD severity stages of chronic obstructive pulmonary disease

Quality of life examination

We used the COPD Assessment Test (CAT) as a complex questionnaire to measure the quality of life [9]. Our patients responded eight questions, scoring the symptoms from 0 (healthy condition) to 5 (severe symptoms). Cough, the amount of sputum, hyperinflation, the load-bearing capacity when climbing stairs, and the level of energy were evaluated subjectively, as well as whether the patients dare to leave home, or whether their illness affected their sleeping habits. In addition, the patients also completed our self-developed questionnaire asking about their smoking and dietary habits. Questionnaires were completed under the coordinators' supervision in the Koranyi facility.

Definition of COPD exacerbation

COPD exacerbation was defined as a significant change of the patient's initial symptoms (dyspnea, cough and sputum production), which is an acute event at a level exceeding the daily variability of symptoms, leading to a change in therapy [8].

The 6-minute walk test (6MWT)

During the 6-minute walk test (6MWD), patients were asked to walk down the aisle for 6 minutes and the maximum walking distance was recorded. 6MWT measures the distance the patient can walk quickly on a hard, flat surface in 6 minutes. This distance value is the result of the test [10].

Body weight, body fat percentage, muscle percentage and body mass index.

While measuring body weight, body fat percentage and muscle percentage we used the OMRON Healthcare BF511 Body Composition Analyser and calculated body mass index. Measuring criteria were empty stomach in the morning, emptied urine bladder and defecation, being in underwear, barefoot and without any metal jewellery. BMI was calculated by dividing body weight by body height squared (kg/m2). Based on body mass index (BMI) values, participants were divided into four groups: <18.5 (underweight), 18.5-24.9 (normal weight), 25-29.9 (overweight) and ≥30.0 kg/m2 (obese) [11].

Body fat percentage is also an important indicator of obesity, as the fat expressed as percentage of body weight, and was used to classify participants into lean (female <24%, male <13%), healthy (female 24-35%, male 13-24%), overweight (female 36-42%, male 25-30%), and obese (female ≥42%, male ≥30%) groups (according to recommendations for those over 60 years) [12].

Blood tests

We conducted fasting blood tests in the central laboratory of the National Koranyi Institute of Pulmonology and measured the serum CRP with high sensitivity (hs) immunoassay method and the lipid profile (total cholesterine, triglyceride, LDL and HDL) standard method. Patients were in clinically stable condition, without fever and respiratory infection throughout the measurements.

Statistical analysis

All statistical analyses were conducted with STATA SE-21.0. Since most of the continuous data did not follow the normal distribution - verified by Sapphiro-Wilk test -, we used non-parametric statistical methods. Continuous variables were represented by medians and interquartile ranges, categorical data were presented with case numbers and proportions. Mann-Whitney tests detected the differences of continuous variables between two groups; in case of more than two groups Kruskal-Wallis ANOVA tests were conducted. Frequency differences of categorical variables were examined by Fisher's exact test. Spearman's correlation tests were used to indicate the relationship between continuous variables. All statistical tests were performed at 95% confidence intervals with significance level $p < 0.05$.

Results

All COPD patients (n=120) were subjected to body composition analysis. Patients' characteristics in median and interquartile range: age: 65 (62-72) years; FEV1 (ref%): 42 (31-53); BMI: 25 (20-31) kg/m², men BMI: 24.7 (20-29), women BMI: 26.8 (21-32). Almost all patients (95%) were smokers, with an average number of 40 years of smoking and an average of 20 cigarettes smoked a day. Malnourished patients had poorer lung function FEV1 (ref%): 29 (27-34) than normal weight FEV1 (ref%): 45 (35-52) or overweight FEV1 (ref%): 42 (35-47), obese patients FEV1 (ref%): 45 (30-56). More than half of patients (52%) reported a weight loss in the last 12 months, with an average of 2.9 kg. Anthropometric characteristics of patients grouped by BMI are detailed in Table 1.

Table 1. Characteristics of the COPD patients by BMI categories

BMI categories (kg/m²)	Underweight <18.5 (n=13)	Normal weight 18.5-25 (n=43)	Overweight 25-30 (n=27)	Obese ≥30.0 (n=37)	p-value
Age (years) (IQR)	61 (57-64)	65.5 (61.26-72)	66 (70-64)	69 (62-73)	0.089
Men (n, %)	8 (61.54)	22 (51.16)	16 (59.26)	14 (37.83)	0.287
Women (n.%)	5 (38.46)	21 (48.84)	11 (40.74)	23(62.16)	0.298
Muscle (%)	44 (33.4-49)	35 (27.7-39.4)	29.4 (27-32.5)	29.2 (25-32.7)	<0.001
Men (%)	46.5 (33-49)	35.4 (28.9-40)	30.7 (26.7-34.4)	32.7 (29.4-33.9)	0.004
Women (%)	40 (33.4-48)	33.3 (27.9-39)	29 (27.7-32)	25.4 (24-29.9)	<0.001
Body fat (%)	11.2 (9-15.9)	25.9 (17.8-34.4)	36 (30.8-41.3)	40 (35-46)	<0.001
Men (%)	10.1 (9-16.1)	18.6 (14.1-32.03)	33.7 (26-38.7)	34.9 (32.2-38.2)	<0.001
Women (%)	15.9 (8-15.9)	29.6 (21-36)	40 (36-42.2)	45.5 (40.7-48.8)	<0.001
AC (cm)	79 (77-81)	92.5 (85-100.5)	105 (90.5-109.75)	114 (102.2-	<0.001
Arm circumference (cm)	23.5 (23-24.5)	26.5 (25-29)	28.75 (24.5-33.9)	32 (29.25-34.12)	<0.001
Total cholesterol (mmol/l)	3.8 (3.8-4.8)	5 (4.32-6.08)	5 (4.25 (5.98)	5.1 (4.45-5.78)	0.064
Triglycerid (mmol/l)	0.8 (0.7-1.4)	1.3 (1-2)	1.5 (1.3-1.7)	1.4 (1-1.9)	0.314
FEV_1 (ref%)	29 (27-34)	45 (35-52)	42 (35.5-47.5)	45 (30.7-56.3)	0.486
FVC (%)	55 (48-62.7)	68 (58.5-81.5)	65.5 (59.25-78)	64.5 (55-76)	0.203
FEV_1/FVC (%)	44 (44-45.7)	49 (42.5-61)	50.5 (41.5-54)	54 (45.5-64.25)	0.392
GOLD stage					
GOLD A (n.%)	1 (7.69)	2 (4.65)	3 (11.11)	1 (2.70)	0.003
GOLD B (n.%)	1 (7.69)	11 (25.58)	3 (11.11)	14 (37.83)	<0.001
GOLD C (n.%)	3 (23.08)	25 (58.15)	17 (62.97)	13 (35.14)	<0.001
GOLD D (n.%)	7 (53.84)	5 (11.62)	4 (14.81)	8 (21.62)	<0.001
CAT (points)	32 (29-36)	28 (23.5-30)	26 (17-29)	24 (16-30)	0.004
6MWD (m)	250 (180-322)	320 (200-410)	320 (252-366)	307 (230-362)	0.098
Exacerbations (in, last 6 months)	1 (0.5-3)	0.5 (0-1)	0.5 (0-1)	1 (0-2)	0.020

Data are presented as median (IQR) or as frequency and percentage; AC: abdomen circumference; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; FEV1: forced expiratory volume in 1 s post-bronchodilator; FVC: forced vital capacity; BMI: body mass index; 6MWD: six-minute walking distance; HDL: high density cholesterol level; LDL: low-density cholesterol level; $p < 0.05$ means the two indicators were significantly correlated

Malnourished patients had a lower workload (6MWD (m) 250 vs. 320; p=0.098) and significantly poorer quality of life (CAT: 32 (29-36) vs. 28 (23.5-30) vs. 24 (16-30); p=0.004) than normal or overweight patients (see at Table 1). The median body fat percentage for men was 28.1 (17.9-35.9) and for women 36.8 (27.1-43.7). 21.7% of female patients had low body fat percentage, 15% had normal, and 63.3% high percentage. The same values of men were 18.4%, 23.3% and 58.3% respectively. The average muscle percentage of men was 33.5 (28.4-37.4) and of women 29.9 (25.1-33.4). Looking at the relationship between body fat percentage and lung function, patients with a higher body fat percentage (women: 36-42%, men: 25-30%) had better lung function results (FEV1), better quality of life (CAT) and fewer exacerbations (see Table 2). Muscle percentage correlated also significantly with the 6-minute walking distance (6MWD: ϱ=0.514; p <0.001) and with the quality of life (CAT: ϱ=-0.344; p <0.001).

Table 2. Characteristics of the COPD patients by body fat percentage categories

BMI categories (kg/m²)	Body fat percentage								p-value
	Lean (n, %)		Healthy (n, %)		Overweight (n, %)		Obese (n, %)		
	Men <13	Women <24	Men 13–24	Women 24–35	Men 25–30	Women 36–42	Men ≥ 30	Women ≥42	
Underweight (n=13)	5 (38.46)	5 (38.46)	2 (15.38)	0	1 (7.69)	0	0	0	<0.001
Normal (n=43)	6 (13.95)	8 (18.60)	7 (16.28)	5 (11.62)	2 (4.65)	8 (18.60)	7 (16.27)	0	<0.001
Overweight (n=27)	0	0	3 (11.11)	2 (7.40)	2 (7.40)	6 (22.22)	11 (40.74)	3 (11.11)	<0.001
Obese (n=37)	0	0	2 (5.40)	2 (5.40)	0	6 (16.21)	12 (32.43)	15 (40.54)	<0.001
CAT (points)	31 (28-36)	28 (27-31)	31 (26-34)	28 (23-29)	23 (5-27)	26 (23-32)	23 (18-26)	29 (23-31)	0.029
6MWD (m)	270 (200-278)	250 (200-325)	325 (285-409)	225 (212-345)	390 (125-390)	284 (220-364)	325 (293-371)	255 (132-307)	0.261
FEV_1 (ref%)	32.5 (27-36)	37 (33-49)	47 (41-53)	49 (30-58)	44 (26-44)	45 (39-60)	41 (30-52)	42 (31-53)	0.237
Exacerbation (n)	1 (0-2)	1 (0-1)	1 (0-1)	1 (0-1)	0 (0-1)	1 (0-1)	1 (0-1)	1 (0-2)	0.139

Data are presented as median (IQR) or as frequency and percentage; CAT: COPD Assessment Test; FEV1: forced expiratory volume in 1 s post-bronchodilator; BMI: body mass index; 6MWD: six-minute walking distance; p <0.05 means the two indicators were significantly correlated

Discussion

The aim of our study was to assess the nutritional status of COPD patients and to describe the interrelations of body composition and the quality of life. We found that underweight COPD patients had poorer lung function, poorer quality of life and lower exercise tolerance than normal/overweight patients and patients with higher muscle percentage had a better quality of life and higher exercise tolerance.

