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Review

Evidence of Improvement of Lower Limb Functioning Using Hydrotherapy on Spinal Cord Injury Patients

Liliana Elena Stanciu [1], Madalina Gabriela Iliescu [1,*], Liliana Vlădăreanu [1], Alexandra Ecaterina Ciota [1], Elena-Valentina Ionescu [1] and Claudia Ileana Mihailov [2]

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[1] Department of Physical Medicine and Rehabilitation, Faculty of Medicine, "Ovidius" University of Constanta, 1 University Alley, Campus–Corp B, 900470 Constanta, Romania

[2] Department of Reumatology, Faculty of Medicine, "Ovidius" University of Constanta, 1 University Alley, Campus–Corp B, 900470 Constanta, Romania

* Correspondence: iliescumadalina@gmail.com

Abstract: Background: Spinal cord injury (SCI) is a devastating problem for modern society, whether it affects young people in the most productive period of their lives or the elderly. The spinal cord injury is currently without curative treatment and the therapeutic intervention aims to minimize secondary complications and maximize residual function through rehabilitation medicine. The main objective of this scientific paper is to determine whether there is evidence in the literature regarding the importance and/or use of hydrotherapy, as part of the therapeutic management of the SCI patient, in order to decrease the degree of spasticity, of pain symptoms, increase or maintain range of motion, improve respiratory, cardiovascular, and metabolic status, as well as improve function and psychological benefits. Methods: Using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) procedures, the following databases were analyzed between 2000 and 2021: Pub Med, Pub Med Central, Science Direct, Scopus, and SpringerLink. Initial keywords: rehabilitation treatment, spinal cord injury. Additional keywords: hydrotherapy, aqua therapy, spasticity, human. For the scientific quality of the included articles, risk of bias was assessed using the Downs and

Black Appraisal Modified Scale. Results: Our research used only four publications as per PRISMA protocol, assessed with Downs and Black Scale. The study models used in the individual studies included in the research are the following: two systematic reviews, one experimental non-randomized control, and one individual semi-structured interview. Due to the low number of studies, despite two of them being reviews, there is the necessity for a more standardized methodology to prove the benefits hydrotherapy for SCI patients for the improvement of lower limb functioning. Conclusion: Hydrotherapy is an important component of the treatment of an SCI patient, despite the limited number of scientific studies that support this aspect. Clinical trials in the future are required.

Keywords: aqua therapy; hydrotherapy; human; rehabilitation treatment; spasticity; spinal cord injury

Introduction

Spinal cord injury is a serious pathology that can cause various aspects of a patient's life to significantly deteriorate. The primary goal of rehabilitation is to improve a patient's functional level and decrease their secondary morbidity. In this paper, we review various secondary long-term complications that can occur after a spinal cord injury (SCI). Some of these include respiratory, cardiovascular, urinary, and intestinal difficulties, as well as spasticity, pain syndromes, pressure ulcers, and fractures [1].

Most of the time, treating patients with spasticity using drugs can lead to side effects. Hydrotherapy can assist in reducing the amount of medication needed as part of rehabilitation treatment [2].

Spasticity is a major issue for SCI patients, also limiting a patient's mobility and affecting their ability to perform various activities of daily life. Spasticity represents a significant challenge, both for the patient and for the rehabilitation team [3].

Medical hydrotherapy has been prescribed since Hippocrates revealed the curative virtues of water hundreds of years ago [4].

During the 1800s, Sebastian Kneipp, the 'founder of hydrotherapy', wrote extensively about the healing effects of water. His research was immediately recognized by healthcare professionals [5].

Hydrotherapy can relieve pain for patients with various conditions [6]. It can also improve their sensory perception by blocking the nociception signals [7,8]. Additionally, warm water can help nourish the body and reduce the effects of lactic acid and other chemicals in the body. It can also decrease the pain threshold and improve muscle relaxation [9,10].

Giesecke defined various aquatic exercise goals, including decreased spasticity, improved respiratory status, increased endurance, and psychological benefits. The temperature of the water is critical because excessively hot or cold temperatures may exacerbate spasticity and can also decrease the effectiveness of certain therapeutic techniques [11]. Warm waters allow individuals with a spinal cord injury to move freely in ways that would be unpleasant and difficult to do on land, and also help avoid the fear of falling. As a result, many physical and functional aims can be accomplished easier [11].

Unfortunately, aquatic therapy is not routinely available to patients with a spinal cord injury. Many of them are vulnerable to colostomies and frequent involuntary incontinence due to neurogenic bowel dysfunction [12].

According to Recio and Cabahug, aqua-based therapy could be effective for treating spinal cord-injured patients with suprapubic and Foley indwelling catheters, tracheostomy tubes, and pressure ulcers [13]. The patients exhibited no signs of dehydration or respiratory problems during the hydrotherapy sessions. Additionally, in patients with suprapubic and indwelling catheters, there was no evidence of a catheter being pulled [13–15].

The primary goal of this study is to determine the importance of hydrotherapy as a part of the therapeutic management of SCI patients on a global scale and whether there is up-to-date literature to confirm our clinical findings.

Methods

To conduct the research, the following databases: PubMed, PubMed Central, Science Direct, Scopus, and SpringerLink were analyzed between 2000 and 2021, according to the PRISMA procedures recommendations for systematic reviews [16]. Initial keywords— rehabilitation treatment, spinal cord injury. Additional keywords—hydrotherapy, aqua therapy, spasticity, human. Downs and Black Appraisal Modified Scale was used to evaluate the scientific quality of the included articles.

Titles and abstracts were read and evaluated for inclusion using the following criteria:

(1) Population (patients with neuromotor or neuromuscular deficits due to spinal cord injury);

(2) Intervention (hydrotherapy/aqua therapy).

Exclusion criteria included publications from previous time periods, publications about hydrotherapy that have as an aim other pathologies than spinal cord injury and publications that do not use English as the publishing language.

The scientifical quality of the studies was evaluated through the modified Downs and Black Scale [17,18], which is a validated and reliable method for assessing the effectiveness of randomized controlled and noncontrolled trials. The modified Downs and Black Scale has 27 items that are focused on five broad categories, namely reporting (10 items), internal validity—bias (7 items), internal validity—confounding (6 items), power (1 item), and external validity. The scale's overall score goes from 0 to 28. A study of superior quality has a higher value of the maximum possible score. The overall quality of the papers was: 80% (very good), 70–79% (good), 50–69% (fair), <50% (weak) (Downs and Black 1998).

For possible addition, one author examined titles and abstracts. Another two separately identified the full text of collected articles, with differences addressed by consensus or arbitration from a third author.

The study initially started with a search in the databases mentioned above for a shorter period, namely 2015–2021. Given the small number of articles eligible for a review to demonstrate the importance of hydrotherapy in the therapeutic management of patients with spinal cord injury, the search period was extended to 2000–2021, keeping the same databases. Unfortunately, the number of items identified as eligible did not increase significantly.

The International Committee of Medical Journal Editors suggested, in 1993, that articles submitted to biomedical journals should follow the same set of guidelines.

We chose articles published in 2000 and after because we supported that during that period the International Committee of Medical Journal Editors' suggestion had been embraced by relevant researchers and had increased the quality of reports [19].

Results

As per Figure 1 and Table 1, the algorithm used in finding and selecting the articles shows that only four remained for the final analysis. The years of publication for the articles were 2004, 2017, 2018, and 2019. The main characteristics of the articles are described in Table 2. For further information, please refer to the bibliography of the mentioned studies.

The first study selected was a study published in 2004 by Kesictas N. et al., where researchers evaluated the effectiveness of aquatic therapy on patients with spinal cord injuries. Twenty-four participants were divided into two groups. The first group received standard aquatic exercise twice a day and the other group received oral baclofen. The participants were also assessed for their muscle spasms and the effects of the treatment on their breathing and movement. The researchers also used the Ashworth scales to measure their progress. The hydrotherapeutic intervention successfully reduced spasticity. There was a statistical improvement in Ashworth scores for both study and control groups ($p < 0.01$ and $p < 0.02$, respectively).

The participants in the hydrotherapy group exhibited a significant increase in their Functional Independence Measurement (FIM) scores. They also showed a decrease in oral baclofen consumption. Regarding spasm severity, the hydrotherapy group showed a significant decrease in spasm severity ($p < 0.02$). The control group's spasm severity was 1.4, and the hydrotherapy group's was 0.7 (respectively, $p < 0.05$, $p < 0.001$).

The researchers also noted that the use of hydrotherapy led to a drop in spasm severity. This is the only clinical trial that we found using our algorithm [1].

The second study selected was a 2017 systematic review made by Chunxiao Li et al. that evaluated the effects of aquatic exercise on the physical function and fitness of people with spinal cord injury. Four studies cited in the 2017 review showed that aquatic exercise combined with physiotherapy improved the participants' functional independence. The programs were either a 10-week program or a 16-week program. A test–retest study conducted on eight participants revealed that underwater walking sessions, three times a week for eight weeks significantly improved their walking ability. A case study also showed that aquatic and land-based exercises can improve their physical fitness. The study showed that the 8-week underwater treadmill program led to a decrease in participants' daily walking heart rate. A 15-week aquatic exercise program was found to improve participants' swimming distance and force critical capacity. However, the program did not enhance the participants' forced vital capacity (FVC) and forced expiratory flow rate. A similar study conducted on a 3-year program showed that combining swimming and physiotherapy sessions helped improve participants' cardiorespiratory efficiency [1].

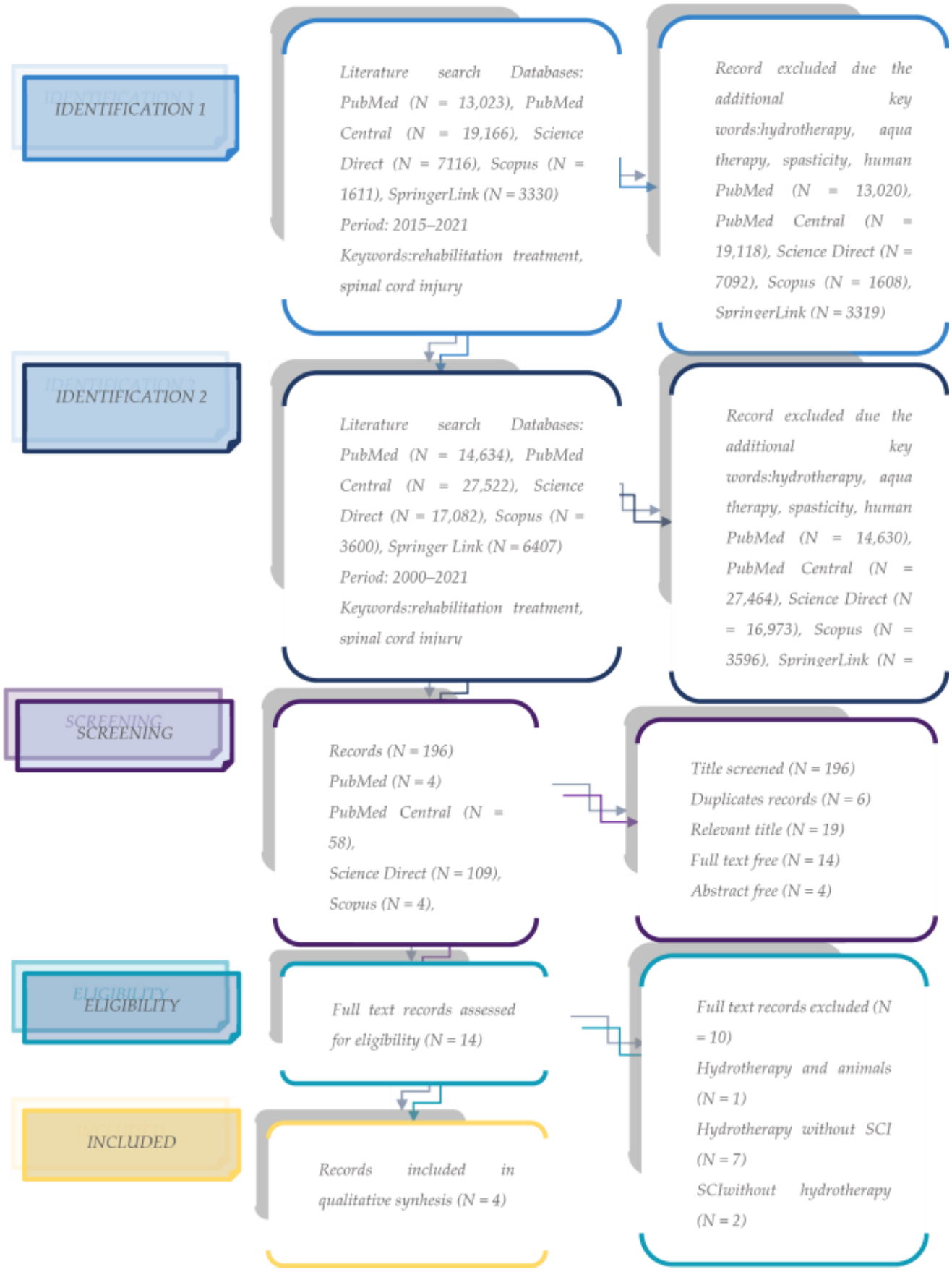

Figure 1. Flow chart of the review process. N—number of records; SCI—spinal cord injury.

The third paper that met the criteria for selection in our review was a review published in 2018 by Terry J. et al. focused on the effects of aquatic exercise on patients with spinal cord injuries. The study analyzed the effects of different types of aquatic exercise on different aspects

of health. It was theorized that the effects of buoyancy and hydrostatic pressure on the patients' lumbopelvic hip complex and force closure improved their underwater walking. Additionally, the effects of buoyancy on the hip swing were proven to counteract the gravity-based effects of walking. The researchers also noted that the energy expenditure of patients decreased during underwater walking sessions [20,21]. The 1998 study conducted by Zamparo and Pagliaro from this 2018 [22] review was limited by its design and did not follow the usual follow-up procedures. As a result, it was not able to provide sufficient evidence supporting the study's findings. While submerged in warm water, an exerciser's heart rate lowers and enhances the thermoregulatory response of the body, which helps prolong the participant's ability to exercise. This benefit is evidenced by the fact that water provides the ideal conductor of heat, which helps the body maintain a low core temperature. This allows the exerciser to endure longer and improve their energy-use efficiency. A study published in 2004 focused on the effects of hydrotherapy on SCI patients. The researchers discovered that a reduction in the dosage of the antiinflammatory drug Baclofen significantly reduced muscle spasticity [22]. According to a 2009 study, the physiological effects of hydrotherapy on various conditions are still not clear. Further studies are needed to analyze the mechanism that influences this treatment's effectiveness. The effects of hydrostatic pressure on the body are similar to those of Boyle's law. Breathing underwater increases the body's pressure, which makes breathing more costly. According to Becker, a patient's vital capacity decreases by about 6% to 9% after performing underwater exercises due to hydrostatic pressure. This effect counters the inspiratory muscle action of underwater exercises. A study conducted in 1980 noted that SCI patients who participated in aquatic exercises improved their respiratory fitness. Other studies also suggest that performing aquatic exercises can improve a person's expiratory muscle strength [22].

The fourth study we selected was a 2019 study made by Andresa R et al. that discussed the use of aquatic therapy by rehabilitation professionals for patients with spinal cord injuries. The researchers stated that aquatic therapy is a useful tool for improving the adherence of patients to their treatment. It does not require a standard technique or design [23].

Table 1. Appraisal of records according to the modified Downs and Black Appraisal Scale.

	Downs and Black Appraisal					
Authors	**Reporting (*n* = 5)**	**External Validity (*n* = 3)**	**Internal Validity (*n* = 3)**	**Power (*n* = 5)**	**Total (*n* =16)**	**Grading % = x/16 × 100**
N. Kesiktas, N. Paker, N. Erdogan, G. Gülsen, D. Biçki, and H. Yilmaz (2004) [1]	5	3	2	4	14	87.5 % (very good)
C. Li, S. Khoo, A. Adnan (2017) [19]	5	3	3	4	15	93.75% (very good)
T. J. Ellapen, H. V. Hammill, M. Swanepoel, G. L. Strydom (2018) [20]	5	3	3	4	15	93.75% (very good)
A. R. Marinho-Buzelli, A. J. Zaluski, A. Mansfield, A. M. Bonnyman, K. E. Musselman (2019) [22]	5	1	3	3	11	68.75% (fair)

N, number; x, sum of Downs and Black appraisal.

Due to the low number of studies supporting the effectiveness of aquatic therapy for patients with spinal cord injuries, further studies are needed to establish a standard procedure for conducting follow-up studies.

In our research, we were able to use only four publications, assessed with Downs and Black Scale as shown in Table 1. Due to the low number of studies, there is need for further studies and standardized methodology to prove the benefits of hydrotherapy for SCI patients.

Table 2. Chronological overview of the characteristics and findings of the records (*n* = 4).

Characteristics of the Study				
Authors	**Type of Study**	**Sample**	**Method**	**Findings**
N. Kesiktas, N. Paker, N. Erdogan, G. Gülsen, D. Biçki, and H. Yilmaz (2004) [1]	Experimental non-randomised control	Hydrotherapy group: 10, mean age 32.13 ± 8.34, gender: 2 females and 8 males, injury time (months): 8.6 ± 5.5, FIM: 52 ± 14.13, Ashworth Score 3 ± 0.92, Oral Baclofen (mg) 96 ± 12, Etiology (accident) 50%. Control group: 10, mean age 33.10 ± 10.71, gender: 3 females and 7 males, injury time (months): 7.70 ± 6.06, FIM: 54.70 ± 18.8, Ashworth Score 2.50 ± 1.18, Oral Baclogen (mg) 100 ± 0, Etiology (accident) 50%.	The hydrotherapy group received 20 min of underwater exercises at 71 º F (21.6 º C) 3 times/week, and also participated in the usual rehabilitation, which included passive range of motion 2 times/day, psychotherapy and oral baclofen for 10 weeks. The control group were able to maintain their usual activities through the conventional rehabilitation program.	The hydrotherapeutic intervention successfully reduced spasticity and oral baclofen doses and raised FIM scores compared to control group.
C. Li, S. Khoo, A. Adnan (2017) [19]	Systematic review	A total of 143 participants with SCI were reported and the sample size of each study ranged from 1 to 60. More male participants were reported than female (male = 91, female = 52). Participants were adults aged between 18 and 63 years. A total of seven of eight studies reported participants' injury levels on the spinal cord (the study by Pachalski and Mekarski did not report the specific injury level). Only 4 studies provided the grade of ASIA impairment scale. There was a big range in terms of postinjury time from 7 months to 28 years. In terms of study design, 3 were controlled clinical trials, 2 single group test–retest designs, 1 randomized controlled trial, 1 single-subject design, and 1 case study.	Eight of 276 studies met the inclusion criteria, of which none showed high research quality. Four studies assessed physical function outcomes and 4 studies evaluated aerobic fitness as outcome measures. Significant improvements on these 2 outcomes were generally found. Other physical or fitness outcomes including body composition, muscular strength, and balance were rarely reported.	There is insufficient evidence to support the efficacy of aquatic exercise on increasing physical function and aerobic fitness among SCI patients. We cannot yet draw any conclusion about the effectiveness of underwater training on body composition, muscular strength, and balance among the study population.

Table 2. *Cont.*

Characteristics of the Study				
Authors	**Type of Study**	**Sample**	**Method**	**Findings**

T. J. Ellapen, H. V. Hammill, M. Swanepoel, G. L. Strydom (2018) [20]	Systematic review	A total of 142 participants were reported (but 83 PWSCI), with sample sizes varying from 1 to 30 and participant age varying from 5 to 70 years. Five studies provided kinanthropometric characteristics, whereas 5 studies considered the number of years injured, and 10 studies described the aquatic exercise intervention. The overall quality of the studies was rated as fair (62.0%).	A literature surveillance was conducted between 1998 and 2017, through the Crossref meta-database and Google Scholar, according to the PRISMA procedures. Key search words were water-therapy, aquatic-therapy, hydrotherapy, spinal cord injury, rehabilitation, human, kinematics, underwater gait, cardiorespiratory, thermoregulation and spasticity. The quality of each paper was evaluated using a modified Downs and Black Appraisal Scale. The participants were recorded pertaining to SCI and hydrotherapy. The outcomes of interest were hydrotherapy interventions, the impact of hydrotherapy on gait kinematics, thermoregulation during water submersion, and cardiorespiratory function of PWSCI.	Hydrotherapy increases PWSCI underwater gait kinematics, cardiorespiratory and thermoregulatory responses and reduces spasticity.
A. R. Marinho-Buzelli, A. J. Zaluski, A. Mansfield, A. M. Bonnyman, K. E. Musselman (2019) [22]	Individual semi-structured interviews	None mentioned.	Six PT (2 male, 4 female), three PTA (female) and 1 KIN (female) participated. The following four themes were identified: (1) multi-system benefits from AT (e.g., from impairment to function, confidence, and enjoyment); (2) application of AT; (3) perceived barriers to implementing AT; and (4) water as an enabler to function on land. All were interviewed regarding their clinical findings while working with SCI patients in aquatic environment.	The participants reported AT was a unique and fickle approach that benefits the multi-dimensional aspects of the health of individuals with SCI/D. They inserted AT very well into their clinical practice despite the barriers professionals and clients face.

AT—Aqua therapy; PWSCI—Patient/SCI patients; SCI—Spinal cord injury; FIM—Functional independence measure; PTs—Physical therapists; PTA—PT assistants; KIN—kinesiologists; ASIA—American Spinal Cord Injury Association.

Discussion

The use of hydrotherapy in the treatment of the SCI patient decreases spasticity, increase underwater functioning of the lower limbs and gait kinematics, increase cardiorespiratory and thermoregulatory status, significant complications that can create a varying degree of disabilities. This therapy has a different mechanism of action on these complications through all the properties that therapeutic water can present: physical, chemical, and mechanical properties [1,20,24].

As it appears in the data from the specialized literature, spasticity can develop months or years after the acute spine cord injury and lead to severe function loss and hospitalization [25]. According to studies, 65–78% of the patients with chronic SCI (≥1 year postinjury) experience spasticity symptoms [26]. Up to 5 years after the spine cord injury, one-third of all patients have difficulties with spasticity [27]. Any medical intervention that leads to an improvement in neurological symptoms is vital for patient therapeutical

management. Future clinical trials are needed to make significant arguments about the role of hydrotherapy.

Risk factors for cardiovascular diseases are increased in SCI patients [28]. SCI patients have considerably reduced daily energy expenditure due to a lack of motor function, as well as fewer opportunities to participate in physical exercise [29]. Additionally, SCI is associated with abnormal blood pressure, heart rate variability, arrhythmias, and a decreased cardiovascular response to exercise, which can restrict physical activity capacity [29]. Improving cardiorespiratory status through hydrotherapy is an important element for the SCI patient's compliance in therapeutic management and the subsequent evolution of cardiac symptoms. Improving cardiovascular status through hydrotherapy is an important element for the SCI patient's compliance with treatment and subsequent cardiac and respiratory symptoms evolution.

The possible effect of increasing underwater functioning of the lower limbs and gait kinematics is an important element that supports the need for future scientific studies on the role of hydrotherapy in the SCI patient.

Design and intervention methods are required for a successful study. They play an essential role in improving the quality of the study and its results.

A significant number of clinical case reports were discovered in the context of the research articles included. Although clinical case reports are not considered objective sources of information, they still play a vital role in the development and dissemination of medical knowledge.

The importance of clinical trials is often neglected. Although they may seem simple, they are conducted through a rigorous process that is governed by ethical principles.

Randomized controlled trials are often used to evaluate the effectiveness of interventions and have been acknowledged as the gold standard because they are considered one of the most powerful types of evidence for use in the development of evidence-based clinical therapeutical programs [30]. However, randomized controlled trials have often been poorly reported in medical journals [31,32].

It is essential to find solutions for implementing randomized controlled trials in medical rehabilitation units, to support the use and effectiveness of balneal factors in the treatment of various orthopedic and neurologic diseases [32], and many literature data with different studies in various pathology can be used as model [33].

The fact that when the PRISMA algorithm was applied the number of articles that could be included in the study in the current review was minimal compared to the vast number of patients with neurological pathology studied is of importance in medical rehabilitation as well as a treatment methodology, and arguments were sought for this aspect. One of the hypotheses is that there is an actual development of other treatments, such as robotic, computerized, or immersion in virtual reality.

The exoskeleton systems represent a new therapeutic approach in the rehabilitation program of these patients and can be successfully used for gait re-education, proposing kinetic models of the exoskeleton with permanent control over the angle, and angular velocity at the level of each joint, as well as over the parameter muscles (muscle parameters), offering the patient continuous passive movement [34,35]. This new therapy, along with the safety offered to the patient during treatment, reduces the therapist's workload and therapy cost [34]. The cost level of a rehabilitation program will always represent a significant concern of any health service so that as many patients as possible can benefit from rehabilitation services. The exoskeleton has much potential to maintain good health and improve the QOL in individuals with SCI. Of course, future studies are needed in this therapeutic sector as well.

Another therapeutic class used in the treatment of spasticity in patients with spinal cord injury in current medical rehabilitation is botulinum toxin. There are standardized schemes that can also be customized for the individual patient's treatment situation and the patient's target muscles, and the treatment is more effective in the first 6 months [36–38].

The strength of the study is as follows: The number of patients with neurological pathology in the field of spinal cord injury is very high, and the disability resulting from this pathology has a significant impact on the quality of life of both the patient and his family. For this reason, it is necessary to investigate all the therapeutic possibilities used to increase independence and maintain the existing functional remainder.

pathologies, with a therapeutic impact on several systems, as discussed in the Section 1. The study was based on the PRISMA research algorithm necessary for conducting systematic reviews. The period included in the research is essential, namely 2000–2021. The databases included in the research have medical scientific value, with broad international recognition: PubMed, PubMed Central, Science Direct, Scopus, and SpringerLink. The subject is of interest both for the medical rehabilitation specialty and for neurological specialists because the therapy studied with an impact on the symptomatology that induces a high degree of disability can lead to a decrease in the doses of medication with a neurological impact, which increases the patient's compliance.

The limitations of the study are as follows: The small number of studies in specialized literature regarding the impact of hydrokinetotherapy on patients with spinal cord injury makes it impossible to carry out a meta-analysis on this subject and very difficult to carry out a review that respects all the stages imposed by the PRISMA algorithm. The fact that the review was not pre-registered in an international prospective register of systematic reviews, such as PROSPERO, and a funnel plot was not made are also a limitation of this review. In addition to the limited number of existing scientific studies, there are other drawbacks, such as heterogeneous methodologies, disparate study populations, and different training programs, which must be overcome in future studies.

Conclusions

Due to the low quality of the controlled studies and the lack of sufficient follow-up randomized controlled trials, there is not enough evidence supporting the importance of hydrotherapy for the functional rehabilitation of patients with spinal cord injury. Hydrotherapy's physiologic effects in orthopedic recovery and neurological disease must be investigated further. Financial support for such studies and the required clinical trials are still an issue. Despite its significant therapeutic benefits, aquatic exercise and balneal rehabilitation are underutilized.

Rehabilitation techniques must represent the main purpose of multiple and important qualitative scientific studies because medical rehabilitation represents a key role in spinal disorders such as SCI.

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The Importance of Rehabilitation Programs Using Balneary Treatments in Patients with Spinal Cord Injury

1. Doinita Oprea [1,2,†], Madalina Gabriela Iliescu [1,2,*], Elena Valentina Ionescu [1,2,*], Liliana Elena Stanciu [1,2], Lucian Petcu [1,†], Sorin Chiriac [2], Andra Maria Stefan [3], Diana Victoria Gidu [4,†], Antoanela Oltean [4], Viorela Mihaela Ciortea [5,†] and Carmen Oprea [1,2]

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[1] Faculty of Medicine, Ovidius University of Constanta, 1 University Alley, Campus-Corp B, 900470 Constanta, Romania

[2] Balneal and Rehabilitation Sanatorium of Techirghiol, 34-40 Dr. Victor Climescu Street, 906100 Constanta, Romania

[3] Emergency County Hospital "St. Andrew" Constanta, 145 Tomis Boulevard, 900001 Constanta, Romania

[4] Faculty of Physical Education and Sport, Ovidius University from Constanta, 1 Cpt. Aviator Alexandru S, erbănescu, 900470 Constanta, Romania

[5] Department of Rehabilitation, "Iuliu Hatieganu" University of Medicine and Pharmacy, 40012 Cluj-Napoca, Romania

* Correspondence: iliescumadalina@gmail.com (M.G.I.); elena_valentina_ionescu@yahoo.com (E.V.I.) † These authors contributed equally to this work.

Abstract: The rehabilitation tools that are designed to improve the function of patients with spinal cord injury (SCI) have various effects. The goals of rehabilitation are to prevent secondary complications, maximize physical functioning, and integrate them into the community. The objective of this study is to evaluate the functional and neurological outcomes of patients with SCI after in-patient rehabilitation in a balneary unit. Methods: one hundred forty-two patients, admitted for primary rehabilitation in a two-year period (2020–2021), aged ≥18 years with SCI, divided into traumatic SCI (T-SCI) and nontraumatic SCI (NT-SCI). The following demographic information was collected: gender, age, studies, occupation, and environment. All patients underwent an initial clinical examination which included diagnosis, causes of SCI, medication, Carmeli score, fall risk, Visual Analogue Scale (VAS) for pain, Functional Independence Measure Motor (FIMm), Functional Independence Measure Cognitive (FIMc), and Functional Independence Measure Total (FIMt). At discharge, the fall risk, VAS, FIMm, FIMc, and FIMt were analyzed. We compared the results between the two groups. Results: T-SCI group was 65 (45.77%) and the NT-SCI group was 77 (54.23%). The study analyzed the effects of rehabilitation on the functional presentation of patients with SCI. It also compared the effects of rehabilitation on T-SCI versus NT-SCI on different outcomes such as age, gender, and clinical–functional impairment. Conclusions: Physical medicine and rehabilitation increase the autonomy of patients. Neurological improvement begins in the first 10 days of complex rehabilitation treatment and is not significantly different between the two groups. The cause of the injury in SCI does not affect the results of the rehabilitation.

Keywords: traumatic; nontraumatic; spinal cord injury; fall risk; VAS; Carmeli; FIM; rehabilitation

1. Introduction

The spinal cord is the main pathway for communicating with the rest of the body. It is damaged by a spinal cord injury (SCI), which disrupts the signals that the brain sends to the body. Most SCIs happen when a blow breaks or dislocates the bones that make up the spine. They can also cause damage by fragments of vertebrae hitting the nerve tissue [1].

SCIs can result from damage to the vertebrae, ligaments, or disks of the spinal column, or to the spinal cord itself, and can be divided into two subgroups on the basis of their etiology: traumatic and nontraumatic. A traumatic spinal cord injury (T-SCI) can stem from

a sudden, traumatic blow to the spine that fractures, dislocates, crushes, or compresses one or more of the vertebrae. It can also result from a gunshot or knife wound that penetrates and cuts the spinal cord. A nontraumatic spinal cord injury (NT-SCI) is a serious injury that can cause significant damage to the cord. It can be caused by various factors such as arthritis, cancer, and disk degeneration [1].

Spinal cord and neck injuries are common causes of disability among young healthy individuals. The cost of care and rehabilitation for these injuries can bring important socioeconomic consequences. Over the past several decades, the mean age of the spinal cord-injured patients has increased, which is attributed to a substantially greater proportion of injuries related to falls in the elderly. Cervical spine injuries, of which approximately one-third occur in the craniocervical junction [2], account for the majority of the spinal injuries, followed by thoracolumbar fractures. Almost half of the spinal injuries result in neurological deficits, often severe and sometimes fatal. Survival is inversely related to the patient's age and neurologic level of injury, with lower overall survival for high quadriplegic patients compared to paraplegic injuries [2].

An SCI is a medical emergency. Immediate treatment can reduce the long-term effects. Treatments may include drug treatment, braces or traction to stabilize the spine, and surgery. Later treatment usually includes pharmaceutical treatment and rehabilitation therapy. Mobility aids and assistive devices may help to get around and do some daily tasks [3].

The quality of life for individuals with an SCI is often influenced by various factors such as physical health, socioeconomic status, and complications [4]. The interventions that are focused on reducing these secondary injuries and complications have a variety of goals. In addition to the conventional drug management, robotic-assisted locomotor training, gait training strategies, specific exercises (including hydrokinetotherapy), functional electrical stimulation devices, and repetitive transcranial magnetic stimulation devices are universally recommended to improve function in persons with SCIs [4].

The primary goals of rehabilitation are the prevention of secondary complications, the maximization of physical functioning, and reintegration into the community [3]. Rehabilitation following an SCI is most effectively undertaken with a multidisciplinary, team-based approach, as follows [3,5]: physical therapists typically focus on lower-extremity function and on difficulties with mobility, occupational therapists address upper-extremity dysfunction and difficulties in activities of daily living, rehabilitation nurses are concerned with the issues of bowel and bladder dysfunction and the management of pressure injuries (pressure ulcers), psychologists deal with the emotional and behavioral concerns of the newly injured patient and with any potential cognitive dysfunction, speech-language pathologists address with issues of communication and swallowing, case managers and social workers are the primary interface between the rehabilitation team, the patient, and his or her family, and the payer source. The rehabilitation team functions under the direction of a physiatrist (a physician who specializes in physical medicine and rehabilitation) or a physician with a subspecialty certification in spinal cord medicine [5,6]. So, an SCI is a serious disease that can cause various aspects of a patient's life to significantly deteriorate. The primary goal of rehabilitation is to improve a patient's functional level and decrease their secondary morbidity. In this paper, we tried to determine the importance of rehabilitation as a part of the therapeutic management of patients with SCIs in order to evaluate the functional and neurological status at admission and discharge, and factors associated with functional status among patients with traumatic and nontraumatic SCIs.

2. Materials and Methods

2.1. Study Model

We performed a retrospective cohort study that analyzed the full medical records of 142 patients in a two-year period (1 January 2020–31 December 2021), admitted for primary rehabilitation in a single center, Balneal and Rehabilitation Sanatorium Techirghiol (BRST), Romania. The unit is representative of Romania's medical rehabilitation services, being a single-specialty hospital with a total of 935 hospital beds and using natural saline water. All the subjects provided the agreement to participate in this study. The study was approved by the Sanatorium Ethical Committee (approval no. 1733 from 2 February 2022) and complied with the revised ethical guidelines of the Declaration of Helsinki. Patients were diagnosed and referred to BRST by the neurologist and the family physician who established the diagnosis.

2.2. Study Population

Patients were selected based on the following inclusion criteria: free consent, based on the explanation and understanding, respectively, of all related procedural steps; age over 18 years; traumatic and nontraumatic spinal cord injury. The exclusion criteria were: neurological diseases of infectious cause during the period of contagion; multiple sclerosis; commitment; decubitus ulcer; pregnancy and/or lactation; uncompensated organ/apparatus sufferings: cardiovascular, hepatic, renal, respiratory; major psycho-organic and/or psychiatric suffering. All patients included in the group followed a standard daily treatment for 10 days which included a major procedure of hydrokinetotherapy in saline water (the water of Techirghiol lake, which is strongly hypertonic, and has a concentration of about 80 g of mineral salt per liter and total mineralization of 52 g/L) and three minor procedures: electrotherapy, massage, and kinesiotherapy [7]. The following demographic information was collected: gender, age, studies, occupation, and environment. All patients underwent an initial clinical examination which included diagnosis, causes of SCI, medication, Carmeli score, fall risk, Visual

Analogue Scale (VAS) for pain, Functional Independence Measure Motor (FIMm), Functional Independence Measure Cognitive (FIMc), and Functional Independence Measure Total (FIMt). At discharge, we analyzed the fall risk, VAS, FIMm, FIMc, and FIMt (the procedures used in the evaluation of the patients in the study are those used in our hospital, according to the internal work protocols, and the protocols are established by the department head doctors, according to the recommendations of the quality control organization in Romania). We compared the results between the two groups.

2.3. Study Objectives

This study was conducted to be a descriptive study about T-SCI and NT-SCI patients, in terms of their demographic and neurological features, the causes of the SCI, the medication, and the Carmeli score in order to investigate the efficient results of rehabilitation. The second aim of the study was to conduct a comparison between T-SCI patients and NT-SCI patients regarding changes in functional status: fall risk, VAS, FIMm, FIMc, and FIMt after 10 days of a complex rehabilitation program.

2.4. Data Analysis

The statistical analysis was performed using International Business Machines Corporation-Statistical Package for the Social Sciences (IBM SPSS) statistics software version 23. Data are presented as mean ± standard deviation (SD) for continuous variables in case of symmetric distributions, median and IQR (Interquartile range) for numerical discrete variables or continuous variables in case of skewed distributions, or as frequencies and percentages for categorical variables. The normality of the continuous data was estimated with Kolmogorov–Smirnov Tests of Normality. For hypotheses testing: Independent Samples Mann–Whitney U test, Independent Samples Median test, Related Samples Wilcoxon Signed Rank Test, Chi-Square Test of association, and the Chi-Squared test for the comparison of two proportions were used depending on the type of analyzed variables. The probability of a Type I error (the significance level α) was set at 0.05. If the test statistic for every conducted test was in the critical region, and the *p*-value was less than or equal to the significance level, we decided to reject the null hypothesis in favor of the alternative hypothesis.

3. Results

3.1. Study Population

Medical records from 142 patients were reviewed; they consisted of 65 (45.77%) T-SCI patients and 77 (54.23%) NT-SCI patients. Demographic characteristics are presented in Table 1. The average age of the T-SCI patients was 47.95 ± 14.67 years and for the NT-SCI patients, 51.56 ± 13.98 years, respectively. Patients with NT-SCI were older than patients with T-SCI (64.9% of the NT-SCI group were more than 50 years). In the T-SCI group, 16 (24.6%) of the patients were females and 49 (75.4%) were males. In the NT-SCI group, 37 (48.1%) of the patients were females and 40 (51.9%) of them were males. The majority of the patients are from a rural environment, 58 (75.3%) from NT-SCI and 42 (64.6%) from T-SCI. When the patients were evaluated on the basis of occupation/profession, which we found in the T-SCI group consisted of 8 working individuals, 34 retired individuals, 1 unemployed, and 22 with disability certificates according to Romanian law, whereas for the patients in the nontraumatic group, we found 17 working individuals, 38 retired individuals, 2 unemployed, and 22 with disability certificates. When the two groups were compared, the percentage of patients who were retired represented the majority in both groups (T-SCI 52.3% and NT-SCI 49.4%). We also analyzed the medication for both groups (Table 1) and we noticed, in the T-SCI groups, most took no medication (40%), followed by antispasmodic (35.38%), and in NT-SCI group, the representative category was antihypertensive (46.75%). Concerning the Carmeli score at admission (Table 1), we found that there were no statistically significant differences between the median values of the Carmeli Score across the categories of etiology (T-SCI/NT-SCI): $p = 0.478 > \alpha = 0.05$ (Independent Samples Median test), and also that the distribution of the Carmeli score was the same across the categories of etiology (T-SCI/NT-SCI): $p = 0.180 > \alpha = 0.05$ (Independent Samples Mann–Whitney U test).

Cause of injury in T-SCI patients (Figure 1) consisted of 28 (43.08%) road/work accidents, 18 (27.69%) direct aggression trauma, and 19 (29.23%) falls. We measured the duration of trauma in weeks (mean 19.41, standard deviation 15.64, minimum 2, maximum 84). Regarding the cause of injury in the NT-SCI (Figure 1) patients, it consisted of syringomyelia 1 (1.30%), transverse myelitis 8 (10.39%), spondylosis with myelopathy 24 (31.17%), cervical disc disorder with myelopathy 15 (19.48%), lumbar disc disorder with myelopathy 9 (11.69%), vertebral fracture other than traumatic 10 (12.99%), medullary/vertebral carcinoma 4 (5.19%), Morb Pott-operated sequelae 2 (2.60%), sequelae operated on the dorsal arteriovenous malformation 2 (2.60%), and poliomyelitis sequelae 2 (2.60%).

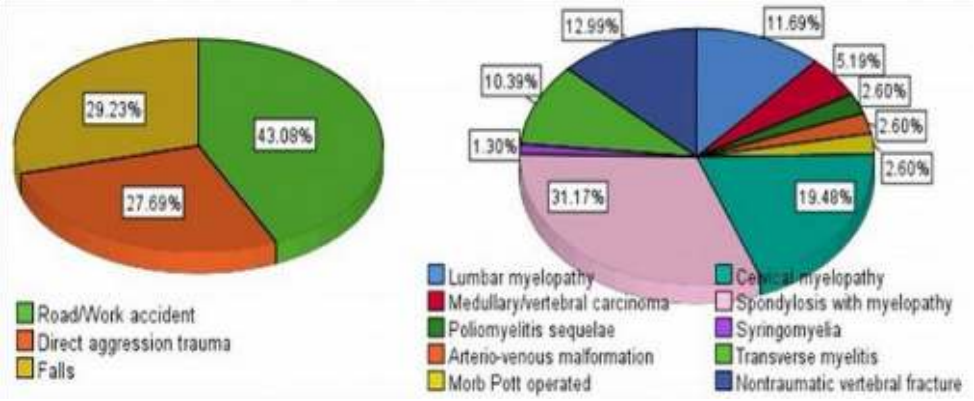

Figure 1. Causes of T-SCI (**left**) and causes of NT-SCI (**right**).

Table 1. Patients characteristics.

Variable	T-SCI (*N = 65)*	NT-SCI (*N = 77)*
Age at admission 18–30		
	8 (12.3%)	6 (7.8%)
30–50	25 (38.5%)	21 (27.3%)
50–90	32 (49.2%)	50 (64.9%)
Sex Male		
	49 (75.4%)	40 (51.9%)
Female	16 (24.6%)	37 (48.1%)
Occupation Unemployed		
	1 (1.5%)	2 (2.6%)
Employed	8 (12.3%)	17 (22.1%)
Retired	34 (52.3%)	38 (49.4%)
Disability Certificate	22 (33.8%)	20 (26.0%)
Environment Urban		
	23 (35.4%)	19 (24.7%)
Rural	42 (64.6%)	58 (75.3%)
Medication No medication	26 (40.00%)	22 (28.57%)
Antispasmodics	23 (35.38%)	10 (12.99%)
Antihypertensive	18 (27.69%)	36 (46.75%)
Lipid-Lowering Drugs	3 (4.62%)	7 (9.09%)
NSAID	2 (3.08%)	3 (3.09%)
Pain relievers	3 (4.62%)	0 (0.00%)
Myorelaxants	8 (12.31%)	6 (7.79%)
Food supplements	16 (24.62%)	11 (14.29%)
Antidiabetics drugs	2 (3.08%)	2 (2.60%)
Psychiatric medication	2 (3.08%)	3 (3.90%)
Anticonvulsants	4 (6.15%)	5 (6.49%)
Carmeli Score		
Mean ± SD	1.78 ± 0.52	1.68 ± 0.64
Median	2.00	2.00
Range	1.00	1.00

Abbreviations: T-SCI: traumatic spinal cord injury; NT-SCI: nontraumatic spinal cord injury; NSAID: nonsteroidal anti-inflammatory drugs; SD: standard deviation.

3.2. Clinical–Functional Status

Regarding the functional status, we analyzed the evolution of the fall risk, VAS, FIMm, FIMc, and FIMt at admission and discharge.

We found statistically significant differences between the median values of each variable across categories of etiology (T-SCI/NT-SCI): $p < 0.001 < \alpha = 0.05$ (Independent Samples Median test), and we also found that the distribution of scores for each variable was not the same across categories of etiology (T-SCI/NT-SCI): $p < 0.001 < \alpha = 0.05$ (Independent Samples Mann–Whitney U test), separately at admission and at discharge. So, comparing the two groups from the point of view of the clinical–functional parameters analyzed in the dynamics, we note that patients with traumatic lesions and nontraumatic

lesions showed a significant increase of FIMm, FIMc, and FIMtm, FIMc, and FIMt ($p < 0.05$). Moreover, the decrease in fall risk and VAS were statistically significant in both groups after 10 days of treatment ($p < 0.05$).

In the following, for each clinical–functional variable (Table 2), we will detail the evolution, specifying for each: minimum (min), maximum (max), mean, median, and IQR.

Table 2. Clinical–functional parameters of T-SCI/NT-SCI.

Etiology		Mean	Median	Standard Deviation	Minimum	Maximum	IQR
T-SCI	Fall risk at admission	3.78	3.00	1.92	1.00	7.00	3.00
	Fall risk at discharge	3.52	3.00	1.97	0.00	7.00	3.00
	VAS at admission	3.95	5.00	2.85	0.00	9.00	5.50
	VAS at discharge	2.42	3.00	1.89	0.00	8.00	2.50
	FIMm at admission	18.12	20.00	6.42	6.00	27.00	12.00
	FIMm at discharge	18.51	21.00	6.24	9.00	27.00	12.00
	FIMc at admission	11.42	12.00	1.48	8.00	13.00	3.00
	FIMc at discharge	11.58	12.00	1.39	8.00	13.00	2.00
	FIMt at admission	29.22	32.00	8.00	13.00	40.00	14.50
	FIMt at discharge	29.65	33.00	7.80	15.00	40.00	15.00
NT-SCI	Fall risk at admission	2.44	2.00	2.18	0.00	8.00	2.00
	Fall risk at discharge	2.27	2.00	2.15	0.00	8.00	2.00
	VAS at admission	5.91	6.00	2.24	0.00	10.00	2.00
	VAS at discharge	3.12	3.00	1.62	0.00	7.00	2.00
	FIMm at admission	21.61	23.00	5.78	8.00	28.00	5.00
	FIMm at discharge	22.12	23.00	5.71	9.00	28.00	4.50
	FIMc at admission	12.08	12.00	1.13	9.00	13.00	1.00
	FIMc at discharge	12.14	13.00	1.13	9.00	13.00	1.00
	FIMt at admission	33.38	35.00	7.43	12.00	41.00	7.00
	FIMt at discharge	33.40	35.00	8.07	4.00	41.00	6.00

Abbreviations: T-SCI: traumatic spinal cord injury; NT-SCI: nontraumatic spinal cord injury; VAS: Visual Analogue Scale; FIMm: Functional Independence Measure Motor; FIMc: Functional Independence Measure Cognitive; FIMt: Functional Independence Measure Total; IQR: interquartile range

Fall risk (Figure 2) at admission was higher in the T-SCI group versus the NT-SCI group, while VAS (Figure 3) at admission was lower in the T-SCI group than in the NT-SCI group. At discharge, both parameters, fall risk and VAS, decreased in both the T-SCI group and the NT-SCI group.

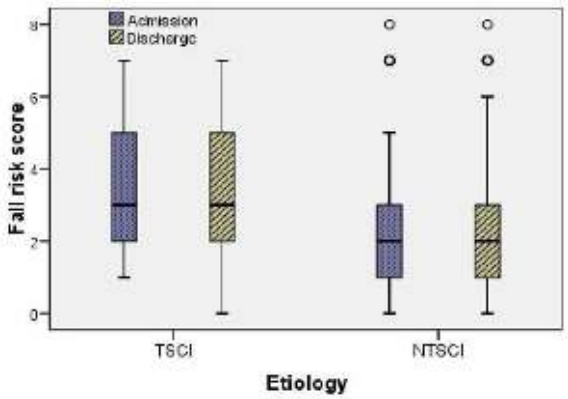

Figure 2. The distribution of fall risk scores across categories of etiology (T-SCI/NT-SCI).

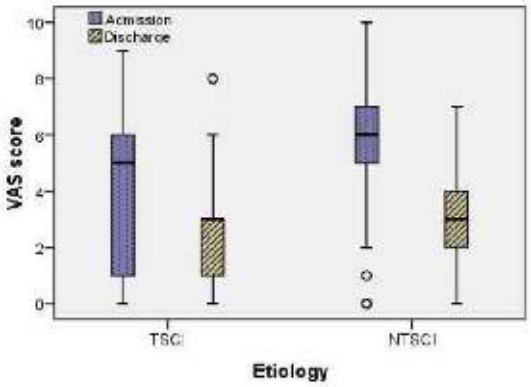

Figure 3. The distribution of VAS scores across categories of etiology (T-SCI/NT-SCI).

In the traumatic group, we analyzed the values of the FIMm scores (Figure 4) at admission and at discharge. In the nontraumatic group, the value of FIMm at admission increased at discharge. We also analyzed the FIMc (Figure 5) at admission and discharge and the results were comparable, with an increase in both groups. The comparison of the admission FIMt scores (Figure 6) between the two groups revealed that the traumatic SCI group had lower scores than the nontraumatic SCI group. Both values, the FIMt scores for T-SCI and FIMt scores for NT-SCI, increased at discharge, which were statistically significant ($p < 0.05$).

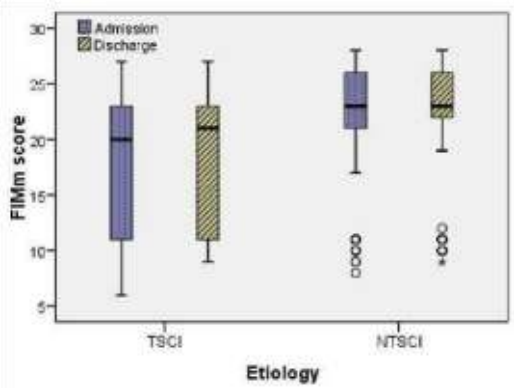

Figure 4. The distribution of FIMm scores cross categories of etiology (T-SCI/NT-SCI).

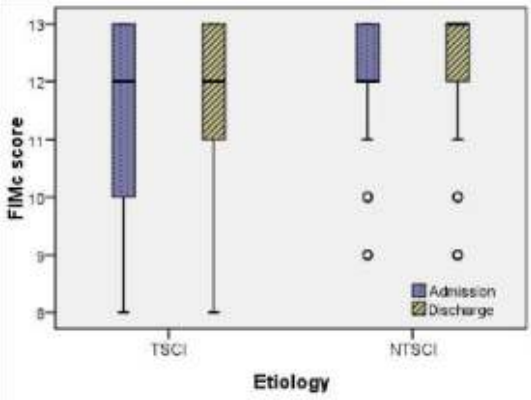

Figure 5. The distribution of FIMc scores across categories of etiology (T-SCI/NT-SCI).

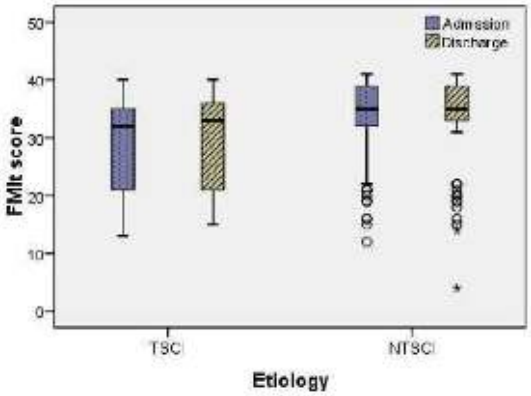

Figure 6. The distribution of FIMt scores across categories of etiology (T-SCI/NT-SCI).

4. Discussion

Rehabilitation following an acute SCI is often considered to improve a person's clinical outcomes. However, the complexity of the interventions used has made their evaluation difficult. Interdisciplinary rehabilitation refers to the treatment of multiple individuals. It can be challenging to determine which intervention can improve a person's recovery. Compared to standard rehabilitation programs, interdisciplinary programs involve the simultaneous care of multiple people. The content of rehabilitation varies depending on the context, jurisdiction, funding, or health care policy. For instance, the type of facility that accepts patients varies depending on the region and population [3].

The conditions of rehabilitation are also affected by the environment and the population. For instance, the BRST rehabilitation center is located in a region that is near the Black Sea. The facility has been providing rehabilitation services to injured individuals for over a century. Its location and services are also influenced by the area's natural cure factors. In our hospital, the daily treatment is standard: hot saline bath prescribed and performed daily as the only major hydrokinetic therapy procedure, at 35 °C, 3–4 electrotherapy procedures (direct current, low-frequency currents/pulses, functional electrical stimulation, ultrasound therapy, extracorporeal shockwave therapy, low-level laser therapy), massage therapy, and physical therapy [7].

A retrospective study was carried out to evaluate the effects of various factors on the functional and psychological outcomes of patients with severe SCIs who were admitted to a rehabilitation program. In our study, 45.77% of the patients were traumatic SCI patients. There are a few studies in the literature about the demography, incidence, and prevalence of this type of patient, and our results are in agreement with the studies already published [8–10]. Similar to the literature [8], in our study, the average age of the traumatic group was lower than the average age of the nontraumatic group. In both groups, the number of men is higher, with a proportion of 75% in the T-SCI group, probably due to the factors that commonly affected these individuals, which included falls from a high place and motor vehicle accidents. Even though in the majority of the studies men are dominant in T-SCI or in NT-SCTI patients' etiology, we found just one study which showed significant differences in the sex distribution between the different etiologies, with women more likely to have a benign tumor than men [11].

Various studies have shown that the majority of patients with severe SCIs who experienced a traumatic event were employed, while in the nontraumatic group, there was a higher number of retired patients [12]. In our study, we found similar results in nonworking, working, or retired patients. Moreover, we found the same ratio of the patients who had a disability certificate according to Romanian law. The majority of the patients are from rural areas, probably due to higher physical demands than in urban areas, as well as higher risks of accidents.

In the study, the leading factor that affected the patients with severe SCIs was road/work accidents (43.08%), followed by falls (29.23), and direct aggression (27.69%), as in other studies [8,10,13].

The major cause of injury in NT-SCI patients consisted of myelopathy with spondylosis or disc disorders, but we also identified the rare cause of NT-SCI, such as poliomyelitis sequelae (2.60%), syringomyelia (1.30%), or Morb Pott-operated sequelae (2.60%) [12,14].

Regarding the American Spinal Injury Association impairment scale (AIS), in the literature we found different results of neurological deficit, probably according to the type of hospitals in which they are treated, or due to the lengths of the disease, whether acute or chronic [15,16]. In meta-analyses and systematic reviews [17,18], we found different reports of patients included in the studies, both in terms of the AIS classification and outpatient status. Perhaps this can be explained due to the social, cultural, and genetic differences between countries, or the access to medical services and rehabilitation strategies. Also analyzing different studies, we concluded that there was a relationship between converting the AIS degree to a better one according to the severity of the SPI or intramedullary lesion length [16]. Moreover, locomotor training improves the AIS grade gait speed to levels sufficient for independent in-home or community ambulation after chronic motor incomplete SCI [19].

In the study, we also looked at the various drugs used by the patients, and the most common type of medication used was a lack of medication in T-SCI, which was a normal result considering that they are younger and healthier, while antihypertensive medication was the most commonly used in NT-SCI [20,21].

It is well known that in SCIs, the most common complication observed in both groups was a urinary tract infection. Mc Kiney et al. compared the complications between traumatic and nontraumatic SCIs and found no statistically significant differences between the two groups regarding urinary tract infections. Moreover, the authors identified other infections such as pneumonia and wound infections. It is important to determine the risk of infections at admission to a hospital because it has an important implication for the rehabilitation outcomes, individualized patient management, and long-term outcome of individuals with NT-SCI. That is why we calculated the Carmeli score, in order to identify patients susceptible to being colonized with multidrug-resistant bacteria at the beginning of the hospitalization. The highest numeric value of the three criteria represents the final value of the Carmeli score (1, 2, or 3). The final score allowed us to classify patients as follows: score 1 (community-acquired infections with microorganisms susceptible to classic antibiotics), score 2 (probably healthcare-associated or community-acquired infections but with a high probability of resistant or multidrug-resistant strains), and score 3 (maximum prediction for nosocomial infections with resistant or multidrug-resistant strains) [22]. In

our study, we found a higher Carmeli score in the T-SCI group (1.78) versus the NT-SCI group (1.68), but without statistical significance. There is no data in the scientific literature regarding this score in SCIs.

An important issue to establish the rehabilitation goals is to evaluate the fall risk. Wilson et al. evaluated the falls in SCIs, acquired brain injury, and a neuromusculoskeletal disease, and the type of disease was not a significant predictor of the fall rate in the multivariable analysis, but these results may be useful when developing and timing fall prevention interventions for inpatient rehabilitation [23,24]. This is consistent with previous reports finding that most falls occur within a few weeks of admission, at 5–7 weeks. We found that the risk of falls is higher ($p < 0.05$) at admission in the T-SCI group, but the risk decreases statistically significantly in both groups ($p < 0.001$) after only 2 weeks of treatment.

We also investigated the pain, using the VAS score [25], and we found that, at admission, there were higher values in NT-SCI ($p < 0.001$), and at discharge, the score decreased in both groups ($p < 0.001$). In previous studies [8,9,26], although spasticity and neuropathic pain are quite common in both the nontraumatic and traumatic groups, these conditions were less prevalent in the nontraumatic group, even if the pain could be visceral, nociceptive, or neuropathic [27].

Regarding the functional status, we evaluated FIMm, FIMc, and FIMt. The results have shown a higher level at admission for NT-SCI for all three parameters, and all increase with a statistical significance ($p < 0.001$) in T-SCI as well as in the NT-SCI group. In our study, we noticed an improvement in the functional outcomes in nonacute SCIs, and the rising of FIM was demonstrated in acute SCIs, where significant differences ($p \leq 0.01$) were found between the groups with regard to the total admission FIM, motor admission FIM, self-care admission, and discharge FIM. [28]. The functional improvement in the rehabilitation of SCIs is sustained by most studies regardless of etiology [10,29–31], even in malignant spinal cord compression [32]. However, a recent study developed in Italy, on 112 patients, showed that nontraumatic lesions could have minor benefits after rehabilitation therapy if compared with traumatic ones. Further research is needed to clarify these issues [33].

4.1. Study Strengths

The patients underwent a complex treatment combined with physical and physiotherapy as well as treatment with balneary factors (salt water from the lake). This is an area in which many studies are needed, as there is currently not much data in the literature. Another strength is the total number of patients in the study, which is representative and the two groups are balanced. The article is produced in compliance with the ethical rules of conception and writing, respecting the deontological aspects of publishing scientific results.

4.2. Study Limitations

This study has several limitations. An important limitation is the retrospective design, which could affect the quality of the data. For instance, data were extracted from medical records that were filled out by clinical staff, and the use of administrative data can also expose the study to various deficiencies. These could include underreporting, misclassification, and missing data. The use of the FIM instead of the Spinal Cord Independence Measure for assessing the functional improvement of patients with spinal cord injury is also a limitation of this study. The retrospective nature/design of the study and the choice of FIM over SCIM for assessing functional independence exposes the study to inherent limitations and deficiencies, including the quality, accuracy, and sensitivity of the data. Moreover, the use of VAS, which is a subjective evaluation, could not give us specific details about the type of pain. The data collected in the dynamics show an improvement of the clinical–functional parameters after only 10 days of treatment, but it could be necessary for a long period of time for treatment or follow-up.

4.3. Facts and Perspectives

The study opens new research perspectives in neurorehabilitation and all the data indicated that the therapeutic and preventive measures should be based on the characteristics of different groups, while public policies aimed at preventing injuries should focus on high-risk populations [34].

5. Conclusions

The physical medicine and rehabilitation increases the autonomy of patients. Neurological improvement begins in the first 10 days of complex rehabilitation treatment and is not significantly different between the two groups. The cause of the injury in the SCI does not affect the results of the rehabilitation.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

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Systematic Review

Main Cations and Cellular Biology of Traumatic Spinal Cord Injury

Constantin Munteanu [1,2,*], Mariana Rotariu [1,*], Marius Turnea [1], Anca Mirela Ionescu [3], Cristina Popescu [2], Aura Spinu [2,3], Elena Valentina Ionescu [4,5], Carmen Oprea [4,5], Roxana Elena T, ucmeanu [4,5], Ligia Gabriela Tătăranu [2], Sînziana Calina Silis, teanu [6] and Gelu Onose [2,3]

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[1] Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Ias, i, 700454 Ias, i, Romania

[2] Neuromuscular Rehabilitation Division, Teaching Emergency Hospital "Bagdasar-Arseni", 041915 Bucharest, Romania

[3] Faculty of Medicine, University of Medicine and Pharmacy "Carol Davila", 020022 Bucharest, Romania

[4] Faculty of Medicine, Ovidius University of Constanta, 900470 Constanta, Romania

[5] Balneal and Rehabilitation Sanatorium of Techirghiol, 906100 Techirghiol, Romania

[6] Faculty of Medicine and Biological Sciences, "Stefan cel Mare" University of Suceava, 720229 Suceava, Romania

* Correspondence: constantin.munteanu.biolog@umfiasi.ro (C.M.); mariana.rotariu@umfiasi.ro (M.R.)

Abstract: Traumatic spinal cord injury is a life-changing condition with a significant socio-economic impact on patients, their relatives, their caregivers, and even the community. Despite considerable medical advances, there is still a lack of options for the effective treatment of these patients. The major complexity and significant disabling potential of the pathophysiology that spinal cord trauma triggers are the main factors that have led to incremental scientific research on this topic, including trying to describe the molecular and cellular mechanisms that regulate spinal cord repair and regeneration. Scientists have identified various practical approaches to promote cell growth and survival, remyelination, and neuroplasticity in this part of the central nervous system. This review focuses on specific detailed aspects of the involvement of cations in the cell biology of such pathology and on the possibility of repairing damaged spinal cord tissue. In this context, the cellular biology of sodium, potassium, lithium, calcium, and magnesium is essential for understanding the related pathophysiology and also the possibilities to counteract the harmful effects of traumatic events. Lithium, sodium, potassium—monovalent cations—and calcium and magnesium—bivalent cations—can influence many protein–protein interactions, gene transcription, ion channel functions, cellular energy processes—phosphorylation, oxidation—inflammation, etc. For data systematization and synthesis, we used the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) methodology, trying to make, as far as possible, some order in seeing the "big forest" instead of "trees". Although we would have expected a large number of articles to address the topic, we were still surprised to find only 51 unique articles after removing duplicates from the 207 articles initially identified. Our article integrates data on many biochemical processes influenced by cations at the molecular level to understand the real possibilities of therapeutic intervention—which must maintain a very narrow balance in cell ion concentrations. Multimolecular, multi-cellular: neuronal cells, glial cells, non-neuronal cells, but also multi-ionic interactions play an important role in the balance between neuro-degenerative pathophysiological processes and the development of effective neuroprotective strategies. This article emphasizes the need for studying cation dynamics as an important future direction.

Keywords: systematic review; cations; sodium; potassium; lithium; calcium; magnesium; iron; traumatic spinal cord injury

3. Introduction

Spinal cord injury (SCI) is a harsh medical incident that frequently generates severe lifelong disability. SCI affects around 2.5 million people worldwide [1]. Traumatic SCI (tSCI) is a devastating neurological-dysfunctional complex disorder caused by various types of mechanical damaging events, such as falls, aggression/violence, and traffic and/or sports accidents [2].

Most human tSCI lesions are caused by the action of traction and/or compression forces on the spinal cord (usually associated with lesions—mainly fractures—of the spinal column). However, SCI can also happen in non-traumatic circumstances such as tumors, infections, and/or spondylosis, etc., [3]. In addition, the related age distribution shows that males are more prone to experiencing early and late adulthood tSCI, while—somewhat surprisingly—females have higher risks for such pathology during adolescence [4]. Currently, we do not yet have the treatment able to cure this pathology [5]; therefore, its therapy is usually focused on supportive [6], mainly neuroprotective aims, and rehabilitative measures [7,8], with, unfortunately, still minimal results.

The spinal cord comprises various cells and nerve fibers in their specific microenvironment. Some of these include the ascending and descending tracts. As a result of a traumatic event, individuals with spinal cord injuries can experience various degrees of disability [9]. The cellular context of a tSCI immersed in a molecular and ionic microenvironment controls the formation of new synapses, the production of extracellular matrix molecules, the regulation of the maintenance and function of the blood–spinal cord barrier, and modulating actions at the synapses, such as the extracellular absorption of glutamate.

The loss of synaptic connections of neurons with pre- and post-synaptic partners can determine the loss of specific function. The therapeutic strategies need to restore these connections, compromised in primary injury, which are described through the toothpaste tube theory (see Figure 1). After trauma, the morphology and gene expression of astrocytes can be seriously affected, and reactive astrocytes can take on new functional roles in the damaged tissue. Together with other types of cells, such as fibroblasts, can form scar-like structures around the damaged area. This process is known to support axon regrowth following spinal cord injury [10]. Detailed information about SCI morph-pathphysiology [11], including biological mechanisms involved in its consequent damages' evolution and (limited) repair mechanisms, is exhaustively presented in numerous research or review papers.

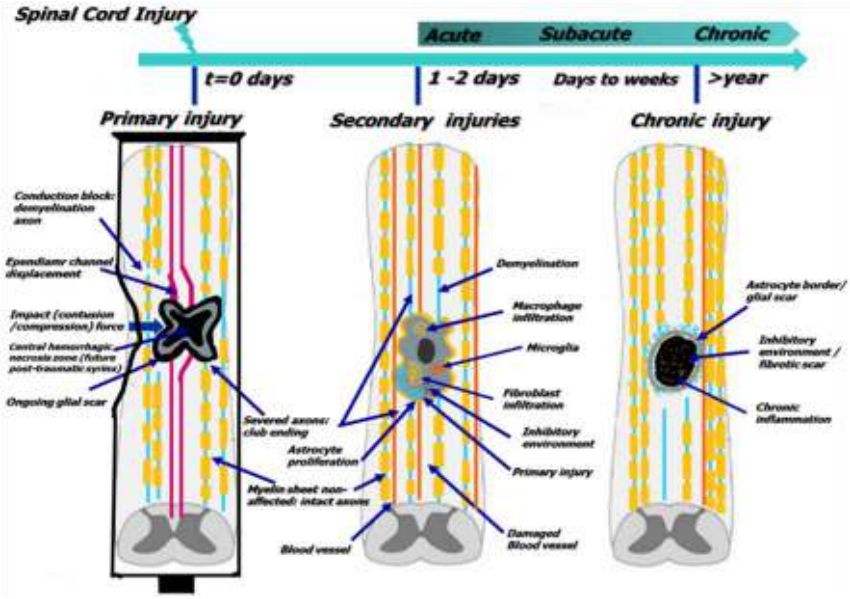

Figure 1. General characteristics of the cellular biology of SCI (preparation based on [12–14]).

4. Materials and Methods

This systematic review is based on the PRISMA methodology, by searching free full-text available papers written in English, which have appeared in the last five years, using specific keywords combinations (Table 1), in the well-known international databases: Elsevier, PubMed, PMC, ISI-Web of Science and Google. In addition, the inclusion criteria were fixed regarding pathology and interventions. Databases interrogation provided, initially, 207 articles. Appling the eligibility selection filters resulted in 51 unique published qualified studies.

Table 1. The keywords combinations used for the contextual search in international databases.

Specific Keywords in Title or Abstract	Elsevier	PubMed	PMC	ISI	Google	Total
"Calcium" AND "Spinal Cord Injury"	21	29	19	6	4	79
"Sodium" AND "Spinal Cord Injury"	9	13	7	8	3	40
"Potassium" AND "Spinal Cord Injury"	4	7	2	3	11	27

"Lithium" AND "Spinal Cord Injury"	5	2	9	7	5	28
"Magnesium" AND "Spinal Cord Injury"	2	3	2	1	7	15
"Iron" AND "Spinal Cord Injury"	0	0	6	0	0	6
"Zinc" AND "Spinal Cord Injury"	0	0	8	0	4	12
Total	41	54	53	25	41	207

5. General Characteristics of the tSCI Cellular Biology

Cells' external and internal microenvironment [10] involves a cloud distribution of various ions, nutrients [15] (for example, sugars, fats, oxygen), and secreted (neurotransmitters, cytokines, hormones, etc.) and excreted molecules (carbon dioxide, metabolic by-products) in an aqueous medium [4]. Lesioned axons can not regrow/regenerate in the adult mammalian central nervous system (CNS) because of multifactorial endo- and exogenous conditions, including developing a post-injury non-permissive environment [16].

A primary injury is caused by a sudden and unexpected impact that dislocates or fractures vertebrae [17]. Although most tSCIs can completely functionally interrupt the spinal cord, some lesions can still allow a limited spinal cord function at/or below the injured level [18]. In addition, according to clinical observations, the primary injury phase can lead to the destruction of neural parenchyma with cellular deaths and loss of axonal network, associated with hemorrhage [16].

The secondary injuries are usually divided into acute, sub-acute, and chronic phases and are characterized by the loss of neurons and glial cells caused by a very extended and puzzling "cascade of secondary events" [19] calcium influx inside the cells, inflammation, ischemia/hypoxia—this complex reactive process affecting even the differential regulation of numerous genes in the spinal cord tissue [4].

In patients with tSCI, the lack of vascular supply can cause various pathophysiological effects, such as cytotoxic and vasogenic oedemas. The lack of water molecules can also cause cell death and promote cell destruction. In addition, the increasing permeability of the blood-spinal cord barrier can also cause the loss of water and ions from the cells [19]. This effect can then lead to the development of vasogenic edema. So, the acute secondary injuries have, as basic lesions, hemorrhage and inflammation. In this stage, a specific pro-inflammatory protein such as IL-6 can be found in the spinal fluid [20] and thereby may be considered a related structural injury biomarker.

In the sub-acute phase, tSCIs encompass three tissue compartments with distinct cellular composition, represented by a central non-neural scar core, an astroglial scar border, and a surrounding zone demarcated by neural tissue [6]. Cell destruction is caused by various factors such as metabolic disturbances [21], ischemia [22]/hypoxia [23].

Astrocytes and microglia are local cells' early responders—activated including toxic debris clearance. In addition, they can recruit pro-inflammatory cells by secreting various cytokines and chemokines. Astrocytes growing in the scar border of severely damaged tissue migrate and assemble into a scar network around the core tissue. The formation of this scar border is usually completed in about 2 to 3 weeks after tSCI. The astrocyte scar borders can protect adjacent neural tissue. Unlike glia limitans borders, astrocyte scar borders are smaller and have few cell layers [6].

The central core of the spinal cord lesions is known as the fibrotic scar. It comprises various immune cells and connective tissues, such as macrophages and stromal fibroblasts [24]. About 70% of the cells that populate the CNS are astrocytes, characterized by a star shape and providing support and physiological insulation to neurons. It is widely believed that the scar plays a dual role in developing SCI as both an inhibitory and protective mechanism. However, the complexity of this role has been underestimated. It is difficult to target the scar in therapeutic applications due to its dynamic nature. This is why it is essential to understand the role of the scar in the development of SCI [24].

The damage caused by spinal cord injury can lead to a cascade of bio-molecular events, which starts with tissue bruising or tearing caused by mechanical damage that leads to the disruption of the nerve fibers and blood vessels, deterioration of nerve and glial cells, ischemia, and tissue edema [25]. Following the initial impact, the various bio-molecular events, such as edema, inflammation, neuronal and glial necrosis, or apoptosis can spread beyond the initial site of injury, but ultimately generate a necrotic zone surrounded by glial/fibrotic scarring. The glial scar has the main role to stabilize the spread of secondary injury and acts as a chronic, physical, and chemo-entrapping barrier that, unfortunately, prevents axonal regeneration (Figure 1) [21].

6. Calcium and Cellular Biology of SCI

Calcium performs many functions in the body. For example, calcium plays an essential role at the neuromuscular level in controlling excitability and releasing neurotransmitters [26]. Calcium also has an enzymatic and hormonal role (second intracellular messenger), mediating cellular responses to many internal or external stimuli [27]. In addition, calcium has a plastic role in the body through its insoluble combinations (skeleton) and a dynamic role through Ca^{2+} ions [28]. There is a close balance between these two forms of calcium: the decrease in blood Ca^{2+} is compensated by mobilization from the bones, and hypercalcemia produces increased storage in the skeleton, vessels, or increased elimination. An average amount of 9.5–10.5 mg% is found in blood plasma in three forms: (a) protein calcium (Ca proteinate = 4 mg%), a non-diffusible form of calcium; (b) ionized calcium (2 mg%), a diffusible form, biologically active; and (c) non-ionized calcium (crystalloid = 4 mg%), also a diffusible form, represented by some complex combinations (i.e., calcium citrate, chelated complex). There is a

constant balance between the fractions of non-diffusible and diffusible forms (ionic, crystalloid). The transition between them is typically achieved quickly, without altering the global level [27].

A significant number of metabolic processes are influenced by small changes in the concentration of extracellular calcium ions. These include excitability of nerve function and neural transmission [29]; secretion of proteins and hormones; coupling between excited cells and effector cells; cell proliferation; blood coagulation; maintaining the stability and permeability of cell membranes; modulation of activity, especially of those enzymes involved in glycogenolysis; mineralization in bone repair [28].

Following spinal cord injury, primary mechanical trauma can cause cell swelling and lysis, increasing the extracellular Ca^{2+} concentration [30]. This phenomenon leads to the development of excitotoxicity (Figure 2). In addition, an increase in extracellular glutamate can also lead to an influx of Ca^{2+} into neurons. The Ca^{2+} pathway is controlled by various subcellular localized channels and pumps proteins. Therefore, these components can also be used to control the dynamics of Ca^{2+} storage [13].

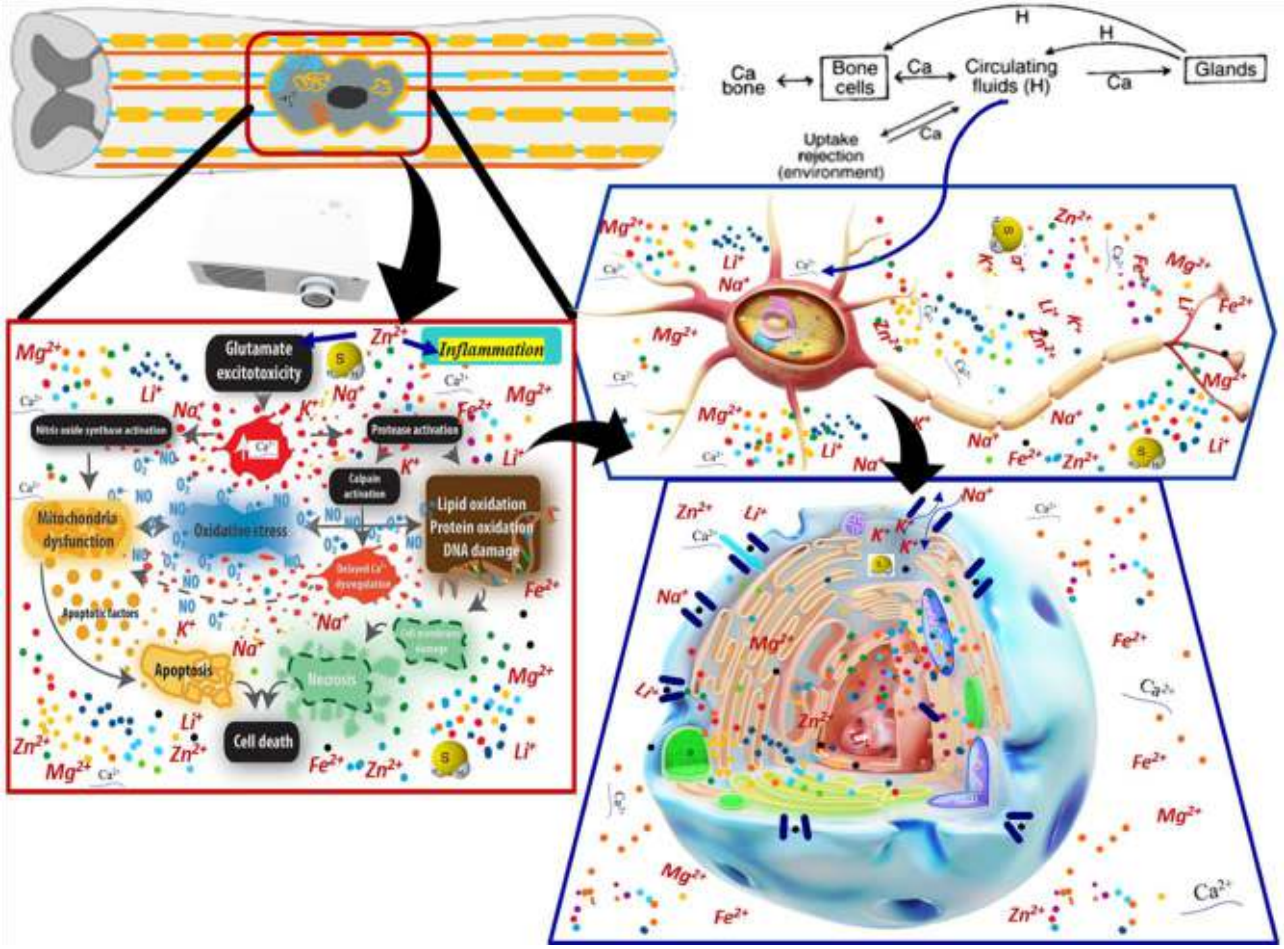

Figure 2. Ionic microenvironment and bio-molecular influenced processes in tSCI. There are presented successive "zoom images" (as follows the black arrows) starting from the tissular view of the injury to the intracellular level of the ionic microenvironment, pointing to the main biological processes influenced by the ions dynamics. The Ca^{2+} pathway is controlled by various subcellular localized channels and pumps proteins. Therefore, these components can also be used to control the dynamics of Ca^{2+} storage (figure preparation is based on [31]).

The differential regulation of genes triggers late injury responses. Some of these effects can affect the cell's metabolism [7]. The increased release of Ca^{2+} and glutamate can also damage the white matter cells and myelin. In addition, the activation of the *N*-methyl-Daspartate receptor (NMDA) can also lead to the accumulation of Ca^{2+} in the cytoplasm. This can cause the death of specific cells in the first week following an injury. Ca^{2+} overload in the mitochondria can also reduce ATP production and decrease the Na^+/K^+ ATPase levels [7]. Ca^{2+} overload in the mitochondria can also cause the production of nitric oxides (NO) and free radicals. It also can lead to mitochondrial permeability transition pores (mPTPs), increasing the water and oxygen influx within a cell's mitochondrial matrix [32].

An increased concentration of Ca^{2+} can also lead to the activation of the poly ADP ribose polymerase-1 (PARP1) enzyme. This protein is a regulatory enzyme that helps maintain DNA integrity during cell death. The depletion of NAD+ by PARP can cause the failure of glycolysis and cell death. It can also induce the release of a cyclic protein known as AIF, leading to cell death [20]. In addition, Ca^{2+} increases the levels of various calcium-dependent enzymes, stimulating the production of lipases and other enzymes [33].

The calcium signaling pathway is a significant component of the survival of neurons [34]. Conversely, after SCI, the disrupted calcium can lead to the onset of neurodegeneration [35]. Ca^{2+} overload is a significant inducer of axonal injury. Ca^{2+} can enter injured axons through mechanopores or nanopores [36]. This accumulation activates Ca^{2+}-dependent enzymes and phosphatases that can cause axonal loss and cytoskeletal disassembly. The upregulation of IP3R is known to cause axonal damage in the spinal cord [37]. Within the first minutes after an injury, a secondary cascade of damage occurs [38]. This stage of injury can last for weeks or months, and its damaging effects are comparable to those of the initial insult. In the first few minutes following spinal cord injury, various processes such as oxidative stress and lipid peroxidation can impair the function of Ca^{2+} pumps and cell membranes. Axons have a Ca^{2+} buffering system that removes the excess Ca^{2+}. However, when the amount of Ca^{2+} gets too toxic, this system can fail [38]. The excessive release of ATP after a spinal cord injury can also be triggered by activating the high-affinity purinergic receptors [39]. This signaling system can also contribute to the Ca^{2+} influx [37]. Ca^{2+} can enter the axoplasm through various pathways [26].

Ca^{2+} dysregulation is a key step in this patho-physiological cascade [38]. Unfortunately, the field of Ca^{2+} dynamics, despite developed electrophysiological and imaging techniques, is not well-supported due to the lack of sufficient evidence supporting its harmful effects [33]. The results of Ca^{2+} overload on the cells can have long-term detrimental effects. Even mild Ca^{2+} elevations can increase calcium levels and trigger the production of reactive oxygen species (ROS) [13]. However, it is not well known if the increased Ca^{2+} levels cause cell death or if it results from the depolarized mitochondria [40].

7. Sodium and Cellular Biology of SCI

Sodium (Na) is a monovalent cation from the same group as Li, K, Rb, and Cs. Sodium is the most important element of the alkaline metals group. In animals, sodium ions generate an electrostatic charge on cell membranes. This allows the transmission of nerve impulses. Its level is controlled by aldosterone. The Na^+ balance is maintained by the diffusion of other substances through the membranes [41].

The total quantity of sodium in the body of an adult male is around 92 g, of which half is located in the extracellular fluid at a concentration of 135–145 mmol/L, about ten percent is found in the intracellular fluid at the concentration of ~10 mmol/L, and the rest is located in the skeleton [42,43]. Different factors, such as metabolic and osmotic factors, interact to maintain the sodium balance [44]. Low sodium levels are uncommon but can usually be caused by certain medications that make people sweat or vomit excessively [45]. The increased calcium and sodium concentration promotes the development of cytotoxic edema and intracellular acidosis. It also triggers the extracellular release of the neurotransmitter glutamate [46].

When glutamate is bound to the kainate or AMPA channels, it can also cause voltagedependent depolarization. This can increase the cell's intracellular Na^+ concentration. Excessive sodium accumulation in the extracellular space can disrupt the cell's osmotic balance [47]. This can increase the cell's water consumption. Free radicals [48] can also disrupt the cellular processes by generating ROS. This can then lead to the cell's programmed death. A high sodium concentration can also lead to the development of edema and intracellular acidosis. It can also cause the excessive accumulation of Ca^{2+} and Na^+ into the cell. Like the K^+ channels, the Na^+ channels are also found within the myocytes and neurons. They are known to generate action potentials and are also affected by SCI [42].

Studies show that Na^+ plays a secondary role in the cell migration process. Electrochemical gradients of sodium (Na^+) can drive the transport of ions and sugars in non-excitable cells. These gradients can also be used to move these solutes across plasma membranes. An increase in the sodium in our body can trigger various signaling cascades that regulate post-translational events. If sodium concentration is elevated in our bodies' fluids and extracellular spaces, our cells have an inward concentration gradient [42]. It generates a membrane potential across plasma membranes and can stimulate long-distance neuronal communication. Besides Na^+ involvement in the generation of action potentials, it can also trigger a signaling cascade that can elicit a mitogenic response. The human body contains thousands of signaling molecules. This intercellular communication serves as the main component of basic cellular activity.

The Na^+/K^+ ATPase is an ion pump that uses the energy produced by the hydrolyzing of an ATP molecule to transport 3 Na^+ out of a cell and 2 K^+ into the cell. Aside from being an ion pump, studies have shown that the Na^+/K^+-ATPase can also function as a signaling transducer and a multi-protein scaffold, activating protein kinase signaling [42].

The Na^+/Ca^{2+} exchanger utilizes the net flux of three or one Ca^{2+} ions to transport the three Na^+ ions across the cell membrane. The resulting Ca^{2+} extrusion can be initiated in two different directions: forward or reverse.

The Na^+/H^+ exchange is involved in the regulation of cell volume. When exposed to hypertonic conditions, cells rapidly lose water and shrink to an equilibrium inside or outside osmolarities [42].

Na^+ channel blockers can help protect neurons by reducing cellular swelling and enhancing membrane integrity. In addition, they can also prevent cellular death caused by the excessive release of glutamate. Riluzole is a neuroprotective drug that blocks the excessive sodium influx into the central nervous system [49]. It is believed that a sodium channel blockade can preserve the white matter of the spinal cord. It can also prevent the activation of the sodium hydrogen antiporter system and reduce glutamate release. It has been shown to reduce the effects of excitotoxicity and neuromodulation in patients with spinal cord injuries [49].

8. Potassium and Cellular Biology of SCI

The human body has about 140 g of potassium, 97% at the intracellular level [50]. The presence of potassium in the cell membrane is known to maintain the normal function of the cell [51]. A tilt in this balance can cause various diseases to develop. Electrolyte balance [52] is crucial for the body's general functioning [48]. The equilibrium between the body's electrolyte and water balance is maintained through a series of homeostatic mechanisms. It is characterized by the existence of both intracellular and extracellular fluid [51]. Homeostasis involves the movement of potassium from inside the cells to the extracellular space. The Na^+/K^+ ATPase pump helps transport potassium and sodium ions across the cell membrane. The pump maintains the equilibrium between the extracellular fluid and the intracellular fluid through a buffering process that involves the hydrolysis of ATP and generates an electrical gradient [53].

Under normal physiological conditions, axons are wrapped with myelin, except for nodes of Ranvier [54]. The depolarization of the nodal axonal membrane and the presence of adequate sodium ions allow for the generation of action potentials. It is believed that the K^+ channels located under the myelin act as an efficient axonal conductor. After recognizing the role of K^+ channels in the pathogenesis of axonal functional loss, potassium channel blockers [36] have been investigated to block these channels and restore axonal conduction in the injured axons [55].