Researchers are increasingly highlighting that patients with COPD require a holistic approach from health care professionals, who need to consider not only basic medical parameters but also other indicators that affect overall well-being, i.e. identifying factors that potentially positively influence their quality of life and incorporate them into a comprehensive treatment programme [13-15]. One of these very important prognostic and modifiable factors is malnutrition, which is common in COPD (up to 40%) and is often associated with poor prognosis [16], poor quality of life, increased exacerbations, longer hospital stays and increased costs

of healthcare [17]. Timely screening and initiation of nutritional therapy can lead to significant improvements in respiratory function, workload tolerance and quality of life, as well as they reduce morbidity and mortality in COPD patients, and low BMI has been shown as an independent risk factor for COPD patients' mortality [18-20]. Our study also highlights that body composition is associated with lung function, exercise tolerance and patients' quality of life, which is in harmony with other previous studies [21, 22]. It is also important to mention that the relationship between body composition and the number of exacerbations is a two-way process, because exacerbation increases the level of inflammatory parameters in the blood e.g. tumour necrosis factor α (TNFα), IL-6, which can lead not only to endothelial dysfunction but also to muscle loss, muscle atrophy and other comorbidities in COPD e.g. diabetes, atherosclerosis, osteoporosis [21-23].

Muscle wasting and malnutrition are independent risk factors for COPD mortality. Research has shown that BMI is not sufficient for multidimensional assessment of COPD patients [21-23], i.e. to the BODE (body mass index, airflow obstruction, dyspnoea, exercise capacity) index, it is recommended to add an assessment of body composition. Body composition can be assessed using simple non-invasive bioelectrical impedance analysis (BIA) body composition analysers [24, 25], a well-estimated and still commonly used method is anthropometric measurement, categorised in 1981 by Bishop et al. in tables of percentile values of carcass volume and skinfold thickness, based on sex and age [26]. A third method is the use of imaging studies (DEXA, dual energy X-ray absorptiometry; CT, computed tomography; MRI, magnetic resonance imaging; ultrasound measurements) [27, 28], which are also proposed to assess the effectiveness of pulmonary rehabilitation [29]. With regular body composition assessment and effective intervention, disease progression can be reduced, abnormal body composition can be managed, which has a positive impact on morbidity, improves treatment efficacy, reduces exacerbations, length and cost of hospital stay, and improves quality of life [3, 21, 29].

Nutritional status plays a significant role in the progression of COPD and diet therapy should therefore be an essential part of treatment. Patients with a good nutritional status are more likely to maintain and improve their health status, which has an impact on their quality of life, while poor nutritional status reduces the chance of survival [30]. The detection of malnutrition and the establishment of an adequate nutritional status are very important for optimal therapeutic management [31, 32]. Weight loss in patients with COPD should be monitored every 6 to 12 months, as an appropriate diet and regular exercise can improve the quality of life of patients.

Half of our study participants (52%) experienced weight loss an average of 2.9 kg in the previous 12 months; this is consistent with previous studies found weight loss in 10-15% of patients with mild COPD and about half of the patients with severe COPD. Notably, severely malnourished participants experienced greater weight loss than those who were mildly malnourished [33].

Conclusions

COPD patients are particularly at risk of developing abnormal nutritional status due to the inflammatory nature of their disease and restricted diet, which can significantly affect their disease prognosis. Thus, attention should be paid not only to the consequences of excessive obesity (metabolic syndrome, hypertension, elevated blood sugar) but also to the consequences of malnutrition (energy and protein deficits). In therapy, the most effective and scientific way of preventing malnutrition is to monitor regularly the body composition.

Limitation of the study

Due to the cross-sectional nature of the study, it is not clear whether further weight loss continued in patients. Limitations of our study include the small number of cases, the cross-sectional study and the single-centre enrolment, therefore further studies and data analyses are needed to provide scientific evidence for the above findings and to disseminate them in widespread practice.

Conflict of interest: The authors have no conflicts of interest.

List of abbreviations
6MWT = six-minute walk test; BMI = body mass index; BODE = body mass index, airflow obstruction, dyspnea, exercise capacity; CAT = COPD Assessment Test; COPD = Chronic Obstructive Pulmonary Disease; CT = computed tomography; DEXA = dual energy X-ray absorptiometry; FEV1 = forced expiratory volume in the first second; FVC = forced vital capacity; GOLD = Global Initiative for Chronic Obstructive Lung Disease; IL-6 = interleukin 6; MRI = magnetic resonance imaging; TNFα = tumour necrosis factor α;

Resumo

Interrilatoj de korpa konsisto kaj vivokvalito en kronika obstrukca pulmomalsano (COPD) estas gravaj, ĉar malalta korpa masa indekso (BMI) kaj muskola atrofio estas bazaj faktoroj de malalta ekzerckapablo kaj havas fidindan prognozan valoron de la progresanta malsano. La celo de nia studo estis taksi kiel korpa konsisto influas la vivokvaliton, ekzercan kapablon kaj spiran funkcion de pacientoj kun COPD.

Ni faris korpan komponan mezuradojn ĉe 120 pacientoj kun COPD de la Nacia Koranyi-Instituto pri Pneŭmologio en Budapeŝto inter la 1-a de februaro 2019 kaj la 1-a de februaro 2020, uzante la analizilon de korpa komponado de OMRON Healthcare BF511. La enketilo pri Taksa Testo de COPD-specifa malsano (CAT) mezuris la kvaliton de vivo; spira funkcio kaj antropometriaj datumoj estis ĉerpitaj el la elektronika sanrekorda sistemo.

Malpezaj pacientoj (10.8%) estis malpli da laborŝarĝo (6MWD (m) 250 kontraŭ 320; p = 0.098) kaj havis signife pli malaltan vivokvaliton (CAT: 32 (29-36) kontraŭ 28 (23.5-30) vs 24 (16-30); p=0.004), ol normalaj aŭ tropezaj pacientoj. Tiuj kun pli alta korpa grasa procento (virinoj: 36-42%, viroj: 25-30%) havis pli bonan pulman funkcion (FEV1) kaj signife pli bonan vivokvaliton (CAT). Muskola procento korelaciis ankaŭ signife kun 6-minuta marŝdistanco (6MWD: ρ=0.514; p <0.001) kaj vivokvalito (CAT: ρ=-0.344; p <0.001).

Niaj rezultoj sugestas, ke mezurado kaj taksado de korpa konsisto estas utilaj por administri COPD-pacientojn kaj devus rutine esti faritaj dum terapio, tial ni proponas aldoni korpan komponan taksadon al la COPD-graveca takso (BODE-indekso).

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LEUKOCYTE AND PLATELET-RICH FIBRIN (LPRF) THERAPY & LEPROSY: THE NEED FOR CAUTION AND RESEARCH AMONG MARGINALISED GROUPS IN LOW RESOURCE SETTINGS

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Abstract

This paper discusses the potential of Leukocyte and Platelet-Rich Fibrin Therapy (LPRF) to help treat ulcers and skin damage associated with leprosy. It warns that although LPRF may be a valuable and cost-effective treatment, it is crucial to understand potential resistance to haematologically based treatments. For physicians and health service staff operating within a biomedical paradigm, folk beliefs resisting such treatments may be inconsequential. However, research and education among marginalised and excluded populations is vital to overcome potential hesitancy and resistance to such treatments.

Keywords: LPRF; Leukocyte and Platelet-Rich Fibrin Therapy; Leprosy; Nepal; Resistance

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Although there has been significant terminological confusion in the field [1], there is a growing evidence base to support the efficacy of Leukocyte and Platelet-Rich Fibrin (LPRF) in wound healing [2-4]. This therapy is relatively new, but has been used extensively in recent years in dental and oral and maxillofacial surgical settings [5]. LPRF has also been found to be effective across a range of other ailments [6-7], including the treatment of both diabetic foot ulcers [8] and chronic venous leg ulcers [9]. Given LPRF's reported success in relation to ulcers, it is perhaps no surprise that it is also currently being trialled in people with leprosy [10-11].

Compared with many treatments LPRF has the advantages of being economical, easy to prepare, and feasible for use in routine non-hospital clinical practices [4]. It is widely anticipated that because of its low cost and ease of use LPRF use will continue to expand rapidly into the future [12].

In many Western countries the marketing of LPRF to patients appears relatively straightforward. One US based dental practice describes LPRF as "basically a bioactive 'band-aid' that is created from your own blood and then placed in your surgery sites to promote healing" [13]. Table One details some of the routine positive health related aspects of LPRF used in marketing materials from another US based dental practice [14].

Table 1: Sample LPRF Health Marketing Material Claims [14]

Only requires a small blood sample	Lower Risk for complications
Virtually Painless	Healing properties
100% natural, 100% you	Promotes Recovery
Biocompatible	Simple Holistic Procedure
No additives, chemicals, or foreign substances	FDA cleared
L-PRF is individually made for you — from you	Reduced risk of allergy or side effects
Latest healing technology	Significantly less recovery time
Improved healing response	

Given the obvious appeal of such marketing it may be easy to overlook patient resistance to LPRF therapy. One of the main religious groups best known for their general refusal of blood donations and many blood based products are Jehovah's Witnesses [15-16]. Although there can be individual variation in attitudes and behaviour that may contrast with a religion's strict doctrinal stances, fresh autologous blood based treatments, such as LPRF, have been identified as acceptable to this religious group [17]. This additional endorsement from this group may to serve to further diminish any concern over patient resistance in some quarters.

However, it is always important to understand patient perspectives and remember that although increasingly mainstream, the prevailing Western biomedical paradigm is not dominant everywhere. Despite both national [18], and international [19] media acclaim around the potential benefits of LPRF it would be naïve to assume universal understanding or acceptance. Medical anthropologists and allied disci-

plines such as health psychology and the sociologies of health have a long tradition of exploring what are often termed such 'folk beliefs' in depth.

In many clinical settings health professionals are very familiar with a modest proportion of the population having a fear of needles or a fear of blood, and the possibility of fainting around blood [20 -25]. However, for many a statement such as the following from a US dental practice website may effectively assuage concerns: 'The procedure to obtain L-PRF is virtually painless — no more so than a routine blood test' [13]. However, an acceptance of such reassurances is far from universal. As well as the actual impact of seeing blood taken for procedures such as LPRF, many cultures place a special emphasis on blood and its symbolic cultural meaning [26-31].