The potassium channels [56] are located on the axons and play a vital role in regulating physiological processes. They are also known to stimulate the production of action potentials. Through demyelination, the K^+ channels can increase their activity, preventing the generation of action potentials. Demyelination also triggers the activation of the Ca^{2+} exchanger, which can increase the intracellular levels of Ca^{2+} [57].

The Kir4.1 channel [58] is a glial-specific potassium channel in the central nervous system's astrocytic maintenance. It is believed that this channel helps maintain the extracellular potassium level. This channel has the highest expression in astrocytes. It contributes to the cell's membrane properties, such as its hyperpolarized resting membrane and low input resistance. Various studies have shown that the downregulation of Kir4.1 can result in multiple physiological and biochemical changes in the central nervous system. A second model of spinal cord injury has also exhibited the effects of Kirnj10 regulation. Studies show that sustained reductions in Kir4.1 protein expression are dependent on the enhancement of DNA methylation. This finding supports the possibility that this protein could be a target for developing effective drugs for treating various disorders [59].

Downregulation of Kir4.1 is common in various central nervous system disorders. In most cases, it occurs in the inner myelin tongue and the perinodal areas of the astrocytes [60]. The loss of Kir4.1 in OPCs and oligodendrocytes can result in various physiological and biochemical changes, such as demyelination and mitochondria damage. The sulfonylurea receptor is a functionalized version of the K^+ channel that is physically coupled to one of the two members of the K^+ family [61].

A shift in the balance of H^+ and potassium occurs when the cell's serum pH increases. A spike in serum potassium can also be caused by increased plasma osmolality, which can pull in some of the cell's water [62].

The body can maintain its normal K^+ balance without altering other metabolic processes. It can also use insulin to push the K^+ into the cells. Furthermore, carbohydrate metabolism can help maintain the concentration of K^+ in the body. The ability of a skeletal muscle to donate some of its stored potassium can help restore the K^+ balance. After experiencing hypoxia, free radicals can trigger the activation of voltage-dependent K^+ channels in vitro. These findings support the idea that activating these channels is one of the earliest responses to oxidative stress. High-potassium diets can also affect the plasma concentration of K^+. Vegetables and fruits with high K^+ content can lead to this effect [62].

Short-term hyperkalemia could be transient or sustained. For example, a person's excessive potassium intake and decreased excretion through diet or infusion can induce hyperkalemia. Other factors contributing to the high potassium excretion level include diminishing the body's mineralocorticoid level or activity. The proper distribution of potassium in the body is essential for maintaining cell function. The body has developed various mechanisms to ensure its adequate distribution [62].

The knowledge about how to manage K^+ imbalance has become more prevalent. Various interventions such as reducing potassium losses and increasing the amount of potassium in the body can be performed to prevent and cure hypokalemia. Balancing the body's potassium level is a strategy that can be used to treat hyperkalemia. This condition can be treated through medications and procedures designed to reduce its level. In addition, understanding the link between an increase in potassium level and oxidative stress can help identify potential causes of various diseases [62].

In severe spinal cord injury cases, the intraspinal networks may no longer function properly as an integrated part of the spinal cord's response to the descending inputs. Several small-molecule compounds have been developed to target key regulators, such as ion channels and receptors, and their pharmacological properties have been well characterized [63]. Following spinal cord injury, the pathways that lead to the formation of potassium-chloride cotransporter (KCC2) in the spinal cord can change its expression. The downregulation of KCC2 can contribute to the development of spasticity following spinal cord injury. After the initial 24 h, the damaged spinal cord can start to recover its capacity to generate sustained depolarizations [64].

9. Iron and Cellular Biology of SCI

Iron is an essential constituent of life, a metal involved in a wide range of physiological functions, including enzymatic reactions, energy production, oxygen transport, protein synthesis, and DNA repair. It is usually included in a stable form in

metalloproteins. Iron can combine with any type of biomolecule and, as such, will adhere to membranes, nucleic acids, proteins, etc. Inorganic iron in redox reactions is also found in iron–sulfur complexes in many enzymes, such as nitrogenase and hydrogenase [65].

Our body generally contains about 4–5 g of iron. Excessive iron is toxic to humans because it reacts with peroxides in the body, producing free radicals. Toxicity occurs when the amount of iron exceeds the transferrin required to bind the free iron. In a complex with protoporphyrin IX, Fe forms the heme, the prosthetic group of proteins: hemoglobin, myoglobin, and cytochromes. Iron is transported by transferrin, a serum glycoprotein that can bind 2 iron atoms and transport them to all tissues. Excess iron is stored in the body by ferritin and is incorporated into apoferritin. Hemosiderin represents histologically amorphous Fe deposits. Iron is an essential component of metabolism due to its property of taking up (passing from ferric to ferrous) or yielding electrons (passing from Fe^{3+} to Fe^{2+}) in a relatively straightforward manner [65].

Iron has a functional dualism at the nervous system level, essential for life but toxic at values that exceed the normal range of variation. At the cellular level, iron is needed for cell growth, but in excess, it causes oxidative stress and cell death. In this context, iron levels are well controlled by the mechanisms of iron homeostasis. The primary protection strategy in the central nervous system is the blood–brain/spinal cord barrier [66].

Iron is essential for several basic cellular processes, including mitochondrial ATP generation and DNA replication, so an iron deficiency in the nervous system affects the division of precursor neuronal cells, astrocytes, and oligodendrocytes. Iron is also needed for several specific functions, such as the synthesis of dopaminergic neurotransmitters and the myelination of axons. Anemia in the early stages later causes mental retardation, and iron deficiency in early development causes neurological abnormalities [67].

Accumulation of iron in the neuronal tissue can cause neurodegeneration. Knowing the ability of iron to donate electrons to oxygen, high iron levels can cause the formation of hydroxyl radicals and hydroxyl ions by the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$ [67]. Elevated iron levels can also generate peroxyl/alkoxyl radicals due to Fe^{2+} lipid peroxidation [68]. Spontaneous oxidation of Fe^{2+} can also produce more superoxide radicals. The resulting ROS and RNS can react with various targets. In most cases, apoptosis occurs due to the influx of Ca^{2+}, which activates enzymes involved in the breakdown of cellular proteins [4].

Iron chelators, which can reduce iron accumulation and promote functional improvement, theorized that treating patients with spinal cord injuries with iron can improve their recovery [69]. A chelating agent could help minimize iron accumulation in the spinal cord. A different study in mice revealed that a chelating agent could improve locomotor function after a spinal cord injury. As the blood in the spinal cord floods the tissues, iron is released from the carriers, such as ferritin and circulating transferrin. The acidic environment can facilitate the release of free iron from these molecules in the post-injury period. It has also been theorized that macrophages play an essential role in the body's recovery by taking up and storing iron [70]. Due to the toxicity of iron and its reactivity, treating patients with spinal cord injuries with iron chelators has been considered a promising strategy to protect the tissue. However, although deferasirox effectively reduced systemic iron stores, it did not decrease intraspinal iron levels [70].

It is believed that the easiest way to chelate iron is through circulating iron levels. Since it does not require the presence of deferasirox to cross a cell membrane, it can be quickly done. After spinal cord injury, the proliferation of new oligodendrocytes leads to new cells within the spinal cord [67].

Iron-induced oxidative stress is hazardous because it causes the release of iron from iron-containing proteins, such as ferritin, heme, or Fe-S combinations, forming a positive feedback loop that exacerbates the toxic effect of iron. It is believed that motor neuron death is a process that leads to the atrophy of the primary motor cortex following spinal cord injury. However, the exact mechanisms of this death are still not known. It is believed that excess iron accumulation in the spinal cord can lead to tissue damage and limit the recovery of individuals following a spinal cord injury [71]. Treating this issue with a chelating systemically could help prevent this accumulation [68].

10. Zinc and Cellular Biology of SCI

Zinc (Zn) is a trace element that plays a crucial role in the various biochemical pathways of the human body [72]. Zinc has antioxidant properties and is known to reduce the production of pro-inflammatory cytokines. Around 2800 proteins can potentially bind to Zn. In addition, it is known to support the activities and efficiency of the immune system [73]. In humans, the average zinc concentration ranges from 650 to 1100 µg/L [72].

The level of zinc concentration that affects the response of the body's immune system is known to depend on its availability [74]. As redistributed from serum to the injured site, the high zinc concentration resulting after an injury can help minimize the inflammatory response's overshooting [75]. It can also contribute to the adequate activation of the downstream target genes [72].

Two main transport proteins are responsible for zinc homeostasis [76]. The zinc transporters ZnT responsible for Zn effluxes and the Zip family are known to increase the cytoplasmic Zn uptake [72]. Due to its rapid distribution into the cellular compartments, zinc is commonly used as a marker for the onset of the inflammatory response following an injury. Therefore, the researchers hypothesized that the zinc concentration in serum could be used to diagnose TSCI based on the patient's neurological status and the time following an injury [75]. The researchers noted that the sudden decrease in serum

concentrations might have been caused by the molecular processes regulating zinc's movement into the body. It was hypothesized that the activation of specific cellular pathways might have triggered these changes. The activation of the NF-kB transcription factor is known to contribute to the polarization of monocytes. Although the effects of Zn on the polarization of monocytes are not yet apparent, it has been suggested that the M2 phenotype could play a beneficial role in treating TSCI [77].

The reduction in extracellular zinc levels following an injury can increase the frequency of glutamate excitotoxicity [78] and promote the growth of oligodendrocytes [72]. The researchers then explored the effects of supplemental extracellular zinc on the Ca^{2+} currents of mice. They found that the zinc concentration in the urine attenuated the impact of the chemical on the vesicular Zn transport [72]. Recent studies suggest that the disruption of calcium homeostasis and the accumulation of zinc and glutamate could trigger the toxicity caused by zinc-induced neurotoxicity. The importance of zinc distribution has been highlighted in the pathogenesis of diseases. Free zinc within cells has been linked to the development of neurotransmitter functions [75].

Zinc promotes functional recovery in contusion spinal cord injury patients by activating the Nrf2/GPX4 defense pathway. In addition, using zinc can decrease the inflammatory infiltrates in the spinal cord [79].

Treating patients with zinc can improve the morphological changes in their cells during ferroptosis [73]. These changes include the collapse of mitochondria and the rupture of the outer membrane potential. After zinc was administered to mice, the intercellular adhesion factors and inflammatory factors exhibited decreased downregulation. The effects of zinc on astrocytes inhibit the damage caused by spinal cord injury. Furthermore, its impact on the expression of related proteins can help improve wound healing. Due to its anti-inflammatory properties, zinc has been considered a conventional antioxidant. Its clinical application as a treatment for nerve injury has been supported by its effects on immune system regulation [77].

Researchers theorize that the inflammatory-related responses could be used to predict the severity of the acute phase of SCI. They noted that the zinc concentration in the blood is inversely correlated with the severity of the response.

Although zinc can promote motor function in people with spinal cord injury, the exact mechanisms that regulate this recovery are not fully understood. The zinc significantly increased the number of 19 cytokines in the site of an SCI lesion. In addition, the results indicate that using zinc increases the production of G-CSF, which could result in decreased levels of neuronal apoptosis following SCI [80].

Previous studies have shown that zinc can improve the function of neurons and reduce oxidative stress. However, the role of zinc in the metabolism of the neurons after SCI is not yet clear [79].

Restoring the mitochondria function is critical in preventing the onset of ROS effects. This process can help the cells maintain their energy-producing capabilities and avoid developing diseases that damage the mitochondria [79]. Zinc is also a vital element that plays a central role in developing various biological processes. It can also contribute to maintaining the nervous system's physiological functions.

11. Magnesium and Cellular Biology of SCI

Magnesium is an essential trace element in the body that intervenes in many critical physiological reactions (metabolism of carbohydrates, lipids, and proteins, neuromuscular excitability, enzymatic activities, cell permeability, blood clotting, etc.). An adult's body contains about 25 g of magnesium (Mg). More than half of this amount is found in the bones, a quarter in the muscles, and the rest is distributed mainly in the heart, liver, kidneys, digestive tract, and plasma. The total magnesium content in an average adult is distributed at 1% extracellularly [81], 31% intracellularly, and 63% in the bones [82]. Magnesium deficiency can cause various symptoms. Some of these include hallucinations, paranoia, muscle weakness, and depression. In addition, magnesium levels can be influenced by multiple factors such as hypercalcemia, primary aldosteronism, and alcoholism. In a followup study, paraplegic individuals with similar symptoms were observed to have increased urinary magnesium [82].

Free and total magnesium ions play critical roles in various cellular functions. They can trigger different regulatory functions through specific ion channels and enzymes. Recent studies have revealed that membrane significant fluxes of Mg^{2+} can affect the cell's metabolic cycles and function [83]. In the central nervous system, Mg^{2+} is known to maintain calcium homeostasis. It is also involved in releasing neurotransmitters and in the transmembrane electrolyte flux. Mg^{2+} can stimulate the excitatory neurotransmitters norepinephrine and serotonin, decreasing the NMDA receptors' action [83].

After spinal cord injury, the recovery of the damaged network requires the remyelination of long tracts. The absence of the architectural framework can impair the formation of new axon growth and free cell migration. The extracellular matrix in the glial scar contains various proteoglycans, restricting axonal regeneration and preventing neurite outgrowth. In addition, these proteoglycans are also known to activate multiple myelin- and neuronrelated signals [84]. Following an injury, in the acute phase, which usually lasts for 3 days, the concentration of magnesium drops [85]. The link between the severity of an injury and magnesium concentration has been investigated. It has been hypothesized that this component could play a role in the secondary injury phase. The group severely affected by the condition exhibited increased plasma protein, blood pressure, and erythrocyte albumin. It also revealed an impairment of redox status. A study conducted in 2000 linked the effects of magnesium and oxidative stress on patients with mild to severe injuries. The researchers noted that the plasma ionized magnesium concentration increased 7 days after the injury [83].

An increase in free hemoglobin can be measured in the central nervous system. It can inhibit the Na/K ATPase activity and lead to neuronal depolarization. This impairs the Na/K ATPase activity and leads to secondary calcium influx [86]. The reduction in total tissue of Mg following an injury could be caused by the influences of extracellular Mg^{2+} on the NMDA receptor [87]. Magnesium can also help restore the concentration of adenosine triphosphate after reperfusion and/or ischemia. However, this process can prevent the long-term regeneration of injured networks [57].

Patients' magnesium level after suffering an injury is inversely predictive of their possible neurological remission. Individuals with low magnesium levels have higher levels of inflammatory cytokines and plasma concentrations of acute-phase proteins. Through its various anti-adrenergic mechanisms, magnesium controls the movement of calcium and inhibits the release of catecholamine from the cell [84]. It also causes a reduction in systemic vascular resistance and helps prevent the accumulation of lactic acid. Contusive spinal cord injuries trigger a pathological cascade that can lead to vasospasm [88].

The onset of acute cell dysfunction and death can be triggered by the destruction of the microvascular supply and the lack of blood flow to the spinal cord. This process involves the formation of aneurysms and microvascular disturbances in the spinal cord.

The onset of hemorrhage and progressive edema following an injury can add to the harsh post-injury environment. This process triggers the proliferation of various cell types and the infiltration of pro-inflammatory cytokines [83]. The activation of phagocytes can also contribute to the cell death of injured cells. In addition, excitotoxic injury can also occur by releasing glutamate through the astrocytes [57].

It has been hypothesized that the loss of endothelial cell function could be caused by the accumulation of calcium or lipid peroxidation, which could be reduced through magnesium treatment. The use of magnesium salt to relieve the vascular spasm of central nervous system vessels has been reported in clinical and experimental settings [88].

12. Lithium and Cellular Biology of SCI

Many of the proposed lithium treatment cellular action mechanisms suggest that it can inhibit the activities of various signaling pathways. Lithium has been shown to inhibit multiple phosphomonoesterases being structurally similar to magnesium [89]. Lithium can improve the microenvironment in the spinal cord and promote the regeneration and survival of motor neurons [90]. Lithium chloride can boost the secretion of brainderived neurotrophic factor (BDNF) in the axons of motor neurons following spinal cord injury [91,92]. Lithium chloride can also help restore the function of the blood–spinal cord barrier after a spinal cord injury [93]. LiCl can also inhibit the apoptotic pathway caused by the GSK-3β activity [94]. The potential targets of lithium are known to require catalyzing metal ions. However, lithium is known to inhibit these targets in a non-competitive manner. The concentrations of Li^+ in the spinal cord and the brain are less than 5 and 2 mmol/L. Lithium levels are influenced by the diet and the medications used for treating diseases. A Na^+-dependent countertransport system regulates the effects of lithium on the plasma membrane. This mechanism involves the stimulation of the Li^+ uptake by external Na^+ and reducing the Li^+ uptake by internal Na^+.

Ion-gated channels are also involved in the distribution of lithium across a cell membrane. The presence of these channels helps regulate the steady-state concentrations of lithium in the cytoplasm. The Na^+/H^+ exchanger is a ubiquitous protein that is commonly found in cells. It carries lithium ions in place of sodium. The Na^+/K^+ –ATPase pump is also involved in the excitability of neurons. The lithium concentration in the cell does not affect the membrane resistance of neurons. It also triggers a decrease in the free potassium concentration. Lithium can also be substituted for potassium or sodium on specific transport proteins, usually carrying potassium or sodium. These proteins can provide a pathway for lithium to enter a cell. The main entrance ways to lithium are through the sodium channels and the Na^+/H^+ exchangers. These channels allow lithium ions to enter the cell without energy consumption [95].

Lithium can also affect the uptake of K^+ into astrocytes by blocking the reuptake of the K^+. It has also been observed that its interaction with the electrogenic Na^+/K^+ pump can trigger membrane hyperpolarization. An increase in the concentration of Li^+ can also cause voltage changes in the microelectrodes sensitive to K^+. It is believed that membrane hyperpolarization is caused by the activation of the Na^+/K^+ pump, 15 mmol/L LiCl can reduce or even abolish this hyperpolarization [96].

The effects of lithium on inositol metabolism have been studied. Both inositol and lipids play a complex role in regulating cellular functions [97]. Activating the catenin signaling pathway promotes cell proliferation in non-neural cells. Lithium also partially blocked the Golgi apparatus fragmentation. The disassembly of the Golgi complex is initiated by activating the GSK-3 signaling pathway. GSK-3 has been known to contribute to the pro-apoptotic signaling activity of cells. The activation of the phosphatidylinositol-3 kinase/Akt-signaling pathway can protect cells from pro-apoptotic stimuli [98].

The regulation of cell survival is a crucial component of normal physiology. It can lead to the accumulation of excess or insufficient cell death, which can result in pathological conditions. Neurotrophic factors and the phosphatidylinositol 3-kinase/Akt pathway can also promote cell survival by blocking the proliferation of apoptosis. The Akt protein is a multi-isoform serine/threonine kinase downstream of the PI 3-K protein. The upstream phosphorylation of Akt initiates its activation by PI-3-dependent kinases. Both glutamate and low potassium-containing cultures can induce apoptosis. It has been observed that lithium can also reverse the effects of these two modes of cell death. A treatment with Li^+ can prevent the induction of low-K+ -induced apoptosis [99].

Lithium's effects on the NGF pathway can be evidenced by rapid cell surface changes and increased protein phosphorylation. Lithium ions can also affect the responses of cells to polypeptide signals. It can increase insulin and epidermal growth factors and inhibit the activation of NGF-dependent signaling [100].

Studies on the effects of lithium ions on the metabolism and synthesis of neurotransmitters have yielded inconsistent results. However, the functional connections between regulatory genes and cis-regulatory sequences have been suggested as critical factors that influence the response of organisms to lithium [95].

The effects of Li^+ on the central nervous system are caused by its interventions in potassium homeostasis. This impairs the ability of the isolated spinal cord to generate spontaneous synaptic activity. It has been hypothesized that Li^+ could compete with K^+ for the binding site of an extracellular binding site. The presence of high concentrations of Li^+ in the cell can decrease the free Na^+ concentration and reduce the stimulation of the Na^+/K^+ pump. After exposing the cells to concentrations of Li^+, the time course of the depolarization is consistent with the effect of the Li^+ on the membrane [95].

Li^+ is known to exert several beneficial effects on axonal regeneration and also has remyelinating effects. Therefore, this molecule could constitute a potential therapeutic remedy for nerve injuries in which axonal lesions and demyelination coexist. Furthermore, there is evidence that the GSK3β could be considered an essential factor in the expression of myelin genes, and they open approaches to treating nerve injuries that use inhibitors of GSK3β such as lithium [101].

13. Discussion

Aside from the endogenous "braking machinery" for regrowth/regeneration afore enumerated, the complex spinal cord microenvironment is one of the main factors that might influence the regeneration and functional recovery of the respective injured nervous tissue. The post-lesional imbalance at the gene-/molecular, cellular, and tissue levels expresses the disturbance of various inhibitory and/or stimulatory physiological and pathological mechanisms. Reversing the perspective, i.e., from the tissue to the molecular point, Fan Baoyou et al., 2018 [102] emphasize that hemorrhage and ischemia/hypoxia can produce drivers of the microenvironment alterations, expressed mainly by demyelination and glial scars (tissue level); differentiation of stem cells, the transformation of the microglia and oligodendrocyte phenotypes, infiltration of macrophages, and activation of astrocytes represent the cellular level of the tSCI damages, and involvement of chemokines, cytokines, (other) pro-inflammatory and, on the other hand, neurotrophic factors correspond to the molecular level of imbalances. To all these above-presented data [102], we consider in our review a distinct level of microenvironment imbalances, the ionic level, as presented in detail in the previous sections.

The primary mechanical damage can disrupt the blood supply to the spinal cord and the topical capillaries. The lack of blood supply but also bleeding into the spinal cord parenchyma can increase the release of specific ions and macromolecules from the cells, likewise edema which enhances the pressure in the surrounding vessels and causes, in a vicious cycle, ischemia. The lack of ATP can cause an ion imbalance, too, and, again, in a vicious cycle, this could also worsen the neural tissue edema caused by water accumulation in the cells [102]. Unfortunately, remyelination can also be delayed or obstructed by cellular debris in the microenvironment. The accumulation of this debris can limit the extent and quality of the process [102].

Ion imbalance is a known factor that can regulate the pathological changes caused by spinal cord injury. After tSCI, the K^+, Na^+, and Ca^{2+} channels are altered. Following tSCI, the levels of Ca^{2+} and Na^+ are upregulated in the cell, while the concentrations of K^+ and Mg^{2+} are upregulated extracellularly. The influx of Na^+ into the cell leads to cytotoxic cellular edema, with water gradually accumulating. On the other hand, previous studies had shown that blocking the Na^+ channel could also lead to detrimental impacts such as the activation of intracellular phospholipases and intracellular acidosis [103].

Ca^{2+} is a vital component of the CNS that participates in various physiological and pathological processes. As previously pointed out, following spinal cord injury, the concentration of Ca^{2+} increases rapidly. High Ca^{2+} levels can cause apoptosis and/or necrosis by increasing the production of free radicals. It can also damage the white matter [104].

The disruption of the myelin of axons' sheath following tSCI can lead to different detrimental effects, too. The K^+ channel's increased activity can cause demyelination, too. Although the voltage-gated K^+ channels are essential for remyelination, they can also be affected by 4-aminopyridine (4-AP), a K^+ channel antagonist [61].

Iron is also a vital component of normal CNS functioning. It can be increased by the influx of red blood cells consecutive to bleeding in the spinal cord. In addition, treating patients with tSCI with deferoxamine can decrease the production of free radicals and promote the survival of the cells. It has also been reported that it prevents the formation of glial scar and reduces the iron ion level [70].

Biomarkers are objectively quantifiable biological characteristics and represent a very attractive and unbiased tool for assessing SCI severity. Furthermore, markers associated with the pathophysiology of acute SCI should aid in monitoring the biological effects of a candidate treatment and also may identify potential targets for novel therapies that reduce secondary damage [105]. A major focus of SCI research is on evaluating the ability of agents to improve recovery after injury. The drug discovery field has adopted the term "perturbagen" to refer to small molecules, peptides, antibodies, oligonucleotides, and so on that, alter a biological process by interfering with one or more molecular targets [106]. To this paradigm, we need to add ions dynamics at the injury site.

14. Conclusions

The concept of the microenvironment imbalance after spinal cord injury explains the various alterations and disturbances at the gene/molecular, subcellular, and tissue levels following tSCI. An interesting parallel might be imagined between the microenvironment of an injured spinal cord and the macroenvironment in which we live, represented by air containing oxygen, carbon dioxide, and different aero-ions. The post-tSCI imbalances at the microenvironment level are like "a storm in our climatic ambient" with considerable damaging force. The universal presence of ions, with their chemical and physical properties, has influenced the evolution of cellular mechanisms and further of living beings. Life started through the capacity of biomolecules to manipulate ions and generate "the cellular weather". Influencing this "weather" can be a desirable intervention in neuro-regeneration. Starting from this paradigm, detailed research on cations dynamics involved in various regeneration mechanisms after SCI is critical in future directions, which might be more feasible in light of the recent spread of biosensors for detecting individual cations.

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15. *Review*

Recent Advances in Molecular Research on Hydrogen Sulfide (H_2S) Role in Diabetes Mellitus (DM)—A Systematic Review

16. **Constantin Munteanu [1,2,*], Mariana Rotariu [1], Marius Turnea [1], Gabriela Dogaru [3,4], Cristina Popescu [2], Aura Spînu [2,5], Ioana Andone [2,5], Ruxandra Postoiu [2], Elena Valentina Ionescu [6,7], Carmen Oprea [6,7], Irina Albadi [6,8] and Gelu Onose [2,5,*]**

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[1] Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Iaș, i, 700115 Iaș, i, Romania; mariana.rotariu@umfiasi.ro (M.R.); marius.turnea@umfiasi.ro (M.T.)

[2] Teaching Emergency Hospital "Bagdasar-Arseni", 041915 Bucharest, Romania; cristina_popescu_recuperare@yahoo.com (C.P.); aura.spinu@umfcd.ro (A.S.); ioana.andone@umfcd.ro (I.A.); postoiu.ruxandra@yahoo.ro (R.P.)

[3] Clinical Rehabilitation Hospital, 400066 Cluj-Napoca, Romania; gabriela.dogaru@umfcluj.ro

[4] Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400347 Cluj-Napoca, Romania

[5] Faculty of Medicine, University of Medicine and Pharmacy "Carol Davila", 050474 Bucharest, Romania

[6] Faculty of Medicine, Ovidius University of Constanta, 900527 Constanta, Romania; elena.ionescu@365.univ-ovidius.ro (E.V.I.); carmen.oprea@365.univ-ovidius.ro (C.O.); irina.albadi@yahoo.com (I.A.)

[7] Balneal and Rehabilitation Sanatorium of Techirghiol, 906100 Techirghiol, Romania

[8] Teaching Emergency County Hospital "Sf. Apostol Andrei" Constanta, 900591 Constanta, Romania

* Correspondence: constantin.munteanu.biolog@umfiasi.ro (C.M.); gelu.onose@umfcd.ro (G.O.)

Abstract: Abundant experimental data suggest that hydrogen sulfide (H_2S) is related to the pathophysiology of Diabetes Mellitus (DM). Multiple molecular mechanisms, including receptors, membrane ion channels, signalingmolecules, enzymes, and transcription factors, are known to be responsible for the H_2S biological actions; however, H_2S is not fully documented as a gaseous signaling molecule interfering with DM and vascular-linked pathology. In recent decades, multiple approaches regarding therapeutic exploitation of H_2S have been identified, either based on H_2S exogenous apport or on its modulated endogenous biosynthesis. This paper aims to synthesize and systematize, as comprehensively as possible, the recent literature-related data regarding the therapeutic/rehabilitative role of H_2S in DM. This review was conducted following the "Preferred reporting items for systematic reviews and meta-analyses" (PRISMA) methodology, interrogating five international medically renowned databases by specific keyword combinations/"syntaxes" used contextually, over the last five years (2017–2021). The respective search/filtered and selection methodology we applied has identified, in the first step, 212 articles. After deploying the next specific quest steps, 51 unique published papers qualified for minute analysis resulted. To these bibliographic resources obtained through the PRISMA methodology, in order to have the best available information coverage, we added 86 papers that were freely found by a direct internet search. Finally, we selected for a connected meta-analysis eight relevant reports that included 1237 human subjects elicited from clinical trial registration platforms. Numerous H_2S releasing/stimulating compounds have been produced, some being used in experimental models. However, very few of them were further advanced in clinical studies, indicating that the development of H_2S as a therapeutic agent is still at the beginning.

Keywords: hydrogen sulfide (H_2S); Diabetes Mellitus (DM); DM vascular-linked pathology; systematic review; oxidative phosphorylation; ROS (Reactive Oxygen Species)

1. Introduction

Diabetes Mellitus (DM) is a non-communicable chronic metabolic disease [1] characterized by prolonged hyperglycemia. Type 1 DM is a chronic condition in which the body's pancreatic β cells, determined by different causes, reduce insulin production. Instead, type

2 DM is caused mainly by lifestyle factors and is characterized by insulin resistance, thus inefficiency on target cells, also with complex pathophysiologic links. The International Diabetes Federation mentions more than 450 million people globally with DM at present,

90% representing Type 2 DM, and estimates an increase to over 600 million in the next 25 years [2]. Therefore, the diabetic "pandemic" seems to be a global public health problem that needs particular attention, considering the deaths and disabilities caused by this disease's complications [3].

Severe and more frequently associated complications of DM may primarily involve the nervous system [4], kidneys [5], and/or eyes [6] due to damage of the microcirculation [7], respectively; and the cardiovascular systems [8], due to macroangiopathy [9]. Cardiomyopathy is responsible for more than 50% of deaths in diabetic patients [10]. Diabetic nephropathy [11] generates kidney injuries and tissue lesions and eventually leads to chronic kidney disease.

In DM, patients with insufficient oxygen supplies due to impaired angiogenesis/ neovascularization [12], refractory wounds and critical ischemic sufferance in limbs are major vascular hazards [11], connected with a malfunction of endothelial cells (ECs) [13].

H_2S, recognized by its smell of rotten eggs, is the simplest thiol (R-SH), a sulfur analog of alcohol, with a high redox potential [14]. However, disentangling H_2S chemistry and biochemistry is much more complicated; H_2S biological actions are dependent on its chemical (reductive and nucleophilic) attributes. H_2S is fast-dissolving in aqueous solutions (due to its fine acid quality), splitting to generate two anionic parts: sulfide (S_2^-) and hydrosulfide (HS^-). About 81.5% of the total H_2S exists as HS^- and S_2^-, and only 18.5% as an undissociated acid at a specific physiological potential of hydrogen (pH) of 7.4 in an aqueous solution, and thus has biological functions similar to other ionic species [3]. As a result, HS^- is an excellent substrate for the Anion Exchanger 1 (AE1) [15]. The acid H_2S/HS^- balance follows the same principle as CO_2/HCO_3^- in the Jacobs–Stewart cycle [16]. H_2S is highly lipophilic—a feature that allows it to freely penetrate all cell membranes without the facilitation of membrane channels, thereby exerting biological activities [17]. Additionally, it has a very high permeability coefficient in human erythrocytes. Related to this capability, it was established that in cases characterized by a lack of extracellular hydration and intracellular dehydration, the pH buffer system of the $Cl-/HS-/H_2S$ is faster than the $Cl^-/HCO_3^-/H_2CO_3$ cycle [18]; however, in severe DM, such a pH buffer, it is much lower than that in healthy persons, and therefore, its protective effect is diminished [19].

H_2S serves as a gasotransmitter in regulating organ development and maintaining homeostasis. Therefore, its abnormal levels are linked with multiple human diseases, such as DM, neurodegenerative diseases, myocardial injuries [20], or ophthalmic pathology [21]. Plasmatic H_2S levels are remarkably lower in diabetic patients [3]. In addition, the levels of H_2S in plasma, urine, and heart tissues are prone to be lower in aging diabetic rodents [22].

The striking lack of uniform data concerning H_2S levels under physiological conditions may contribute to uncertainty about the precise mechanistic roles of H_2S in various physiological processes. Hence, it is essential to improve the specificity of detection and reduce the threshold limits of related techniques, which may be helpful in achieving more accurate information regarding the physiological distribution of H_2S in the blood, cells, and tissues, as well as more consistent knowledge on its typical tasks at an intimate level based on more comprehensive data on its oxidative phosphorylation activities [23] to satisfy cellular energy requirements, and perhaps other biological involvements [3].

2. Materials and Methods

This systematic literature review is based on the PRISMA methodology, by searching free full-text available papers written in English, which have appeared in the last five years, by specific keywords combinations (Table 1), in the well known international databases: National Center for Biotechnology Information (NCBI)/PubMed, PubMed Central (PMC), Elsevier, and Web of Science. Cochrane and PEDro databases returned no results.

Table 1. The keyword combinations used for the contextual searches in the international databases.

Keywords in Title, Abstract or Author-Specified Keywords	Elsevier	PubMed	PMC	ISI	Total
"Hydrogen sulfide" AND "Diabetes"	8	63	45	59	175
"H2S" AND "Diabetes"	1	14	8	14	37
Total	**9**	**77**	**53**	**73**	**212**

The scientific impact of each article was established using a customized quantification formula to obtain a PEDro score. We considered eligible the works that received a score of at least 4 ("fair quality = PEDro score 4–5").

To evaluate the impact of hydrogen sulfide therapeutic interventions in DM, we searched on https://clinicaltrials.gov, https://trialsearch.who.int/, and https://www. clinicaltrialsregister.eu, (accessed on 1 January 2022), for clinical trials using as items Diabetes Mellitus and H_2S or hydrogen sulfide. The inclusion criteria were fixed regarding patients with Diabetes, age: 18 to the elderly, of all genders. Exclusion criteria correspond to study dates: not before 2017. In addition, a meta-analysis was included to analyze the different pathologies associated with diabetes and the number of subjects.

3. Results

3.1. Search and Filtering Results

Databases interrogation provided, initially, 212 articles. Appling the PRISMA selection filters and scoring resulted in 51 unique published qualified studies. We added another 94 free papers found based on Google Search by a direct internet search (Figure 1), which were highly related to our research (Table 2).

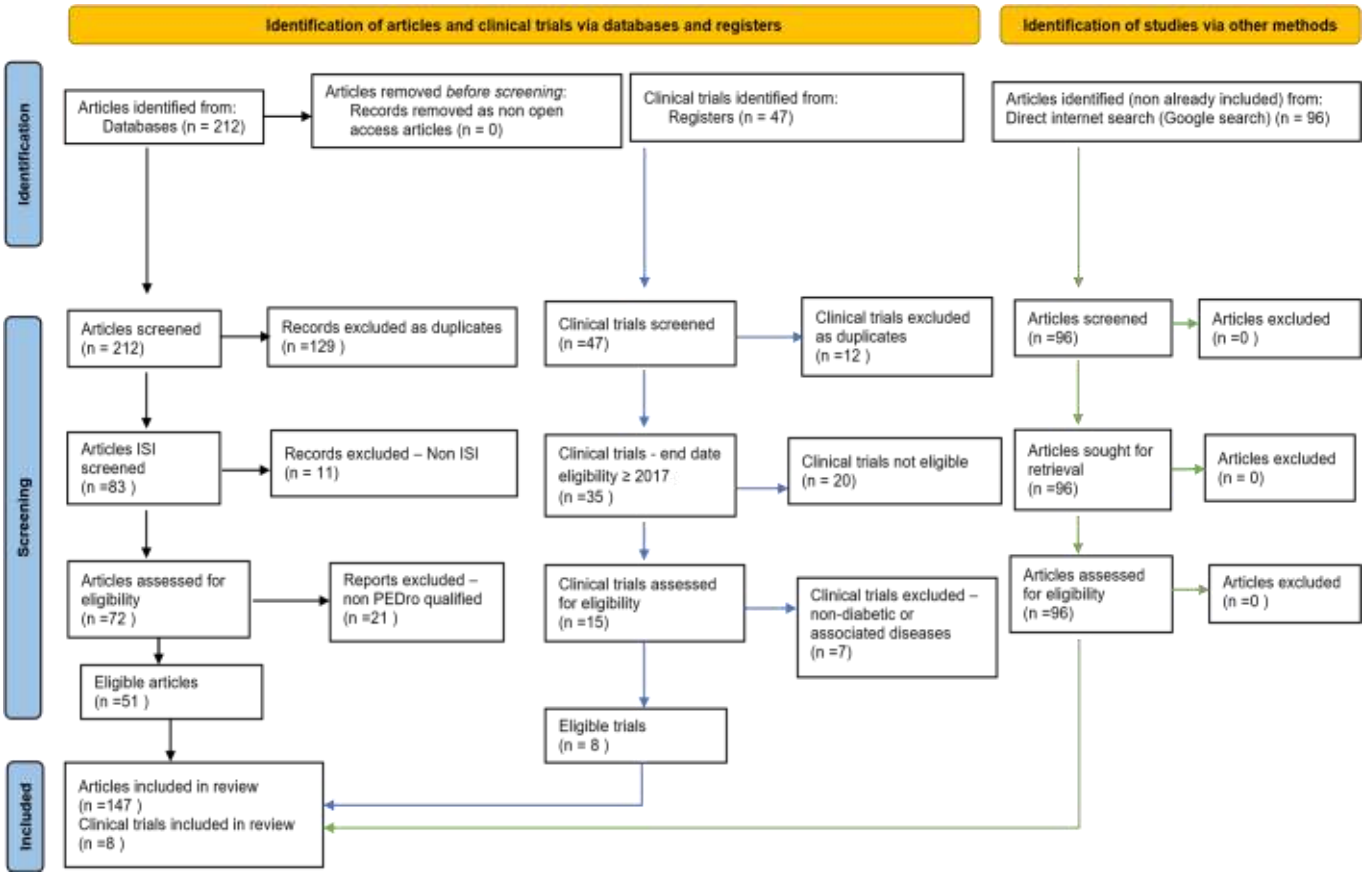

Figure 1. Our adapted PRISMA-type of the flow diagram.

Table 2. PRISMA resulting conceptual skeleton structure of the article's organization approach.

Physiological Properties of H_2S

Authors	Ref. No.	Subject-Data
(Sun, 2021)	[3]	An Updated Insight Into Molecular Mechanism of H_2S in Cardiomyopathy
(George, 2018)	[6]	Treating inflammation and oxidative stress with H_2S during age-related macular degeneration
(Zou, 2017)	[10]	H_2S ameliorates cognitive dysfunction in streptozotocin-induced diabetic rats
(Rey, 2021)	[11]	Mitochondrial metabolism as target of the neuroprotective role of erythropoietin in Parkinson's disease.
(Testai, 2021)	[12]	Modulation of EndMT by H_2S in the Prevention of Cardiovascular Fibrosis
(Ciccone, 2021)	[13]	Endothelium as a Source and Target of H_2S to Improve Its Trophism and Function
(Wu, 2017)	[16]	Exogenous H_2S facilitating ubiquitin aggregates clearance via autophagy
(Hu, 2017)	[20]	Chelerythrine Attenuates Renal Ischemia/Reperfusion-induced Myocardial Injury
(Kar, 2019)	[22]	H_2S -mediated regulation of cell death signaling ameliorates adverse cardiac remodeling
(Jeong, 2020)	[24]	Protective effect of H_2S on oxidative stress-induced neurodegenerative diseases
(Luo, 2019)	[25]	H_2S upregulates renal AQP-2 protein expression and promotes urine concentration
(Yang, 2019)	[26]	Exogenous H_2S mitigates myocardial fibrosis through suppression of Wnt pathway

(Liu, 2018)	[27]	H_2S attenuates myocardial fibrosis through the JAK/STAT signaling pathway
(Sun, 2019)	[28]	Exogenous H_2S reduces the acetylation levels of mitochondrial respiratory enzymes
(Roa-Coria, 2019)	[29]	Possible involvement of peripheral TRP channels in the H_2S-induced hyperalgesia
(Yang, 2017)	[30]	Exogenous H_2S regulates endoplasmic reticulum-mitochondria crosstalk to inhibit apoptosis
(Zhao, 2021)	[31]	H_2S Plays an Important Role in Diabetic Cardiomyopathy
(Liu, 2017)	[32]	H_2S modulating mitochondrial morphology to promote mitophagy in endothelial cells
(Qiu, 2018)	[33]	Alpha-lipoic acid regulates the autophagy of vascular smooth muscle cells elevating H_2S level
(Li, 2017)	[34]	H_2S reduced renal tissue fibrosis by regulating autophagy in diabetic rats
(Yu, 2020)	[35]	Exogenous H_2S Induces Hrd1 S-sulfhydration and Prevents CD36 Translocation via VAMP3
(Kar, 2019)	[36]	H_2S Ameliorates Homocysteine-Induced Cardiac Remodeling and Dysfunction
(Dominic, 2021)	[37]	Decreased availability of nitric oxide and H_2S is a hallmark of COVID-19
(Loiselle, 2020)	[38]	H_2S and hepatic lipid metabolism-a critical pairing for liver health
(Ma, 2017)	[39]	Exogenous H_2S Ameliorates Diabetes-Associated Cognitive Decline
(Jiang, 2020)	[40]	H_2S Ameliorates Lung Ischemia-Reperfusion Injury Through SIRT1 Signaling Pathway
(Wu, 2019)	[41]	H_2S Inhibits High Glucose-Induced Neuronal Senescence by Improving Autophagic Flux
Pathophysiological Properties H_2S		
Authors	**Ref. No.**	**Subject-Data**
(Citi, 2021)	[7]	Role of H2S in endothelial dysfunction: Pathophysiology and therapeutic approaches
(Kang, 2020)	[14]	H_2S as a Potential Alternative for the Treatment of Myocardial Fibrosis
(Sun, 2019)	[42]	H_2S and Subsequent Liver Injury
(Szabo, 2017)	[43]	Pharmacological Modulation of H_2S Levels

Table 2. *Cont.*

Pathophysiological Properties H_2S		
(Sun, 2020)	[44]	The Link Between Inflammation and H_2S
(Zheng, 2020)	[45]	H_2S protects against diabetes-accelerated atherosclerosis by preventing the activation of NLRP3
(Jia, 2020)	[46]	H_2S mitigates myocardial inflammation by inhibiting nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome activation in diabetic rats
(Li, 2017)	[47]	H_2S improves renal fibrosis in STZ-induced diabetic rats by ameliorating TGF-beta 1 expression
(Kar, 20190	[48]	Exercise Training Promotes Cardiac H_2S Biosynthesis and Mitigates Pyroptosis
(Li, 2019)	[49]	Exogenous H_2S protects against high glucose-induced apoptosis and oxidative stress
H_2S—Role in Diabetes Mellitus and Associated Vascular Pathology		
Authors	**Ref. No.**	**Subject-Data**
(Gheibi, 2020)	[8]	Regulation of carbohydrate metabolism by NO and H_2S: Implications in diabetes
(Zhang, 2021)	[50]	H_2S regulates insulin secretion and insulin resistance in diabetes mellitus

(Chen, 2021)	[51]	Role of H_2S in the Endocrine System
(Gheibi, 2019)	[52]	Effects of H_2S on Carbohydrate Metabolism in Obese Type 2 Diabetic Rats
(Luo, 2017)	[53]	The Role of Exogenous H_2S in Free Fatty Acids Induced Inflammation in Macrophages
(Comas, 2021)	[54]	The Impact of H_2S on Obesity-Associated Metabolic Disturbances
(Suzuki, 2017)	[55]	Clinical Implication of Plasma H2S Levels in Japanese Patients with Type 2 Diabetes
(Zhou, 2019)	[56]	H_2S Prevents Elastin Loss and Attenuates Calcification Induced by High Glucose
H_2S—As a Natural Therapeutic Factor in DM		
Authors	**Ref. No.**	**Subject-Data**
(Melino, 2019)	[2]	Natural H_2S Donors from *Allium* sp. as a Nutraceutical Approach in Type 2 Diabetes
(Sashi, 2019)	[5]	H_2S inhibits Ca^{2+}-induced mitochondrial permeability transition pore opening
(Yang, 2017)	[21]	H_2S Releasing/Stimulating Reagents
(John, 2017)	[57]	GYY4137, an H_2S Donor Modulates miR194-Dependent Collagen Realignment
(Bitar, 2018)	[58]	H_2S Donor NaHS Improves Metabolism and Reduces Muscle Atrophy in Type 2 Diabetes
(Ding, 2017)	[59]	High Glucose Induces Mouse Mesangial Cell Overproliferation via Inhibition of H_2S Synthesis

Our search on clinical trial registration platforms showed that despite the vast number of trials (25,969) on DM, only 46 included H_2S/hydrogen sulfide. In addition, 12 were duplicates, and 26 were performed/ended before 2017. Therefore, we selected/filtred for our meta-analysis eight relevant reports that included 1237 subjects.

3.2. Physiological Properties of H_2S

H_2S influences many cellular processes (Figure 2) through a broad spectrum of signaling molecules, reacting with superoxide anions, hypochlorite, hydrogen peroxide, peroxynitrite, metals, thiol derivatives, and NO [60]. Moreover, with its aforementioned high rate of the anionic chemical state in aqueous solution, there are reported antioxidant properties of H_2S that can mitigate oxidative stress-induced dysfunctions. It also acts through the potassium (KATP/K^+) and calcium (Ca^{2+}) ion channels to increase (antioxidant) glutathione (GSH) levels. GSH, Gpx (glutathione peroxidase), and superoxide dismutase (SOD) neutralize H_2O_2-induced oxidative damage in mitochondria. To be specified that ROS (Reactive Oxygen Species) are formed within the oxidative phosphorylation process (and in excessive quantities in such a process' inefficiency, leading to oxidative stress and affecting mitochondrial metabolism) [11], and attenuation of mitochondrial ROS release results in completely preserved insulin sensitivity despite a high-fat diet [24].

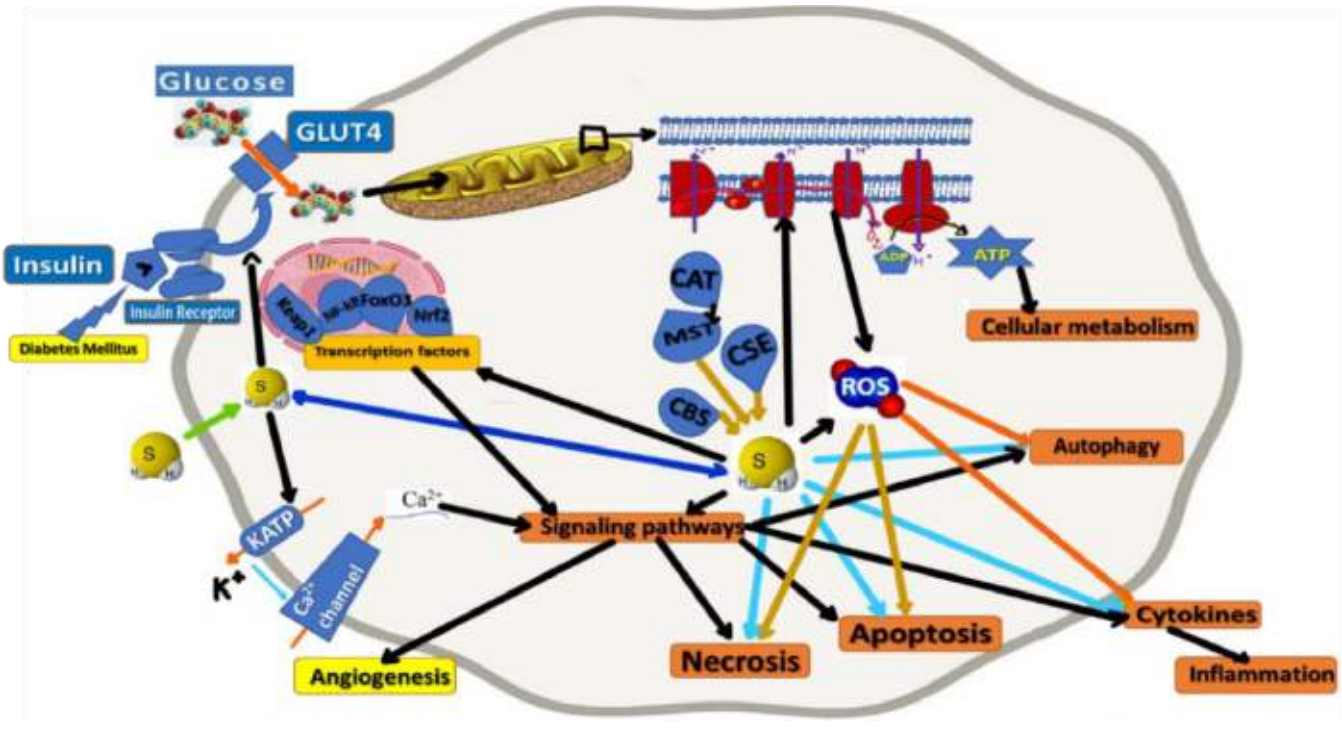

Figure 2. Intimate mechanisms as molecular therapeutic/rehabilitative targets of H_2S in the case of cells influenced by DM (which impacts glucose uptake, affecting the relation of insulin signal transmission pathway with the cell glucose uptake). Intimate connections are indicated through black arrows, while increasing influences are marked by green arrows and inhibiting or reducing impacts of H2S through blue arrows. Finally, the biosynthesis pathways are stated, represented by cystathionin-β-synthase (CBS), cystathionin-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST), the latter connected with cysteine aminotransferase (CAT).

H_2S is also endogenously produced, like nitric oxide (NO) and carbon monoxide (CO), which are similar gasotransmitters. H_2S has been experimentally shown to be involved in the bio-molecular regulation of vital physiological processes such as the inflammatory response, apoptosis, oxidative stress, and angiogenesis. The brain, liver, kidney, and other organs produce H_2S [7]. The cellular biogenesis of H_2S is based on the desulfuration of cysteine or homocysteine, a process involving mainly three enzymes: cystathionin-β-synthase (CBS), cystathionin-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST) [61]. H_2S biogenesis at the mitochondria level implies cysteine aminotransferase (CAT) that catalyzes L-cysteine and glutamate to 3-mercaptopyruvate and α-ketoglutarate. Furthermore, 3-mercaptopyruvate is metabolized to pyruvate and H_2S via 3-mercaptopyruvate sulfurtransferase (3-MST) [24].

MicroRNAs are factors involved in the upregulation of CSE expression. It was also found that some currently used drugs, including angiotensin-converting enzyme (ACE) inhibitors [62], statins [14], calcium channel antagonists, aspirin, and metformin vitamin D3 [42], and many others, may increase the biogenesis of H_2S. From this list, statins, for example, can increase H_2S synthesis via Akt-mediated control of CSE or suppress H_2S degradation by decreasing coenzyme Q level, a sulfide quinone reductase cofactor [57].

Several routes could eliminate the H_2S. Firstly, H_2S can be transformed into thiosulfate by mitochondrial oxidative modification, or further converted into sulfite and sulfate. Next, cytosolic methylation is another pathway used to transform H_2S to dimethylsulfide by thiol S-methyltransferase. Finally, the excessive H_2S could be scavenged by Metallo- or disulfide-containing molecules or glutathione disulfide and could also be released by the lungs [63].

Exogenously supplied or endogenously generated, H_2S can be stored at the cellular level as bound sulfane, a reductant labile sulfur (e.g., persulfide, polysulfide, and proteinassociated sulfur, among others) [14]. Human erythrocytes are about ~5 billion per mL of blood, and each has over 270 million hemoglobin molecules that can uptake H_2S, effectively controlling its clearance. This distribution ensures the maintenance of the physiological plasma and tissue concentration of free H_2S in the range of 15 to 150 nM. In addition, the high lipid and water solubility of H_2S allow quick passage through the alveolar membrane, which assures an equilibrium between blood and the alveolar air level of H_2S [18].

The potential of H_2S metabolite products as biomarkers is appreciated since the plasmatic and urinary levels of H_2S may reflect renal disease severity, such as chronic kidney disease [25]. Therefore, excessive exposure to H_2S can lead to cellular toxicity, orchestrate pathological processes, and increase the risk of various diseases [64]. H_2S is one of the most toxic poisons, and is even more harmful than cyanide on a mole-to-mole basis. A solution of dissolved H_2S diminishes the activity of mitochondrial cytochrome c oxidase at a concentration ranging from 10 to 30 µM. In vivo studies have shown that in rodents and large mammals, severe depression of the medullary respiratory neurons and/or cardiac contractility by infusion or inhaling H_2S at concentrations yield plasma concentrations of gaseous H_2S between 2 and 5 µM.

H_2S can attenuate matrix deposition and myocardial fibrosis [26] and improve MMP/TIMP disorder. The mechanism of H_2S protection against diabetic myocardial fibrosis depends on the down-regulation of JAK/STAT and TGF-β1 (transforming growth factor) signaling [27].

Many physiological and pathophysiological properties regarding antioxidation, apoptosis, or inflammation of H_2S are mediated through transcription factors such as Nrf2 (nuclear factor-E2-related factor), FoxO3 (Forkhead box O), and NF-kB (Nuclear factor kappa-light-chain-enhancer of activated B cells) [22]. The epigenetic role of H_2S is unveiled by Brg1 (Brahma-related gene 1) expression modulation at the promoter region, decreasing the ATP-dependent chromatin remodeling complex's transcriptional level, which inhibits vascular smooth muscle cell proliferation. Moreover, H_2S may reduce the lysine acetylation of enzymes involved in fatty acid β-oxidation and glucose oxidation in diabetic statuses [28], exerting a beneficial effect on cardiac energy substrate utilization [65].

H_2S is known to regulate various physiological functions, such as decreasing blood pressure, acting on various targets, including ion channels, such as ATP-sensitive potassium channels (KATP) [66], voltage-gated potassium channels (Kv7) [67], transient receptor potential channels (TRPV) [29], or L/T-type Ca^{2+} channels [68], mitoKATP/Kv7 channels [69]. By activating ATP-sensitive K^+ channels, H_2S lowers blood pressure, protects the heart from ischemia and reperfusion injury, inhibits insulin secretion in pancreatic β cells, and exerts anti-apoptotic, anti-inflammatory, and anti-nociceptive effects [70]. KATP channels also play a crucial role in insulin secretion in pancreatic cells, where the opening of the channels by H_2S decreases insulin secretion. Both endogenous and exogenous H_2S inhibits insulin secretion from cells by activating KATP channels and inhibiting L-type voltage-dependent calcium channels. In addition, by inhibiting glucose transporter-4 (GLUT-4), H_2S inhibits insulin-stimulated glucose uptake in adipocytes, indicating that H_2S decreases the insulin sensitivity of adipocytes [71].

During hyperglycemia, elevated levels of H_2S can open the KATP channels in the islets cell membrane, which can cause high hyperpolarization and lower insulin secretion. This effect is caused by several biochemical processes that inhibit insulin secretion [69].

The endoplasmic reticulum (ER) is the cytoplasmic location where proteins are synthesized. It maintains Ca^{2+} homeostasis and participates in protein folding [72]. The molecular markers of stress include C/EBP homologous protein, cleaved caspase-12, and the glucose-controlled protein 78 (GRP78). It has been observed that chronic ER stress can trigger DM, Alzheimer's disease, and other neurodegenerative disorders [73], engaging ER stress-induced apoptosis [10].

Studies on diabetic cardiomyopathy have shown that the effects of H_2S on the endoplasmic reticulum stress are related to its reduction in levels of mitochondria apoptotic proteins [30,31]. The endoplasmic reticulum's interaction with mitochondria is regulated by the ROS pathway [74]. Mitofusin-2 is a critical protein that can bridge the endoplasmic reticulum and mitochondria. It plays a role in the fusion and fission of mitochondria. It is believed that Mfn-2 is involved in the cardiac system's mitochondria function and is triggered by oxidative stress [30]. The high levels of Mfn-2 can also induce cardiomyocyte apoptosis [32].

The mitochondria control energy homeostasis and regulate ROS production [75]. Mitochondria play a significant role in the mechanism of fatty acids β-oxidation. On the other hand, mitochondrial dysregulations occur in insulin resistance. The number of mitochondria in hepatocytes decreases in CSE-deficit cells. Hyperglycaemia leads to the generation of mitochondria superoxide, which causes the synthesis of oxidants and endothelial dysfunction. H_2S works as an electron donor to the respiratory chain and plays a therapeutic role in DM and associated vascular diseases.

Mitochondrial DNA (mtDNA) content levels are significantly reduced in CSE-gene knockout mice. This depletion can be reversed by exogenous H_2S gain [76]. H_2S can provoke mtDNA replication and mitochondrial biogenesis by suppressing mitochondrial transcription factor A (TFAM) methylation. In contrast, H_2S may stimulate cardiac mitochondrial biogenesis by activating the AMPK (5^0 AMP-activated protein kinase) [33] PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) pathway [77]. Sulfhydration of AMPK and PP2A (protein phosphatase 2A) [78], which leads to AMPK activation and PP2A inhibition, respectively, has been proposed as a mechanism that may be involved in H_2S-mediated stimulation of unstressed mitochondrial biogenesis [14].

H_2S is a gasotransmitter with discovered roles in cellular signaling, which can also be stored as bound endosulfan, known to play a variety of physiological functions [79]. Some of these include vasodilation [80], anti-apoptosis [81], anti-inflammation [82], cell survival/death [15], cell proliferation/hypertrophy [83], endoplasmic reticulum stress [84], antioxidative stress [32], mitochondrial bioenergetics/biogenesis [50], blood pressure reduction [85], and cell differentiation [86]. H_2S ameliorates diabetic complications, including endothelial dysfunction [87], nephropathy [34], retinopathy [88], and cardiovascular diseases.

H_2S could also increase the apoptosis of islet cells and inhibit the programmed cell death of pancreatic cells by blocking the ERK (extracellular signal-regulated) protein kinase [33]. It has also been shown to inhibit the anti-inflammatory or antioxidant signaling pathways of pancreatic cells. Injecting H_2S into STZ-induced diabetic rats can improve the status of their diabetes by blocking the PKC/ERK$\frac{1}{2}$ signaling pathway. The effects of blocking the JAK/STAT signaling pathway are also linked to the H_2S' anti-apoptotic effects [58]. The myocardial expressions of pro-fibrotic factors, such as MMP-2 (matrix metalloprotease 2), TIMP-2 (tissue inhibitor of metalloproteinase 2), transforming growth factor (TGF)-β1/SMAD family member 3 (Smad3) signaling pathway, and collagens are strikingly changed in diabetic rats. Many studies have shown that suppressing the STAT3 pathway can improve the physiological effects of H_2S. It can also contribute to the cardioprotective effects of H_2S by reducing the levels of ROS in the body [89].

H_2S can also activate the soluble guanyl cyclase (sGC) by directing its interaction with the cGMP phosphodiesterase (PDE). In addition, this molecule can trigger the activation of the cyclic GMP-protein kinase G pathway [90]. It can also trigger the re-translation of eIF2 (eukaryotic initiation factor 2) [91] by increasing the phosphorylation of protein phosphatase-1 [27].

H_2S can also convert the -SH group of cysteine into a -SSH group, which can alter the activities of various enzymes such as the F_1F_0-ATPase pump, KATP channels, and the phosphatase and tensin homolog (PTEN) [14]. This can lead to the disappearance of certain protein S-sulfates. S-sulfhydration is a post-translational process that produces a hydropersulfide moiety or polysulfide in specific body regions. It is known to regulate the cellular functions of H_2S [92]. HMG-CoA reductase [35] is an enzyme involved in the ubiquitination of various substrate proteins, such as Hrd1. H_2S induces the degradation of VAMP3 (vesicle-associated membrane protein 3), which controls exocytosis by Hrd1 S-sulfhidration. It is also known to trigger the translocation of CD36, which can cause lipid toxicity in the body [58].

Cytokines are small molecules that help the cell produce pro-inflammatory signals. Increased production of cytokines in the serum and heart muscle is a common feature of cardiovascular disease involving cell death. The JAK/STAT signaling pathway is a vital pathway for cytokine signal transduction and a pleiotropic cascade involved in growth hormone receptors' activity and regulates various physiological and pathological processes, including proliferation, differentiation, apoptosis, and cellular immunity, and inflammation. In addition, this pathway can also increase the expression of TGF and type III collagen [93].

The pro-inflammatory cytokine TNFα can also induce apoptosis and necrosis. The stimulation of TNFα in the liver and macrophages can increase the secretion of H_2S. It has been observed that the treatment with LPS leads to an increase in the production of both IL-6 and TNFα, an epigenetic regulation mechanism [94].

In addition, treating patients with H_2S can decrease the production of neutrophils in the myocardium and contribute to the development of anti-apoptotic signaling. Neutrophils are recruited into the myocardium to express IL-1β and TNFα. H_2S reduces these immunity cells, correlated with promoted Bcl-2 anti-apoptotic signaling, decreases cytokine release, and preserves cardiac function [95].

VEGF is a pro-angiogenic cytokine that promotes endothelial cell survival. In the case of acute coronary syndrome, the reduction of VEGF leads to the depletion of microvessels. On the other hand, H_2S can prevent coronary artery disease and improve the survival of endothelial cells [96].

TGF is a critical cytokine in the development of cardiac remodeling. Myofibroblasts can promote the growth and deposition of collagen in the body. However, the presence of H_2S can inhibit the signaling cascade in myofibroblasts and limit the proliferation and survival of these cells. The cytoprotective effects of H_2S on cell death appear to act through cell types other than cardiomyocytes, where it influences TGFβ expression and inhibits the signaling cascade in fibroblasts. This restricts the differentiation and proliferation of fibroblasts into myofibroblasts and prevents the deposition of collagen in the heart [22].

H_2S and NO are physiological and pathological factors that have been extensively studied lately. They have been linked to the development of diabetes and heart failure [92]. The exposure of mice to H_2S can stimulate the production of NO through the activation of the eNOS pathway [97]. This can result in the development of more severe cardiac dilatation. Treating patients with H_2S using CSE overexpression can also improve the function and structure of their hearts after undergoing transverse aortic contraction. This therapy activates the eNOS-NO-cGMP pathway [36] and can prevent hepatic and myocardial ischemia-reperfusion injury [98–100].

3.3. H_2S in Pharmacology and Pathophysiology

H_2S levels are decreased in several conditions (e.g., DM, ischemia, and aging), even in COVID-19 [37], and are elevated in other statuses (e.g., inflammation, critical biological disbalances, and cancer). In recent decades, multiple approaches to the therapeutic exploitation of H_2S have been identified, either based on H_2S exogenous apport or decreased H_2S biosynthesis [43]. Inhibition and stimulation of H_2S synthesis have been suggested as potential interventions in DM.

Treating patients with H_2S can improve the recovery of liver and myocardial ischemiareperfusion injury. It can also restore the damaged endothelium-dependent relaxation caused by NO depletion [13]. H_2S is known to produce nitroxyl, a one-electron reduction of NO. It can also help restore the relaxation caused by the depletion of NO [101].

Various studies have also shown that H_2S can promote the development of new blood vessels. Most of these studies were focused on the effects of VEGF on the angiogenic response [11]. Silencing of CSE by siRNA can also decrease the impact of VEGF-induced angiogenesis. It can also stimulate the activity of various cellular signaling pathways, such as the eNOS-NO-c pathway and the K1A2T7P signal transducer. H_2S promotes angiogenesis by increasing the activity of endothelial nitric oxide synthesis (eNOS), phosphatidylinositol 3 (PI3)-kinase/protein kinase B (AKT), p38/MAPK, K1A2T7P, signal transducer and activator of transcription 3 (STAT3), and sirtuin 1 (SIRT1)/VEGF/cyclic guanosine 5^0-monophosphate (cGMP) cascade [102].

Although diabetes has been known to impair the development of new blood vessels [13], the mechanism of this process is not yet precise. The absence of vascular perfusion leads to diabetes-induced angiogenesis. H_2S rescues the migration of HUVECs in mice with hyperglycemia-induced migration. The effects of this condition on the pro-angiogenic and bio-energetic properties were also studied. H_2S improves the revascularization of diabetic mice through increasing NO bioavailability and promotes the development of vascular progenitors [86].

Under pathological conditions, the levels of H_2S and its production enzymes are significantly altered [103]. This can lead to the development of various cardiac disorders [38]. In addition, H_2S increases the filtration rate and kidney blood flow [62] and generates an increase in the excretion of certain nutrients, such as K^+ and Na^+. The role of the RenalAangiotensin system (RAS) [22] is well established in the pathogenesis of various diseases. It plays a central role in regulating physiological function and possesses neuronal control of the circulatory system. H_2S is also known to interact with the zinc metalloproteinase, a zinc metalloproteinase. In addition, studies show that this protein can reduce the activity of the angiotensin-converting enzyme (ACE) in human endothelial cells.

Myocyte stretching releases angiotensin II (ANG II) [3], increases p53 binding to the ANG II promoter and the AT1 (angiotensin II type 1) receptor, and results in a four- to seven-fold increase in apoptosis. Adding Zn^{2+} to the diet lowered the ACE mRNA level and reduced ROS production. H_2S could also alter RAS signaling, interacting with the ACE, a zinc metalloproteinase [62]. A dose-dependent drop in ACE activity in human endothelial cells after treatment with H_2S was observed. Supplementation of H_2S in DM rats reversed RAS activation and reduced ROS production. H_2S could alter RAS signaling, reducing oxidative stress [22].

As a neuromodulator, H_2S can improve the effects of diabetes on the central nervous system (CNS). Due to the impact of diabetes on the CNS, it is considered a leading cause of cognitive decline [39]. H_2S can also reduce the risk of cognitive decline and microvascular complications [94]. An equilibrated balance of oxidative stress/antioxidants is essential for maintaining cellular function. When this is disturbed, the other molecules, such as deoxyribonucleic acid, lipid, and protein oxidize, imprinting a pathological condition, like diabetes [24]. Oxidative stress comes from the overproduction of reactive oxygen and nitrogen species. The main source of ROS is the mitochondria [99]. Oxidative stress in diabetic patients determines dysfunctions during insulin secretion in the nervous system, and thus, neurodegeneration, such as diabetic peripheral neuropathy (DPN), occurs [104].

The pancreatic β cell is the most essential metabolically active part of the body, where metabolites take place for energy synthesis at the high glucose concentration level. H_2S displays antioxidant effects by directly silencing reactive oxygen species (ROS) via a hydrosulfide anion (HS-), a powerful one-electron chemical reductant dissociated from H_2S in a physiological fluid. H_2S can improve the function of the mitochondria, which is a type of respiratory chain that produces oxygen [105]. Overproduction of

reactive nitrogen and oxygen species can lead to oxidative stress. The free radicals produced by these species can be suppressed by antioxidant molecules [14]. H_2S can also decrease ROS production by suppressing the copper/zinc superoxide activation. In addition, it can also prevent the degradation of antioxidant enzymes and proteins [63].

Autophagy is emerging as a critical cellular stress response that is involved in a variety of disease states. Autophagy is a highly conserved self-feeding pathway that degrades macromolecules and damaged organelles to maintain intracellular homeostasis. It has been shown that H_2S is a regulator of autophagy. Generally, autophagy serves a dual purpose: it may play a cytoprotective or harmful role in the body, hanging on the type and severity of the lesion it causes [22]. A certain degree of autophagic activity is essential in promoting tissue homeostasis and cell survival. However, excessive autophagic activity can contribute to apoptosis on the other side. In addition, autophagy dysfunction is involved in diabetic cardiomyopathy [106].

Autophagy is a well-coordinated, multi-stage process regulated by autophagy-related genetic products and proteins, such as Beclin1 and P62. Exogenous H_2S facilitates the elimination of autophagosome contents, which improves autophagy. The promotional effects of exogenous H_2S on autophagy may be essential for decreased ROS production. In addition, there are studies that ubiquitin aggregate clearance is mainly dependent on autophagy, and disruption of autophagy results in the accumulation of ubiquitin aggregates in cells [33].

H_2S has its regulatory role in autophagy during the development and progression of numerous diseases, such as diabetes, heart failure, or Parkinson's disease [16]. Exogenous H_2S reduces the ubiquitination level. Recent studies have found that Keap-1 is crucial in eliminating ubiquitin proteins [107]. Keap-1 can be a critical factor in the protective role of exogenous H_2S on ubiquitin aggregate clearance via autophagy [16]. Exogenous H_2S upregulates the expression of Keap-1. Reported data show that Keap-1 regulates the translocation of Nrf2, a negative regulator of ROS production. However, exogenous H_2S had no significant effects on the translocation of Nrf2 to the nucleus. A recent study demonstrated that H_2S suppressed diabetes-accelerated atherosclerosis via Nrf2 [108].

H_2S rectifies high glucose/palmitate-induced excessive autophagy in endothelial cells. The Nrf2-ROS signaling pathway can trigger this effect. However, exogenous H_2S inhibits mitochondrial apoptosis and promotes mitochondrial autophagy, thus protecting endothelial cells against apoptosis induced by high glucose and palmitate. Therefore, it has been hypothesized that H_2S can promote the normal development of the diabetic endothelial system by suppressing the excessive autophagy that occurs following stressful events [44]. The optimal window of autophagy is maintained in response to stressful events.

However, if it is excessive, autophagy is maladaptive, leading to cell death [22].

Some studies showed that H_2S upregulates autophagy and others that H_2S inhibits autophagy. H_2S plays diverse roles in autophagy depending on the tissue and disease. For example, H_2S could downregulate LC3BII and Beclin-1 protein expression and upregulate p62 protein expression in VSMCs (vascular smooth muscle cells) under HG (high glucose) conditions, which could be reversed by rapamycin, an autophagy activator. Furthermore, NaHS decreased the autophagy induced by HG in VSMCs. Similarly, ALA (Alpha-lipoic acid) could also inhibit autophagy in VSMCs under HG conditions via the AMPK/mTOR signaling pathway. Autophagy is regulated by many signaling pathways, among them the AMPK/mTOR signal pathway being crucial. The activation of the AMPK/mTOR pathway in DM has been widely studied. Increased AMPK phosphorylation and decreased mTOR phosphorylation activate autophagy [33]. H_2S also downregulates autophagy via the AMPK/mTOR signaling pathway [33].

An essential regulator of inflammation associated with metabolic syndrome is the nucleotide-binding domain, leucine-rich-containing family, pyrin domain containing-3 (NLRP3) inflammasome, which activates caspase-1, after interacting with the adaptor protein apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC). Induction of phosphorylation of the p65 subunit of NF-κB resulting in NF-κB signaling activation is a prerequisite for transcriptional activation of NLPR3 [109]. Cleavage, processing, and secretion of pro-inflammatory cytokines IL-1β and IL-18 result from NFκB-mediated activation of NLRP3 inflammasome and subsequent caspase-1 activation [45].

H_2S can exert anti-inflammatory effects against free fatty acid (FFA)-induced inflammation and apoptosis in macrophages by suppressing TLR4/NF-κB-stimulated NLRP3 inflammasome activation. H_2S can thus prevent FFA-overload-mediated insulin resistance and type 2 DM [110].

H_2S exerted an anti-inflammatory role in diabetic myocardia by downregulation of Thioredoxin-interacting protein (TXNIP)-mediated NLRP3 inflammasome activation. H_2S alleviated hyperglycemia-mediated myocardial inflammation in type 1 DM. The mechanism may involve inhibiting TXNIP-mediated NLRP3 inflammasome activation, which might serve as an efficient, targeted therapy in diabetic cardiomyocytes [46].

Pyroptosis is a type of cell death with several characteristics that make it different from other forms of cell death. This type of cell death relies on the canonical pathway, dependent on caspase-1 [47], and the non-canonical pathway, reliant on caspase-11 [111]. Since pyroptosis is known to trigger the inflammatory response that contributes to chronic inflammatory diseases, it has shifted its focus away from the body's natural defenses. The downstream inflammatory markers of pyroptosis are associated with toxic shock, nephropathy, and pathogen defense. The NLRP3 (NOD-like receptor protein 3) inflammasome activates caspase-1 in the canonical pyroptosis pathway. NLRP3 also localizes to the mitochondria and supplies high ROS production, but it can be inhibited by

H_2S [48].

The Mfn-2 protein is known to promote early apoptotic events in the mitochondria. The fragmentation of the mitochondria network can also lead to the development of these events. It has also been shown that Mfn-2 can prevent the transfer of Ca^{2+} from

the endoplasmic reticulum to the adjacent mitochondria. High glucose levels can also promote the growth of H9C2 cells through the increased expression of Mfn-2- and siRNA-mediated Mfn-2 silencing [30,49].

Necrosis is a version of cell death that occurs following severe injury. It is programmed to utilize the TNF receptor and the RIPK1/RIPK3 necrosome. The effects of H_2S on necroptosis and necrosis are limited. However, recent studies have shown that treating cardiomyocytes with high glucose levels can inhibit these markers [112].

A non-canonical death pathway known as MPT (mitochondrial permeability transition pore) is also initiated by Ca^{2+} and ROS. These stressors open the nonspecific MPT pore in the mitochondrial inner membrane, dissipate inner membrane potential, and rupture both mitochondrial membranes through osmotic swelling. The absence of the outer membrane can prevent the formation of apoptotic bodies. Instead, cell death occurs through the accumulation of necrosis. Data show that H_2S protects against MPT-driven necrosis in the heart and brain [113].

3.4. H_2S and Insulin Secretion and Sensitivity

Insulin resistance and compromised insulin secretion lead to impaired glucose metabolism, which contributes to the development of Diabetes [17]. H_2S could be produced endogenously in the pancreatic island's β cells, liver, fat, skeletal muscle, and hypothalamus and regulates local and systemic carbohydrates metabolism [51]. Specifically, H_2S is reported to suppress insulin secretion and promote or reduce islet β-cell apoptosis. It influences insulin sensitivity. H_2S also suppresses glucose uptake and glycogen storage and promotes or inhibits gluconeogenesis, mitochondrial bioenergetics [50], and mitochondrial biogenesis in the liver [52]. This gas also promotes glucose uptake into adipocytes in the fat tissue, while other studies have reported inhibiting this process. H_2S has been shown, as well, to increase adipogenesis, inhibit lipolysis, and regulate adiponectin and MCP-1 secretion in adipocytes [53]. H_2S increases glucose absorption in skeletal muscle, improves insulin sensitivity and modulates circadian clock genes in myocytes. The hypothalamic CBS (cystathionin-β-synthase)/H_2S pathway reduces obesity [54] and improves insulin sensitivity through brain–adipose interactions. Most studies have shown that plasmatic H_2S levels are lower in diabetic patients [50,55].

H_2S can influence insulin secretion and modulate circulating glucose levels. H_2S administration to β cell lines attenuates insulin secretion triggered by a high glucose concentration. High levels of H_2S can also decrease the secretion of insulin. It can also cause the membrane to become polarized and inhibit the KATP channel's independent signaling. H_2S can also inhibit insulin secretion by affecting various biochemical processes: activation of KATP channels, inhibition of ATP synthesis, and inactivation of L-type voltagedependent Ca^{2+} channels [114].

The effects of hyperglycemia on insulin secretion can vary depending on the phase of diabetes development. During the early stages of the disease, increasing levels of H_2S can protect islet cells from further damage. During the development of diabetes, an increase in H_2S can inhibit the secretion of insulin and reduce the overload of islet cells. This can also trigger an increase in ER stress response [50].

Inhibitory effects of sodium hydrosulfide (NaSH, 10 μM^{-1} mM) and L-cysteine (0.1–10 mM) on glucose (10 mM)-induced insulin secretion has been observed in both isolated mouse islets and pancreatic β cell lines, an effect that was not observed at a low glucose concentration (3 mM) [115]. One of the mechanisms through which H_2S inhibits insulin secretion is through the opening of KATP channels, as the inhibitory effects of NaSH and L-cysteine on insulin secretion were reproduced after using tolbutamide (a KATP blocker), α-ketoisocaproate (a mitochondrial fuel), and high K^+ condition (30 mmol/L). Interactions between H_2S with KATP channels seems to be mediated through functional manipulation, probably by decreasing selective cysteine residues of the KATP channel protein, independent of cytosolic second messengers. It has been suggested that the Ssulfhydration of KATP channels is a mechanism by which H_2S could influence insulin secretion [56].

Hepatic insulin resistance reveals the failure of insulin to inhibit glycogenolysis and gluconeogenesis in the liver to maintain normal plasma glucose levels. The enzymes CSE, CBS, and 3-MST, responsible for endogenous H_2S, are found in the liver. The effects of diabetes mellitus and its related pathologies on the H_2S production system in the liver are controversial. Compared with nondiabetic rats, H_2S production and CSE and CBS mRNA levels in the liver were increased in STZ diabetic rats, while insulin treatment reversed these effects [114]. H_2S regulates glucose uptake, glycogen storage, and gluconeogenesis H_2S is a key component of liver glucose metabolism [50].

3.5. H_2S and Neurological Dysfunctions as Diabetes Associated Diseases

Neurological research concerning diabetes patients with complications such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis present changes in the central nervous system because of the high blood glucose levels (HbA1c) correlated with poor cognitive function. Oxidative stress plays a part in inhibiting insulin signaling, which is necessary for brain function [110]. The regulation of Schwann cells, aggregation of sorbitol during signaling of the polyol pathway, inactivation of Na^+/K^+ signaling, and hyperglycemia-induced oxidative stress are causative factors of neuropathy in the brain. About 50% of diabetic patients are affected by neurological disorders, the most common comorbidities of DM. In addition, some neurodegenerative diseases, like Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), coincide in the central nervous system (CNS) in diabetic patients because of oxidative stress [24].

H_2S inhibits Aβ-induced neuronal apoptosis by regulating mitochondrial function. In addition, H_2S could inhibit the expression of IL-23/IL-17 axis and mitochondrial apoptotic proteins to alleviate the cognitive decline caused by DM [32].
The repercussions of hyperglycemia-induced oxidative stress on neurons vary in T1DM and T2DM, and DPN patients with T2DM have a low capacity to control hyperglycemia. In the peripheral nerves of T2DM patients, oxidative stress is increased from proximal to distal parts, passes from DRG to the sciatic nerve, and decreases the metabolism under the glycolytic and tricarboxylic acid cycle [29]. In AD, cholinergic homeostasis is hampered by downregulation of insulin/insulin growth factor (IGF) resistance, leading to downregulation of target genes. There is evidence that 40% of patients with DM develop PD, and glucose is impaired at an early stage. However, H_2S can mitigate the effects of oxidative stress on nerve cells.

3.6. H_2S and Cardio-Vascular Dysfunctions as Diabetes Associated Diseases

Cardiovascular complications frequently cause hospitalization and death among diabetic patients. The early-onset diastolic dysfunction is the main characteristic of diabetes cardiomyopathy (DCM), an independent complication of diabetes, secondary to myocardial fibrosis [30]. DCM is a type of cardiomyopathy of unknown etiology, responsible for 75% of idiopathic dilated cardiomyopathy cases among diabetic patients. DCM characteristics are represented by impaired myocardial insulin signaling, mitochondrial dysfunction, overstimulation of the sympathetic nervous system, oxidative stress, increased inflammation, coronary microcirculation dysfunction, and inadequate immune response. These pathophysiological changes lead to fibrosis, hypertrophy, cardiac diastolic and/or systolic dysfunction, and ultimately, heart failure [35].
One of the alarming structural characteristics of DCM is represented by the overproduction and deposition of myocardial interstitial collagen, which leads to cardiac interstitial fibrosis, myocardial rigidity, and cardiac dysfunction. Although the precise mechanism of these changes has not been fully elucidated, the available literature suggests the cellular implication of oxidative stress, cell apoptosis, autophagy, inflammation, and endoplasmic reticulum stress are the main triggers [16].
Homocysteine transsulfuration produces H_2S, a gaseous signaling molecule with a cardioprotective role that is capable of preventing cardiac remodeling, cell death, and pyroptosis. Research activity in vitro and on mouse models demonstrated that H_2S inhibits caspase-1 activity and IL-1 secretion, with important suppression activity on pyroptosis in ischemic cardiomyopathy [116]. H_2S has reducing hypertensive effects and has an important protective role in cardiomyopathy models. Moreover, H_2S is an essential signaling molecule of the cardiovascular system with physiological and pathological mechanisms in ensuring homeostasis [80].
Accelerated atherosclerosis is a common cardiovascular complication in diabetic patients [117]. With a much higher incidence than in non-diabetic patients, atherosclerosis has an earlier onset and a higher mortality rate. Unfortunately, there is no proven treatment capable of slowing down atherosclerosis in DM. On the other hand, H_2S has important effects on atherosclerotic plaque stabilization and on hyperglycemia-induced endothelial dysfunction, being capable of ischemia-reperfusion injury, myocardial infarction, and heart failure prevention [118].
Different pathological states of the venous and/or arterial system characterize the vascular dysfunction or vascular disease, a pathology capable of inducing adverse cardiovascular events. We mention atherosclerosis, arterial remodeling, thrombosis, and restenosis, among these pathologies. The main cardiovascular risk factors (diabetes, obesity, hypertension, aging) are responsible for vascular dysfunction through mechanisms such as oxidative stress, an essential target in therapeutic and preventive strategies. Cellular oxidation is a tightly regulated process involving both pro- and antioxidant systems from different cellular compartments in physiological conditions [87].
Results from current literature support the multiple beneficial roles of H_2S in diabetic cardiovascular complications. First, H_2S slows down the onset and improves the prognosis of diabetic cardiomyopathy. Second, H_2S treatment ameliorates high-fat diet (HFD)induced cardiac dysfunction through sulfide levels restoration; activation of adiponectinAMPK signaling and decrease in HFD-induced ER stress secondary to H_2S underlines its protective effects. Third, adiponectin's essential cardiovascular protective role strengthens the correlation between low adiponectin levels and high cardiovascular risk. Fourth, adiponectin uses the AMPK to deliver its metabolic regulatory effects. AMPK increases the expression of GLUT4, which stimulates glucose transport and modulates fatty acid oxidation and cardiac lipid accumulation through the phosphorylation and inhibition of acetyl-coenzyme [50].
In recent studies, H_2S was shown to be involved in the regulation of various vascular conditions, such as nephropathy, retinopathy, and neuropathy. H_2S-releasing agents could potentially be used as a treatment for diabetes-related endothelial dysfunction. They could help restore the function of the vascular endothelial cells [7].
Many authors also noted that the use of H_2S-releasing agents could be beneficial for treating diabetes by blocking the formation of advanced glycation end products (AGEs), which can lead to the development of vascular complications, and contribute to the degradation of the endothelium's functionality. The rats that were treated with H_2S-releasing agents exhibited a decrease in their vascular oxidation stress levels. This beneficial effect was also partially explained by the compound's ability to increase the NO level [7].

3.7. H_2S and Renal Dysfunctions as Diabetes Associated Diseases

In renal physiology, H_2S induces vasodilation and increases renal blood flow and glomerular filtration rate, resulting in an indirect increase in the urinary excretion of Na^+ and K^+. In addition, H_2S exhibits an inhibitory effect on specific Na^+ and K^+ kidney transporters, thus further increasing the excretion of such electrolytes into the urine. Furthermore, H_2S acts as an oxygen sensor

in the renal system, especially in the medulla. Moreover, H_2S is found to inhibit renin release in rat models of renovascular hypertension. Hypertension-related nephropathy, a consequence of long-term hypertension, is the second leading cause of chronic kidney disease in the world. The blood pressure-lowering [119] actions of exogenous H_2S donors have been demonstrated in spontaneously hypertensive rats, angiotensin II-induced hypertension, N^wnitro- L-argininemethyl ester (L-NAME)induced hypertension, and renovascular hypertension [120]. Furthermore, renal protective effects of H_2S are observed in hypertensive animal models [121].

In Diabetic Nephropathy (DN) [122], the increased expression of TGF-β1 has been shown to promote the accumulation of ECMs such as collagens and fibronectin, apoptosis, dedifferentiation of podocytes, and epithelial-mesenchymal transition of proximal tubules, all of which are considered to facilitate renal hypertrophy and dysfunction. ERK$\frac{1}{2}$, a member of the MAPK family, may be expressed in mesangial cells in the condition of high glucose. ERK$\frac{1}{2}$ may upregulate TGF-β1 expression. Dysregulation of matrix metalloproteinases, (MMPs) or tissue inhibitors (TIMPs), are involved in the mechanism of renal fibrosis. MMPs are responsible for extracellular matrix degradation. MMPs and TIMPs construct a time-and-space-dependent system [68]. Treatment with H_2S could attenuate the progression of renal dysfunction in diabetic rats. The protective effects of H_2S are correlated with TGF-β1 signaling through the $ERK_{1/2}$ pathway [47].