A reluctance to embrace Western biomedical haematological processes may be particularly acute in marginalised and illiterate populations, with extremely poor occupational, social and economic status. One example of such groups that may now potentially encounter procedures such as LPRF are patients with leprosy [10,32,33]. This disease is inequitably distributed across populations, with it routinely being more common amongst the poorest and most excluded ethnic and cultural groups. In Nepal for example the majority of the population with leprosy are Dalit, formerly known as the Untouchables. The Dalit population are a highly stigmatised and excluded group [34-38]. Anecdotal concerns around interventions such as LPRF are already emerging among some members of this highly marginalised population. Reluctance, hesitation and refusal among such marginalised groups must not be ignored or brushed aside.

High quality research is required to explore the evidence base for LPRF as an intervention among patients with leprosy. However, research is also required to examine fears, attitudes, beliefs and lay understandings around this form of haemotologically based treatment. Finally it is also essential to explore and develop community based education programs to encourage acceptance and utilisation of such potentially crucial therapeutic interventions. The involvement of marginalised communities in developing such resources, as well their involvement in peer education, is vital. Such resources and ways of working are important for two reasons. Firstly, because of both the relative low cost and ease with which such interventions can be used in relatively rural and remote settings. Secondly, because despite the WHO's misguided millennium designation of it having been eliminated [39,40], the incidence of leprosy in countries such as Nepal is increasing [41].

Resumo

Ĉi tiu artikolo diskutas la potencialon de Leŭkocitoj- kaj Trombocitoj-Riĉa Fibrinterapio (LPRF) por helpi trakti ulcerojn kaj haŭtajn damaĝojn asociitajn kun lepro. Ĝi avertas, ke kvankam LPRF povas esti valora kaj kostefika traktado, estas grave kompreni eblan reziston al hematologie bazitaj traktadoj. Por kuracistoj kaj sanservopersonaro funkciantaj ene de biomedicina paradigmo, popolkredoj rezistantaj tiajn traktadojn povas esti malgravaj. Tamen, esplorado kaj edukado inter marĝeniĝintaj kaj ekskluditaj populacioj estas esencaj por venki eblan hezitemon kaj reziston al tiaj traktadoj.

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A CHILD WITH OTITIC HYDROCEPHALUS

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Abstract

Acute otitis media (AOM) is a widespread disease which may lead to extra- and intracranial complications. Intracranial complications of AOM are currently still potentially health and life threatening even with proper treatment. Otitic hydrocephalus is a rare intracranial complication of otitis media. It is characterized by elevated cerebrospinal fluid (CSF) pressure with normal CSF biochemistry and without any focal neurologic abnormality other than those related to the increased intracranial pressure. The precise mechanism underlying the development of otitic hydrocephalus is unknown. Eradication of ear disease and lowering of the elevated intracranial pressure are the goals of the therapy. The authors of this essay will introduce a 7 years old boy with otitic hydrocephalus and review the literature. It can be concluded that, in patients with acute or chronic ear infections, complaints of headache, blurred vision, diplopia, or photophobia may be a heralding sign of an intracranial complication. On the other hand, MRI is very important for detection of the complications of otitis media. Contrast-enhanced MRI and magnetic resonance venography must be immediately performed in patients with neurological symptoms such as nerve palsy, neck stiffness, or confusion.

Keywords: acute otitis media, increased intracranial pressure, otitic hydrocephalus, neurological effects

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Background

Otitic hydrocephalus manifests as signs and symptoms indicative of increased intracranial pressure. This condition can arise as a rare complication of acute otitis media, chronic otitis media, or otologic surgery (1,2). In a series of 100 cases of intracranial complications secondary to infectious ear disease, Gower and McGuire found that only 5 patients had otitic hydrocephalus (3). In a 15-year study of intracranial complications of otitis media, de Oliveira Penido et al did not find any case of otitic hydrocephalus (4).

Case presentation

A 7 years old Iranian boy with no obvious previous medical history, no significant past otological history or other neurological problems, was admitted with 10 days of headache, unilateral eye gaze, photophobia and fever. His symptoms initiated with headache and then, after 5 days, fever, photophobia and diplopia added. He had no complains of nausea, rhinorrhea, cough or otalgia. No changes in his level of consciousness was reported. He didn't have any previous recurrent otitis media or sinusitis. And also, he had no history of head trauma and or recent upper respiratory tract infection.

On physical examination at the time of admission, blood pressure was 100/75 mmHg, Pulse rate 100/ minutes and temperature was 38.8. There was no conjunctival injection. The funduscopic examination was normal. his right eye had inward deviation and outward movement restriction with normal pupil size and normal light response of pupil was significant, which was remarkable as the 6th cranial nerve palsy (abducens nerve palsy (CN VI palsy)) (fig.1). He had decreased range of motion in right eye scored as -4 and other eye examinations were in normal range. He examined for Brown syndrome, which was negative. No uveitis was seen with slit lamp examination. tympanic membrane was intact and normal. All other physical examinations were normal.

Fig. 1

His audiogram demonstrated normal hearing in both ears on pure tone averages.

In his laboratory tests, he had Normal complete blood count (CBC), C-reactive protein(CRP) 46, erythrocyte sedimentation rate(ESR) 127. Blood culture was positive with strep viridans. A lumbar puncture was done. So, CSF analysis: WBC: 7 PMN, RBC:547, Glu:62, Pro:24, Smear: normal, Culture: negative. Cerebrospinal opening pressure was normal.

In his rheumatologic workups, a high lupus anticoagulant antibody was reported, while an-

tiphospholipid Ab (IgM, IgG), HLA-B5 and B51, Anti b2 globulin, FANA, Anticardiolipin Ab (IgM, IgG), Anti b2 glycoprotein Ab (IgM, IgG), PANCA, CANCA, ACE, Protein C & S, Factor V Leiden, Ferritin, IL-6 and Procalcitonin were all within normal laboratory range. A chest X-ray was done, which was normal. Normal echocardiogram with normal anatomy of valves with no vegetation.

A tentative differential diagnosis of acute bacterial meningitis complicated by cerebral edema, acute hydrocephalus or cerebral abscess, cerebrovascular events and intracranial hemorrhages was made.

To resolve the diagnostic dilemma, Magnetic Resonance Image (MRI) of brain with and without intravenous contrast ordered. Intravenous Ceftriaxone (50mg/kg/dose 4 times a day), vancomycin (20 mg/kg/dose 4 times a day) and acyclovir (10mg/kg/dose three times a day) started with the first impression of meningitis.

Brain MRI confirmed Mild mucosal thickening of sphenoid. Fluid retention at right mastoid air cell with apparent intra septa as well as abnormal signal intensity at petrosal apex which is noted for ruling out of petrosal apititis. Right side otomastoiditis was notable. Fig.2-6

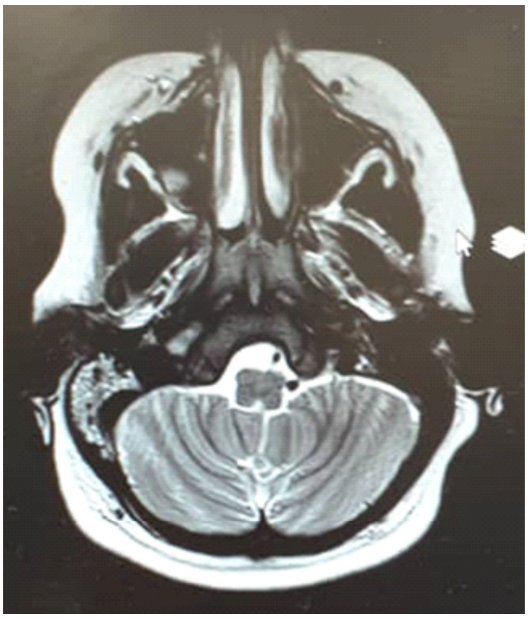

Fig. 2

Fig. 3

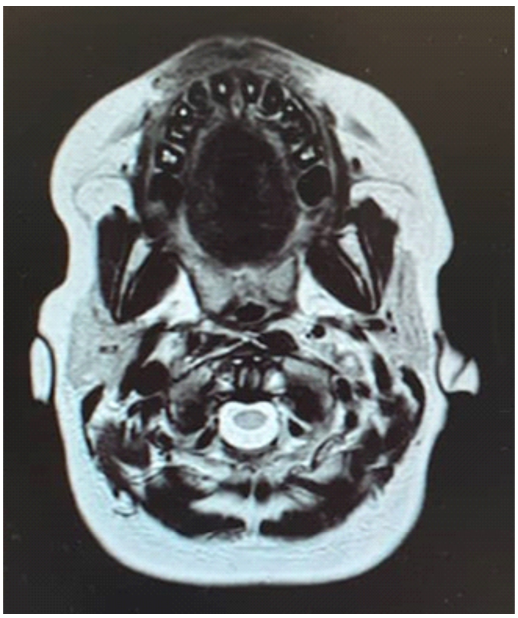

Fig. 4

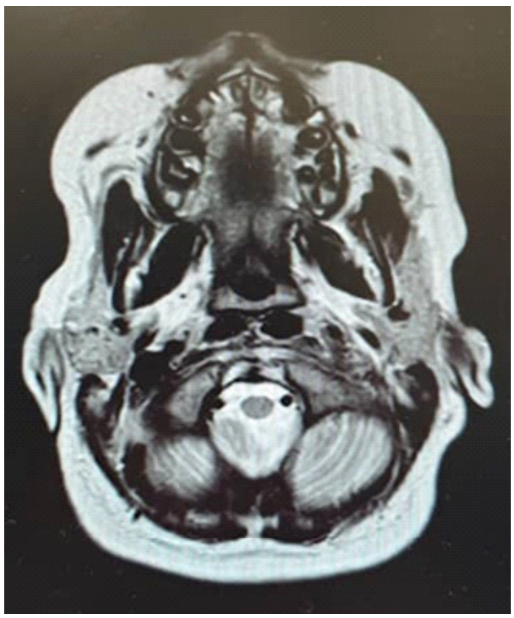

Fig. 5

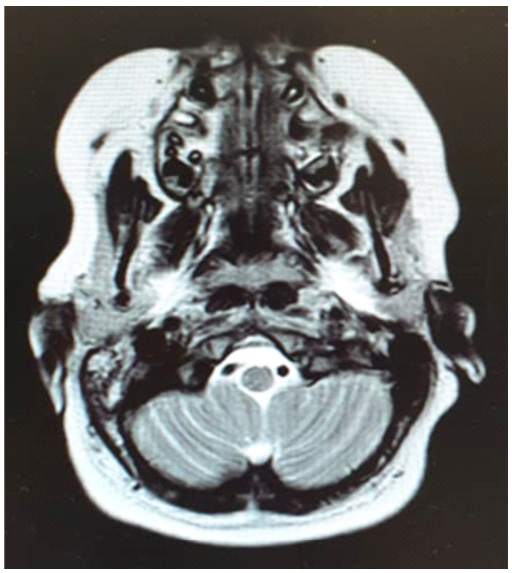

Fig. 6

Brain MRV showed Central filling defect in left sigmoid sinus. Left jugular bulb and proximal part of left internal jugular vein were compatible with CVT (central venous thrombosis).

Right temporal bone CT scan with and without intravenous contrast was done, in which right side chronic otitis media with sclerosis and mastoidial cells turbidity (loss of pneumatisation of the mastoid air cells) and thickening of temporal membrane reported.

In order to prevent life-threatening complications such as thrombosis of the sinous, the patient was given Enoxaparin 1mg/kg/dose (max 40mg) twice a day, subcutaneous injection, started. His antibiotics changed to Linezolid and Meropenem and continued for 14 days. An intravenous Methylprednisolone with dose of 1mg/kg/day and oral Acetazolamide prescribed.

After 5 days of IV antibiotics, a complete recovery of pyrexia was obtained, and 10 days after the initiation of treatment the neurological examination showed a partial recovery of the sixth nerve palsy. After 3 weeks of admission His inflammatory markers returned to normal.

Discussion and Conclusion

Acute otitis media (AOM) is a widespread disease which may lead to extra- and intracranial complications. Intracranial complications of AOM are currently still potentially health and life threatening even with proper treatment. The use of antibiotics has led to reduction in the incidence of intra- and extracranial complications from approximately 17%to 1%(5). Because these complications have declined markedly since the advent of antibiotics, many contemporary otolaryngologists have been unexposed to these complications. Furthermore, patients' symptoms are now often masked, so patients appear remarkably well despite the presence of potentially fatal complications (10). Severe complications of AOM such as subperiostal abscess, Bezold's abscess, facial nerve paralysis, osteomyelitis, meningitis, lateral sinus thrombosis (LST), extradural abscess, subdural empyema, brain abscess and otitic hydrocephalus(OH) have become very rare today (6-8).

The classical syndrome of otitic hydrocephalus consists of the signs and symptoms of elevated CSF pressure. It is more common among children and adolescents. The onset may delay several weeks after the acute ear disease or after years of chronic ear disease. (9). There are no focal neurological findings other than papilledema and occasionally 6th nerve palsy. In affected patients, the results of cerebrospinal fluid (CSF) bio-chemistry studies on lumbar puncture are normal.

The precise mechanism underlying the development of otitic hydrocephalus is unknown. Because superior sagittal sinus thrombosis should be associated with more neurologic deficits than are found in otitic hydrocephalus, it is postulated that a mural non-obstructing thrombus extending to the superior sagittal sinus impedes CSF resorption by pacchionian bodies and results intracranial hypertension (11). An alternative mechanism proposes that the presence of thrombus in the lateral sinus leads to impeded venous drainage into the neck, especially if the thrombus occurs in a dominant lateral sinus

(12). An increase in the intra cranial pressure may then be produced either by direct transmission of the raised venous pressure to the CSF or by impending the function of the arachnoid villi (13). However, ligation of the internal jugular vein in the neck does not cause hydrocephalus; thus, that mechanism must be the suspect. If a dominant venous sinus becomes obstructed, in the presence of inadequate cross communication at the trochlea, venous drainage may be sufficiently impaired to cause raised intracranial pressure. Probably because of this anatomical variation, otitic hydrocephalus is expected to be seen more commonly in right-sided ear disease (14).

Radiologic imaging may help us to identify the possible main physiologic mechanisms of otitic hydrocephalus. In this regard, as Magnetic resonance imaging allows for superior evaluation of the venous sinuses, it is the imaging modality of choice (2). for the determination of intra-sinus thrombosis, Magnetic resonance venography (MRV) can be used, which could not be diagnosed by classic venography (15).

Eradication of ear disease and lowering of the elevated intracranial pressure are the goals of the therapy. Acute cases of ear disease may be resolved spontaneously. Due to some vital merits persisting middle ear infection has to be treated. Surgical procedures must consist of cleaning the disease completely from middle ear and mastoid and draining the perisinus abscess, if present. Nowadays, to control suppurative thrombophlebitis complete mastoidectomy with evacuation of all middle ear and mastoid disease, drainage of the perisinus abscess, and clot removal, along with high-dose appropriate antimicrobial medications administered intravenously, is considered to be adequate. Those cases, in which sepsis continues, despite an adequate surgical procedure and appropriate intravenous antibiotic therapy, Ligation of the internal jugular vein is reserved (2). Raised intracranial pressure may be treated by the use of steroids, diuretics such as acetazolamide, and hyperosmolar dehydrating agents (mannitol). Repeated lumbar puncture has been advocated, but this is not risk free in the presence of raised intracranial pressure. Furthermore, in some articles, repeated LPs are referred to have only historic importance (10,15,16). Because otitic hydrocephalus occurs regularly in conjunction with lateral sinus thrombosis, anticoagulation therapy may be considered as a therapeutic option.

All in all, necessitating a high index of suspicion, Otitic hydrocephalus is an uncommon complication of otitis media, with potentially significant morbidities. In patients with acute or chronic ear infections, complaints of headache, blurred vision, diplopia, or photophobia may be a heralding sign of an intracranial complication.

And also, MRI is very important for detection of the complications of otitis media. Contrast-enhanced MRI and magnetic resonance venography must be immediately performed in patients with neurological symptoms such as nerve palsy, neck stiffness, or confusion.

Declaration

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Resumo

Akuta meza otito (AMO) estas ofta malsano, kiu povas konduki al ekster- kaj intrakraniaj

komplikaĵoj. Intrakraniaj komplikaĵoj de AMO ankaŭ nuntempe estas ebla minacoj por sano kaj vivo eĉ dum adekvata kuracado. Otita hidrocefalo estas malofta intrakrania komplikaĵo de AMO-meza. Ĝi estas karakterizita per altigita premo de cerebrospina likvaĵo (CSL) kun normala CSL-biokemio kaj sen iu fokusa neŭrologia anomalio krom tiuj en rilato al la pliigita intrakrania premo. La preciza mekanismo de la evoluo de otita hidrocefalo estas nekonata. Elradikigo de orelmalsano kaj malpliigo de la altigita intrakrania premo estas la celoj de la terapio. La aŭtoroj de ĉi tiu eseo prezentos 7-jaran knabon kun otita hidrocefalo kaj recenzos la literaturon. Oni povas konkludi, ke ĉe pacientoj kun akraj aŭ kronikaj orelinfektoj, plendoj de kapdoloro, neklara vizio, diplopio aŭ fotofobio povas esti anonca signo de intrakrania komplikaĵo. Aliflanke, magneta resonanca bildigo (MRB) estas tre grava por detekto de la komplikaĵoj de AMO. Kontrast-plifortigita MRB kaj magneta resonanca venografio devas esti tuj faritaj en pacientoj kun neŭrologiaj simptomoj kiel ekzemple nerva paralizo, kolo-rigideco aŭ konfuzo.

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LIFE STYLE AND STRUCTURE DIFFERENCES BETWEEN SPECIES OF FRESHWATER FISH

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Abstract

It has been recommended that people eat fish rich in unsaturated fats at least twice a week to reduce the risk of heart disease. Fish consumption is significant, mainly from fish living in ocean saltwater. However, in countries without sea like Hungary, the richness of freshwater fish has developed a wide range of cooking techniques for fish with different nutrition. We suspect that muscle structure differences have not yet been investigated. The difference in fatty acid composition of African catfish and Siberian sturgeon is known, but no morphological studies have been performed on their muscle structure. The aim of this study was to compare the structure differences between freshwater fish with different lifestyles. The organization of muscle structure was monitored in meat by means of cytochemistry combined with scanning electron microscopic studies on tissues of two different species, and the techno-functional parameters measured. The filleted muscles of African catfish (Clarias gariepinus) and Siberian sturgeon (Acipenser baerii) were compared after fresh and fast freeze. The associated complex structure of muscle in both species appeared different. One is a tightly closed muscle mass, while the other is a soft structure, which shows a different degree of softness of the meat after baking. In both species, the right muscle structure is beneficial under extreme environmental conditions. The different skeletal structure in fish needs altered processing, which we wish to continue with further testing and to prepare tasty food for consumers and use in dietetics.

Keywords: freshwater fish, muscle tissue structure, ingredients, scanning electron microscope

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Introduction

Fish consumption on Earth is significant, mainly from saltwater fish living in ocean important source of nutrients. In the EU, the fish consumption per capita is the second highest in the world around 2 kg/capita/year but about 6.7 kg/capita/year in Hungary [1], and some individual EU Member States have among the highest rates in the world. However, in countries without sea, such as Hungary, the richness of freshwater fish has developed a wide range of cooking techniques for fish with different nutrition.

One of the popular and well-breeding fish is the Siberian sturgeon or Lena Hungary is a native fish as it migrated from the Black sea after the ice age (Figure1) [2]. It is cold-blooded species. Today, its main stock is found in the rivers of Eastern Europe and western Siberia. The meat of the sturgeon is extremely valuable because its taste is excellent and is threaded with cartilage. They moved to Hungary in 1981 and they have been settled in our fishing lakes these days. The Siberian sturgeon weighs about 65 kg, they are long-lived, up to 60 years, and they reach sexual maturity rather late [3].

Another favorite fish is the African catfish (*Clarias gariepinus* Burchell). This species is native to Africa and Asia Minor, and it can be farmed and found everywhere. Its adaptability is high, not picky about food and can develop properly in polluted environments [4]. It is native to Inland waters of Africa, but it is living in Netherland and Hungary too. The catfish is perfectly adapting to high stocking densities, thanks to its auxiliary respiratory system it can withstand even persistently low oxygen levels. Furthermore, it is a heat tolerant fish but it dies at temperatures below 15 oC or in a microbiologically infected environment [2]. The meat of the catfish is fiber-free, low-fat, it has an excellent taste and is well transportable due to its elongated body, similar to that of eel or ling.

Although these are well-transportable fish, their meat, and the quality of the meat products made from them has several factors. The age, gender, posture and feed of the animal primarily determine the quality of the meat. It plays an outstanding role in the chemical composition of raw meats, physical properties such as color, texture, and techno-functional properties. In addition, it is important how we prepare, how we store to preserve the quality for a long time.

Millions of people live with minor or major disorders, of which metabolic diseases are the most serious, the number of those is currently on the rise. Many people prefer white and easily digestible meat, which is an inexpensive source of protein from chicken but we forget about fish. Freshwater fish is barely available for purchase high and therefore consumption is especially low in countries where sea fishing is not possible.