3.8. H_2S Exogenous Sources as Possible Therapeutic Interventions in Diabetes or Related Diseases

H_2S is, on the one hand, a therapeutic natural gas [17] that is found in mofettic joints with carbon dioxide [123], in sulfurous waters [124], with appraised medical effects in balneotherapy [4], and, on the other hand, an endogenous gaseous signal substance in the organisms. Therefore, experimental animals can be exposed to an H_2S-rich environment to observe this gas's physiological effects or toxicity. Reports show that when mice were exposed to 80 ppm of H_2S for 6 h, their oxygen intake dropped by ~50%, and the metabolic rate and core body temperature were also seriously decreased into a suspended animation state. Notably, lowering metabolic demand could help reduce tissue/cellular damage caused by trauma. However, a later study of other larger species indicated that H_2S only exerted thermoregulatory effects. In diabetes, H_2S could promote glucose uptake by ameliorating insulin resistance and reducing renal injury [125].

Peloid or therapeutic mud is a maturated mud with healing properties, composed of a complex intermixture of fine-grained natural substances of geologic and/or biologic origins, water, and standard organic composites from biological metabolic activity. Sapropelic muds or sapropels are found at the bottom of salt waters, originating from the action of microorganisms on flora and fauna of the water basin [17]. The gaseous phase of sapropelic mud results from the biochemical processes involved in the mud formation (peloidogenesis): H_2S, CO_2, NH_4, CH_4, O_2, and Rn. H_2S has been reported as an active molecule of the mud, which can be absorbed through the skin [126], exerting numerous pharmacological effects. Under the action of mud, there is a harmonic stimulation in all glands to increase the enzymatic and synthetic activity, while maintaining the specificity of each. Usually, mud therapy is contraindicated in diabetic patients without glycemic control. Future research is necessary to elucidate the implications of mud therapy on diabetes [127].

Although less rigorously described in the scientific literature, H_2S is commonly used in the context of balneotherapy, where H_2S inhalation occurs as humans are soaking in H_2Scontaining sulfurous waters, with at least 1 mg/L of H_2S. Hydrogen sulfide delivery into the body probably occurs via inhalation and absorption through the skin [128] or, in specific cases, when patients are sitting in closed rooms with H_2S donors and H_2S fountains of H_2S-containing thermal water placed in the middle of the room, where a sensor/ventilation feedback system regulates the H_2S concentration in the air of the room [124]. Small-scale preclinical studies demonstrate the beneficial effects of H_2S delivery via sulfurous waters [129]. In addition, exploratory clinical studies suggest the anti-inflammatory effects of ultrasonic nebulization with sulfurous water in asthmatic patients. However, the potential therapeutic effect of these approaches has not been studied in appropriately powered, randomized clinical trials [130].

One of the potential problems with all forms of H_2S delivery, but especially with H_2S inhalation, relates to the issue of possible overdosing and consequent intoxication. Although the inhibitory effect of H_2S on mitochondrial Complex IV is reversible and therefore supporting therapy can result in patient recovery in some cases, there are currently no well-characterized pharmacological antidotes to H_2S intoxication [131].

Under physiological pH, H_2S is in a specific equilibrium with HS- in aqueous solutions. The HS- and H_2S are in an 81 to 19% report. Inorganic sulfide salts, such as sodium sulfide (Na_2S) and sodium hydrosulfide (NaHS), are frequently used as H_2S equivalents in many kinds of research [132]. These salts are fast H_2S donors, as they produce H_2S after being dissolved in aqueous solutions [21].

The rapid volatilization of H_2S can cause it to escape from the buffers. This phenomenon could explain the discrepancy between the physiological responses required to trigger physiological responses in tissues and blood [133].

Many studies have used NaHS as a standard H_2S donor. For example, it was shown that NaHS could alleviate amyloid beta-peptide (Ab)-induced neural lesion in an Alzheimer's disease cellular model. Furthermore, in hypoxic skin damage, NaHS could exert anti-inflammatory effects through inhibition of reactive oxygen species (ROS)-activated NF-kB/cyclooxygenase (COX)-2 [134].

Allicin is commonly used as a sulfur-containing compound in garlic. It can be considered an active H_2S pool. In aqueous solutions, it can transform various sulfur-containing combinations into H_2S. In contrast, the diallyl disulfide (DADS) produces only a limited amount of H_2S after a slow reaction with GSH. This process can be initiated by forming a cyclic disulfide [2].

3.9. Synthetic Slow-Releasing H_2S Donors

The types of donor that can be considered controlled are those with various release mechanisms [135]. Since using H_2S gas or sulfide salts in studies has been deemed dangerous, researchers have focused on synthetic molecules releasing H_2S [21]. For example, GYY4137 is a Lawesson's reagent that can be used as a slow and safe source of H2S. However, it is not as effective as an aqueous solution and can only be administered on animals.

Another commonly used method is using sulfur-containing dithiolethione [132].

Thiomolybdate salts are thiol transfer reagents in organic synthesis [21]. The four sulfur atoms they present in their structures make them excellent copper chelators. Ammonium tetrathiomolybdate (TTM) can release H_2S under strongly acidic conditions. As such, it is possible to use TTM as an inorganic complex-based H_2S donor. TTM is a slow H_2S releaser. It was discovered that acidic pH increase TTM's H_2S release [43].

In the last years, several ROS-activated H_2S donors were designed. For instance, carbonyl sulfide can be released through a cyclic anhydrase reaction. This process can be sped up by carbonic anhydrase. The tandem reaction will remove carbonyl sulfide (COS), as well as quinone and amine byproducts [136].

Further studies reveal that donors can also release H_2S through the intervention of an endogenous H_2O_2 in their cells. This method is similar to the cyclic anhydrase reaction. COS can easily undergo hydrolysis to produce H_2S if carbonic anhydrase (CA) is presented. However, studies showed that CA is unnecessary for the donors' H_2S release, as H_2O_2 can also trigger the rapid H_2S release from COS [21]. The effects of oxidizing stress on the cell viability of donated human tissue were studied [76]. It was revealed that these individuals exhibited the most effective outcomes of oxidizing stress on their cells [137].

Recently were also communicated a series of esterase-activated H_2S donors. Association of esterase-activated donors with NSAID can form hybrid anti-inflammatory and anti-oxidative combined drugs that reduce NSAID-induced gastric damage [21].

Researchers also discovered nitroreductase-activated donors that could be used to release H_2S [21]. A type of H_2S-producing material is the polyNTA. This substance contains N-thiocarboxyanhydrides and undergoes a ring-opening reaction to donate H_2S [21]. A PEG-ADT (5-4-hydroxyphenyl-3H-1,2-dithiole-3-thione-conjugated with polyethylene glycol) can also be used to generate H_2S. Cell imaging studies also showed that PEG-ADT could enter cells through the endolysosome and last in the cytoplasm [21,43].

3.10. H_2S-Stimulating Agents

Aside from H_2S donors, some compounds can also stimulate the production of H_2S in vivo. For instance, the amino group L-cysteine is an essential substrate for the enzymes that produce H_2S. When acetylated, the resulting product N-acetyl-L-cysteine can increase the production of H_2S [138]. Two other cysteine derivatives, S-allyl-L-cysteine and Spropargyl-L-cysteine, can be used as CSE substrates to generate H_2S [139].

Vitamin D [11] is known to promote the growth and remodeling of bones [21]. In addition, researchers discovered that vitamin D could increase the concentration of H_2S in the liver and kidney. Notably, it was found that cholecalciferol, known as VD3, could increase tissue H_2S concentration in mouse heart, brain, and kidney. Meanwhile, another report suggested that VD3 could upregulate glucose transporter type 4 (GLUT4) and decrease glycemia in diabetes through stimulation of CSE expression and H_2S generation [21,50].

3.11. Clinical Studies on H_2S Donors/Exogenous Sources in Diabetes or Related Diseases—Meta-Analysis

Presentation of clinical studies on H_2S donors/exogenous sources in diabetes or related diseases (Figure 3, Table 3)

- https://clinicaltrials.gov
  - X 17,498 Studies found for: Diabetes Mellitus

X 2 Studies found for: H_2S/Hydrogen sulfide|Diabetes Mellitus

X 18 Studies found for: hydrogen sulfide

- https://trialsearch.who.int
  - X 6430 trials found: Diabetes Mellitus

X 0 Studies found for: H_2S/Hydrogen sulfide|Diabetes Mellitus X 25 trials found for: hydrogen sulfide

- https://www.clinicaltrialsregister.eu
  - X 2041 trials found: Diabetes Mellitus

X 0 Studies found for: H_2S/Hydrogen sulfide|Diabetes Mellitus

X 1 trial found for: hydrogen sulfide

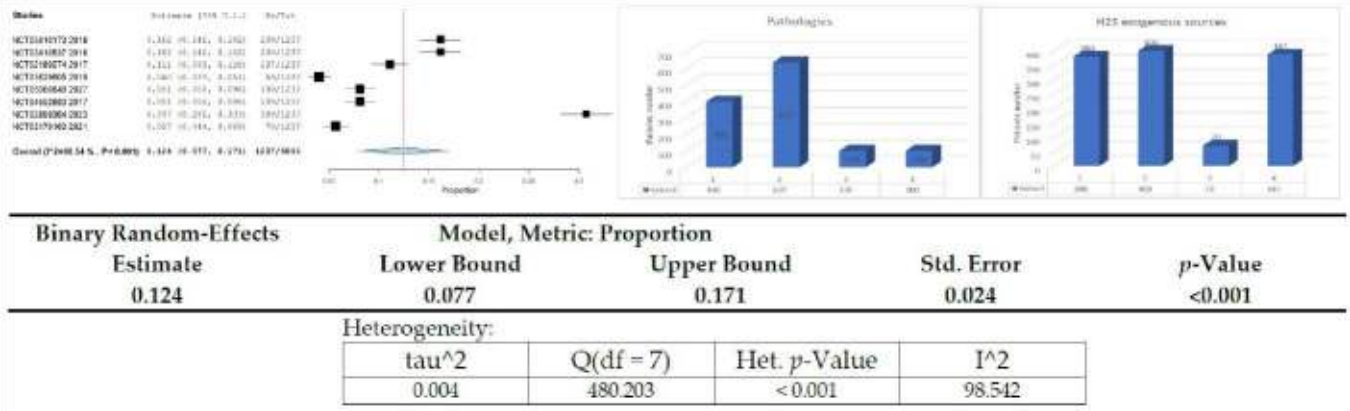

Figure 3. Forest plot related to patients number included in each clinical trial selected for this study.

Table 3. The clinical trials that satisfied all the previous filtering criteria/PRISMA stages selected for qualitative synthesis were included in our meta-analysis to determine the using frequency of the hydrogen sulfide-based interventions for DM or related associated diseases.

No.	Study	Start Year	END Year	N-TotalSubjects	DiabetesMellitus	Cardiovascular/ AssociatedDisease	Neurodegenerative/ AssociatedDisease	Respiratory/ AssociatedDisease	SodiumThiosulfate	Taurine	Captopril/Enalapril/ Hydrochlorothiazide	Observational
0	AXIS LEGEND				1	2	3	4	1	2	3	4
1	NCT03410173	2017	2018	200	200					200		
2	NCT03410537	2017	2018	200	200					200		
3	NCT02180074	2013	2017	137		137						137
4	NCT03829605	2019	2019	50		50						50
5	NCT05060848	2021	2027	100			100					100
6	NCT04952883	2016	2017	100				100				100
7	NCT02899364	2018	2023	380		380			380			
8	NCT03179163	2016	2021	70		70					70	
	TOTAL			1237	400	637	100	100	380	400	70	387
	%			100	32.34	51.50	8.08	8.08	30.72	32.34	5.66	31.29

4. Discussion

DM has become a significant risk factor for human health. The worldwide incidence of this disease has steadily increased due to higher rates of obesity and bad lifestyle habits. It is also associated with several fatal complications, such as cardiovascular illnesses, which account for most of the morbidity and mortality in the diabetic population. Furthermore, plasma H_2S levels are negatively linked with HbA1c, duration of this sickness, and systolic and diastolic blood pressures [24].

It must be additionally specified that the pathogeny of DM has a common ground with the ischemia that generates a stroke: in both pathologic states, there is a depletion of ATP, the primary energy provider of metabolic processes [140], and an increase in the oxidative stress at the cellular level. Interestingly, H_2S is indicated as a therapeutic intervention in these two pathological conditions [141].

On the other hand, H_2S has been previously considered, inclusive in occupational medicine, as a poisonous and occasionally lethal toxic gas [66] formed from the decomposition of various organic materials. Therefore, it might also represent an industrial safety hazard, too, as it is colorless. However, H_2S is a toxic byproduct of microbial metabolism in the atmosphere, depending on its concentration. The human nose could detect H_2S at a level of 0.1 ppm [3].

H_2S is a widely used reducing agent that has unique chemical properties. It can be availed to target in this purpose various cellular and molecular components due to its nucleophilic nature. In addition, it can rapidly lose its chemical identity under multiple conditions, such as a tissue bath. For instance, under aerobic conditions, the half-life of H_2S is about 2.0 min in human hepatic cells, 2.8 min in kidney tissues, and 10.0 min in brain homogenates [27].

H_2S is a metabolite of sulfur amino acids in mammals, aside from SO_2 (sulfur dioxide) and Taurine. Taurine methionine, cysteine, and homocysteine are the four most common sulfur-containing amino acids, but only methionine and cysteine are incorporated into proteins. Taurine was first isolated about 150 years ago from ox (Taurus) bile. Although taurine can be produced in vivo from cysteine, with the enzymatic help of cysteine dioxygenase, it is mainly acquired from dietary sources, such as meat, eggs, and seafood. The mention of Taurine in the discussion session is determined by the fact that the only two clinical trials found, within our meta-analysis, in the above-mentioned searching platforms, address as primary pathologic condition DM, and H_2S as the intervention, and these studies indicate Taurine as a drug used.

A wide range of interventions can extend the lifespan and healthspan of H_2S, including dietary restriction. This is done through the removal of certain nutrients, such as amino acids. One of the most common molecular factors that can affect the longevity of people is the altered metabolism of certain amino acids, such as methionine and cysteine, and the increased production capacity of H_2S [142]. It is also believed that the presence of H_2S can delay the onset of aging by blocking the activation of the silent information regulator of the transcription 1 protein (SIRT1). In some studies, it is suggested that the use of dietary restriction for a specific duration can increase the production of H_2S in rats [143].

As pointed out in the meta-analysis, most patients presented associated cardiovascular diseases (637, representing 51.50% of the total number). It must be emphasized that the main interventions proposed in the eligible clinical trials include Taurine (400 patients representing 32.34% of the total number) and sodium thiosulfate (380 patients representing 30.72% of the total number). It must also be underlined that there was no drug used for 387 patients/subjects (representing 31.29% of the total number), but only the measurement of the H_2S plasma level.

Various analytical methods have been used to determine the concentration of H_2S in blood and other tissues, such as fluorescent tools, colorimetry, spectrophotometric analysis, headspace gas determination, polarography, and liquid chromatography-mass spectrometry. Yet, different analysis methods have obtained very diverse intervals of H_2S concentrations. Furthermore, the levels of H_2S within tissues and plasma are also significantly different, ranging from 15 nM [18]—for instance, in human plasma—to 300 µM in animal tissues [8] in vivo. These high discrepancies—including as regards to sensitivity and specificity items—can be challenging and, at the same time, require both cautiousness in integrating the related data and must be worthy of further minute research in this domain [18].

In very recent studies, it has been shown that H_2S can provide therapeutic effects in COVID-19 too. In addition, it is well known that DM is one of the comorbidities which dramatically increases the risk for aggravation of SARS-CoV-2 infection. “Cytokine storm” is a dominant paradigm in explaining the pathogenesis of COVID-19, being involved in many signaling pathways H_2S influences, including the immune system functioning. Therefore, linking DM to H2S and COVID-19 is an interesting and justified quest direction [37,144–147].

H_2S: entrance in the organism, its plasma levels, signaling, metabolism, and their regulation, and also its pathogenic roles, represent topics that warrant an enhanced quest focus. At the same time, being a constituent of sapropelic muds, sulfurous mineral waters, and solfatara—natural sanogenic resources used in balneology—another scientific and practical goal is to promote them based on current, thorough evidence acquired through adequate research activities. Although H_2S biology and medical usefulness have expanded over the last decades, many related issues/hurdles remain to be further explored, explained, and hopefully overcome [17].

5. Conclusions

The available data in this field have revealed interesting sulfur-related biological mechanisms, potentially impacting DM pathophysiology and treatment (as considered, too, empirically in older balneological approaches). Hopefully, future studies will clarify many still poorly known and/or debatable aspects of the subject we approached and pave the way to a better turn to good account, including from bench side to bedside, of the interesting and subtle biological properties of H_2S.

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R.P. contributed to writing and reviewing the significant parts of the manuscript. C.M., M.R., M.T., A.S., G.D., C.P., I.A. (Ioana Andone), R.P., E.V.I., C.O., I.A. (Irina Albadi) and G.O. contributed to data analysis, drafting or revising the article, finalizing the version to be published, and agreeing to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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Review

18. Lithium Biological Action Mechanisms after Ischemic Stroke

Constantin Munteanu [1,2,*], Mariana Rotariu [1], Marius Turnea [1], Ligia Gabriela Tătăranu [3], Gabriela Dogaru [4,5], Cristina Popescu [2], Aura Spînu [2,3], Ioana Andone [2,3], Elena Valentina Ionescu [6,7], Roxana Elena Țucmeanu [6,7], Carmen Oprea [6,7], Alin Țucmeanu [6], Carmen Nistor Cseppento [8,*], Sînziana Calina Silișteanu [9] and Gelu Onose [2,3]

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[1] Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Iași, 700454 Iași, Romania
[2] Teaching Emergency Hospital "Bagdasar-Arseni", 041915 Bucharest, Romania
[3] Faculty of Medicine, University of Medicine and Pharmacy "Carol Davila", 020022 Bucharest, Romania
[4] Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj-Napoca, Romania
[5] Clinical Rehabilitation Hospital, 400437 Cluj-Napoca, Romania
[6] Faculty of Medicine, Ovidius University of Constanta, 900470 Constanta, Romania
[7] Balneal and Rehabilitation Sanatorium of Techirghiol, 906100 Techirghiol, Romania
[8] Faculty of Medicine, University of Oradea, 410073 Oradea, Romania
[9] Faculty of Medicine and Biological Sciences, "Stefan cel Mare" University of Suceava, 720229 Suceava, Romania
* Correspondence: constantin.munteanu.biolog@umfiasi.ro (C.M.); delia_cseppento@yahoo.com (C.N.C.)

Abstract: Lithium is a source of great scientific interest because although it has such a simple structure, relatively easy-to-analyze chemistry, and well-established physical properties, the plethora of effects on biological systems—which influence numerous cellular and molecular processes through not entirely explained mechanisms of action—generate a mystery that modern science is still trying to decipher. Lithium has multiple effects on neurotransmitter-mediated receptor signaling, ion transport, signaling cascades, hormonal regulation, circadian rhythm, and gene expression. The biochemical mechanisms of lithium action appear to be multifactorial and interrelated with the functioning of several enzymes, hormones, vitamins, and growth and transformation factors. The widespread and chaotic marketing of lithium salts in potions and mineral waters, always at inadequate concentrations for various diseases, has contributed to the general disillusionment with empirical medical hypotheses about the therapeutic role of lithium. Lithium salts were first used therapeutically in 1850 to relieve the symptoms of gout, rheumatism, and kidney stones. In 1949, Cade was credited with discovering the sedative effect of lithium salts in the state of manic agitation, but frequent cases of intoxication accompanied the therapy. In the 1960s, lithium was shown to prevent manic and also depressive recurrences. This prophylactic effect was first demonstrated in an openlabel study using the "mirror" method and was later (after 1970) confirmed by several placebocontrolled double-blind studies. Lithium prophylaxis was similarly effective in bipolar and also unipolar patients. In 1967, the therapeutic value of lithemia was determined, included in the range of 0.5–1.5 mEq/l. Recently, new therapeutic perspectives on lithium are connected with improved neurological outcomes after ischemic stroke. The effects of lithium on the development and maintenance of neuroprotection can be divided into two categories: short-term effects and long-term effects. Unfortunately, the existing studies do not fully explain the lithium biological action mechanisms after ischemic stroke.

Keywords: review; lithium; ischemic stroke

1. Introduction

Stroke is confirmed as the second leading cause of death [1] and the third leading cause of disability [2] worldwide, involving motor, sensory, perceptual, and cognitive dysfunctions [3]. Of the main two types of stroke: hemorrhagic and ischemic, the second

is the most frequent type of stroke and accounts for over 80% of all cases [4]. It is characterized by the blockage of blood vessels in the brain. The exact cause of an obstruction of blood vessels in the brain can vary, being attributable to different factors such as plaque buildup in the arteries or embolisms in the heart. Brain injury following obstruction of blood flow can be caused by the impairment of the blood flow in a brain region. Regions with low cerebral blood flow can become irreversibly damaged. Areas within the penumbra with less severe blood flow obstruction are referred to as ischemic penumbra. These regions are considered structurally intact and can function normally. Over time, their characteristics may change and they may develop into an established infarct or a spontaneous recovery [4].

The brain is able to maintain its homeostasis by regulating the transport of water and solute across its various cellular barriers. These include the blood–brain barrier (BBB), the neuroglial cell membranes, and the choroid plexus epithelia. Cerebral edema is a type of cellular pathology that affects the brain's extracellular and intracellular spaces. The brain contains four different fluid compartments. These include the blood, the cerebrospinal fluid, the interstitial fluid, and the intracellular fluid. In the central nervous system, over 70% of the fluid is composed of the intracellular fluid compartment. The different cellular barriers that surround these four fluid compartments help maintain the proper solute and volume composition of these compartments, which are vital for the functioning of the brain [5]. Ischemic stroke occurs when the blood flow in the brain is reduced due to an obstruction of an artery. This condition usually involves thrombosis or an embolism. The reduction in the flow of blood can lead to a loss of neural function, and this can cause tissue damage [4].

The level of potassium and calcium in the fluid is different from that of the interstitial and cerebrospinal fluid. The ion gradients in the brain can also affect the concentration of nutrients in the cells (Figure 1). In order to maintain the concentration of nutrients in the cells, the level of potassium is balanced by the activation of certain energy-dependent ion pumps. These include the Na^+/K^+-ATPase and the Ca^{2+}-ATPase. The Na^+-K^+-ATPase prevents the accumulation of water and solute in the cells, which can lead to cell swelling and the loss of their cytoskeletal integrity. The activity of the Na^+/K^+-ATPase can also generate the necessary electrochemical gradients for the transportation of water and ion in the brain [6]. The first fluid compartment that is affected by an ischemic insult is the intracellular fluid. The various energy-dependent processes that regulate the position and volume of the fluid are known to cause cerebral edema. As in other tissues, water is in a thermodynamic equilibrium across the membranes of all brain cells. This means that the osmotic concentration of both extracellular and cytoplasmic fluids is equal [7].

Lithium used at therapeutic doses can exert neuroprotective effects towards the detrimental excitotoxic effects of glutamate [8]. Lithium can stimulate the level of certain cell survival factors, these include the Bcl-2 and the thermal shock protein 70 [9]. It was also shown that lithium can block the activity of the p38 kinase [8], as well as the glutamateassociated JNK activity [10].

By inhibiting glycogen synthase kinase-3β (GSK-3β), lithium influences neurogenesis, exerting multiple actions that are supportive in explaining its therapeutical properties [11]. Upregulation of endothelial Wnt/β-catenin signaling based on GSK-3β inhibition can represent a therapeutic manipulation of BBB integrity as a potential strategy for combating BBB breakdown in the early stages of ischemic stroke [12].

In addition to neuroprotective effects, the inhibition of GSK-3β can also trigger the activation of a long list of transcription factors, such as cyclic AMP response element binding protein (CREB), nuclear factor-κB (NF-κB), activating protein-1 (AP-1), heat-shock factor-1 (HSF-1),β-catenin, T-cell factor (Tcf)/lymphoid enhancer factor (Lef), and p53. CREB, for example, is a transcription factor involved in learning and memory and promotes the expression of Bcl-2 as well as brain-derived neurotrophic factor (BDNF) [13]. By inhibiting the pro-apoptotic effects of so many molecules, lithium was shown to be beneficial.

Considering the complexity of the many biochemical processes influenced by lithium, its very narrow therapeutic range and side effects are understandable when not dosed [14].

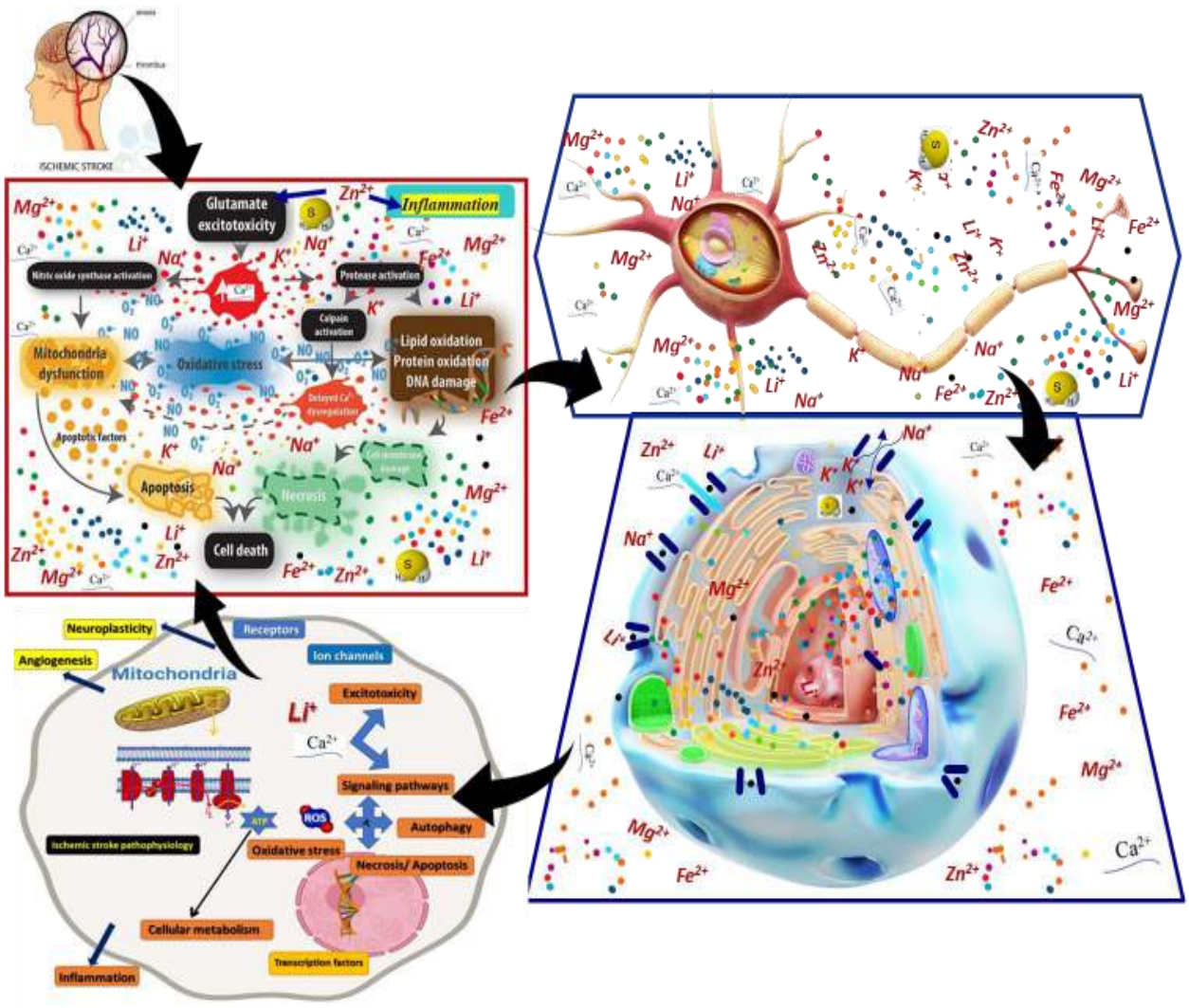

Figure 1. Ionic microenvironment and bio-molecular influenced processes in stroke. Successive "zoom images" (as follows the black arrows) are presented, starting from the tissular representation view to the intra-cellular level of the ionic microenvironment, pointing to the main biological processes influenced by lithium. The Li^+ dynamics are controlled by various subcellular localized channels and pump proteins.

2. Materials and Methods

This review is based on searching free full-text available papers written in English, found in well-known international databases: Elsevier, PubMed, PMC, ISI—Web of Science, and on Google. In addition, the inclusion criteria were fixed regarding pathology and interventions using specific keywords in the title or abstract: "Lithium" AND "Stroke".

3. Ischemic Stroke Pathological Context and Lithium Interventions

In patients with focal ischemic conditions, such as stroke and intracerebral hemorrhage, the presence of thrombin can trigger the formation of gaps in the blood–brain barrier. This condition can also affect the integrity of the vascular endothelium. In addition to this, the presence of vascular angiogenic factor—vascular endothelial growth factor (VEGF)—can also promote the development of vasogenic edema. In addition to these mechanisms, other factors such as the activation of matrix metalloproteinases can also trigger the development of vasogenic edema. When the membrane of the capillary base is damaged, it can lead to the formation of vasogenic edema [15].

Following a stroke, the effects of this condition can lead to the degeneration and death of neurons, loss of motor abilities [16], frequent occurrence of cognitive impairment, depression [1], and changes in the volume of grey matter in the brain—difficult-to-solve issues for treatment and rehabilitation [17]. Excitotoxicity, oxidative stress, and inflammation are the main factors that contribute to this process [18]. The reduction in the volume of grey matter is known to increase the risk of cognitive impairment and dementia [19]. Another common clinical outcome that can result from a stroke is post-stroke depression. There is also a potential link between the volume of grey matter and many other clinical outcomes. Although there is a recovery period window following a stroke, the prevalence of cognitive improvement following a stroke is low [20].

In addition to the pathological changes in the ischemic brain, the presence of additional neuropathological alterations, such as the activation of the hypothalamic–pituitary– adrenal (HPA) axis, can also trigger various physiological changes. The presence of these alterations can contribute to the development of brain injury by affecting the various aspects of stroke progression. These abnormalities are often reflected in the multi-system complications that can affect the clinical course of a stroke [21].

Currently, the only available and approved treatments for post-stroke sequelae are acute thrombolysis [22] and mechanical thrombectomy [23]. Lithium may reverse the grey matter atrophy by stimulating neurogenesis [24]. Several neuroprotective agents, including lithium, have been shown to stabilize the blood–brain barrier (BBB) as part of their neuroprotective mechanisms [25]. The endothelium is a critical component of the BBB function, and it plays a role in regulating its properties. The Wnt/catenin signaling is responsible for the regulation of the barrier's properties. Lithium is known to upregulate the activity of the β-catenin gene by inhibiting the GSK-3β enzyme [26].

Lithium promotes acute post-stroke neuronal survival [27]. Lithium has been shown to promote neurogenesis [28], protect against cell apoptosis through autophagy induction [29], and increase the global volume of grey matter [30]. These features make it an ideal candidate for further studies on the effects of lithium on cognitive recovery following a stroke and other neurological disorders. A meta-analysis of the data collected from bipolar populations revealed that there is a positive correlation between lithium use and the volume of grey matter. The effectiveness of lithium in stroke also involves lithium–endothelium interactions [31] by improving vascular or cerebrovascular autoregulation of blood flow, dynamic endothelial barrier permeability, and vasorelaxation capacity [32].

Hypothermia can provide various benefits in rehabilitation and neurocritical care [4]. Hypothermia treatment can affect the regulation of the expression of the insulin-like growth factor 1 gene [33]. It has been known that lithium chloride can prevent hypothermia by inhibiting the Tau hyperphosphorylation and enhancing the expression of catenin protein. The body temperature of patients following an ischemic brain stroke can increase significantly in the following 6 to 72 h [34]. This phenomenon is known to be an indicator of the outcome of the disease. In vitro studies suggest that hypothermia can be used to control the expression of the insulin-like growth factor 1 gene [35].

In cultures, the presence of lithium significantly increased the number of stem cells as well as their proliferation and differentiation. It also reduced the number of macrophages and microglia. Lithium has dual effects on the differentiation and proliferation of stem cells in the central nervous system. It is believed that lithium activates the receptors of the BDNF [36]. This suggests that the activation of this receptor could be a signal that promotes the growth of the stem cells. The effects of lithium on the generation of neurons by the hippocampal neural stem cells have been studied. The presence of BDNF significantly increases the number of neurons that are derived from this stem cell. Several studies also suggest that the presence of this protein in the precursor cells can hamper the survival and proliferation of these cells. Other molecular pathways that could play a role in the development of neurons include the activation of the ERK/Bresor and GSK-3 inhibition. It was also confirmed that the use of lithium does not alter the level of glial differentiation [24].

4. Data on Lithium Biological Action Mechanisms after Ischemic Stroke

Lithium, discovered in 1817 by Arfvedson, is found in tiny amounts in all soil types. It is especially prominent in the luteic (clay) fraction and less so in the organic soil fraction, and appears in amounts between 7 and 200 μg/g. Lithium concentrations in groundwater reach up to 500 μg/L. In some natural mineral waters, a lithium concentration of up to 8 mg/L is reached [11]. In some regions, drinking water also provides significant amounts of the element [37]. Lithium is also found in varying amounts in food: the main food sources are grains and vegetables. The intake of lithium in the human diet depends on the location and type of food consumed and varies in quantity between 650 and 3100 μg [11]. The basal serum level of lithium in adults is in the range of 7 to 28 μg/L, corresponding to daily lithium intakes of 385–1540 μg. The concentration of lithium in the hair reflects the average intake of available lithium over time and is a non-invasive way to determine the required amount of lithium in the diet [11].

Lithium was initially used to treat depression during the 19th century. In 1949, the antimanic effects of lithium were confirmed through a double-blind study. In the 1960s, it was discovered that lithium could prevent the recurrence of depression and mania [1]. During the 1970s, significant controversy emerged regarding the safety of lithium. It was reported that long-term use of lithium could cause kidney damage. However, a multicenter study by international researchers revealed that long-term lithium treatment could lower suicide and mortality rates, but also can reduce the risk of stroke [38].

In the form of soluble salts, lithium is absorbed by the small intestine through the Na^+ channels. It is then transported to the kidneys and is completely degradable. The level of lithium in body water is similar to that of the extracellular and internal levels. The levels of lithium in the serum are influenced by the amount of lithium that an individual takes [39]. Adults taking 0.25 mM (1.74 mg) of lithium as chloride per day for several weeks express a mean serum lithium concentrations increased from a baseline concentration of lithium [40].

Lithium can be transported through membranes in five different ways. Of these, passive flow is the most important route of entry for lithium into cells, and co-transport of sodium-lithium for the expulsion of lithium from cells. The five components of Li^+ transport are: 1. The Na^+-dependent antiport transport system transports Li^+ in both directions through the plasma membrane; 2. The Na^+/K^+ pump mediates the uptake of Li^+ but not its release from cells with physiological Na^+ and K^+ content. Both Na^+ and external K^+ inhibit the uptake of Li^+ via the pump into choline environments; 3. Li^+ can enter the cell through the voltage-dependent Na^+ channel. The entry of lithium in this way is stimulated with veratridine and scorpion toxin, the stimulation being blocked by tetrodotoxin; 4. The residual pathways comprise a saturable component, which is comparable to the basal Na^+ uptake; and 5. An ouabain-resistant component that causes an expulsion of Li^+ against the electrochemical gradient in choline environments [11].

Lithium has a simple structure, and its bioactive properties have been known for over a hundred years. It has been used to treat bipolar disorder, also known as manic-depressive illness. It has also been used to treat various mental disorders like depression and anxiety. Studies have shown that lithium can protect nerve cells from multiple attacks, such as toxins, stress, ischemia, and injury [41]. In addition to its mood-stabilizing effects, lithium has other physiological properties that can affect the development of various tissues. Lithium has been shown to exert a pleiotropic neuroprotective effect on different neurological conditions by triggering various pro-survival mechanisms. These mechanisms mainly affect the regulation of inflammation, neurogenesis, and apoptosis [42].

Both in vitro and in vivo data revealed that lithium exerts various effects on neurotransmitters [43] and receptor-mediated signaling regulation. In addition, data showed that lithium could influence the development of gene expression and hormonal regulation. Although the molecular mechanisms by which lithium exerts all its effects are still unknown, its various effects are believed to be linked to the multiple interactions with molecular signaling components. Lithium's acute effects are mainly mediated by inhibiting certain enzymes involved in the regulation of two signaling pathways. These include the protein kinase C and the glycogen synthase kinase 3β [13]. During chronic lithium administration, the expression of different genes is severely affected. Chronic lithium administration significantly increases the expression of a neuroprotective protein known as Bcl2, with some of the lithium effects being mediated by its neurotrophic properties [44].

In 1971, it was discovered that the levels of inositol in rats were reduced by lithium. Due to its neurotropic properties, lithium is now regarded as a promising drug for treating various diseases [45]. It is also known that it can block the activity of certain enzymes that are involved in the regulation of the myo-inositol monophosphate cycle and glycogen synthase kinase 3β. The same activity is related to lithium's influences on diabetes and cancer [11].

In addition to being useful in treating bipolar disorder, lithium can also be used to prevent the development of other processes, such as hematopoiesis and the synthesis of glucose. Several enzymes are known to be potential targets of lithium's action. Various studies suggest that the effects of lithium on the signaling pathways that are involved in the development of complex physiological and behavioral traits can be inhibited by lithium. These include the cyclic AMP formation, the G protein, and the inositol phosphate metabolism [11].

Lithium is known to be an effective inhibitor of the multiple structurally related magnesium-dependent phosphomonoesterases. It can be found in concentrations that are therapeutically relevant for these enzymes. The G protein family is a ubiquitous group of proteins that play a critical role in the regulation of various physiological and behavioral traits. These include the development of mood and appetite. The first direct evidence that lithium can be used as a target of its actions was provided by a study conducted by Avissar and colleagues, which revealed that the presence of lithium significantly decreased the binding of isoproterenol and carbochol to various G-proteins in the cerebral cortex of rats [11].

Lithium is known to stimulate neurogenesis and exert neuroprotective effects by inhibiting glycogen synthase kinase-3 (GSK-3β) [46]. It also modulates the activity of various neuroprotective factors, such as B-cell lymphoma-2 and heat shock protein 70. It can also downregulate pro-apoptotic factors. Lithium has been shown to reduce the death of neurons, activate the cyclooxygenase-2 pathway, and maintain BBB integrity [9]. In addition, the presence of GSK-3β can also cause the activation of certain nuclear factors. For instance, it can phosphorylate the Wnt/β-catenin and the nuclear factor of activated T cells. Inhibiting the activity of GSK-3β can also influence various other transcription factors, such as the AMP-activated protein and the mitogen-activated protein [9].

Studies have shown that chronic lithium treatment at therapeutic doses provided complete protection against the effects of glutamate-induced excitotoxicity in various types of neurons [47,48]. These neuroprotective effects were independent of the activity of inositol monophosphatase. However, they were associated with the downregulation of the calcium influx through the NMDA receptor. It is not yet clear if this aspect of lithium's action requires GSK-3β inhibition [9].

Lithium is also able to block the pro-apoptotic effects of certain molecules, such as p53 and Bax [49]. It inhibits the activation of caspase-3 [50] and the cleavage of laminin B1 [49]. In addition, by increasing the activity of the serine-threonine Akt-1 kinase, lithium can also prevent the development of neurons that are prone to apoptosis. For instance,

by increasing the levels of phosphorylated and active Akt-1 in the cerebellar neurons, they were able to respond to higher levels of toxic chemicals [51].

The presence of lithium significantly increased the activity of the PI3K and the kinase activity, which reversed the loss of the latter's activity and cell viability. In addition, it was shown that the treatment of patients with acute lithium exposure protected cortical neurons from the effects of glutamate [9].

It has been known that lithium can increase the levels of nerve growth factor (NGF) in the hippocampus, amygdala, and the frontal cortex. However, no effects were detected on the levels of NGF in the hypothalamus, striatum, or midbrain [9].

In an analysis of the effects of lithium on the expression of VEGF, it was revealed that the drug promoted the development of vascular cells through a pathway that is independent of the PI3K/GSK-3 receptor. These findings support the idea that the neuroprotective effects of lithium are partially mediated by the activation of the VEGF [9].

A new member of the FGF superfamily, fibroblast growth factor-21 is known to regulate the development of fat and glucose metabolism. It is also known to target the liver and pancreatic islets. It is a potential therapeutic target for diseases such as diabetes and obesity [9].

Numerous studies have shown that lithium can induce the development of neurogenesis. In a rodent model of striatal injury, lithium exposure induced neurogenesis by increasing the number of neurons and their phenotypes [9]. However, in older rats, the effects of lithium on the development of neurogenesis were not as significant [9]. Chronic lithium treatment significantly increased the differentiation of neural stem cells in the hippocampus. In studies on human brain cells, lithium was shown to stimulate the development of nestin-positive stem cells [9]. The effects of lithium on the development of neurogenesis were shown to be significant when it came to increasing the number of neurons that were derived from transplanted cells. It was also shown that the drug inhibited the activation of the macrophages and the PI3K/Akt pathway. In addition, it was revealed that lithium prevented the development of the Notch gene in Chinese hamster oocytes [9].

Due to the properties of lithium and its multiple mechanisms of action, it has been used to investigate the possible neuroprotective effects of this drug on various conditions. Some of these include Parkinson's disease [52], Alzheimer's disease, and Down syndrome. In addition, it has been shown that lithium can improve the cognitive performance of patients following a stroke [9].

In studies on mice, lithium was shown to reduce the volume of lesions following injury induction [53]. It was also able to prevent the development of neuroinflammation caused by the activation of the cyclooxygenase-2 and the activation of the microglial. In addition, it was able to maintain the integrity of the blood-brain barrier by inhibiting the expression of the matrix metallopeptidase-9 [9].

In studies on mice, lithium was shown to reduce the levels of amyloid precursor protein (APP) in the brains following injury [54]. It was also able to prevent the development of neuroinflammation caused by the activation of the matrix metalloproteinase [55]. In studies on the effects of lithium on spatial learning and memory, it was discovered that it can reduce the loss of hippocampal volume caused by brain injury [56]. This evidence supports the idea that lithium can prevent the development of negative downstream effects, such as memory deficits. It also shows that the drug can reduce the levels of tau phosphorylation and APP accumulation [9].