The living systems, animal and plant cells are highly complex colloidal systems. In the cytosol containing carbohydrates and salts due to this, the cell sap behaves like a colloidal solution during freezing. During slow freezing, extracellularly crystals form from the water and some of the cellular fluid diffuses into the extracellular space and freezes into the crystal nodule formed there. In medium-fast freezing speed changes the size of the water crystal during quick freezing, heat removal is faster than cell liquid diffusion, resulting in intracellular freezing inside the cell. During quick freezing, heat removal is faster than cell sap diffusion, resulting in intracellular freezing of the cell liquid [5, 6]. The ice crystals form at temperatures between -0.6 -2.2 oC. This rapid crossing of the temperature range eliminates the formation of large ice crystals. This reduces tissue and cellular destruction.

The biological fats are solid in texture differ from oils which are liquid at ambient temperatures. But chemically there is a little difference since the substances are composed predominantly of esters of glycerol with fatty acids, so called triacylglycerol (TG). Biologically in the living we used synonymously the lipid term that

those are including substances all important fat-soluble soluble in food and nutrition. Their function is distinguished as structural fats, metabolic fats, storage fats or fats in transit in the body [7, 8 and 9]. The fatty components can be made within the body from carbohydrates consumed in the diet. Exceptions are the essential fatty acids (EFAs) that those fatty acids the human and other animals must ingest because the body requires for good health but cannot synthetize them [10] which two of the fatty acids are only known to be essential for humans, the alpha-linolenic acid (omega-3 fatty acid) and the linolenic acid (an omega-6 fatty acid) [11, 12].

The value of fish meat with lipids is for preventing, protecting and improving many diseases, it is also our natural source of proteins, minerals and ions. Atrial fibrillation is a dangerous condition that tends to strike the elderly and can lead to stroke or heart failure. Omega-3 fatty acid intake related to other dietary factors known to reduce coronary heart disease risk assuming moderate consumption.

Western diets are deficient in omega-3 fatty acids, and need to add excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established [13, 14, 15]. The omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory as well as autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert suppressive effects.

Hungary is rich with freshwater fish in rivers but the consumption of fish meat lower than the European average, in 2007: 3.8 kg/capita/year, in 2016 increased to 6.7 kg/capita/year. There are some popular freshwater farms in Hungary but different in feeding and they are living in different ecological area with altered genetic background and lifestyle. These two fish, sturgeon and catfish, are also relatively easy to breed in our country, despite the fact that they come from a very different habitat in terms of their ancient origin, however, they retain their genetic and phenotype well.

Based on it, we hypothesized, that the right muscle structure is depending from processing of freezing technology of the meat before cooking and the results being beneficial under extreme environmental conditions.

The aim of this study was to compare the techno-functional parameters and structure differences of muscle between freshwater fish with different lifestyles. We would like to show the differences of molecular components from farmed freshwater fish in Hungary, whose original habitat is alive but popular among consumers.

Material and methods

Studied species

African catfish (*Clarias gariepinus*) and Siberian sturgeon (*Acipenser baerii*) were compared after fresh- (precooled), slow- and rapid- freezing (Figure 2).

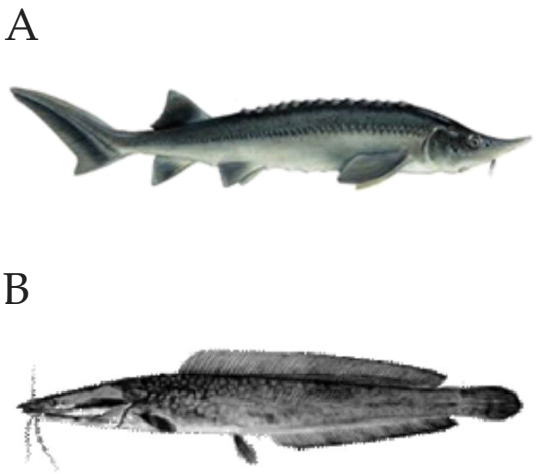

Fig.1. The Siberian sturgeon (*Acipenser Baerii*) (A) and African catfish (*Clarias gariepinus Burchell*) (B) are very popular and well breeding in Hungary.

Photo by https://www.dreamstime.com/photos-images/siberian-sturgeon.htmlandhttps://stock.adobe.com/search?k=%22 clarias+gariepinus%22

Fig.2. Meat of African catfish (1, 2 and 3) and Siberian sturgeon (4, 5 and 6). Photo by Zoltán Répás

After the processing of cleaning and filleting of precooled fish, small part of muscle was fresh-freezing (precooled) at 4 oC (Figure 2: 1, 4) or fast-freezing at -40 oC (Figure 2: 2, 5) or slow-freezing at -20 oC (Figure 2: 3, 6). After freezing the pieces of tissues were washed in phosphate buffer containing 4.0 % saccharose first.

Techno-functional parameters

Measurement of water holding capacity

The test was done by Grau and Hamm' filter press method [16], (n = 10 /fish). From the samples, the weight of tiny pea-grain tissues was determined, wrapped in filter paper, then placed between two glass sheets and pressed for 5 minutes with 1 kg weight. After that, the mass of fish meat was back and calculated from the data.

Determination of the water-binding ability

The determination of the water-binding ability was performed using a cooking probe. The size of the samples was cut into pieces of 3x5 cm (n = 10 /fish), measured its weight and placed it in a heat-treatable bag and sealed it with vacuum and foil baking. The heat treatment at 75 oC lasted 10 minutes which we brought back to room temperature. The weight was measured before packaging and after heat treatment. After heat treatment, the water was carefully soaked on the surface of the samples and then was calculated the water-binding ability [8].

Scanning electron microscopy analysis

The skeletal muscles of two types of fish were collected and the anatomy analyzed with a stereo microscopy. Here the main aim was to study high resolution ultrastructure with scanning electron microscopy (SEM). The tissues were treated in phosphate buffer (PBS, pH 6.7) containing 4.0% saccharose to keep the physiological condition of cells, and continued with 30% saccharose at 4 oC during 2 hours in PBS (pH 6.7). Small pieces of fish samples were fixed in 2.5% glutaraldehyde containing PBS (pH 6.7) supplemented with 30% saccharose during 2 hours. After post-fixation in 1% OsO4 for 1 hour, the samples were dehydrated in aqueous solutions of increasing ethanol concentrations, critical point dried, mounted on specimen stubs, covered with 15 nm chromium by a Quorum Q150T ES sputter and observed in a JEOL JSM-

7100F/LV scanning electron microscope by using 3 kV accelerating voltage.

Analysis and documentation of the samples were carried out with a program of scanning electron microscope and Microsoft programs.

Organoleptic analysis

The sensory examination was carried out by 17 independent students from the University of Szeged Faculty of Engineering. The fish products examined were different types of frozen and cold smoked fish. A total of six samples were evaluated: the pre-chilled (EH), slow (SF) and fast frozen (FF) fish of the two tested species. The evaluation criteria are texture, taste, and smell. The evaluation was based on a textual assessment and a scoring method, which ranged from 0 to 10 points. The higher the score a sample received, the soft it tasted and smelled better. Text opinions were evaluated according to their content as positive or rather negative.

Results

Techno-functional parameters

Techno-functional property includes water holding capacity, water-binding ability and emulsification of the pH.

We measured the proximal techno-functional parameters of muscle in Siberian sturgeon and African catfish (Figure 1 and 2; Table1). The water holding capacity, was highest in precooled muscle in both fish has well as the water-binding ability. However, the boiling loss was high after fast freezing in sturgeon and catfish too. Mass of meat was highest after cooling back (Table1), it is opposite what was expected, with slow freezing method in both fish.

In the African catfish the techno-functional parameters are rather positive characters but in Siberian sturgeon the physiological taste, consistency, flavors and essence were much more pronounced in organoleptic analysis. This was preliminary experiments (not shown).

Table 1. Proximal techno-functional parameters of muscle in Siberian sturgeon and African catfish. Measured by Zoltán Répás

Parameters	Sample	Siberian sturgeon	SEM ±	African catfish	SEM
Water holding capacity, %	Precooled	88,90	4,5	82,37	3,95
	Slow-freezing	76,01	3,47	81,98	4.60
	Fast-freezing	74,63	4,89	81,65	4.58
Water-binding ability, %	Precooled	84,42	2,74	88,98	5,84
	Slow-freezing	87,62	1,86	88,33	3.95
	Fast-freezing	80,72	1,02	88,70	3,76
Boiling loss, %	Precooled	15,58	0,97	11,02	0,49
	Slow-freezing	12,37	0,83	11,67	1,49
	Fast-freezing	19,28	1,42	11,30	1,58
Mass changed, %	Precooled	98,83	-	99,55	-
	Slow-freezing	98,27	-	99,58	-
	Fast-freezing	98,42	-	99,42	-
Mass change, after smoking, %	Precooled	92,99	-	97,31	-
	Slow-freezing	94,99	-	96,67	-
	Fast-freezing	92,24	-	97,51	-
Mass after cooling back, %	Precooled	84,40	-	91,17	-
	Slow-freezing	88,00	-	90,79	-
	Fast-freezing	85,33	-	90,02	-

Structure of skeletal muscle in freshwater fish using scanning electron microscopy

Methods were developed for studying with scanning electron microscope (SEM) to investigate and compare the muscle structure by molecular scale between two different originated freshwater fish.

The structures of skeletal muscle are differing between Siberian sturgeon (Acipenser sp.) and African catfish (Clarias sp.) in Figure1. The stiffness and the color of muscle appeared to be different in both species (Figure 2) of freshwater fish.

Earlier, the scoring assessment of organoleptic analysis gave a high score in both species. In this investigation, we determined the molecular structure of muscle of Siberian sturgeon and African catfish by SEM method.

African catfish has a tightly closed muscle mass with darker red color, while the Siberian sturgeon has a light rose color (Figure 2, 1-3). Muscle of the sturgeon shows more softness (Figure 2, 4-6). The precooled and slow-freeze muscle structure was very similar comparing to the own controls (precooled) in species. But in the sturgeon samples there are several small drops of fat comparing to catfish (Figure 3 left side comparing to the catfish in precooled samples). The bars on SEM photos of Siberian sturgeon are 10 μm on the left column and 100 μm on right side, but opposite on photos of African catfish, bars are100 μm on the left column and 10 μm on right side in Figure 3. The precooled and slow-freeze patterns were not different in photos of the sturgeon as well as of catfish.

The molecular structure appeared to be altered after fast-freeze variously depending on the molecular structure (Figure 3).

We have some knowledge that the slow-freeze can change the muscle structure because ice crystals come into being and this process can influence the tissues structure but not in freshwater fish [17]. This work is first to demonstrate the difference between freshwater fish can keep the original muscle structure in altered habitats.

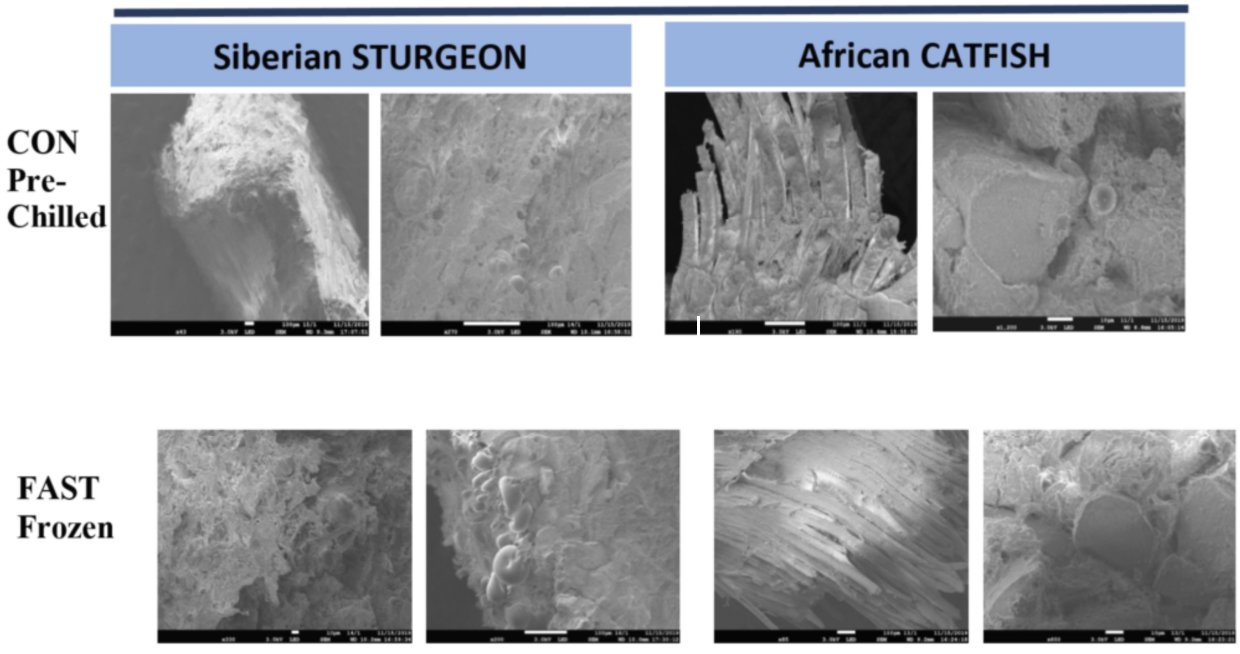

Fig.3. Analysis by scanning electron microscope of muscle tissues of Siberian sturgeon and African catfish after pre-chilled and fast-frozen. CON = control, Pre-Chilled tissues; FAST= fast-frozen tissues. Investigated by Dr. Ildikó Domonkos.

However, we had not so much knowledge about this progress in freshwater fish until now. Our results revealed that both the fast-freeze and slow-freeze can altered the molecular structure but may be the precooled tissues is maintaining only the original structure of myocytes under short intervals.

The scanning electron microscopy method is an advantageous choice for the detection of quality differences in meat because they supported the organoleptic tests, the thousands of years of experience in the processing of meat with visible tissue changes on the sections of Figure 3.

In the case of processing of fast-freezing in Siberian sturgeon, but the slow-freezing process in African catfish was found of the organoleptic characteristics of the final product; they are positively influenced by the freezing process. In the future we are able to carry out deeper analysis at molecular level with high resolution.

Organoleptic analysis

The SEM technique was an advantageous possibility for the deeper analysis of macromolecular systems in the structure of muscle cells and fibers.

The texture of fresh and fried muscle of catfish and sturgeon fillets significantly differ from that of the roasted fillets in meat because they supported the organoleptic tests, the thousands of years of experience in the processing of meat with visible tissue changes on the sections.

In the case of processing of fast-freezing in Siberian sturgeon, but the slow-freezing process in African catfish was found of the organoleptic characteristics of all parameters in the final product, in smoked fish of them; they are positively influence by the right freezing process.

After fried with oil, the techno-functional parameters of the African catfish were rather positive characters with slow freezing, but Siberian sturgeon with fast-freezing was the best in substance, taste and essence.

This study is the first which has an advantage in the industrial freshwater fish meat processing.

Discussion

The purpose was to demonstrate that the (1) muscle tissues can be studied at high resolution in freshwater fish by nanotechnology methods at molecular level, (2) the skeletal muscle structure can be different depending from originated of animal and (3) living style. (4) The organoleptic analysis of freshwater fish can be reflected the connection between these molecular differences in the muscle.

Fiber's, structural elements as well as the lipid content of muscle are different between these species demonstrated Pelvic et al. (2019) [18]. The knowledge of the molecular structure and composition of meat is important for the precise setting of functional parameters. After frying the slices in oil, both the African catfish slow-freeze, and Siberian sturgeon fast-freeze are best in substance, consistency, taste and essence. The sturgeon was better 5% in taste comparing to catfish only, but substance and essence were closed in scoring assessment of organoleptic analysis (n= 17 students, unpublished data). This analysis with SEM needs to be repeated more times.

The closed fibrous structure of the African catfish needs the destructive effect of large ice crystals due to slow freezing. This and the lipid content led to the improvement of the tissue structure associated with the beneficial pleasure value.

These two fish are also relatively easy to breed in our country, despite the fact that they come from a very different habitat in terms of their ancient origin, however, they retain their genetic and phenotype well.

The structural changes in Sturgeon due to rapid freezing and higher lipid content proved to be sufficient to increase the enjoyment value of the product. Consumption a fish, as a good meat, will be raised most likely, due to the com-

plete values of fish meat addition to daily diets in different diseases [19, 20, 21].

In the future, we are able to use to developed new methods to compare tissue structure of freshwater fish by SEM technique. The knowledge of the molecular structure and composition of meat is important for the precise setting of functional parameters.

The PUFA ratio of polyunsaturated fatty acids is very high in fish oil, including n-3 fatty acids, which have a primary role in preventing the development of cardiovascular diseases. In the Biological Research Center of Szeged (Hungary) Professor Tibor Farkas MD biochemist was the first to determine the content of carp (Cyprinus carpio L.) n-3 fatty acids in the 1970s. He drew first attention to the physiological significance of this and then began to develop it in medicine. After that, research in this direction was also started in saltwater fish. The unsaturated fatty acids reduce LDL levels and they increase the protective HDL cholesterol levels in our body. Also beneficial for the heart and vascular system [20, 21].

Organoleptic values were excellent both in species but altered freezing parameters.

Consuming freshwater fish helps to maintain some diets with different meals in healthy and non-healthy people to regenerate our body and prevent diseases.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Resumo

Oni rekomendis, ke homoj manĝu fiŝojn riĉaj je nesaturitaj grasoj almenaŭ dufoje semajne por redukti la riskon de kormalsano. Fiŝokonsumo estas signifa, plejparte de fiŝoj vivantaj en oceana salakvo. Tamen, en landoj sen maro kiel Hungario, la riĉeco de dolĉakvaj fiŝoj evoluigis larĝan gamon de kuirteknikoj por fiŝoj kun malsama nutrado. Ni suspektas, ke muskolstrukturaj diferencoj ankoraŭ ne estis esploritaj. La diferenco en grasacida konsisto de afrika anariko kaj siberia sturgo estas konata, sed neniuj morfologiaj studoj estis faritaj pri ilia muskola strukturo. La celo de ĉi tiu studo estis kompari la strukturdiferencojn inter dolĉakvaj fiŝoj kun malsamaj vivstiloj. La organizo de muskola strukturo estis monitorita en viando per citokemio kombinita kun skanaj elektronaj mikroskopaj studoj sur histoj de du malsamaj specioj, kaj la tekno-funkciaj parametroj mezuritaj. La filetaj muskoloj de afrika anariko (Clarias gariepinus) kaj siberia sturgo (Acipenser baerii) estis komparitaj post freŝa kaj rapida frostigo. La rilata kompleksa strukturo de muskolo en ambaŭ specioj ŝajnis malsama. Unu estas forte fermita muskola maso, dum la alia estas mola strukturo, kiu montras malsaman gradon de moleco de la viando post bakado. En ambaŭ specioj, la ĝusta muskola strukturo estas utila sub ekstremaj mediaj kondiĉoj. La malsama skeleta strukturo en fiŝoj bezonas ŝanĝitan prilaboradon, kiun ni deziras daŭrigi kun pliaj provoj kaj prepari bongustajn manĝaĵojn por konsumantoj kaj uzon en dietetiko.

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AN EXAMINATION OF PORTRAYALS OF SMOKING IN NOIR GRAPHIC/ COMIC NOVELS

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Abstract

Global mortality from tobacco related diseases continues to rise. Most smokers start smoking in adolescence and early adulthood. Graphic novels have a particular appeal to this age group, and are increasingly read by females and males. Prior research examining a range of graphic novels had noted high rates of portrayals of smoking in noir and cyber-punk novels. This research focussed on a random sample of noir graphic novels to explore this finding in more depth. A quantitative content analysis of ten noir graphic novels was conducted. Although there was substantial variability in the number of portrayals of smoking, smoking imagery was prevalent throughout all of the novels examined. Of particular note was the high rate of portrayals of women smoking. This is an issue given the rise in smoking rates in young women and girls in some Western countries. Some examples of substitution and relegation of tobacco products was noted, and tobacco control advocates should seek to work with artists to promote omission, substitution and relegation of such imagery. Further research is suggested to explore in more depth the characterisation of smokers in such graphic novels, and the ongoing recurrence of such imagery in contemporary graphic noir novels.

Keywords: smoking, graphic novel, comic book, adolescents, young adults, noir

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Introduction

Smoking remains the world's leading cause of preventable mortality and morbidity, resulting in up to 8 million deaths per annum [1-3]. The financial burden of smoking is also a significant factor that should not be under-estimated [4]. Although smoking rates have decreased in many countries they remain unacceptably high, despite widespread adoption internationally of key elements of the World Health Organization's (WHO) Framework Convention on Tobacco Control (FCTC) [5,6]. This includes the widespread adoption by many countries of bans on sponsorship and advertising of tobacco products.

Given the continued adoption of smoking by a significant minority of adolescents and young adults it is important to explore the provenance of pro-smoking influences in this cohort. It must be remembered that the majority of smokers start smoking in their teens or early twenties [7,8]. This period is also critical for identity formation. As such it is opportune to explore media representations of smoking, especially given aspects of performativity, social representation/ group identity and modelling in smoking behaviours.