In animal models of stroke, the upregulation of GSK-3β was revealed to be a neuroprotective mechanism. This mechanism was also linked to the induction of BDNF, which plays a role in the development and maintenance of neurons. In addition, the increased expression of VEGF in the vascular endothelium was found to induce neurovascular remodeling. The inhibition of the N-methyl-D-aspartate receptor, which is a critical factor in the development and maintenance of neurons, was shown to decrease glutamate excitotoxicity. It also inhibited the regulation of autophagy, which is a process that contributes to the survival and functioning of neurons [57].

Metalloproteinases play a role in the development and maintenance of atherosclerosis by participating in the inflammatory process and the degradation of the extracellular matrix. When the production of this metalloproteinase by macrophages is restricted, it can lead to the formation of a thrombus. By suppressing the production of this metalloproteinase, lithium treatment was found to reduce the level of this enzyme. The development and maintenance of atherosclerosis can also be influenced by the presence of vascular smooth muscle cells (VSMCs), known to play a role in the proliferation and migration of the arterial wall. They are also involved in the abnormal neovascularization of the plaque. In a study, lithium inhibited the migration and proliferation of VSMCs in an animal model. It also reduced the cholesterol and blood glucose levels in the animals. These findings suggest that lithium can help decrease the detrimental effects of hyperlipidemia and diabetes on stroke events [58]. The above mechanisms may contribute to the beneficial effects of lithium on cerebral ischemia and atherosclerosis. In a study, lithium was shown to reduce the accumulation of atherosclerotic plaque in mice [59]. Another study revealed that the combination of lithium and captopril reduced the risk of stroke and improved the survival of rats that were prone to experiencing severe stroke [59].

Doeppner et al. examined lithium in a rat MCA occlusion model. They noted that when lithium was administered within 6 h of onset, reduced infarct volumes, edema, leukocyte infiltration, and microglia activation were present [27]. They reported that lithium increased levels of miR-124, resulting in the degradation of the RE1-silencing transcription factor and thus leading to postischemic neuroplasticity. This effect was independent of glycogen synthase kinase 3β (GSK3β) [60].

Lithium is a non-selective inhibitor of the glycogen synthase kinase-3β. It can also stimulate neurogenesis, promote the production of neurotrophins, and prevent post-injury inflammation. The imbalance between the production of reactive oxygen and the effectiveness of the antioxidant system can lead to detrimental effects on cells. The downstream target of Nrf-2 and other antioxidative enzymes, such as glutathione and superoxide dismutase, play a significant role in protecting cells from the effects of oxidative stress. They also regulate the levels of antioxidants and cytoprotective genes. Nrf-2 is a component of the cell's antioxidant system that is associated with Kelch-like ECH protein 1. Upon stimulation, it is transferred to the nucleus, where it plays a role in the transcription of various antioxidants and phase II genes [61].

The effects of lithium on the activity of phospholipase A2 in the brain were also studied. They revealed that chronic lithium administration significantly decreased the turnover of arachidonic acid in various brain phospholipids. In addition, this effect can also block the activity of the brain PLA2 in signal transduction. The study also revealed that chronic treatment with lithium significantly increased the levels of protein and mRNA for calmodulin, which are both sensitive and insensitive to the drug. It was also observed that the presence of lithium in rats also decreased the levels of Gi2 and Gi1. Although the effects of lithium on the two G-proteins were not seen in response to short-term treatment, they were observed in response to chronic treatment. The presence of chronic lithium has been known to affect the activity of the protein kinase C and the glycogen synthase-3β in signal transduction pathways. These two are known to be involved in the regulation of synaptic function. By linking the expression of these two genes to the actions of lithium, a strategy can be developed to identify the signature genes that are involved in lithium treatment [62].

The complex effects of lithium on the development and maintenance of neuroprotection are known to be important for its therapeutic effect. These include its ability to stabilize the activities of the neurons and support neural plasticity. It is also believed that the effects of lithium on the modulation of neurotransmitters can contribute to neuroprotection [63]. Lithium also affects the signals that are delivered to the cytoskeleton, which are known to contribute to neural plasticity. These include the AMP-dependent kinase, the protein kinase C, and the glycogen synthase-3β. These three kinases are known to be involved in the regulation of mood recovery and stabilization. Lithium achieves its antiapoptotic function through the protein-mediated signaling pathway Kinase B (Akt). Akt is a serine-threonine kinase and a proto-oncogene that has a phospholipid-binding domain used to anchor them to the plasma membrane [11].

Although it is believed that lithium can replace sodium in the sodium-transport system, the biological significance of this process is still unclear. It is currently not possible to determine the concentration of lithium in excitable cells. Due to the presence of certain ion channels that can easily accept lithium, it has been theorized that there should be a tenfold increase in the concentration of this chemical in excitable cells. In order to maintain its normal potential, this concept should be examined by taking into account the Nernst equation. Lithium concentration in cells does not correspond to the Nernst equation's prediction, instead, it is lower than the concentration in extracellular and blood fluids [39].

Excitotoxicity is a process that can contribute to the development of atrophy and damage to neurons following an ischemic event [37]. Neurons require oxygen to produce adenosine triphosphate, which is a vital component of the brain's energy supply. During an ischemic event, the lack of oxygen can prevent the production of new ATP. The Na^+/K^+ATPase is a vital component of the brain's energy supply and is involved in the ionic gradient across the cell membranes. During an ischemic event, the lack of oxygen can prevent the production of new ATP. The proper function of this enzyme is required to prevent the unwanted depolarization of the cell. Due to the limited number of ATP stores in the brain, the transport system is unable to maintain cell membranes' ionic gradient. The reduction of the number of neurons' excitatory neurotransmitters can lead to the release of these chemicals. In addition to this, the lack of reuptake can also lead to the over-expression of certain chemicals in the neurotransmitters. These include glutamate, which can cause the activation of certain post-synaptic receptors [63]. Over-expression of these receptors can lead to the activation of a certain type of cell-damaging protein known as the Ca^{2+} influx. This process can cause cell structure to be destroyed through the degradation of the cell's calcium-dependent protein kinases. Although this process is considered to be the main cause of infarcts during the acute phase, its role in the development of other conditions is not clear [63].

Two types of non-oxidative substances (NOSs) known as calcium-sensitive forms are commonly activated in neurons when exposed to high levels of Ca^{2+}. Following an ischemic insult, the activity of these two types of compounds is increased, which can lead to the development of more toxic chemicals such as superoxide and nitric oxide. In addition to this, a variety of other processes can also affect the development of superoxide. One of these is the activation of the xanthine dehydrogenation enzyme, which is carried out through the mitochondria's electron transport chain activity [57]. When superoxide is combined with nitric oxide, it forms peroxynitrite, which can cause DNA damage. This process can trigger DNA repair mechanisms, which are known to be involved in the development of post-ischemia apoptosis in

models. In addition to this, the presence of reactive oxygen species can also affect the proton gradient across the cell membranes. This can cause the release of signals that are related to cell apoptosis. There is also evidence linking lithium treatment to the pyroptosis mechanisms and oxidative stress, through the Nrf2-HO-1 pathway [64].

The inflammatory response to an acute cerebral infarction is a dual mechanism, detrimental, but also a protective factor that can determine the outcome of the stroke. Activation of various immune cells such as B cells, T cells, and macrophages can help in reducing the damage caused by an ischemic stroke [65]. In addition, cerebral ischemia can trigger the production of cytokines and neutrophil infiltration. The c-Jun N-terminal protein kinases (JNKs) are involved in the regulation of various processes, such as the formation of non-transcriptional and inflammatory responses, as well as the activation of the apoptosis and necrosis signaling pathways. Li^+ has neuroprotective properties for these threats [66].

The effects of Li on inflammation have been investigated. Fewer cytokine-secreting cells (interleukin [IL]-6, IL-10, IL-2, and interferon [IFN]-γ) were found in bipolar patients after chronic Li treatment, suggesting that Li may normalize immune activation [67]. When activated, the presence of oxidizing stress can stimulate the development of an inflammatory cascade in the parenchyma. This process can trigger the release of various types of inflammatory mediators, such as chemokines and cytokines. Dedicated studies on the development of these conditions are focused on the treatment of tumor necrosis factor α and IL-1 [68]. IL-1 is known to be a proinflammatory cytokine that can be involved in the development of post-ischemia damage in the brain. In studies, it has been shown that inhibiting or knocking out the activity of this cytokine can reduce the number of neurons injured in the reperfusion phase [69]. In addition to this, in vitro studies suggest that it can also induce cell apoptosis through the activation of nitric oxide-induced processes [70]. It has been theorized that tumor necrosis factor can regulate the activity of apoptosis following an ischemic insult. In studies, it has been shown that knockout animals that were treated with tumor necrosis factor showed better survival compared to those that were not treated [71]. Other studies suggest that this factor can also be involved in the restructuring and neuroprotective processes following stroke [72].

The inflammasome, which is a component of the central nervous system's response known to intervene in the development and progression of ischemic stroke. The function of the inflammasome is to recognize various danger signals that are associated with the development of lesions in the nervous system. Existing theories suggest that the interaction between the inflammasome and various other molecules, such as lysosomal rupture and potassium efflux, can trigger the accumulation of reactive oxygen species (ROS) [1].

Autophagy is a regulated process that involves the recycling and degradation of damaged macromolecules to maintain cellular homeostasis [73]. In neurons, the regulation of the synthesis and degradation of protein is very important for the development of synaptic plasticity and cell growth [74]. Various studies have shown that lithium can promote autophagy activation in different central nervous system cells [75]. These include neurons, astrocytes, macrophages, and capillary endothelial cells [31] upon an ischemic event. Autophagy can play a dual role related to the removal of damaged or senescent cells and in the protection of the brain following an injury [76] by preventing the development of downstream apoptosis. This is because the degradation of damaged mitochondria through mitophagy can help prevent downstream apoptosis. Unfortunately, excessive autophagy can lead to the destruction of various cellular functions. It can also be detrimental to neurons, which eventually leads to cell death. In addition, evidence has shown that the activation of cerebral IR could cause this process to negatively affect the neurons. It is known that prolonged cell stress can trigger excessive activation of the autophagy process. This could explain why the reduction of this process in the presence of chronic IR-induced hippocampal neuronal damage can help alleviate cognitive impairment [74]. One way to prevent the development of downstream apoptosis following an injury is by using lithium chloride [2].

Apoptosis can be mediated through the activation of various pathways, such as those that are dependent on the presence of a certain type of protein known as caspase-3. In neurons, the activation of this protein can be triggered by the release of a certain type of chemical by the mitochondria. This process then leads to the breakdown of the structural proteins. It is not clear how the activation of the JNK and AIF can lead to cell death. However, it is believed that the activation of these two factors can cause structural and DNA damage that ultimately leads to cell death. In order to prevent the proliferative process from taking place, certain B cells can be kept in check. These include B-cell lymphoma 2 and nuclear factor NF-kB [11].

Circadian (daily) rhythms exist in all eukaryotes and are conducted by endogenous, self-sustaining biological oscillators; in the absence of external time interventions, the rhythm will last for almost 24 h. The effects of lithium under synchronous conditions are specifically variable, with significant delays or phase advances. The effects also depend on the time of its application, so the administration of lithium hydroxybutyrate in the evening for 10 days stabilizes circadian rhythms in rats, in contrast to an administration during the morning. Inositol phospho-metabolism has been proposed as a potential component of both the circadian oscillator and blue light photo-transduction [11]. The therapeutic action of lithium salts appears to be the result of a combination of events that alter neuronal activity at multiple levels (Figure 2). Three interacting mechanisms appear to be more affected [11]:

(1)The modulation of neurotransmitters by lithium ions seems to change the ratio between their excitatory and inhibitory activities and the decrease of glutamatergic activity may contribute to the effect of neuroprotective lithium [77];
(2)Lithium ions modulate signals with an impact on the cytoskeleton, a dynamic system that contributes to multilevel neuronal plasticity, including glycogen synthetase kinase-3β, cAMP-dependent protein kinase, and protein kinase C;
(3)Lithium ions alter signaling activities involving secondary messengers, transcription factors, and therefore gene expression.

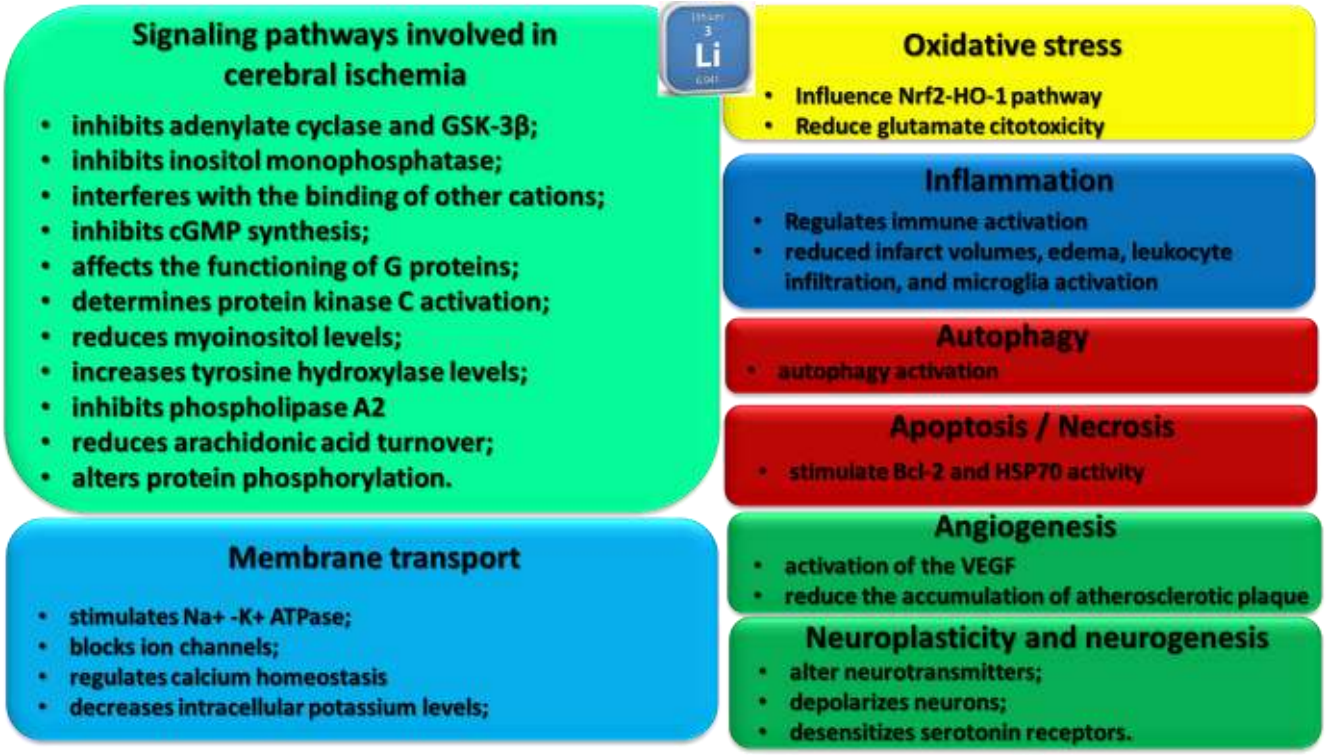

Figure 2. Synthetic overview of the lithium effects in various cellular functions correlated with possible therapeutic interventions in ischemic stroke, such as membrane transport, signaling pathways, neuroplasticity, neurogenesis, angigenesis, apoptosis, necrosis, autophagy, inflammation, and oxidative stress.

5. Discussion

The properties of lithium are well-characterized. It is an 80–100% bioavailable compound, and it can be administered orally. It can reach its peak serum concentration in around two to four hours. It is also water-soluble, which makes it ideal for dissolving in water [11]. Although the effects of lithium on mood are known to be beneficial, it is also known to have side effects such as polyuria and polydipsia [78]. These side effects can be caused by the lack of thyroid function. It is also believed that the side effects of lithium are caused by the actions of its effects on the regulation of certain enzymes in the kidney and the thyroid gland [79]. The effects of lithium on the development and maintenance of diabetes-insipidus-like conditions are also known to have significant effects on the levels of vasopressin mRNA in the supraoptic hypothalamic and paraventricular nuclei [80].
Lithium is known to have various metabolic, neurological, and cognitive side effects. Neurotoxicity is a significant factor that limits its use. The presence of lithium poisoning can trigger the accumulation of iron in the brain due to the disruption of the tau cascade. This process hinders the iron efflux from the brain cells and increases the hydroxyl radicals produced by the iron. Lithium has been implicated in various outcomes, such as its effect on the retention of fluids in the hypothalamus [81].
Patients taking lithium are prone to experiencing various types of tremors. These are usually triggered by the initiation or up-titration of the drug's lithium dose. The usual therapy for patients with this condition is to reduce their lithium dosage to around 0.8 to 1.2 mg/L. This is considered as a fine postural tremor with a higher frequency than other types of tremors. It can also be characterized as an irregular, non-rhythmic tremor of the lower extremities. In some cases, abnormal movement abnormalities such as dysarthria, aphasia, and choreoathetosis can also be caused by severe lithium intoxication [82].

Studies that investigated the effects of lithium on cognitive outcomes revealed that prolonged lithium use was associated with small cognitive impairments [83]. These findings support the idea that prolonged lithium use can affect the development of mental abilities. However, these studies did not find a difference between the short and long-term users of lithium. The effects of lithium on learning and verbal memory were also investigated. The results of the meta-analysis revealed that prolonged lithium use was associated with small cognitive impairments. One study revealed that lithium responders scored higher on a measure of executive function known as the Wisconsin Card Sorting Test [84]. Another study found that lithium use did not affect the cognitive functioning of individuals within two years [20]. However, it did suggest that the effects of lithium on verbal learning could be beneficial. A third study revealed that continued lithium use could reduce the incidence of dementia [85]. The rate of dementia was lower after patients started taking lithium. Lithium chloride can improve spatial learning and memory by increasing GSK3 and Akt phosphorylation [74].

In response to the monoamine hypothesis, which hypothesizes that depression is caused by a lack of dopaminergic signaling pathways, studies have been conducted to identify the potential effects of lithium on these pathways. In a study, the authors noted that chronic lithium use could reduce the release of potassium-mediated dopamine [86]. This suggests that the effects of lithium on this pathway could explain the mood-stabilizing effect of its use. In another study, the researchers found that the treatment of cells with lithium prevented Ca^{2+}-influx. They believe that the drug's effect could be mediated by the downregulation of the subunit phosphorylation [87].

Further downstream, treatment with lithium also decreases the activation of calpain, which is a Ca^{2+}-dependent protein in the apoptotic cascade. Given its neuroprotective properties, researchers conducted a study on the effects of lithium on the functioning of the brain in a model of ischemic conditions. They found that the rats that were treated with lithium before the middle cerebral artery occlusion had better functional outcomes
[88].

In studies on animal models, the effects of lithium on the release of glutamate were observed [89]. These findings suggest that excitotoxicity could be one of the mechanisms by which lithium can cause adverse effects. On the other hand, the effects of lithium on GABAergic signaling are also known to affect the release of glutamate and dopamine [90]. In the brains of rats, chronic lithium use significantly upregulated the levels of glutamic acid decarboxylase and gamma globulin (GABA) and decreased the concentration of dopamine [86]. In addition, acute lithium use significantly increased the levels of this substance in the central nervous system [47].

In downstream signaling, lithium changes the activity of adenylyl cyclase, which is a transcription factor that is involved in regulating the expression of Bcl-2 and BDNF [91]. Long-term lithium use can decrease the activity of PKC [47]. It has also been shown that the drug can decrease the expression of myristoyl alanine-rich C-kinase substrates [92]. It has also been shown that chronic lithium use can increase the levels of Bcl-2. These findings suggest that the drug's effects could be related to the development of functional outcomes. In clinical and preclinical models, the effects of lithium on the levels of BDNF were observed [93].

It has been theorized that the effects of lithium on the activity of PKC could be related to the treatment of mania [88,94]. In addition, it has been shown that the drug can also decrease the activity of the glycogen synthase kinase-3β [95]. This activity is regulated by direct competition with the binding of Akt and Mg^{2+} [96]. The activation of the Akt pathway is a critical factor that contributes to the survival of neurons following a stroke. It can be triggered by the non-phosphorylation of the GSK3 protein, which leads to its degradation [96]. This process is also triggered by the degradation of the β-catenin substrate. The dual effects of lithium are related to the different functions of the IPPase [96]. For instance, it can directly inhibit the activity of the drug as a competitive inhibitor of the potassium channel, or it can indirectly affect the activity of the drug through the promotion of its phosphorylation [96]. The Akt pathway is not always involved in the neuroprotective effects of certain neuroprotectants [97]. Other studies have shown that it can promote the production of Bcl-2 and other protein components. It can also prevent the activation of p53 and suppress the inflammatory factors c-jun and c-fos [98]. In addition to activating the pathway, lithium can also reduce the size of the infarct by inhibiting the activity of other cellular signaling pathways [99].

It was found that the volume of grey matter in the paralimbic and cingulate cortex was significantly higher in the lithium-treated group compared to the control group [100]. In another study, the researchers found that the volume of grey matter in the subgenual cingulate gyrus and the anterior postcentral gyrus was significantly higher in the lithiumtreated group compared to the control group [101]. Hajek and colleagues found that the volume of hippocampal volumes was significantly higher in the patients who were treated with lithium [102]. A study conducted by Benedetti and colleagues revealed that the use of lithium was associated with the volume of grey matter from the frontal lobe [103]. A meta-analysis conducted by Bora et al. revealed that the prevalence of lithium users was associated with the volume of grey matter in the anterior cingulate cortex [104].

The proliferative processes in neurons that culminate in observable changes to the volume of grey matter following a stroke can be triggered by the presence of cerebral infarction [105]. In a study, the presence of this type of injury was associated with a decrease in the volume of grey matter [19]. Other studies also suggest a negative correlation between

the global grey matter volume and white matter lesions [106]. Due to the effects of lithium on the development of neurogenesis and the remodeling pathways involved in the spontaneous recovery following a stroke, it is possible that the effects of lithium on the volume of grey matter can be observed in a post-stroke population [107].

The effects of lithium on the global volume of grey matter were analyzed in patients. The patients who were given at least 300 mg of lithium daily had a positive change in their grey matter volume. Following a stroke, patients usually experience changes in their mood and cognition, such as post-stroke depression and frontal vascular impairment. Kempton and colleagues also noted that the prevalence of lithium users was associated with the volume of grey matter in a population [108]. A meta-analysis conducted by the researchers revealed that the use of lithium was associated with the global volume of grey matter [101]. Moore and colleagues also revealed that the use of lithium was associated with the cortex grey matter volume [109]. These studies also noted that the effects of lithium on the development of functional outcomes were observed. In healthy individuals, the use of lithium was associated with an increase in regional grey matter volumes [110].

The activity of numerous enzymes, such as the adenylate cyclase and the GSK-3 level is directly affected by the availability of cytosolic magnesium [111]. However, if lithium is present, these same enzymes might be driven to overcompensate by the presence of this substance. For instance, if the level of lithium is high, these enzymes might perform a large corrective response. The members of the lithium-sensitive and magnesium-dependent phosphatases, such as the IPPase and the IMPase, have the potential to form subtle components of a low-Mg failsafe system. In effect, these enzymes are part of a system that can activate different types of failsafe systems. These include the multiple sub-mechanisms that are known to enhance the activity of the inositol phosphates and other related sugar phosphates. It is believed that these two components can help maintain the cell's energy levels by regulating the interaction between different cellular functions [112].

One of the most common types of sequelae following an ischemic stroke is cognitive impairment. Another common clinical outcome following a stroke is depression. Depression has been known to negatively affect the quality of life of stroke survivors, and it can also lead to disability and worse functional outcomes [107].

Although many stroke survivors experience spontaneous recovery, this doesn't necessarily mean that they have recovered their pre-stroke functions. It can be mediated by the recovery of compensatory behaviors and baseline functions. Although lithium is widely regarded as a mood stabilizer, its effects on cognition are not known to be definitive. The use of lithium can also negatively affect the cognitive performance of individuals. These effects may be transient and may be affected by the underlying cognitive changes caused by the disease state. Although it has been known that lithium can improve the symptoms of depression, it is not yet clear if its effects on cognitive performance can also be beneficial [107].

Lithium poisonings are most often associated with drug interactions or infections. Patients who are being treated with lithium may develop polynucleosis, which was first observed during their biological exam. The treatment of lithium poisoning is similar to that of cytotoxin. One of the most common complications of lithium exposure is polyuria. Lithium intoxication can also lead to the development of diabetes insipidus. This condition can cause dehydration and increase lithium retention. GSk3β is known to increase the risk of developing diabetes insipidus [113].

The effects of GSk3β inhibition on hair growth are also known to be associated with the development of alopecia. Although this complication is not considered a life-threatening condition, it can still have a negative impact on the quality of life of the patient [113].

6. Conclusions

The therapeutic effects of lithium are indirectly related to its ability to activate a cascade of fail-safe pathways, designed to protect cells from a variety of threats such as posttraumatic brain injury and depletion of the triphosphate level. Lithium mimics the lowered level of the cellular environment in a way that makes it incredibly effective at protecting cells. These systems are designed to protect neuronal cells from conditions that are detrimental to their survival, such as low levels of magnesium. These systems would be able to regulate, limit, and restore the functions of neurons. Being able to use these systems allows lithium to exert its therapeutic effects. Acting through competition with the magnesium, Li can mimic the effects of low levels of Mg.

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Mud therapy and rehabilitation - scientific relevance in the last six ye ars (2015 – 2020)
Systematic literature review and meta-analysis based on the PRISM A paradigm

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MUNTEANU Constantin[1,2,3], ROTARIU Mariana[1,3], DOGARU Gabriela [1,5,6], IONESCU Elena Valentina [7,8], CIOBANU Vlad[9], ONOSE Gelu [2,3]

Editor: Mihail HOTETEU, Romanian Association of Balneology, hoteteu@yahoo.com

Keywords: mud-therapy, pelotherapy, peloidotherapy, fango therapy, rehabilitation, balneotherapy,

Reviews ˙Systematic REVIEW

1. Romanian Association of Balneology, Bucharest, Romania
2. Teaching Emergency Hospital ”Bagdasar-Arseni”, Bucharest, Romania
3. Faculty of Medical Bioengineering, University of Medicine and Pharmacy “Grigore T. Popa”, D, L, Romania
4. Faculty of Medicine, Department of Physical and Rehabilitation Medicine, University of Medicine and Pharmacy ”Carol Davila”, Bucharest, Romania
5. Faculty of Medicine, University of Medicine and Pharmacy “D+XLOX, LHJDQX”, Cluj-Napoca, Romania
6. Clinical Rehabilitation Hospital Cluj-Napoca, Romania
7. Balneal and Rehabilitation Sanatorium TechirghiolQDWVQR& D5RPDQLD
8. Faculty of Medicine, QDWVQR&IR\WLVUHYLQ8´VXLGLY2³DRomania
9. Computer Science Department, University Politehnica of Bucharest, Romania

Abstract

Background. Balneotherapy is a stimulation - adaptation treatment method applied in the forms of bathing, drinking, and inhalation cures performed with natural therapeutic factors, a method which is acting in three main ways: thermally, mechanically, and chemically. Mud or peloids are natural therapeutic factors formed by natural processes under the influence of biological and geological phenomena, which in a finely dissolved state and mixed with water (mud) are used in medical practice in the form of baths or local procedures.

Objective. This systematic review aims to rigorously select related articles and identify within their content, the main possible uses of therapeutic mud and physiological mechanisms, to see the main region of scientific interest for pelotherapy, and to discuss the value of mud therapy in rehabilitation medicine.

Methods. The working method is based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We searched for open-access articles published in English, between January 2015 and December 2020, from the following databases: Cochrane, Elsevier, NCBI/PubMed, NCBI/PMC, PEDro, and ISI Web of Knowledge/Science (the latter was also used to identify ISI indexed articles). The contextually searched syntax used was ”Pelotherapy/Peloidotherapy/Mud-therapy/ /Fango-therapy AND Rehabilitation”. The selected articles were analyzed in detail regarding pathologies addressed by mud therapy and country scientific relevance for this therapeutic method. The meta-analysis proceeded was designated to estimate the prevalence of various pathologies in the use of mud therapy.

Results. Our search identified, first, **394** articles. Based on the successive filtering stages and, respectively, on the classification criteria of the Physiotherapy Evidence Database (PEDro), we finally identified/retained and analyzed **68** articles. Although, in principle, a rigorous method – and we have followed the PRISMA type paradigm – there still might be some missing works of our related article selection. On the other hand, to augment/ consolidate our documentation base, we have used also **40** papers freely found in the literature, and even – aiming, too, at an as exhaustive knowledge underpinning as possible – derogatively, we have also considered some articles which, probably being very new, couldn't yet have reached the PEDro threshold score we have settled.

Conclusions. This paper overviews the current state-of-the-art knowledge in the approach of peloidotherapy in rehabilitation, with a focal point on the therapeutic properties of peloids.

Authors:

Prof. dr. Gelu Onose, Ph.D.
Senior Physician in Physical and Rehabilitation Medicine (PRM) and Gerontology & Geriatrics (GG)
Head of the P(neural-muscular)RM Discipline/ Clinic Division - the National
Reference Centre for NeuroRehabilitation - and of its RDI Nucleus, and RDI (ex) Director, at the Teaching Emergency Hospital "Bagdasar-Arseni"

Lecturer dr. Constantin Munteanu, Ph.D. Priority research areas: Bioclimatology, Hydrothermotherapy, and Physiology, President of the Romanian Association of Balneology,
Vice President of the Romanian Society of Physical Medicine, Rehabilitation and Balneoclimatology,

Assoc. prof. dr. Mariana Rotariu, Ph.D. - Kinesiology, Psychomotricity, psychomotor education and reeducation, Kinesitherapy. Kinesiprophilaxy, Massage. Complementary techniques, Occupational therapy, Rehabilitation, and techniques of functional reeducation

Assist. prof. dr. Gabriela Dogaru, PhD
Priority research areas: balneoclimatology, including the study of carbonated mineral waters and mofettes, molecular and cellular medicine, e-health systems, neurological and cardiovascular rehabilitation, development of spa, and rehabilitation tourism.

Assoc. prof. dr. Elena Valentina IONESCU, PhD
Priority research areas: Bioclimatology, Hydrothermotherapy, and Medical

➢ INTRODUCTION

Collectively, the spa economy is estimated at $94 billion, with a consistent growth perspective in the coming decades. The general context of spa environments can support a holistic approach to health promotion (1). Basic components of health resort interventions - spa therapies (2) - are balneotherapy (3) and climatotherapy. In many countries, treatments involving natural mineral waters, gases, or peloids (4) are referred to as balneotherapy. Routes of application include bathing, inhalations, drinking (Crenotherapy), etc. (5), often encompassing rehabilitation objectives/procedures (6)(7). According to the definition given by the "International Society of Medical Hydrology", muds (peloids) and clays (8) are "substances formed in natural conditions under the influence of geological processes and which, mixed with water (9), are used in medical practice in the form of baths or local procedures (10). Muds are earths or rocks of a pasty consistency, used as therapeutic remedies or cosmetics (11) from remote times. Some beneficial effects of mud are known empirically from antiquity, others have been described and studied quite recently, some have remained today in the stage of summary explanation. Mud treatment is also called pelotherapy (9), peloidotherapy, or sometimes – a largely – fango therapy. Pelotherapy should be used only as directed and under medical supervision (8).

From ancient times people used mud, when they had it at their disposal, for healing or beautification (12). Archaeological artifacts support such a hypothesis and there are no problems in understanding these data since the instinct to seek healing remedies also exists for the animal world, argued by new data also (13). Even if images in which the pig rolls in the mud for comfort seem legendary, or from a fairy-tale, we can only admit that such behavior is explainable in the equation of an adaptive benefit. The use of mud has its origins in antiquity. In ancient Egypt, the sick were anointed with the mud brought by the Nile during the floods.

The most common therapeutic muds are deposits in saline basins - black sulfurous muds, which form on the bottom of marine estuaries, bays, and continental salt lakes. The activity of quality evaluation for the resources of natural therapeutic substances begins by taking samples at source and ends with their complex interdisciplinary characterization, being developed indications and contraindications for prophylaxis, therapy and/or rehabilitation by conducting a complete pharmacodynamic study.

In one of his works, Pliny (23-79 AD) recommends the use of mud, a recommendation made by Dioskorides (50 AD) in his works and later also by Galen (131-2020 AD). In antiquity, Cleopatra's behavior is historically emphasized, considered a beauty of her time, in terms of bodily maintenance, an aspect directly correlated with skin health. The beneficial effects of the mud and salt from the Dead Sea (11) led her to ask Marc Antonius to conquer this region to benefit from this remedy. Therefore, the use of Dead Sea (14) mud for therapeutic purposes dates back thousands of years, from the time of the Roman Empire.

We have more accurate data on the use of mud from the 14th century, in a paper that talks about the use of therapeutic mud from Albano and Battaglia, and Fallopius describes in 1564 the technique of using mud. Later, mud therapy (pelotherapy/peloidotherapy) is used in France (seventeenth century) and Germany (eighteenth century). In Russia, the cradle of pelotherapy is the Crimean peninsula.

Last but not least, Romania is a country famous for its balneary resorts. For renowned mud deposits,

Techirghiol, Sovata, Ocna Sibiului, Mangalia, Amara are the main resorts promoted for mud therapy.

The complex interdisciplinary characterization of the natural therapeutic factors necessarily includes interpretation of physicochemical analyzes and microbiological (15)(16) examinations. **Classification of muds** (17)

Clay (18) has been used by humans since before recorded history to accomplish basic but fundamental healthcare purposes. The clay plates of Nippur, Mesopotamia, which date back to about 2500 BC, contain a reference to the use of clays for therapeutic purposes, including the treatment of wounds and the inhibition of hemorrhages. Also, the famous Papyrus Ebers dated about 1600 BC describes the treatment of some diseases using clay-based medicines. Since then, from Ancient Greece, Roman times, medieval times, renaissance times to contemporary times, there exist reports on the therapeutic, nutritional and cosmetic uses of clay and clay-based products (8). There are several types of clay and within them, the socalled healing clay (19) and edible clay had been used and are still being used, by man for therapeutic, nutritional, and cosmetic purposes. Edible clay is a particular type of healing clay which use is limited to internal application through ingestion, for instance, of cookies made of clay/ /potable water dispersion/suspension. Conceptually healing clay besides the oral use for internal health benefits of the digestive tract (20) can also be used in external or topical applications as clay and mineral water paste called mud or peloid for the treatment of muscle-skeletal and dermatological disorders (8)(21).

Peloid is a maturated mud or muddy dispersion with healing and/or cosmetic properties, composed of a complex mixture of fine-grained natural materials of geologic and/or biologic origins, mineral water or seawater, and common organic compounds from biological metabolic activity.

NATURAL peloids are formed by depositing more or less decayed, rotten, overripe, organic, and inorganic substances in bayous, sea lagoons, saltwater and freshwater lakes, rivers, marshes, mineral water spring mires, and volcanic areas (22).

ARTIFICIAL peloids are natural clays that are refined and significantly changed before their use (23).

According to their origin:

ORGANIC PELOIDS comprise those peloids consisting of over 10% of organic substances:

- ✓ peat (peat soil, elevated and leveled peat) (24)(25)
- organic mud (bitumen mud, sapropel and Gyttja)(8)

INORGANIC PELOIDS (8) *comprise:*

- ✓ mineral peloids (clay, loam, tufa)
- ✓ volcanic peloids (8)

Depending on the formation conditions and the chemical composition, the therapeutic muds are classified into:

a) **Sapropelic muds -** are represented by black deposits, rich in colloidal iron hydrosulfide, have a plastic and greasy appearance, are found on the bottom of salt waters originating from the action of microorganisms on the flora and fauna of the aquatic basin to which minerals or inorganic substances that come from the soil of the lake basin are associated. Their content in organic substances is greater than 10% to the weight of the dry matter - continental lake, lagoon;

The flora consists of microphyte and macrophyte algae in which *Cladophora vagabunda*, *Cladophora cristalina*, predominates, algae that grow only in saltwater.

The aquatic fauna is represented by the species Artemia salina 10 - 12 mm, red arthropods. The carcasses of Artemia salina, by bacterial decomposition, together with the alga *Cladophora cristalina*, form the sapropelic mud. Sapropelic muds are black deposits underwater sediments rich in colloidal iron hydrosulfide, plastic and greasy.

b) **Mineral and vegeto-mineral muds** appear by sedimentation of the salts of some springs with sulfidic, ferruginous, carbogazeos character. Mineral muds - arise by the sedimentation of salts of carbonaceous, calcium, ferruginous or sulfurous springs;

c) **Peat** (*humus vegetabilis lutosa)* - organic, brown mud. Peat muds result from the microbiological decomposition of the remains vegetables accumulated on the bottom of some swamps. Their content in organic substances is also greater than 10% of the weight of the dry matter (24). Peat is formed in the following ways:

- ➢ natural formation through physicochemical and microbiological processes at the contact of the mineral water with the clay bed, around the natural emergents, resulting in the content of organic substances in dry mud below 10%;
- ➢ directed formation through microbiological and physicochemical processes at the contact of the mineral water with the clay bed - artificial basins - resulting in the content of organic substances in dry mud below 10%;
- ➢ incomplete transformation of the vegetal material in conditions of advanced humidity - swamps, resulting in the content of organic substances in dry mud of over 10%;
- ➢ salty clays and marls, sedimented in arid land conditions - lagoons from the biological past;
- ➢ calcareous tuffs, formed by chemical precipitation - around natural carbon dioxide emergencies.

Fangotherapy - is a particular type of pelotherapy that involves the use of fango name of Italian origin attributed to muddy natural peloids deposited from thermal springs as happens in the case of the Euganean volcanic region, in Italy, where important Thermal Resorts exist (8).

Chemical composition of peloids (26)(27)

From a physicochemical point of view, the mud is a heterogeneous system (28) consisting of a liquid phase containing water and water-soluble mineral salts, a solid phase containing mineral and organic substances, and a gaseous phase containing hydrogen sulfide (29). The ionic balance of mud is also reflected in its pH. In general, the mud is alkaline pH = 9 – 10 (30).

The therapeutic effect of mud is given by the combination of its physical and chemical properties (31).

LIQUID PHASE

It is the solution of imbibition - the aqueous solution of organic and inorganic substances

Depending on its proportion muds are divided into:

- ➢ poorly hydrated with moisture content <37%
- ➢ medium hydrated by weight humidity = 37-40%
- ➢ strongly hydrated with a moisture content> 40% The liquid phase results from the water of the lakes in the basin of which the mud is produced rainfall, and impact of mineral waters. Depending on the predominant ions (32), it may have a different character: sulfated, carbonated, or mixed, and with the following content:

- Water, anions, cations (33),
- Oligoelements (ug/kg) (28)

1. with pharmacological effect (Fe, Co, J, Br, and B)
2. involved in the enzymatic processes (J, Fe, Cu, Mo, Zn, Co, Mn, Ni, Ba, Sr, Cd)
3. non-essential/toxic elements (As, Pb, Hg, V, and F)
4. not yet elucidated biological role (Ti, Zr, Ir, Cs).

• Biologically active substances - protein hydrolysates, amino acids, enzymes (34).

GASEOUS PHASE - results from the physicochemical and biochemical processes involved in the mud formation (peloidogenesis): H_2S, CO_2, NH_4, CH_4, O_2, Rn (8) Hydrogen sulfide (H_2S) is also an endogenous gas with important physiological functions (35). Endogenous hydrogen sulfide has been reported to function as a neuromodulator in the brain and within the vasculature; the main functions of H_2S are vasodilation and promoting new vessel growth (36).

SOLID PHASE

- Crystal framework (peloid skeleton) – *determines the mechanical structure*
- Oxides (SiO2, Al2O3, Fe2O3, CaO, MgO, Na2O, K2O, TiO2, MnO, P2O5), Na2O/CaO < 1
- Colloid complex - *plastic hydrophilic basis which absorbs moisture and defines thermal properties*
- Inorganic component Fe(HS)3, Fe(OH)3, Al(OH)3, H2SiO3

- Organic component – humic substances: humin, humic and fulvic acids (34)

Humic substances are generally considered to have occurred during the humidification process of the organic material. Their color varies from yellow to black, molecular mass from 2000 to over 300,000 Da, have a C content between 45 and 62%, O between 30 and 48%, and free acidity between 500 and 1400 mEq% (37). The heterogeneity of the molecular weight of many individual compounds with different chemical compositions containing biogenic elements (C, N, O, P, S) in carboxyl, phenol, alcohol, peptide, amide, and other functional groups causes humic substances to be polydisperse, heterogeneous, biophilic and multifunctional (37).

The mud can be fractionated using the variation of pH and polarity of solvents and humic extracts can be characterized spectrophotometrically based on absorption in the wavelength range 340-700 nm, humic acids, and fulvic acids being differentiated based on solubility and molecular weight. Briefly, the mud is treated with a KOH solution, obtaining the alkaline extract of soluble humates and fulvates and the precipitation of insoluble humin(37). **Physical properties of mud** (38)(27)

- ✓ **Specific weight** - depends on the components **mineral substances > organic substances**, is maximum for strongly mineralized muds, minimum for peat muds
- ✓ **Hydropexy:** water absorption and retention capacity, practical value - peloids with low hydropexy (mineral mud) are used only for packaging, those with medium hydropexy (sapropelic mud) and high (peat mud) can also be used for baths.
- ✓ **Plasticity**: the property of stretching and molding on the surface of the body - is given by the solids in their content; low plasticity (mineral mud), medium (peat), and high (sapropelic) (25)
- ✓ **Thermopexy:** heat absorption and storage - peloids have a high ability to retain heat (39)(40).
- ✓ **Consistency** – it depends on the viscosity conditions the frictional force that is exerted on the skin when applied (40)
- ✓ **Specific heat** - represents no. of calories to raise the heat of one gram of mud by one degree (40)(41)(42)
- ✓ **Granulometry -** the degree of mud dispersion and the size of the solid particles (41)

Methods of mud applications

- ✓ **Mud baths,** full, half body, or arm-leg - in tubs with heated lake water in which 10 - 12 kg of mud are added, progressively increasing its concentration up to 10 - 25%. The mixture can be heated to 42 ° C (43). In the case of patients with cardiovascular diseases, the temperature should not exceed 37ºC, and for those with inflammatory rheumatic diseases below 36ºC. The duration of the bath is 20 ´- 40´. The bath must be followed by a shower at 37º - 38ºC and a rest of at least one hour. A cure consists of 12-15 daily baths.
- ✓ **Mud Packages applications:** peloids are applied to specific areas of the body (44)
- ✓ **Mud anointing** - is the oldest method that involves applying cold mud on the patient's skin, after it has been heated 10-15, in a thin layer, on limited areas or the whole body. The patient should then be exposed to the sun, in an upright position, until the mud dries, approximately 30 - 60´, followed by a bath of 10´ - 15´ in sea or lake water accompanied by movement and a short shower with cold water and bed rest for at least an hour. It is a method of stressing the mechanisms of thermoregulation, neuroendocrine stimulation, and adaptation processes by alternating hot-cold contrasting factors.
- ✓ **Mud wraps** - consist of applying mud in a layer of 1 - 2 cm heated to 38º - 46ºC on a limited region or the entire body surface for 20´ - 40´.
- ✓ **Poultices** - applications with mud at different temperatures on limited regions of the body (24).
- ✓ **Gynecological applications** - in the form of vaginal swabs with mud at 39º - 40ºC for 2 hours or vaginal irrigation with mud dissolved in lake water and heated to 37ºC (43).
- ✓ **Mud massage** - stretching and mud massage

Application method:

1. After application, the body gets freed of mud with the assistance of a warm shower (taken under the water in temperatures of 37-38 ºC).
2. The patient gets dried of immediately and rests for half an hour to an hour.
3. Afterward, the patient either takes a light walk or receives a massage, or is taken into an adequate exercise program, if necessary.