An established literature already exists detailing malignant links between Big Tobacco and the media dating back to the 1920s [9,10]. Significant payments have been noted by the tobacco industry to ensure the placement of their products in popular films with particular appeal to teenagers and young adults including Superman II (Marlboro cigarettes by Philip Morris) and Supergirl (Eve cigarettes by Liggett) [9]. A significant body of research has already identified the impact of smoking portrayals in movies and TV on tobacco use among young people [11-14].

To date little research has examined the portrayal of smoking in graphic/ comic novels, despite an extensive literature focussing on tobacco adverts in magazines [15-18]. This is perhaps odd given the appeal of such graphic novels to young adults [19-21], including 'reluctant readers' [22], at a time of crucial behavioural choices, including smoking initiation. Historically such publications may have been located firmly on the margins [23], however in recent years they have moved increasingly into the mainstream [24]. The appeal of graphic novels has also increased beyond their traditionally male dominated audience [25].

Perhaps one of the most high profile uses of a modern graphic novel in the field of Public Health was developed by the Centers for Disease Control and Prevention (CDC) to promote emergency preparedness among students and young adults: Preparedness 101: Zombie Pandemic [26]. Such was the success of this initiative that the CDC's website crashed under the number of hits it received as the campaign went viral [27]. Despite this literature examining this and similar interventions has noted only limited success [28,29], and unanticipated outcomes [27,30,31].

It should be acknowledged that a developing academic literature exists about graphic novels in the fields of education, media studies and health/ medicine. In the health domain graphic novels have been examined around such topics as: healthy eating [32-34]; cancer screening [35]; HIV/STD prevention [36,37]; and thanatology [38]. Other research on graphic novels in health has examined issues such as: the portrayal of disability/ impairment [39,40]; depictions of violence [41] and self-harm [42]; physician education [43,44]; healthy decision making [45]; and the treatment of subthreshold depressive symptomatology [46].

Of most relevance to this research are four studies that have examined portrayals of smoking in graphic novels. The first two of these involved an examination of portrayals of smoking in contemporary Japanese Manga graphic novels [47,48]. Both of these studies noted high levels of depictions of smoking. A more recent examination found that although there had been a decline in such portrayals over time, they still remained routine in Manga [49]. Given the now

almost global popularity of Manga, such examinations are vital as Bouissou has identified that 'to export comics is also to export ideology and values system' [50]. Graphic novels, similar to any media perform the three 'Rs' of cultural transmission, in that they can reflect, reinforce and reproduce specific cultural patterns and behaviours such as smoking.

Manga represents a very different breed of comics and graphic novels to what many of the older generation may traditionally associate with this format. Traditionally 'safe' products associated with this genre, such as Disney's Mickey Mouse and products from the Ecole Franco-Belge such as Tintin and Asterix, have little or nothing in common with contemporary Manga [51-53]. Manga is designed to be shocking and has purposefully opposed societal and cultural norms across a wide range of topics [50,54]. The appeal of such depictions in Manga, it has been suggested, is a direct result of the sterility imposed by censorship: 'the American cartoons, after struggling with the stringent constraints of the Comics Code for half-a-century, gradually became emasculated. The readers were tired of no violence, no sex, no police-bashing, no smoking and no- nothing' [50].

The fourth study of smoking portrayals in graphic novels was based on a random selection of ten graphic novels available in the main library of a provincial Irish city [55]. This research noted three key findings. Firstly, that there was a significant volume of portrayals of smoking in such graphic novels. Secondly, it identified that there was significant variation in the sample chosen, with several having none or very few such images, compared to others that included in excess of 250. Third, this research noted a dramatic gender imbalance in smoking portrayals. This research noted that for every panel that showed a woman smoking there were almost 17 panels showing men smoking. This research also identified two distinct genres of graphic novel that appeared to depict smoking on a significant scale. These were the cyber-punk and noir genres of graphic novel [55].

In light of the ongoing popularity of the noir setting [56], troublesome global rates of smoking among women [57], and the growing appeal of graphic novels to female audiences [23], this research therefore focussed on the noir genre of graphic novel. In Houghton & Houghton's research outlined above, the most frequent depiction of female smoking was in a noir graphic novel featuring the trope of the femme fatal [55]. Critics often view literary noir as a specifically hardboiled American art form produced by archetypal writers such as Hammett [58-61], Chandler [62-66], and Cain [67-69] during the 1930s to the 1950s [70]. These authors produced iconic characters such as Chandler's Philip Marlowe, and Hammett's Sam Spade that had, and continue to have, a significant influence on media forms. Film noirs, also perceived as a typically American art form, are usually thought to have been produced in Hollywood during a two decade period beginning with John Huston's The Maltese Falcon in 1941, starring Humphrey Bogart, and concluding with Orson Welles's Touch of Evil in 1958, which starred Marlene Dietrich [71].

Noir graphic novels usually follow this time period, routinely portraying the dark, gritty and seedy underworld of a corrupt society . Typical tropes include the femme fatale, private investigators, disgruntled and alienated police officers, vigilantes, and host of villains with malign intent. In response to the controversy they caused noir comics were suppressed during the 1950s and beyond and the fate of this genre seemed certain to be extinction. However, there has been a notable current resurgence in crime and noir comics/ graphic novels, that has in turn spawned its own literature [71,72].

Method

A random selection of ten noir graphic novels was selected chosen from an amalgamated

list created from four leading online web sites listing popular crime noir graphic novels [73-76]. A quantitative content analysis of examples of smoking and smoking paraphernalia was then conducted on these 10 novels [77-79]. A coding schedule was developed to record the gender of smokers (male, female, mixed). Analysis was conducted on the novels on a panel by panel basis. Details of the texts selected and the results can be seen in Table 1.

Results

Examination of the ten noir graphic novels identified 534 panels containing examples of smoking. 60.3% (322) of these smoking images featured just males, while 38.5% (205) only related to women (see Table One). There was substantial variation in the average number of panels portraying smoking per page, which varied from a high of one smoking related panel every 2.1 pages in Sleeper [80], to a low of an average of one smoking related panel every 8.5 pages in Hawaiian Dick: Byrd of Paradise [81]. Examples of such smoking imagery may be seen in Figure One.

Tab.1. Analysis of Portrayals of Smoking By Gender in a Random Selection of Ten Noir Graphic Novels

Title	Pages (including cover)	Male/s Smoking Panels	Female/s Smoking Panels	Male/s & Female/s Smoking Panels	Total Smoking Panels
Batman: Year One [82]	132	18	2	5	25
Sleeper [80]	292	28	113	0	141
Gotham Central Book One [83]	244	80	0	0	80
Sin City- A Dame To Kill For [84]	212	27	17	1	45
Hawaiian Dick: Byrd of Paradise [81]	136	12	3	1	16
Criminal: Coward [85]	152	15	39	0	54
Criminal: The Sinners [86]	148	26	0	0	26
Batman: Broken City [87]	146	20	10	0	30
Batman: The Long Halloween [88]	389	48	17	0	65
100 Bullets: First Shot, Last Call [89]	132	48	4	0	52
Total	1983	322	205	7	534

Discussion

Noir graphic novels contain a substantial number of portrayals of smoking. Given that a significant proportion of readers of graphic novels are routinely youths and young adults this is particularly problematic. As noted above, this age is a crucial stage in social identity formation and as such these representations are important. It should be noted that none of the representations of smoking portrayed it negatively in terms of its adverse impacts on health, or on the basis of any other factors such as finances, or environmental impacts.

Prior research examining portrayals of smoking by gender in a mixed genre selection of graphic novels noted a ratio of almost 1:17 in female to male portrayals of smoking [55]. The ratio of such female to male portrayals in this research was dramatically different at approximately 2:3. Given the increase in girls and young women reading graphic novels, this preponderance of smoking imagery associated with women in the noir graphic is potentially highly problematic.

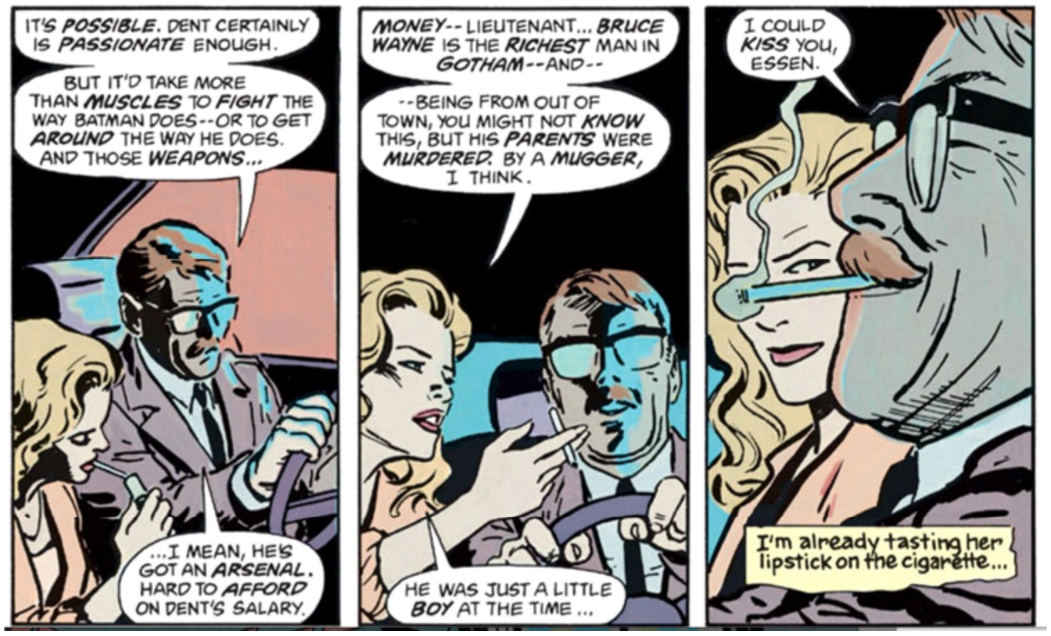

Fig.1. Examples of Panels Containing Smoking From Batman: Year One [82]

The sensual undertones in Figure One, and the more overtly sexualised smoking imagery in Figure Two, clearly demonstrate the link portrayed between sex and smoking in some noir graphic novels. Perhaps of more concern is the portrayal of such smoking in attractive, self-confident, assertive women. Such depictions must serve to increase the appeal of smoking to girls and young women.