Main peloid actions:

- ➢ cardiovascular system - stimulation of cardiovascular reactions with accentuation of hemodynamics by central and peripheral mechanisms. Immediate mobilization of blood deposits and their involvement in the general circulation takes place; cutaneous vasodilation → increases cardiac output (45)
- ➢ nervous system - sedative or exciting effects on the CNS and SNV by sedating pain receptors (46).
- ➢ The mud bath determines the increase of the antimicrobial defense capacity by increasing the phagocytic power of the leukocytes - highlighted by the changes of the opsono-cytophagic index
- ➢ digestive: determines the balance of gastric secretion, increases biliary secretion
- ➢ renal: decreases renal secretion and diuresis (when perspiration is stimulated)
- ➢ tissue: tissue hydrophilicity changes → increases resorption capacity (chronic inflammation, bursitis)
- ➢ immune system - regulation of immunoglobulin values (47), an increase of non-specific immunity (9)(48).

- The endocrine system. The enzymatic and metabolic changes that appeared in the endocrine glands after peloidotherapy differ according to the specific secretion of the gland, according to the functional stage in which the gland is located, and are related to the type of therapeutic application. Under the action of mud, there is a harmonic stimulation in all glands in the sense of increasing the enzymatic and synthetic activity while maintaining the specificity of each. Correlated with the activity of endocrine functional harmonization is the stimulation of the activity of the hypothalamic-pituitary-adrenal axis translated by optimizing the plasma levels of β-endorphins. Glands with internal secretion - ovarian, pituitary function are stimulated.
- The endocrine mechanism is also involved in inducing the anti-inflammatory (49) effects of pelotherapy by modulating the activity of the hypothalamic-pituitaryadrenal axis and by the general balancing of the endocrine balance, persistent effects, and post-cure.
- Reactivity to chemical mediators - acetylcholine and adrenaline - as well as to ions correlated with vegetative activity - Ca, Mg, K - is increased after the external cure with mud, and the vegetative tone tends regulation/normalization, which leads to remediation of vegetative stigmas.

Therapeutic effects of mud (50)

Through its composition and thermopexy properties, the mud determines:

- ✓ stimulation of peripheral mechanoreceptors - triggers reflex mechanisms with favorable effects;
- ✓ subsequently follows skin vasodilatation (neurohumoral phase, with input made directly through the thermal factor) - benefits the absorption of elements present in the mud;
- ✓ bacteriostatic and bactericidal effects (51)(34)
- ✓ exchanges of energy and substances (thermoregulation of peripheral circulation, excretion, stimulation of skin repair processes) (2);
- ✓ general thermoregulation stress resulting in related optimized adaptive physiology and or therapeuticrehabilitative responses (2);
- ✓ vitamin D synthesis;
- ✓ regulating the balance of homeostasis through immune, endocrine, and neurovegetative mechanisms (47)(52);
- ✓ better oxygenation of tissues (an increase of SO2% in peripheral blood, O2 binding capacity, and oxyhemoglobin).

The thermal effect of peloidotherapy determines:

- ✓ pain relief (53);
- ✓ decreased muscle contractions (54);
- ✓ anti-inflammatory effects - the higher the application temperature, the higher the tonic immuno-stimulatory and cardiovascular effects, the application is possible due to the special mud property of thermopexy.

All of these develop local and general remote effects, such as analgesic effect, anti-inflammatory, muscle relaxant, detoxifying, neuroendocrine, to regulate immunity (55) and because the (including) mud based thermotherapy acts, as well, by enhancing the elasticity of the conjunctive-collagen major musculo-entheso-articular constituents – respectively through reducing their stiffness, which is associated to many conditions affecting such structures and/or to (just) aging – this, together with the above mentioned muscle decontracturant and analgesic effects, results also in an important link to facilitating rehabilitation: with more supple, relaxed and pain freer segments of the body, is a biological state prone to better outcomes of kinesiotherapy/ physical exercise, with consequent augmented functional mobility recover, and thus, an overall improvement of the quality of life, too.

Pharmacodynamic properties

Being a biological material used in human therapy, the analysis is performed according to the methods indicated in the "Romanian Pharmacopoeia" with some additions taken from the working methods used for soil analysis. The obtained result is presented in the form of the physicochemical analysis bulletin.

When applying peloids, a series of processes take place at the level of the skin:

- ✓ sensitive reception of mud qualities/properties and their transmission to the upper stages of integration and control;
- ✓ exchange of energy and substance with the peloid environment;
- ✓ fulfillment of adaptive functions: circulatory (peripheral circulation of thermoregulation), secretory (sweating), protective (keratinogenesis, melanogenesis, hydrolipid film formation);
- ✓ integration of skin - mud application effects in the general physiology of the body: thermoregulation, vitamin D synthesis, optimization of homeostatic balances: immune, endocrine, and neurovegetative of the body (17).

Applying mud to the entire surface of the skin triggers local tissue and general functional reactions, inhibiting or activating some enzyme systems and intermediate metabolites. The mud spa treatment produces favorable effects that persist for a long time by changing the ability to respond adaptively to various stimuli.

This procedure is a demanding one as the application time, density and temperature are higher, respectively the higher the salt concentration of the dilution saltwater. Even in healthy, robust patients, this procedure is especially demanding for the cardiovascular system. That is why it is contraindicated especially in hyper and hypertensive patients.

Peloiodotherapy is indicated for the following conditions:

- degenerative rheumatism with different locations: spine (spondylosis, simple discopathy, chronic lumbago (56), peripheral (coxarthrosis, gonarthrosis) (57)
- osteoartritis (58)(59)(60)(61)(62)(63)(64)(65)(66)(67),
- inflammatory rheumatism (54)
- ab-articular rheumatism (68)
- fibromyalgia (69)(70)
- musculoskeletal disorders (MSDs) (71)(72)
- post-traumatic sequelae (73)
- carpal tunnel syndrome (74)
- central and peripheral nervous system damage (75)
- psoriasis (76)(34)
- chronic eczema, chronic wounds (77)
- chronic urticaria
- obesity (78)
- pituitary dwarf
- rickets or children's weakness
- gynecological chronic inflammation, secondary sterility, post-tuberculosis sequelae

The ability of therapeutic mud to effectively restore reproductive function has long been known and does not cause doubt. In mud resort centers for the treatment of infertility applications and mud baths, vaginal and rectal tampons with mud heated to the desired temperature. The effect of mud therapy will be more visible if it is combined with physical therapy (79) and gynecological massage, with medical microclyms, reflexology, and homeopathy.

Mud treatment is indicated for patients with chronic inflammatory processes of different origins, with chronic adnexitis and peri-adnexitis, with different adhesions, with parameterized residues, with chronic endocervicitis and colitis, with some forms of infertility, with miscarriage.

Mud treatment is useful in case of insufficiency of ovarian function and mild forms of uterine underdevelopment. Patients with severe genital lesions at Trichomonas may go for a spa treatment. Amenorrhea and dysmenorrhea - menstrual disorders - are also indications for mud therapy.

Contraindications

- febrile/infectious or acute/subacute conditions - other than those for which the treatment is performed;
- cardiovascular diseases;
- respiratory diseases: exacerbation of COPD, asthma with recent seizures, bronchiectasis, recent respiratory infections;
- dermatological diseases: bedsores, suppurative wounds, eczema in the developmental stage, psoriatic erythroderma.

Mud therapy is contraindicated if the patient suffers from asthma, diabetes, ulcers, any form of cancer, or hepatitis, kidney, or cardiovascular disease.

➢ METHOD

Search Strategy. To fundament the above-mentioned data synthesis we have achieved a related systematic literature review and meta-analysis. Accordingly, we have searched for relevant *open access* works, in 6 international databases: including Cochrane[1], Elsevier[2], NCBI/PubMed[3], NCBI/PMC[4], PEDro[5] , and ISI Web of Knowledge/Science[6], published from January 2015 until December 2020. The contextually quested key words combinations/ syntaxes used in this respect were: ”Pelotherapy /Peloidotherapy /Mud-therapy /Fangotherapy AND Rehabilitation”.

The eligible articles were analyzed in detail regarding pathologies addressed by mud therapy/ rehabilitation – following a (Preferred Reporting Items for Systematic Reviews and Meta-Analyses – **PRISMA**[7] – type filter/ selection methodology – see Fig. 1) with additional country scientific relevance specified for this therapeutic method. The meta-analysis proceeded was designated to estimate the prevalence of various pathologies in the use of mud therapy/ rehabilitation.

Inclusion and Exclusion Criteria

Any relevant article that reported clinical or physical/chemical or biological information regarding peloids/mud therapy/ rehabilitation was included in the analysis. All articles with any design (reviews, randomized controlled trials, non-randomized controlled trials, case-control studies, cross-sectional studies), if eligible according to the above-mentioned selection methodology, were included.

Articles were excluded if they didn’t reach – using a weighted, own, PEDro inspired scoring classification – at least a score of 4 – ”fair quality” or mare points.

➢ RESULTS

Our search identified, first, **394** articles. Based on the successive filtering stages and, respectively, on the classification criteria of the Physiotherapy Evidence Database (PEDro), we finally identified/retained and analyzed **68** articles (see Fig. 1).

Although, in principle, a rigorous method – and we have followed the PRISMA type paradigm – there still might be some missing works of our related article selection. On the other hand, to augment/ consolidate our documentation base, we have used also **40** papers freely found in the literature, and even – aiming, too, at an as exhaustive knowledge underpinning as possible – derogatively, we have also considered some articles

[1] https://www.cochrane.org/
[2] https://www.elsevier.com/
[3] https://pubmed.ncbi.nlm.nih.gov/
[4] https://www.ncbi.nlm.nih.gov/pmc/
[5] https://pedro.org.au/
[6] http://apps.webofknowledge.com/WOS_GeneralSearch
[7] http://www.prisma-statement.org/

which, probably being very new, couldn't yet have reached the PEDro threshold score we have settled.

Meta-analysis (80)

Afferent with the final step, we have done also a related Meta-analysis, **26** studies were included in this step, which count **15123** human subjects in total. The smallest sample size was **32** and the largest was **6281**. The metaanalysis (80) investigated the beneficial effects of mud therapy on specific indicated diseases.

Our obtained **forest plot**, also known as a **blobbogram**, is a graphical display of estimated results from several scientific studies addressing the same question, along with the overall results. Sometimes trials are just looking at different concepts. Statistical heterogeneity is apparent only after the analysis of the results. Heterogeneity may be judged graphically (by looking at the forest plot) and be measured statistically. In a forest plot from the systematic review, the error bars for each trial include the summary result, which suggests that statistical heterogeneity is not a problem and that the message is a consistent one (fig 3). To determine whether significant heterogeneity exists, a P-value high for the $\chi 2$ test of heterogeneity is good news because it suggests that the heterogeneity is insignificant and that one can go ahead and summarise the results (81).

- **Relevant topical data regarding mud therapy in, Physical and Rehabilitation Medicine** (**PRM**) **and Balneology**

In vivo experimental models (82) based on murine osteoarthritis or rheumatoid arthritis, to establish the actions of Hévíz mineral water and mud, Hungary (83) or Jeju Magma-Seawater, Korea (82) – by a modern methodological approach using functional tests and morphological analysis – provided favorable EvidenceBased data of Complementary and Alternative Medicine effects of these natural therapeutic-rehabilitative factors, that can be observed clinically in OA patients, too. Articular diseases are the most frequent causes of chronic pain and related disability. For instance, rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial inflammation and progressive structural joint damage. Osteoarthritis is a degenerative process of the articular cartilage associated with hypertrophic changes in the bone (84). The new insights in the pathogenesis of osteoarthritis (OA) reveal the implications of adipocytokines. In the last years, balneological research was directed towards the study of specific inflammatory cytokines' involvement in musculoskeletal disorders. Correlations between peloid therapy and inflammatory pathways, at a molecular level, are established and new insights into the world of chondrocytes and osteoblasts are brought to light (85).

Peat obtained from Taean-gun, Chungcheongnam-do, Korea, can be considered as a therapeutic option for pain relief of knee OA patients. Although short-term peat intervention did not have significant effects on serum cartilage oligomeric matrix protein, long-term follow-up assessment using several parameters, including pain and OA biomarkers (86), is needed. The reduction in knee joint varus/valgus range of motion and the increase in gait velocity after peat intervention are meaningful results as effects of peat intervention on gait parameters (87). Experts agree that the Spa therapies are effective in controlling the symptoms and objective signs of disease in patients with musculoskeletal disorders, as well as in ensuring a decrease in the recruitment of NSAIDs and analgesics (88).

The hormetic effects of balneotherapy are related to different factors. The main factor that is common to all types of mineral-medicinal waters and muds is heat. The anti-aging properties of mud therapy (69) are an exciting concept for prevention and slowing the aging process throughout the entire body and other dermatological diseases. Therapeutic spas and baths offer an atmosphere of health and physical fitness by their chemical, thermal, mechanical, and endocrine actions. They also provide relaxation and stress relief. Mud therapy is effective individually or as a complement to other medical therapies. Recently, the concept of thermal mud therapy has been changing. During the aging process, endocrine changes result in a decline in endocrine function involving the responsiveness of tissues as well as reduced hormone secretion from peripheral glands. This is coupled with modifications in the central mechanisms controlling the temporal organization of hormone release, with a dampening of circadian hormonal and non-hormonal rhythms. Compared to younger individuals, healthy older individuals have alterations in body composition and a decline in functional status: decreased muscle mass, increased fat mass, and decreased strength. Even with healthy aging, there are changes in endocrine systems, including estrogen (menopause), testosterone (andropause), growth hormone/insulin-like growth factor-I axis (somatopause), hypothalamic-pituitary-thyroid axis, hypothalamic-pituitary-cortisol axis, and dehydroepiandrosterone and its sulfate (adrenopause) (89). H2S – which is also a biochemical component within the mud, as well as radon (90) – induces a wide range of physiological responses such as blood pressure modulation, neuromodulator in the brain and within the vasculature, protective against ischemic reperfusion injury, and anti-inflammatory reactions (36). H2S is an endogenous gasotransmitter, and, as such, it can be absorbed by numerous routes; it can penetrate the skin and mucosae and can therefore act at the cell level both in the skin (29) and in internal organs. Organic components of muds were demonstrated to have biological effects contributing to the healing mechanisms, but their medical significance is not still fully understood (91).

Techirghiol sapropelic mud is one of the natural therapeutic factors of medical use in Romania, being represented by the deposit on the bottom of lake Techirghiol; it has been and still is a subject to ongoing medical research because of the need of its medical use for scientific evidence-based medicine (92). One of the interesting papers regarding Techirghiol mud's postresorptive effects, refer to the plasmatic level variations of leptin show a different response depending on the balneal

application type. Warm mud application leads to a significant decrease of the leptin level which translates into the remission of inflammation and rheumatic pain. Cold mud application as a contrasting therapy leads to an increase of the leptin values at the end of the treatment, an increase that is not statistically significant. The lack of leptin variation in the case of the group that underwent constant therapy is due to the thermoregulation function that is highly strained during the cold application, while the thermal comfort during baths neutral from a thermal point of view stabilizes the hypothalamus function and does not request major neuroendocrine answers. Recent data indicate that the hypothalamus is targeted by leptin actions, leptin which crosses the hematoencephalic barrier and interacts with the leptin receptor in the arcuate nucleus of the hypothalamus, thereby controlling the thyroid-stimulating hormone, the melanocyte-stimulating hormone, and gamma-aminobutyric acid, which will eventually modulate the metabolic answers of the body (93).

- **DISCUSSION**

The use of mud by humans for medicinal and wellness purposes is most probably as old as mankind.

Balneotherapy is an effective complementary approach in the management of several low-grade inflammations and stress-related pathologies, especially rheumatic and metabolic conditions. However, despite the demonstrated clinical and symptomatic benefits of these therapies, their role in modern medicine is still controversial, mainly because the biological mechanisms underlying these benefits have not yet been completely elucidated. In the context of these pathologies, further studies are necessary to clarify the mechanisms of effects involving the stress response and, consequently, its interaction with the inflammatory response (42).

Thermal muds have been used in many spas for the treatment of different diseases (medical uses) as well as to clean and beautify the skin, in different forms/wellness such as mud baths, masks, and cataplasms (94). DPSIR (Drivers-Pressures-State change-Impacts-Responses) (95) framework can be used also to analyze touristic activities, including mud uses, to identify a set of key indicators with weightings for health tourism destinations by using an advanced analytic hierarchy process (AHP) method, derived from the official, academic, and professional opinions of the experts (96).

Yet, in the literature, mineralogical and chemical compositions and the possible toxicity of the peloids (97) need to be investigated and compared with some limits to determine whether they have any health benefits and potential applications for pelotherapeutic treatments (26). On the other hand, the therapeutic Euganean thermal mud is a unique product of the Euganean Thermal District (Italy) that represents the largest and oldest thermal center in Europe (54). The application of the therapeutic mud, whose beneficial effects have been documented since Ancient Roman times, is recognized by the Italian Health System as a healing treatment for arthrorheumatic diseases. The beneficial mud is obtained by a specific maturation procedure that can be considered as an ancient biotechnological process. This process is now coded by a protocol to be followed to obtain the “Mature Mud AOC” certification. The mud maturation process begins when virgin clay, obtained from the lakes of Arquà Petrarca (Padova, Italy), is laid in open-air tanks or silos of the different thermal Spas, and maintained there for a period of at least two months, constantly covered by a layer of flowing thermal water, at the indicated temperature of 38–40 ◦C. This maturation procedure allows a microbial community, mainly represented by cyanobacteria, to grow on the mud surface. Microorganisms, embedded in a thick polysaccharidic matrix, generate a green biofilm that indicates the correct mud maturation. Before using the mud for therapies, this is mixed and put in tanks in which thermal water at nearly 60 ◦C is present, to reduce the natural microbial load and maintain the fluidity of the product (98). Additionally, Techirghiol represents ancient golf of the Black Sea, with sapropelic mud extracted from the lake, associated with marine climatic characteristics in a combined heliomarin and thalassotherapy cures. In BRST are treated patients with a wide range of diseases, most of them with osteoarticular and neurological pathology, both adults and children. Yearly, in Balneal and Rehabilitation Sanatorium from Techirghiol, are admitted more than ten thousand patients. The patients are admitted for a period of 12 days up to 30 days and they receive complex rehabilitation treatment: hydro-kinetic-therapy in the salted water of the pool, alternated with warm mud baths or hot mud wrapping, or cold mud ointment, and then swim in the lake, completed with massage, electrotherapy, and kinesiotherapy. All patients underwent an initial clinical examination and then the physician filled up a questionnaire, which includes personal data, information about the disease requiring admission if the patient has in the medical history any balneal treatment and what were the results, and finally the group of affections to which fit the existing symptoms (99).

Delivery of drugs on/into/through the skin enables either local or systemic actions and improvement of poor biopharmaceutics profiles of drugs administered via other administration paths, and becomes a useful strategy in situations in which other administration routes are not possible or inadvisable. Pelotherapy is the topical administration of hot-muds or peloids, with optimal rheological and thermal properties composed of clay minerals and mineral-medicinal water aimed at treating arthro-rheumatic issues, bone-muscle traumatic damage, and dermatological pathologies. Most of the important properties attributed to clays for dermo-cosmetic applications are related to their surface properties (surface area, cation exchange capacity, layer charge, among others); rheological properties (thixotropy, rheopecty, viscosity, plasticity); and other physical and mechanical properties including particle size and shape, color, softness, opacity, reflectance, iridescence, and so on (21).

The paucity of evidence about cost-effectiveness and economic evaluation of conservative, nonpharmacological and non-

surgical rehabilitative interventions for the range of lower limb musculoskeletal complaints is a concern for one found study (100). Balneotherapy could be beneficial in treating patients suffering from low back pain, but there is still not enough quality scientific evidence (101).

Mud produces important beneficial effects including antiinflammatory and anti-microbial activity which might explain in part the therapeutic properties of mud packs against chronic inflammatory skin disorders. Besides, treatment of keratinocytes with mud extract led to a significant increase of ATP levels as well as mRNA expression of genes involved in cell protection and longevity. The mud could serve as a natural anti-oxidant and moisturizing anti-aging agent with important cosmeceutical applications (34).

Figures and tables

Articles	Keywords	Web of Science	Cochrane	Elsevier	PubMed	PMC	PEDro	Total
Articles found in the first phase	"Mud therapy"	44	1	7	69	41	15	177
	"Peloidotherapy"	13	4	2	7	5	0	31
	"Pelotherapy"	52	2	2	14	29	0	99
	"Fango therapy"	0	1	0	0	0	0	1
	"Mud therapy" + "rehabilitation"	11	1	5	20	21	0	58
	"Peloidotherapy" + "rehabilitation"	2	0	1	3	2	0	8
	"Pelotherapy" + "rehabilitation"	3	0	0	4	13	0	20
	"Fango therapy" + "rehabilitation"	0	0	0	0	0	0	0
	TOTAL	125	9	17	117	111	15	394

TABLE 1 Step I: numerical search results.

Kinesiotherapy (therapeutic exercises including stretching, isotonic, isometric, and isokinetic strengthening), is effective in improving pain, joint stiffness, functional mobility and muscle strength in patients with knee OA. There are new data that emphasize the efficiency of the combined effect of geotherapy with kinesiotherapy in reducing pain for patients with osteoarthritis (102).

- **Conclusions**

This paper overviews the current state-of-the-art knowledge in the approach of peloidotherapy in rehabilitation, with a focal point on the therapeutic properties of peloids. Most of the thermal spas around the world recommend their mud baths or local mud cataplasm applications, as they recognize therapeutic results through their anti-inflammatory, analgesic, and antiseptic effects on musculoskeletal and dermatologic pathologies, which are increasingly supported by clinical trials (103). Guidelines and studies reviewed are recognizing the beneficial effects and mechanisms of action of mud therapy (12). Osteoarthrosis is the most addressed pathology (104)(105).

The works we have evaluated show that almost half of the thereof included patients originate from Romania. Our systematic review and meta-analysis have emphasized an additional, corollary, conclusion, too: the often connected/ synergistic, therapeutic, and rehabilitative effects of the balneary interventions (46), including mud procedures based, prove and strengthen the Romanian successfully paradigm of a unitary/ sole specialty: Physical and Rehabilitation Medicine & Balneology – and this is reflected also in the new focus and title of our publication ”Balneo and PRM Research Journal”.

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This study, being a systematic review, did not require written consent from the patients involved.

All authors have equally contributed to this article.

- **Declaration of interests**

Authors declare no competing interests. The approval of the Ethics Commission of „Bagdasar-Arseni Hospital” in Bucharest (N.O. 9910/17.03.2021) was obtained for this article.

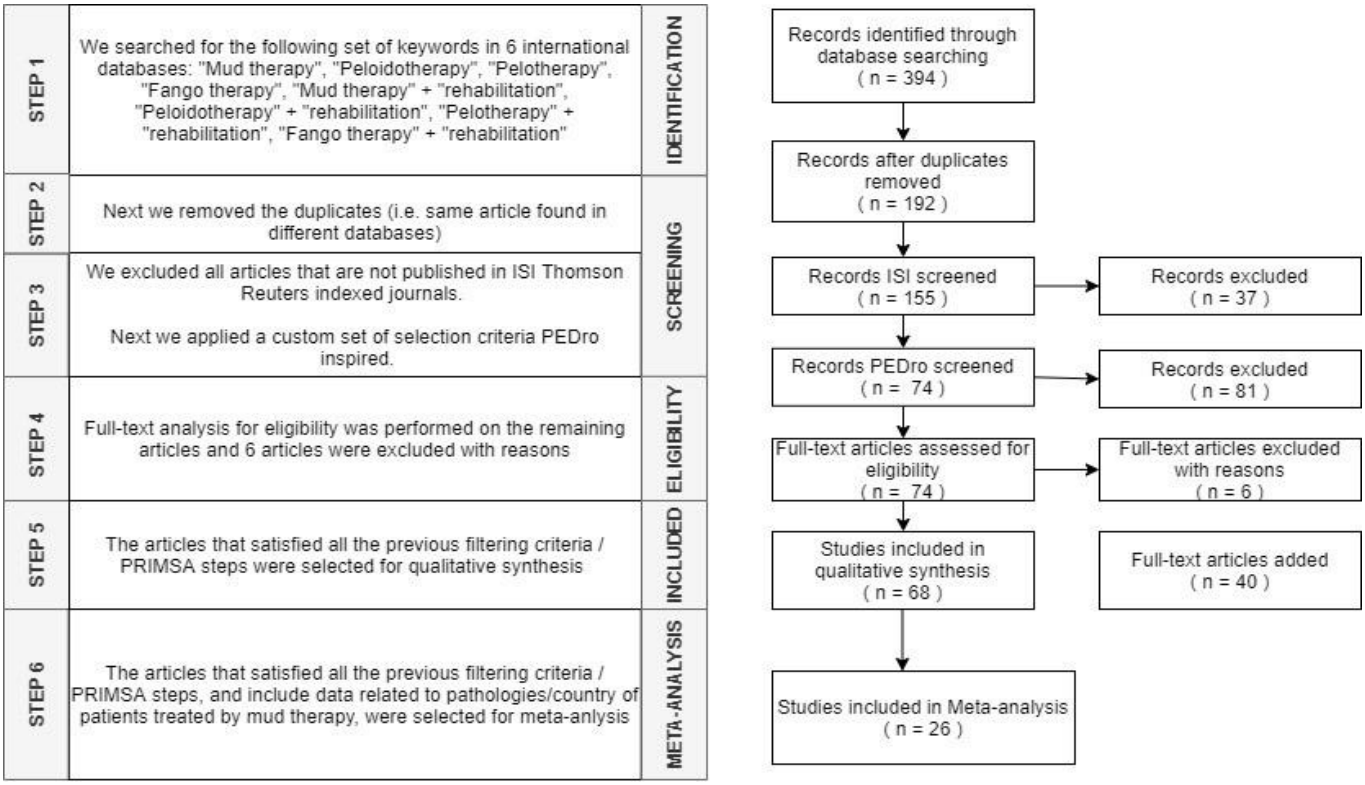
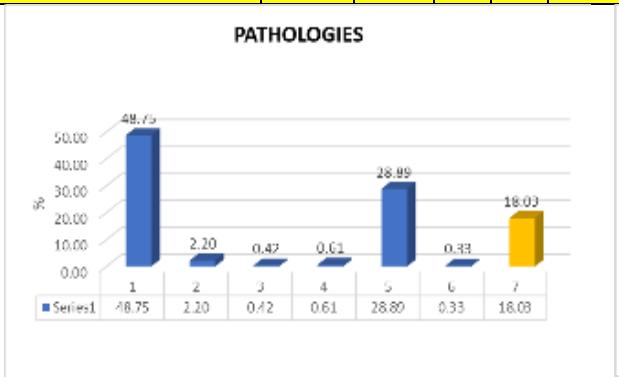


Figure 1: Flow-chart depicting the literature search and selection strategy. After applying the inclusion and exclusion criteria

TABLE 2 OpenMeta-Analyst source files related to pathologies/country patients treated by mud therapy

Study	Year	N - total patients	OA	Rheumatoid arthritis	Osteoporosis	Algic symptoms	Low Back Pain	Musculoskeletal disorders	Control	Romania	Spain	Hungary	Turkey	France	Italy	OTHER COUNTRY
AXIS LEGEND			1	2	3	4	5	6	7	1	2	3	4	5	6	7
Karagülle (3)	2015	404	0	0	0	0	404	0	202	0	0	0	0	360	0	44
Morer (5)	2016	1118	599	308	64	0	147	0	535	0	0	397	156	0	0	721
Fioravanti (55)	2015	95	95	0	0	0	0	0	46	0	0	0	0	0	95	0
Fioravanti (106)	2015	103	53	0	0	0	0	0	50	0	0	0	0	0	103	0
Yücesoy (57)	2018	139	0	0	0	0	139	0	139	0	0	0	139	0	0	0
Forestier (58)	2016	2233	1721	0	0	0	0	0	512	0	132	117	330	642	378	634
Özkuk (32)	2017	50	50	0	0	0	0	0	50	0	0	0	50	0	0	0
Pascarelli (107)	2016	103	53	0	0	0	0	0	50	0	0	0	0	0	103	0
Eröksüz (70)	2019	50	0	0	0	0	0	50	50	0	0	0	50	0	0	0
Gungen (44)	2016	59	34	0	0	0	0	0	25	0	0	0	59	0	0	0
Valckenaere (108)	2018	185	0	0	0	92	0	0	93	0	0	0	0	185	0	0
Gyarmati (83)	2017	47	47	0	0	0	0	0	24	0	0	47	0	0	0	0
Tenti (31)	2020	212	212	0	0	0	0	0	212	0	0	0	0	0	212	0
Cantista (7)	2020	120	60	0	0	0	0	0	60	0	0	0	0	0	0	120
Alfier (102)	2020	48	48	24	0	0	0	0	24	0	0	0	0	0	0	48

Hahm (53)	**2020**	**32**	**0**	**0**	**0**	**0**	**16**	**0**	**16**	**0**	**0**	**0**	**0**	**0**	**0**	**32**
Aksoy (47)	**2017**	**63**	**33**	**0**	**0**	**0**	**0**	**0**	**30**	**0**	**0**	**0**	**63**	**0**	**0**	**0**
Kim (87)	**2020**	**41**	**22**	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**0**	**0**	**0**	**0**	**0**	**41**
Ionescu (85)	**2017**	**46**	**23**	**0**	**0**	**0**	**0**	**0**	**23**	**46**	**0**	**0**	**0**	**0**	**0**	**0**
López (101)	**2019**	**1390**	**0**	**0**	**0**	**0**	**1390**	**0**	**0**	**0**	**0**	**71**	**81**	**1192**	**0**	**46**
Xiang (60)	**2016**	**1010**	**1010**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**132**	**53**	**236**	**451**	**80**	**58**
Gálvez (62)	**2018**	**36**	**36**	**0**	**0**	**0**	**0**	**0**	**36**	**0**	**36**	**0**	**0**	**0**	**0**	**0**
Iliescu (99)	**2018**	**6281**	**2744**	**0**	**0**	**0**	**2273**	**0**	**0**	**6281**	**0**	**0**	**0**	**0**	**0**	**0**
Varzaityte (65)	**2019**	**92**	**32**	**0**	**0**	**0**	**0**	**0**	**60**	**0**	**0**	**0**	**0**	**0**	**0**	**92**
Király (66)	**2019**	**60**	**60**	**0**	**0**	**0**	**0**	**0**	**60**	**0**	**0**	**60**	**0**	**0**	**0**	**0**
Hou (104)	**2020**	**1106**	**440**	**0**	**0**	**0**	**0**	**0**	**411**	**0**	**121**	**53**	**151**	**451**	**290**	**50**
TOTAL		**15123**	**7372**	**332**	**64**	**92**	**4369**	**50**	**2727**	**6327**	**421**	**798**	**1315**	**3281**	**1261**	**1886**
%		**100**	**48.75**	**2.20**	**0.42**	**0.61**	**28.89**	**0.33**	**18.03**	**41.84**	**2.78**	**5.28**	**8.70**	**21.70**	**8.34**	**12.47**



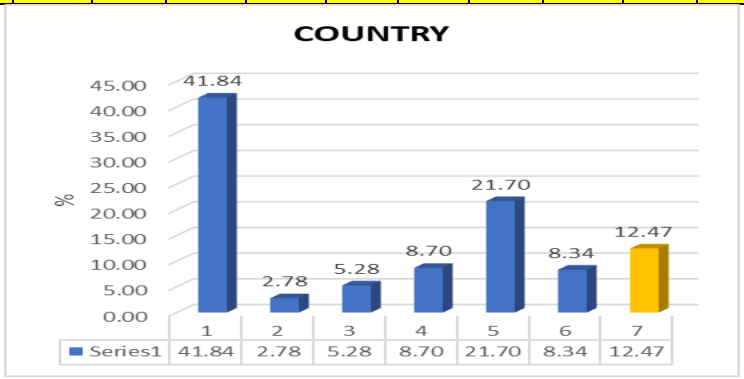
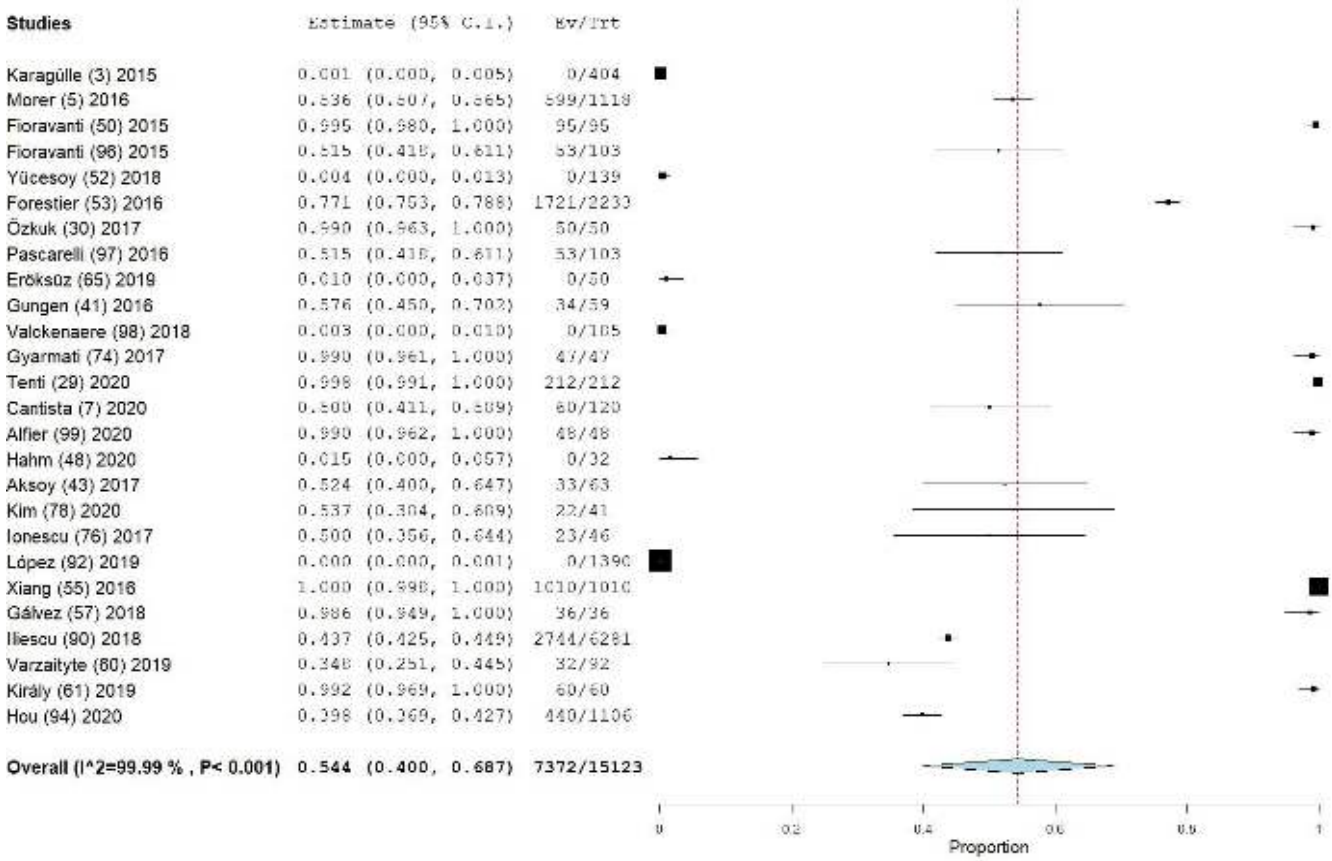


Figure 2: Pathologies and Country frequencies in mud therapy studies (see LEGEND in Table 3)



Binary Random-Effects Model Metric: Proportion

Model Results

Estimate	Lower bound	Upper bound	Std. error	p-Value
0.544	**0.400**	**0.687**	**0.073**	**< 0.001**
Heterogeneity	**tau^2**	**Q(df=25)**	**Het. p-Value**	**I^2**
	0.138	**1447636.745**	**< 0.001**	**99.995**

Figure 3: Studies **Heterogeneity** - forest-plot (80)

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WATER, NATURE, TECHIRGHIOL – LONG-TERM THERAPEUTIC BENEFITS FOR PATIENTS WITH DEGENERATIVE LOW BACK PAIN

M. G. ILIESCU[a,b], A. A. LUPU[a,c], E. V. IONESCU[a,b]*, I. TICA[a,c]*, R. E. ALMASAN[b], C. OPREA[a,b], D. M. ILIESCU[a]

[a]*Faculty of Medicine, 'Ovidius' University of Constanta, University Alley, Campus – Corp B, 900 470 Constanta, Romania*

[b]*Balneal and Rehabilitation Sanatorium of Techirghiol, 34–40 Dr. Victor Climescu Street, 906 100 Techirghiol, Romania*

[c]*Emergency Country Hospital 'St. Andrew' Constanta, 145 Tomis Street, 900 591 Constanta, Romania*

E-mail: *elena_valentina_ionescu@yahoo.com*; *irinatica@gmail.com*

Abstract. Exercise programs in water is an effective therapy for chronic low back pain. The aim of our study is to evaluate the impact of hydro-kinetotherapy in the salty water of Techirghiol lake, on patients with degenerative low back pain. We evaluated 50 patients admitted to Balneal and Rehabilitation Sanatorium of Techirghiol, before and after 2 weeks of therapy. The patients were divided into 2 groups, one group had hydro-kinetotherapy in the lake (hydro-kinetotherapy group – HKT), and the other group had no kinetotherapy (control group – CG). Both groups had electrotherapy (low, medium and high frequency currents, magnetic therapy, ultrasound therapy, laser therapy) and massage. Each patient had to complete a questionnaire regarding lumbar disability (Roland-Morris questionnaire), and during the physical examination we completed the Back Performance Scale and Visual Analogue Scale. We discovered statistically significant differences between the 2 values for both groups, but the values were more significant for the hydro-kinetotherapy group of patients (p value < 0.02). Our study reached the same results as the other studies, so hydro-kinetotherapy in salty water among special rehabilitation treatment improve the lumbar disability and also decrease the painful syndromes and dysfunction for patients with degenerative lumbar pathology.

Keywords: lumbar pain, Techirghiol lake, exercise.

AIMS AND BACKGROUND

Techirghiol Lake is located in Romania, in Dobrogea District, at 16 km from Constanta, near Eforie Nord on Lake Techirghiol shore, in a region with a mean altitude of about 200–300 m, situated between the lower Danube River and the Black Sea. The lake is in a temperate continental climatic area with an important influence

of the sea (Fig. 1). The depression occupied by the lake was formed as fluvial-maritime lagoon during the last glacial periods[1].

* For correspondence.

Fig. 1. Techirghiol lake (our private photo)

Fig. 2. Sapropelic Mud from Techirghiol lake (our private photo)

The water of the lake has an important characteristic due to the variable concentrations of salt, which varies between insignificant salinity to 60, even 85 g/l. Water of the lake is greenish due to the numerous microorganisms and algae. The main alga that grows here is Cladophora. The pH of the water is alkaline between 7.8 and 8.3. Beside the alga, Techirghiol lake has a very prolific phyllopod crustacean,

named Artemia salina which lives in the water during the warm season, from April until September[1]. Artemia salina feeds on micro plankton and contributes to the formation of mud (Fig. 2), depositing on the bottom of the lake after completing its life cycle[1,2]. The average annual temperatures range between 11°C along the Danube to 11.8°C on the coast and less than 10°C in the higher parts of the region[1]. The sapropelic mud, among the salty water, is an important natural factor that has a high curative importance. The natural factors from Techirghiol area are extensively researched in various studies, especially in terms of therapeutic benefits for the patients treated in the Balneal and Rehabilitation Sanatorium of Techirghiol. Water may have therapeutic properties under different circumstances, such as Techirghiol lake water, but it can sometimes be a vehicle for transmitting different germs[3,4].

Low back pain is an important factor causing long-term disability. Although there are low back pain episodes which resolve rapidly, there are a lot of patients that suffer of chronic low back pain. Chronicity is due to the duration of over 12 weeks of pain. This has a significant impact on patient quality of life[5]. The prevalence of low back pain is increasing, and it is considered that approximately over 80% of the population suffer from this condition. It is considered the fifth most common reason for visiting a physician and it is also the most common cause of disability in patients between 45 and 65 years old and it seems that the prevalence of these pathology is higher in women[5]. In terms of risk factors the most commonly and frequently are: high levels of psychological distress, low levels of physical activity, smoking, obesity. Chronic low back pain has multiple causes, divided into 3 main categories: mechanical low back pain (90%), non-mechanical spinal conditions and visceral disease. Mechanical low back pain is defined as pain caused by trauma or deformities of anatomic structures. These conditions are aggravated due to inappropriate physical activity. Causes of mechanical low back pain are: lumbar strain, sprain, degenerative processes, herniated disc, spinal stenosis, osteoporotic compression fracture, spondylolisthesis, traumatic fracture, congenital disorders, severe scoliosis, severe kyphosis, transitional vertebrae, spondylosis, internal disc disruption, presumed instability[5].