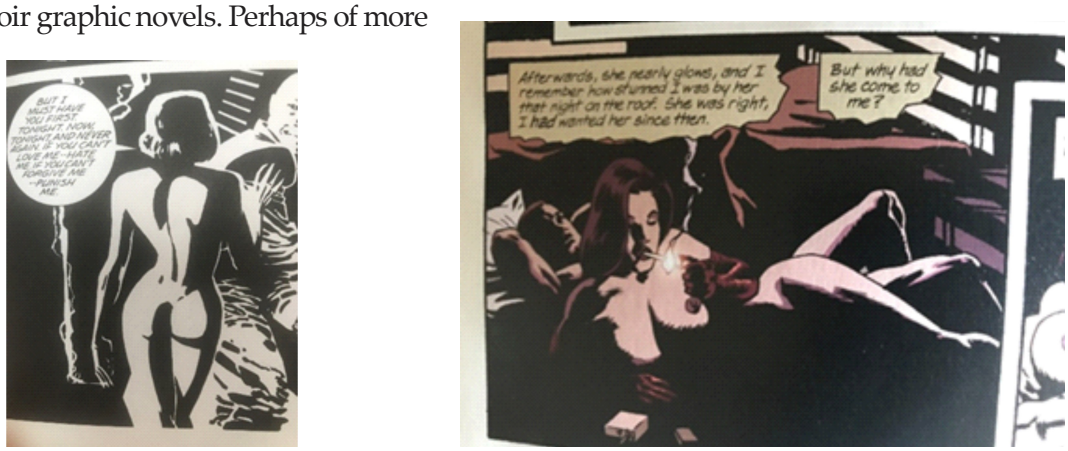

Fig.2. Examples of the Femme Fatal & More Sexualised Smoking Imagery [84,80]

It is interesting to note that the noir novel Hawaiian Dick Vol. 1: Byrd of Paradise featured examples of what may be termed substitution and relegation [81]. Figure Three clearly demonstrates how a cigarette, cigar, or pipe can be substituted for another object, in this case a drinking straw. This panel filled the entire page, a rare event in graphic novels, and as such the replacement is significant. Figure Three also features what may be termed relegation. Many graphic novels contain a selection of extra material after the main story which routinely feature scripts, initial drafts of pictures, and unused art. The picture showing the figure smoking in a doorway, although completed, was never used. Although still included in the extra material section, it may be significant that this image was relegated to subsidiary material in the novel which included the least number of smoking related panels per page [81]. Tobacco control advocates might usefully strive to develop links with those working in the arts and graphics fields to disseminate their messages on how such portrayals may serve to not only reflect smoking use, but may also reinforce and reproduce such use. Omission of such imagery is the primary aim, however, substitution and relegation may be useful tactics to minimise harm.

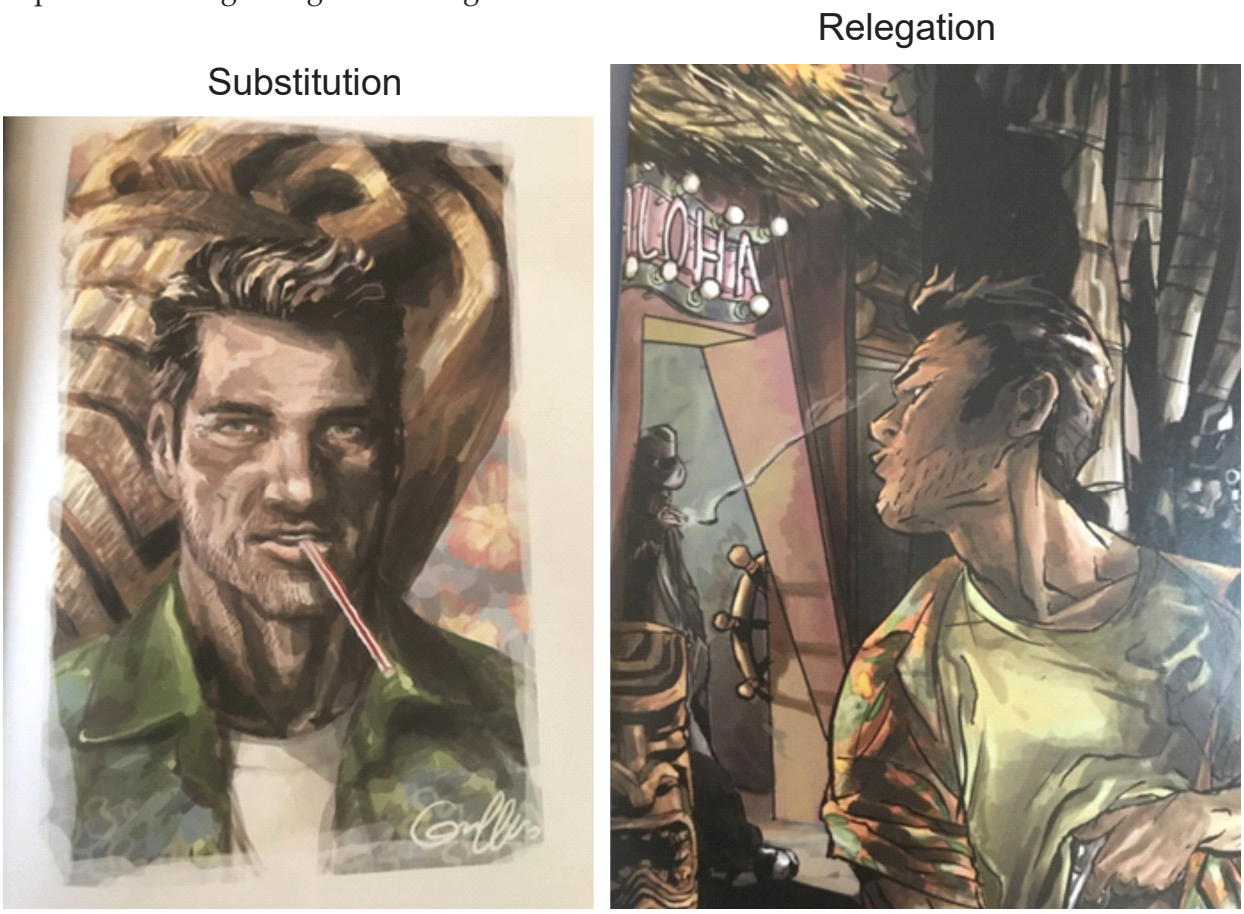

Fig.3. Examples of Substitution & Relegation From Hawaiian Dick Vol. 1: Byrd of Paradise [81]

Conclusion

Noir graphic novels are highly problematic in terms of their consistent portrayal of smoking. This research indicates that this genre of novels also routinely include a far higher proportion of women smoking than graphic novels generally [55]. In terms of women's health this is an important issue, particularly given the positive portrayal of such female smokers. Further research may usefully explore both how the noir genre continues to be linked so substantively to smoking. Further research should also explore the characters associated with smoking to move beyond quantitative content analysis, and facilitate a more nuanced ana-

lysis. Further research might also explore related issues, including: possible changes over time; association between depictions of the consumption of both alcohol and tobacco; character type shown smoking; cigarettes v's cigars and other forms of tobacco consumption.

Compliance with Ethical Standards

Conflict of Interest: Ms Daisy Houghton declares that she has no conflict of interest. Dr Frank Houghton declares that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Resumo

Tutmonda morteco de tabakrilataj malsanoj daŭre altiĝas. Plej multaj fumantoj komencas fumi en adoleskeco kaj frua plenaĝeco. Grafikaj romanoj havas specialan allogon al tiu aĝoklaso, kaj estas ĉiam pli legitaj fare de inoj kaj maskloj. Antaŭa esplorado ekzamenanta gamon da grafikaj romanoj notis altajn tarifojn de portretadoj de fumado en noir- kaj ciber-punkaj romanoj. Ĉi tiu esplorado temigis hazardan specimenon de noir-grafikaj romanoj por esplori ĉi tiun trovon pli profunde. Kvanta enhavanalizo de dek noir-grafikaj romanoj estis farita. Kvankam ekzistis granda ŝanĝebleco en la nombro da portretadoj de fumado, fuma figuraĵo estis ĝenerala ĉie en ĉiuj la romanoj ekzamenitaj. Aparte rimarkis la altan indicon de portretadoj de virinoj fumantaj. Ĉi tio estas problemo pro la pliiĝo de fumado de junaj virinoj kaj knabinoj en iuj okcidentaj landoj. Kelkaj ekzemploj de anstataŭigo kaj malavanco de tabakvaroj notiĝis, kaj tabakkontrolaktivuloj devus serĉi labori kun artistoj por antaŭenigi preterlason, anstataŭigon kaj malavancon de tia figuraĵo. Plia esplorado estas proponita esplori en pli profunde la karakterizadon de fumantoj en tiaj grafikaj romanoj, kaj la daŭranta ripetiĝo de tia figuraĵo en nuntempaj grafikaj noir-romanoj.

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FOR AUTHORS

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FILE FORMAT

Manuscripts should be saved in native format, offered by text processing software (preferably .docx, .doc, .odt). All figures (apart from being embed in text, or marked) should be saved and submitted in native separate files (.jpeg, .cdr, .cpt, .svg, .tiff) with resolution suitable for printing typically 300 dpi (or greater) for color photographs and at least 400 dpi (or greater) for black-white and gray scale drawings.

TEXT

Layout of the manuscript should be left as simple as it is possible. Text has to be in single column format. Use Times New Roman 12 with 1.5 interline throughout the manuscript and avoid unnecessary formatting. Paragraphs have to be clearly separated from each other. Italic and bold face fonts can be used just as subscripts and superscripts etc. Options in word-processing software for text justification and word hyphenation must not be used.

Mathematical formula should be written in single line format (e.g. (2+2)^-2/[(2*2)-2]=2/x^-2) or written in LaTeX®Chemical formulas should be provided in a chemical drawing software format (rarely a high resolution .tif or .jpeg can be accepted but at the redaction discretion).

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Should not be exceedingly long and should state the main research goal and methodology.

ABSTRACT

The abstract should be written in English and Esperanto, left short and state briefly research goal, results, and the most important conclusions. As *Medicina Internacia Revuo* is readily willing to accept papers from authors who speak neither Eng-

lish or Esperanto, this task might be fulfilled by the editorial board on these special occasions. References in the abstract should be avoided. Abstract should be prepared in a way allowing its presentation as a stand-alone note. The maximum number of characters is 250.

KEYWORDS

Up to six keywords can be defined, and should allow others to find the article in search forms.

REFERENCES STYLE

Vancouver, example:

Smith M, Kowalski A, Johanson MK. Medicinal activity of *Bacopa monnieri*. Med Int Rev. 2013; 1(2): 54-62.

INSTRUCTIONS FOR CITATIONS OF REFERENCES IN THE JOURNAL

In the text, sequential numbers of citations should be in order of appearence **(NOT ALPHABETICALLY)** in parentheses.

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NATURE

HAVE YOU EVER SEEN...

Geastrum fimbriatum (photo Jacek Lelek)

Phallus impudicus (photo Jacek Lelek)

*Calvatia gigantea (*photo Jacek Lelek)

Amanita strobiliformis (photo Bożena Muszyńska)