Hydrotherapy or aquatic exercise is a very useful treatment for patients with low pain or with other musculoskeletal or neurological conditions. Recent studies revealed positive effects for middle-age and elderly people[6]. This research showed that patients with chronic low back pain are obese, have a deficiency of mobility and endurance. Exercise programs in water are an effective therapy for chronic low back pain. These programs, according to the recent studies, reduce the incidence of painful episodes, increase muscle strength, increase flexibility of the spine and of the whole body, and improve the quality of life of patients with this pathology. Aquatic exercise is facile because all body weight is discharged into water and is an easier way to exercise for patients with low back pain. Each program can be improved and adapted

for each patient[7]. These studies[6–10] had evaluated patients with low back pain who performed aquatic exercise in freshwater, but we proposed to evaluate patients who performed hydro-kinetotherapy in the salty water of Techirghiol lake and also performed rehabilitation treatment.

EXPERIMENTAL

The aim of our study is to evaluate the impact of hydro-kinetotherapy in the salty water of Techirghiol lake, during summer time, on patients with degenerative low back pain. We evaluated 50 patients with degenerative low back pain, admitted to Balneal and Rehabilitation Sanatorium of Techirghiol, for a period of 2 weeks. Each patient received an informed consent and oral instructions about the study and the protocol. The patients were evaluated paraclinically and clinically before and after 2 weeks of therapy. The patients were divided into 2 groups, one group had hydro-kinetotherapy in the lake (hydro-kinetotherapy group – HKT), and the other group had no kinetotherapy (control group – CG). Both groups had electrotherapy (low, medium and high frequency currents, magnetic therapy, ultrasound therapy, laser therapy) and massage. Each patient had to complete a questionnaire regarding lumbar disability (Roland-Morris questionnaire – RM), and during the physical examination we completed the Back performance scale (BPS) and Visual analogue scale (VAS). These parameters were completed before and after the treatment for each patient. The study groups had been analysed by age, gender, provenance, pathology, history of disease, frequency of balneal treatment, body mass index. We included in the study patients with low back pain, with a history of pain of minimum 12 weeks, with degenerative causes of low back pain. Exclusion criteria were: major rheumatologic, neurologic, cardiovascular, respiratory, renal, digestive conditions, presence of inflammatory syndrome (elevation of: erythrocyte sedimentation rate, fibrinogen), malignant diseases, presence of any psychiatric disorder, pregnancy or recent childbirth, any sign or symptom that might suggest serious medical illness, any skin disorders.

Patients from the hydro-kinetotherapy group had to perform a specific program in the salty water of Techirghiol lake. The exercises were done in the lake during the summer time, the temperature of the water was approximately 23–25°C and the temperature outside was within 28–32°C. Each hydro-kinetotherapy session lasted 50 min, the patients were closely supervised by a kinetotherapist. Each session included 10 min of warm-up, 15–20 min of aerobic exercises, 10 min of resistance exercises and 10 min of stretching exercises.

Statistical interpretation of the results of the study was performed using SPSS 22.0 programme.

RESULTS AND DISCUSSION

Analysing the groups from the study we find that from the control group (CG) out of 25 patients, 16 were female (64%) and 9 were male (36%), and from the hydrokinetotherapy group (HKT), 13 were female (52%) and 12 were male (48%), which confirms that the prevalence of this pathology is higher for women[5]. Regarding the age of the patients, in the control group (CG) patients were between 45 and 67 years old, with a mean of 58.72 years, and in the second group (HKT) patients were between 44 and 65 years old, with a mean of 56.36 years (Table 1).

Table 1. Age of patients in the two groups from the study

Group		*N*	Minimum	Maximum	Mean	Std. deviation
CG	Age	25	45	67	58.72	6.275
HKT	Age	25	44	65	56.36	6.645

Majority of the patients are from urban provenance, as shown in Table 2, the whole control group and 21 patients from the hydro-kinetotherapy group. **Table 2**. Characteristics of the CG and HKT group of patients from the experimental study

Characteristics of the groups		Group				*p**
		CG		HKT		
		count	%	count	%	
	total	25		25		
Patient gender	woman	16	64.0	13	52.0	0.390
	men	9	36.0	12	48.0	0.390
Pathology	spondylosis	15	60.0	13	52.0	0.569
	spondylolisthesis	3	12.0	3	12.0	1.000
	lumbar discopathy phase II	3	12.0	5	20.0	0.440
	Lumbar disc herniation	4	16.0	4	16.0	1.000
Provenance	Urban	25	100.0	21	84.0	0.037
	Rural	0	0.0	4	16.0	0.037
History of disease	0–6 months	0	0.0	2	8.0	0.149
	> 6 months	25	100.0	23	92.0	0.149
Frequency of balneal treatment	biannual	0	0.0	0	0.0	–
	annual	9	36.0	15	60.0	0.089
	occasional	13	52.0	0	0.0	0.000
	first time	3	12.0	10	40.0	0.024
BMI interval	normal	9	36.0	3	12.0	0.047
	obesity	16	64.0	22	88.0	0.047

*Chi-squared test for the comparison of two proportions.

Regarding the average age of the disease, almost all the patients had the low back pain pathology over 6 months, all patients form the control group and 23 patients form the second group of patients. In terms of the distribution of patients by frequency of balneal treatment we discovered that no patients did the rehabilitation treatment biannual, as indicated, patients that had each year treatment were 9 patients (36%) from the first group and 15 patients (60%) from the second group, occasional treatment did 13 patients (52%) from the first group and no patient from the second group, and for the first time we had 3 patients (12%) from the first group and 10 patients (40%) from the second group. Analysing the body mass index (BMI) of the patients in the study we had only 9 patients (36%) from the first group and 3 patients (12%) from the second group which had a normal BMI, the other patients were overweight or with obesity. This is a worrying number of patients with obesity, which confirms once again the previous studies[5] regarding the fact that obesity is a risk factor for low back pain. Analysing the groups regarding the pathology of the patients, we noticed that spondylosis had 60% of patients from the control group and 52% of patients from the hydro-kinetotherapy group, lumbar disc herniation is the same in both groups 16% of patients, spondylolisthesis 12% of patients in both groups, and lumbar discopathy is 12% in the first group and 20% (Ref. 8) in the second group. We do not have a pattern regarding degenerative low back pain, as we mentioned above there are no studies conducted on the prevalence of the causes of low back pain and we have few patients in our group in order to conclude that this could be the pattern of the prevalence of degenerative low back pain (Table 2). In terms of demographic characteristics there are no statistically significant differences between female proportion from both groups or any other categories (males, provenance, history of disease, pathologies, frequency of treatment, etc.) from the demographic characterisation table (Table 2).

In our study we measured VAS for pain in the first day of treatment and in the last one. VAS is a commonly used scale used to monitor the patient pain or the response to a pain treatment. It is a subjective measurement, the patient respond with a mark between 1 and 10 regarding the level of pain that he has. 1 is for no pain, and 10 is for severe, insupportable pain. Analysing VAS scores for groups in the study, we noticed that the distribution of VAS scores before treatment (VAS1) ($p = 0.370$), and the distribution of VAS scores after treatment (VAS2) ($p = 0.547$) is the same in the control group and in the hydro-kinetotherapy group, so there are no significant differences between the scores obtain for the VAS scores (VAS1 and VAS2) measured in the two groups (Figs 3 and 4, Table 3).

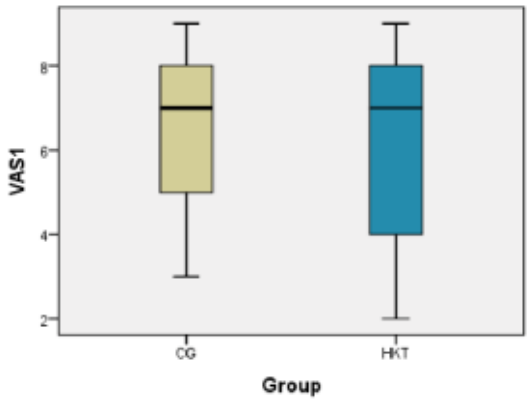

Fig. 3. Box-Plot representation of VAS1 values for the two groups from the study

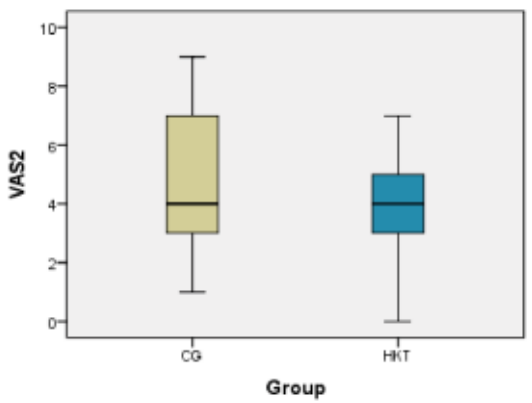

Fig. 4. Box-Plot representation of VAS 2 values for the two groups from the study

Table 3. *p* Values for the VAS scores, RM values and BPS values for the CG and HKT group of patients from the experimental study

Values of VAS, RM and BPS	Differences between CG and HKT (*p* values)	CG (*p* values)	HKT (*p* values)
VAS1	0.370		
VAS2	0.547		
Median differences between VAS1 and VAS2		<0.001	<0.001
RM1	0.619		
RM2	<0.020		
BPS1	0.600		
BPS2	0.401		

Median differences between BPS1 and BPS2	0.546	<0.001

But if we analyse the VAS scores before and after the treatment separately for each group we noticed that also for the control group and for the hydro-kinetotherapy group there are statistically significant differences between mean VAS scores before and after the treatment ($p < 0.001$) as it can be observed from the Box-Plot representation (Figs 5 and 6, Table 3), VAS scores after the treatment are significantly lower than VAS scores values before treatment (Table 3).

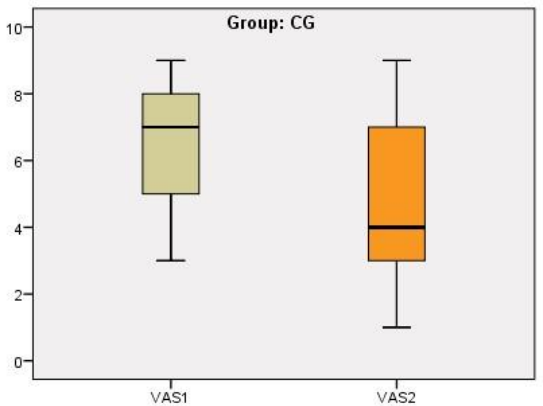

Fig. 5. Box-Plot representation of VAS1 and VAS2 scores for group CG

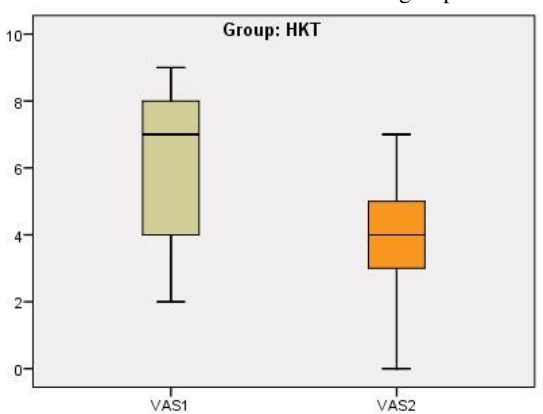

Fig. 6. Box-Plot representation of VAS1 and VAS2 scores for group HKT

In our study we used a disability questionnaire specific for lumbar pathologies the Roland Morris (RM) questionnaire. The patients from the study groups completed this questionnaire before and after the treatment. Roland-Morris questionnaire has 24 affirmations regarding diverse activities that each one is doing as a regular basic (if the pain is almost all day, if the pain affects the sleeping hours, or affects the appetite,

if the pain appears when sitting or standing, or lifting, etc.). Analysing the Roland Morris questionnaire before (RM1) and after the treatment (RM2) for the two groups of patients, we discovered statistically significant differences between the 2 values for both groups, but the values were more significant for the hydro-kinetotherapy group of patients (p value < 0.02) (Figs 7 and 8, Table 3).

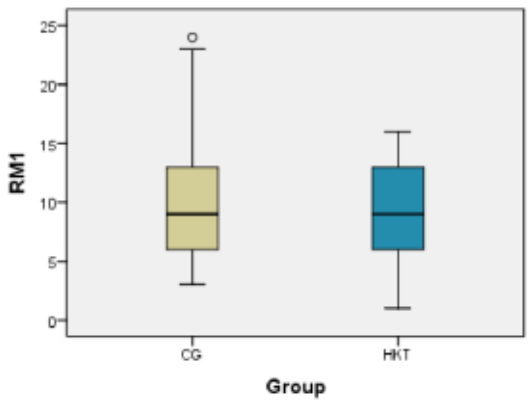

Fig. 7. Box-plot representation of RM1 values for the two groups of our study

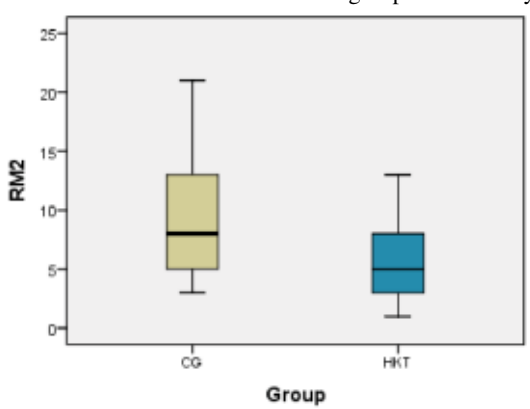

Fig. 8. Box-plot representation of RM2 values for the two groups

In order to evaluate the mobility of each patient, we used the Back performance scale before (BPS1) and after the treatment (BPS2). This scale is composed by 5 tests: sock test, pick-up test, roll-up test, fingertip-to floor test and lift test. All tests require mobility of the trunk and there are performed from sitting, standing or lying positions[11]. For each test the patient has a task, for example for the sock test, the patient is sitting on a firm bench, with the feet not reaching the floor, and he has to grab the toes with fingertips of both hands. For each test the patient gets a score from 0 to 3. We analysed the results for each group in comparison and we discovered that

the distribution of BPS scores before the treatment ($p = 0.600$) and the distribution of BPS scores after the treatment ($p = 0.401$) is the same for each group in the study, so there are no significant differences between the scores of BPS measured for the two groups (Figs 9 and 10, Table 3).

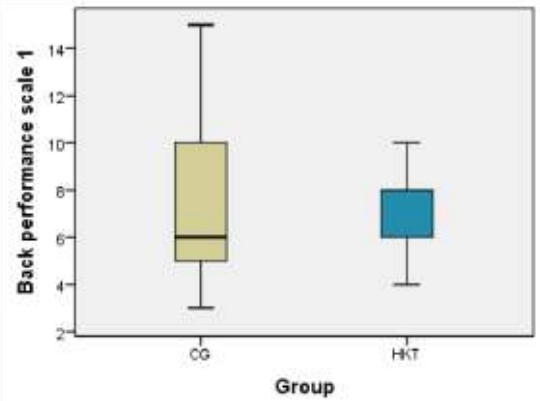

Fig. 9. Box-plot representation of BPS1 scores for CG and HKT groups

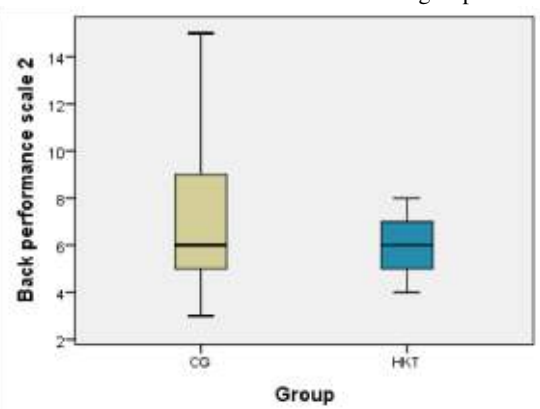

Fig. 10. Box-plot representation of BPS2 scores for CG and HKT groups

We also evaluated BPS scores separately for each group and we discovered that for the control group there is no statistically significant differences between the median values of the BPS scores before and after the treatment ($p = 0.546$) (Table 3). For the hydro-kinetotherapy group there are statistically significant differences between the median values of the BPS score before and after treatment ($p < 0.001$) (Table 3), and as seen from the associated Box-Plot representation, BPS scores after treatment are significantly lower than the BPS score before treatment (Figs 11 and 12, Table 3).

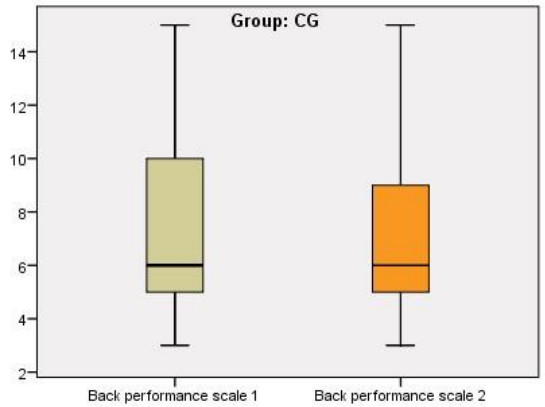

Fig. 11. Box-plot representation for BPS1 and BPS2 scores for CG group

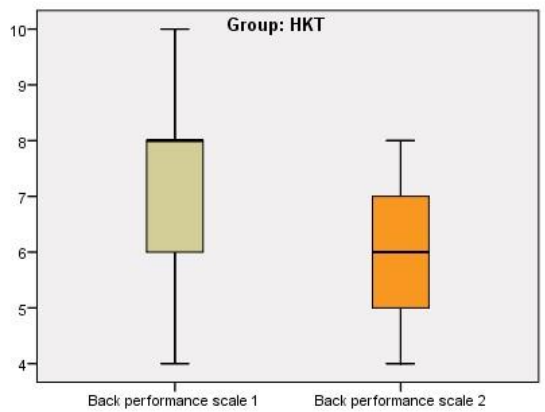

Fig. 12. Box-plot representation for BPS1 and BPS2 scores for HKT group

CONCLUSIONS

As previous studies conducted which analysed the lumbar disability (with RM questionnaire)[9–12], our study reached the same results as the others, so hydrokinetotherapy in salty water among special rehabilitation treatment improve the lumbar disability for patients with degenerative lumbar pathology. Although we did not have statistically significant differences between the two groups regarding the VAS scores before and after the treatment, we had significant differences between the 2 values of VAS scores for each group (CG and HKT group), that highlights the fact that rehabilitation treatment without any physical activities decreases the level of pain in patients with degenerative low back pain. Regarding the BPS scores, although we did not have statistically significant differences between the two groups,

we noticed that only in the hydro-kinetotherapy group we had statistically significant differences between BPS scores before and after the treatment, which highlights that aquatic exercises in the salty water of Techirghiol Lake improve the mobility of patients with degenerative low back pain. Until now there were no studies[13] conducted on aquatic exercises performed in natural salty water and we believe that it is an important natural factor that it is provided to us in this region[14].

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ADIPONECTIN CORRELATION WITH BIOCLINICAL BENEFITS OF USING NATURAL THERAPEUTIC FACTORS IN KNEE OSTEOARTHRITIS

E.V. Ionescu[1], I. Tica[2*], C. Oprea[1], D.M. Iliescu[2], L.C. Petcu[4], M.G. Iliescu[3]

[1]Balnear and Rehabilitation Sanatorium of Techirghiol, "Ovidius" University of Constanta, [2]Faculty of Medicine - Medical Department, [3]Departament of Rehabilitation Balneophysiokinetotherapy, [4]Faculty of Dentar Medicine - Departament of Biophysics, Biostatistics and Medical Informatics, Constanta, Romania

Abstract

Context and objective. The new insights in the pathogenesis of osteoarthritis (OA) reveal the implications of adipocytokines. This study aims to analyze the correlations between the serum value of adiponectin and the clinical rehabilitation effects in patients diagnosed with knee OA, admitted and treated in the complex balneal resort of Techirghiol lake.

Subjects and methods. The prospective randomized clinical study included 23 patients in the study group, diagnosed with knee OA according to ACR criteria, and a matching control group of 23 subjects. Serum level of adiponectin (using Elisa technique), uric acid, triglycerides, cholesterol, HDL-cholesterol and clinical response using a visual analog scale (VAS) were evaluated in all patients on their admission day and after 10 days of balneal treatment. Control group benefited from the same procedures except for cold mud therapy and mineral water baths.

Results. Plasma adiponectin levels (23.73±6.44 ng/dL) were statistically higher ($p<0.05$) in the study group compared to the control group (18.15±6.49 ng/dL). The mean VAS in both groups was decreased ($p<0.005$) compared to the initial moment.

Conclusions. Cold peloidotherapy combined with physical therapy and balneal factors induces serum adiponectin elevation and improves knee pain in OA. Therapeutic properties of Techirghiol mud still need further research.

Key words: adiponectin, osteoarthritis, mud, knee, VAS, Techirghiol.

INTRODUCTION

In the past years, in medical literature, emphasis has been placed on implication of adipocytokines in inflammatory processes of the osteoarticular system as well as in osteoporosis (1). Both white and brown adipose tissue is producing interleukin (IL)-1 β, tumor necrosis factor (TNF)- α, IL-6, IL-8, IL-17, basic fibroblast growth factor, vascular endothelial growth factor (VEGF), leptin, resistin, adiponectin and probably many others.

Infrapatellar fat pad (IPFP), fat tissue situated intracapsular and extrasynovial in the knee joints, rich in vessels, nerve fibers, immune cells, produces all the above mentioned adipocytokines which can play a role in the initiation and progression of knee osteoarthritis (OA)(2, 3).

OA is a multifactorial degenerative joint disease characterised by damaged articular cartilage, modified subchondral bone architecture, formation of bone exostosis and inflammation of the synovia.

Even if OA is the most common so-called "non inflammatory" rheumatic disease, according to new research, inflammation plays an important role in its etiology, along with obesity, age, previous injuries etc. (4, 5).

In the last years, balneological research was directed towards the study of specific inflammatory cytokines involvement in musculo-skeletal disorders. Correlations between peloid therapy and inflammatory pathways, at molecular level, are established and new insights in the world of chondrocytes and osteoblasts are brought to light (6).

In general, adiponectin is considered a benefic adipokine because of its systemic anti-inflammatory effect based on reduction of T cell activation, of the seric levels of IL 6 and TNF-α, of its antiatherosclerotic properties etc. (7).

Medical data is contradictory regarding the role of adiponectin in osteoarticular disorders. There is proof that adiponectin decreases both in the serum and in synovial fluid along with the aggravation of OA (8) and also with the radiologic deterioration of hand OA. (9). Contrary to all that, there are other opinions

*Correspondence to: Irina Tica MD, "Ovidius" University of Constanta, Faculty of Medicine, Medical Department, 1A Cpt. Dobrila Eug street, bl. ST4, sc C ap 48, Constanta, 900512, Romania, E-mail: irinatica6@gmail.com

that adiponectin is involved in the pathogenesis of OA with a proinflammatory effect at chondrocytes level and also in the degeneration of interstitial cartilaginous matrix (10-12). In chondrocytes, *in vitro*, this adipokine induces pro-inflammatory mediators such as nitric oxide, IL-6 and matrix metalloproteinase with catabolic effects (13, 14). Furthermore, one study revealed a higher level of adiponectin in the IPFP of the patients with knee OA, especially in advanced stages, than in the subcutaneous tissue (15).

Mud, a balneotherapeutic agent used for centuries, preserves its mysteries regarding its implications in the pathophysiology of osteoarticular system. The complex biochemical composition of Techirghiol peloid, with its rich microbial flora, represents a valuable therapeutic option in musculoskeletal disorders.

In an already published paper we obtained proof of the influence of warm mud applications over the serum levels of adiponectin in patients with knee OA (16).

This study aims to evaluate the serum level of adiponectin and the rehabilitation benefits using a visual analogous scale for pain (VAS) under the effect of natural therapeutic factors after 10 procedures of cold mud applications (CMA) in knee OA. We also aim to find out if there are any correlations between adiponectin and uric acid, considered a pro-inflammatory articular factor, and if balnear treatment affects adiponectin's cardiovascular protective role, regarding lipid profile.

PATIENTS, MATERIAL AND METHOD

In our prospective randomized clinical study, we included 2 groups: the study group consisting in 23 patients, between 30 and 70 years old, diagnosed with knee OA, according to ACR criteria (17) and a matching control group of 23 patients. The patients were hospitalized in the Balnear and Rehabilitation Sanatorium of Techirghiol between 01.06.2016-01.09.2016.

All subjects signed a special informed consent with the agreement to participate in this study and approval of the sanatorium ethical committee was obtained.

Patients from the study group (cold mud applications group-CMAG) performed 10 complex physical and balnear therapy procedures, in Techirghiol specific area, consisting in hydrotherapy (general cold mud baths and salt bath in the lake Techirghiol), heliotherapy, electrotherapy (current of low, medium, high frequency, laser therapy, ultrasonotherapy, phototherapy), massage therapy, kinesiology; while the patients in control group (CG) performed the same therapy except for specific hydrotherapy (mud baths or salt baths in Techirghiol lake).

Serum adiponectin, uric acid, cholesterol, HDL-cholesterol, triglycerides and clinical response after natural treatment with sapropelic mud were evaluated in all patients as follows. All patients were evaluated on their admission day and after 10 days of balnear treatment. Blood samples were collected at admission, before treatment and at discharge, both determinations respecting an overnight 12 h digestive rest. Serum adiponectin was determined at admission and after 10 days of treatment. Specific reagents were used for the determination of human adiponectin using ELISA technique produced by "DRG Instruments GmbH", Germany, with normal reference values of adiponectin according to BMI in Table 1 (however, the producer considered that every laboratory should establish their own values).

Table 1. Adiponectin reference values according to BMI

Gender	BMI (kg/m^2)	Mean (μg/mL)	SD (μg/mL)
Men	< 25	15.9	4.0
	25-30	13.8	4.0
	> 30	13.3	2.8
	Total	14.5	3.9
Women	< 25	18.6	5.4
	25-30	18.9	8.6
	> 30	16.4	3.8
	Total	18.2	6.1

In parallel, the groups were analyzed in terms of age, gender, average age of the disease, body mass index and severity of pain in the knee joints assessed by visual analogue scale (VAS).

Exclusion criteria were: patients with erythrocyte sedimentation rate (ESR) > 40 mm/h; patients with skin disorders (chronic or acute), including skin lesions; with serious conditions such as: cardiovascular, respiratory, digestive, renal, neurological; cancer; presence of connective tissue disorders. Previous treatments were restricted as follows: NSAIDs and steroids at least 4 weeks before study entry; surgery (arthroplasty, arthroscopy, abrasion, etc.) performed at least 3 months before the study; administration of corticosteroid intra/periarticular at least 6 weeks before starting the trial or hyaluronic acid with at least 6 months before the study; treatment with opioids for at least 4 weeks before study entry; physiotherapy, acupuncture, TENS, massage and

physical therapy at least 4 weeks before study entry; administration of sedatives, hypnotics, anticonvulsant and muscle relaxant at least 2 weeks before study entry.

Statistical interpretation of the results was performed by processing data using IBM SPSS 18.0. The results were analysed with mathematical methods in order to obtain an average of the values, standard deviation and also the standard error of the media (descriptive statistics in order to characterise discrete and defined continuous variables); parametric statistical tests (t-Test to compare media of two independent samples and t-Test to compare media of paired samples).

Normal distribution was confirmed for all studied variables by Shapiro-Wilk test ($p > \alpha = 0.05$). By assessing Skewness and Kurtosis coefficients resulted that distributions are symmetrical and they do not present an abnormal vaulting.

Correlations were considered according to the values of the r correlation coefficient (adapted after D.E. Hinkle, W. Wiersma si G.S. Jurs, 1988, p.118).

The values of adiponectin, VAS, cholesterol, HDL-cholesterol, triglycerides, uric acid measured in the first day of treatment were registered as "Adiponectin 1", "VAS 1" etc. At the end of treatment, after 10 days of balneotherapy, the values were registered as "Adiponectin 10", "VAS 10" etc.

Statistical measurements were presented in Table 1.

RESULTS

Presentation of the study groups

The two groups contained 23 patients each and were considered statistically similar in terms of demographic features and of associated co-morbidities, such as metabolic syndrome and diabetes which might have had an impact over adiponectin values (18).

- sex (60.9% females in CG, 65.2% females in CMAG, p>0.05),

- age (medium value in CG=56.17±12.2 years *vs.* 55.17±8.28 years in CMAG, p=0.747>0.05),

- medium value of BMI (29±4.64 in CG *vs.* 27.69±4.33 in CMAG; p=0.328>0.05),

- medium value of VAS scale at admission (5.43±2.52 *vs.* 5.45±1.87, p=0.974>0.05),

- values of adiponectin 1 (17.90 ±8.04 ng/dL in CG *vs.* 21.46±10.31 ng/dL in CMAG, p=0.198>0.05). Adiponectin values were correlated with BMI in both groups ($p < 0.001$, $r = -0.690$, negative correlation in CG and $p = 0.002$, $r = -0.609$, negative correlation on CMAG),

- values of glycemia in day 1 (CG: medium value 111.60 ±19.21 mg/dL *vs.* 118.52±34.20 mg/dL, p = 0.40>0.05,

- proportion of diabetic patients (considered both from anamnesis and after a cut-off value of glycemia of 126 mg/dL, twice registered) was 21.7% in CG *vs.* 26.1% in CMAG, ($\lambda 2$=0.00, p>0.05),

- length of disease (in CG: 8.7% of the patients had a duration of 1-6 months, 17.4% had a duration of 6-12 months, 43.5% had a duration of 1-5 years, 13% had a duration of 5-10 years and 17.4% of more than 10 years, in the CMAG the percentages are: 4.3%, 13%, 52.2%, 17.4% and 13 %. All proportions compared revealed a p>0.05),

- regarding the presence of metabolic syndrome the control group was statistically similar with the study group ($\lambda 2$ =0.392; p>0.05),

- both cholesterol and HDL –cholesterol and uric acid values did not differ significantly between groups.

We divided time of illness in the following time intervals: 1 – 6 months, 6 months – 1 year, 1 to 5 years, 5 to 10 years and more than 10 years. Occurrence of pain was considered the time of OA onset (the main feature of knee OA). We found that the patients suffering from pain for 1 up to 5 years were in a bigger number than in the other time intervals.

In CMAG, adiponectin 10 (23.73±6.44 ng/dL) is significantly higher than in day 1 (20.24±9.90 ng/mL) (t = -2.919; df = 22; Mdiff = -3.482; p = 0.008).

The difference between adiponectin 10 in CMAG and the one in CG was significant, also, in favor of the CMAG (t = -2.922; df = 44; Mdiff = -5.573; p = 0.005), see Figure 1.

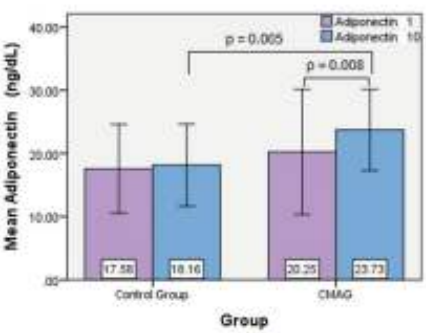

Figure 1. Values of Adiponectin 1 and 10 in both groups.

We found no statistical difference between the values of adiponectin 1 and adiponectin 10 in CG (17.58±7.02 ng/mL *vs.* 18.15±6.49 ng/dL.; t = -0.461; df = 22; Mdiff = -0.573; p = 0.649>0.05).

Adiponectin significant negative correlation with BMI persists in the 10th day of treatment.

VAS, a tool for measuring subjective pain that cannot be measured directly, was performed both in the research project baseline, day 1, and after 10 days of treatment.

Even if the difference between VAS 1 and VAS 10 is significant in each group (t = 4.657; df = 22; Mdiff = 1.5; p < 0.001 in CG, t = 6.28; df = 22; Mdiff = 1.69; p < 0.001 in CMAG), there is no difference between VAS 10 in CG and the one in CMAG (t = -0.267; df = 44; Mdiff = 0.17; p = 0.791).

DISCUSSION

The variety of the natural therapeutic agents used in balneotherapy, the influence of seasons and of the climate - different from the one of the patients' residence place, and of the changes in diet and daily activity, make strong scientific studies difficult in the field of balneology. Because of all that, debate exists in medical literature regarding the benefits of mud therapy.

In our study most of the patients were suffering from knee pain for 1 up to 5 years. It seems that patients with symptoms for less than 1 year are underestimating the knee OA evolution and prognosis, while those with more than 5 years of suffering are somehow "adapted and consoled" with the situation.

VAS is a psychometric response scale that can be used in questionnaires. It is a tool for quantifying subjective characteristics or attitudes that cannot be measured directly. When responding to a VAS item, respondents specify their level of agreement to a statement that indicates a position along a continuous line between 2 end points (pain with intensity from 1 up to 10, for example). In our study VAS was significantly higher on the 10th day of treatment in both groups. This is, once more, the proof of balneal treatment efficiency in knee osteoarthritis.

Our study brings proof not only of mud therapy efficiency but also of its possible correlations with adiponectin.

If up to 5-6 years ago, medical data sustained the pro-inflammatory effect of adiponectin, with a deleterious effect over the cartilage (19-21) and just a few considering that adiponectin may have a protective role in the progression of OA, proving that both the serum and the synovial level of adiponectin decrease significantly with the severity of osteoarthritis (22), or that adiponectin modulates cartilage destruction in chondrocytes (up-regulating TIMP-2 and down-regulating IL-1beta- induced MMP-13) (23), new studies (24-26) are sustaining the beneficial effect of adiponectin over joint cartilage. Under the same light, the study of Zheng S *et al.* published in 2016 in Scandinavian Journal of Rheumatology sustaines that serum adiponectin is significantly and negatively associated with radiologic severity of knee osteoarthritis(27).

Looking at our results, we found a statistically significant variation of adiponectin after 10 days of complex balneal therapy in the study group compared to control group. Cold mud bath induced a significant increase in serum adiponectin values both related to value of adiponectin 1 in CMAG and to the adiponectin 10 of the control group. Based on our previous study, in which we found that hot peloid bath induced significant decrease in serum levels of adiponectin value after 10 days (16), we suppose that thermoregulation interferes with therapeutical results of peloid baths, along with other similar results from medical literature (28, 29). This is why we will extend our study with repeated dosage of serum levels of adiponectin at 3 months/ 6 months after balneal contrastant treatment, because we have a new work hypothesis.

We did not find a significant correlation between adiponectin values and the levels of uric acid, HDL-cholesterol, triglycerides in either group.

The results of this study, that adiponectin values are higher in case of cold mud applications than in the control group, are in contrast with our previous results (16), in which hot mud baths reduced the value of adiponectin. At the same time the significant negative correlation between VAS scale and adiponectin level is pleading for a protective role of adiponectin over knee osteoarthritis. So, we consider that thermoregulation is involved, and further research is needed to establish the relation between adiponectin, reduction of pain and cold peloid treatment with Techirghiol mud.

In conclusion, although the study included a small number of cases, it allowed the issuance of plausible assumptions on understanding the beneficial effects of Techirghiol peloid on osteo-articular degenerative disorders.

In our study we find a significant elevation of adiponectin level after 10 cold sapropelic mud applications in the study group as related to the control group.

Even if prevention of obesity related to pro-inflammatory cytokines' production is still relying on physical activity, reduction of fat body mass and healthy diet, new data about control of adipokines and their effect over osteoarthritis may represent the base of new pharmacological approaches in this disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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BIOLOGICAL VARIATION UNDER BALNEAL TREATMENT OF SERUM LEVEL OF LEPTIN IN RELATION WITH BODY MASS INDEX IN CASE OF PATIENTS SUFFERING FROM KNEE OSTEOARTHRITIS

E. V. IONESCU[a,b], C. OPREA[a], E. R. ALMASAN[a], L. E. STANCIU[a], M. G. ILIESCU[b,c10]

[a]*Balneal and Rehabilitation Sanatorium, Techirghiol, Romania*
[b]*University 'Ovidius', Constanta, Romania* [c]*Rehabilitation Hospital, Eforie Nord, Romania E-mail*: *iliescumadalina@gmail.com*

Abstract. Taking into account recent studies regarding the pathogenic mechanisms of osteoarthritis (OA) of the knee, in the present study we intend to identify an interdependence relationship between leptin and the body mass index (BMI) in this pathology. 78 subjects participated in the study, all of which had been diagnosed with OA of the knee and undergoing a balneal treatment at the Balneal and Rehabilitation Sanatorium in Techirghiol. They were organised in 3 groups, based on the therapeutic regimen: the control group (CG), the group taking warm mud baths (WMB) and the group taking cold mud baths (CMB). Leptin, an adiponectin involved in the osteoarthritis pathogeny and described in recent studies as key-mediator that correlates obesity and OA was dosed in the moment of inclusion in the research project, day 1, and also after 10 days of treatment. The values obtained were correlated with those of the BMI. The plasmatic level variations of leptin show a different response depending on the balneal application type. Warm mud application leads to a significant decrease of the leptin level which translates into the remission of inflammation and rheumatic pain. In the case of the control group, we notice a major statistical remission that we could interpret as well as a remission of inflammation and rheumatic pain, even though these patients were not treated using natural methods. Cold mud application as a constant therapy (cold baths) leads to an increase of the leptin values at the end of the treatment, increase which is not statistically significant.

Keywords: osteoarthritis, leptin, balneal treatment, obesity.

AIMS AND BACKGROUND

The question arises whether arthrosis, through the hypo-mobility and sedentariness it induces, favours obesity or if the situation is reverse: obesity favours the onset of

[10] For correspondence.

arthrosis. Studies rather seem to indicate the latter hypothesis, showing that by reducing the body mass, the arthrosis process slows down. The association between arthrosis and obesity is more obvious in female patients and affects mostly the knee (bilateral affection) and less the hip. This association does not affect at all the hands. The first and easiest explanation regards mechanical overstrain, but on itself it does not explain the differences between gonarthrosis (strongly linked to obesity) and coxarthrosis (for which no obvious association exists). Lately, there have been discussions about the preponderant involvement of metabolic factors and not mechanical ones. Therefore, one tries to identify the existence of an interdependence relation between leptin and body mass index, this being also the subject of our study. Peloid therapy interacts with pathogenic mechanism of osteoarthritis, but the way in which the change in disease is produced is still unclear. The data suggest that mud therapy influences adipokines, which already has enough evidence that would be involved in the pathogenic mechanism of OA (Ref. 1). Leptin produced in the adipose cells crosses the hematoencephalic barrier and interacts with the leptin receptor in the arcuate nucleus of the hypothalamus thereby controlling and modulating through different mediators the metabolic answer[2].

EXPERIMENTAL

Seventy eight subjects participated in the study, all of which had been diagnosed with osteoarthritis of the knee and undergoing a balneal treatment at the Balneal and Rehabilitation Sanatorium in Techirghiol. They were organised in 3 groups, based on the therapeutic regimen: the control group (CG), 23 patients treated with plants baths, three adjuvant procedures and one session of kineto-therapy and massage therapy per day; the group taking warm mud baths (WMB), 32 patients that underwent treatment with one mud bath per day alternated with one salty bath per day (in the pool or in the tub), three adjuvant procedures and one session of kineto-therapy and massage therapy per day; the group taking cold mud baths (CMB), 23 patients that underwent cold mud embrocation (progressive general heliotherapy, mud embrocation, lake dipping), three adjuvant procedures and one session of kineto-therapy and massage therapy per day.

Leptin, a 16 kDa non-glicolised hormone, secreted mainly by adipocytes, has been dosed in the moment of inclusion in the research project, day 1, and also after 10 days of treatment. The blood samples were collected both times by respecting the 12-hour fast and according to the time of the day, being known that leptin varies circadianly[3–5].

We registered the body weight (kg) and the waist measurement (cm) of each subject, based on which we calculated the BMI, distributing the patients in 5 weight classes as follows: normal weight (BMI = 18–25), overweight (BMI = 25–30),

obesity class I (BMI = 30–35), obesity class II (BMI = 35–40) and morbid obesity (BMI > 40), taking into consideration that obesity together with other laboratory investigations predicts the existence of metabolic syndrome X and that obesity is a proven risk factor of knee OA (Refs 6–8).

RESULTS AND DISCUSSION

By analysing the values from the first day of leptin determination according to BMI classes (Table 1), we notice an increase of leptin values accompanied by an increase of BMI in all the three groups.

Table 1. Average values of leptin 1 and BMI classes

Lot	BMI classes		*N*	Minimum	Maximum	Average	Standard deviation
Control normal weight group		leptin 1	4	7.20	15.00	11.4250	3.28062
overweight		leptin 1	8	8.20	34.20	17.5125	9.45160
	obesity (class I)	leptin 1	9	4.90	46.60	19.6000	14.90914
	obesity (class II)	leptin 1	2	15.20	41.40	28.3000	18.52620
WMB group normal weight		leptin 1	10	3.90	36.60	14.9700	11.26223
	overweight	leptin 1	8	6.20	43.00	22.6250	13.09981
	obesity (class I)	leptin 1	7	11.50	46.40	27.9143	11.96849
	obesity (class II)	leptin 1	5	21.90	48.60	41.0600	10.98285
	morbid obesity	leptin 1	2	51.60	53.30	52.4500	1.20208
CMB group normal weight		leptin 1	7	3.50	15.70	8.8143	5.12069
	overweight	leptin 1	10	3.70	28.50	13.2000	8.73028
	obesity (class I)	leptin 1	5	5.20	21.60	12.7800	7.54135
	obesity (class II)	leptin 1	1	12.30	12.30	12.3000	.

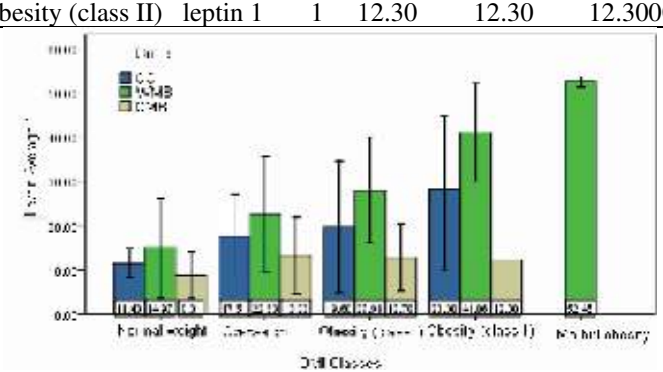

Fig. 1. Graphic representation of leptin 1 according to BMI classes

When including the subjects into the study, we notice (Fig. 1) that there is a direct proportional relation between leptin levels and patients weight quantified in

the BMI classes, the best example being the increased average value of leptin (52.45 ng/ml) registered in the case of patients suffering from morbid obesity.

Obesity is linked to increased risk of developing a range of chronic diseases, including OA. The biomechanical overstrain effect on the cartilage can partially explain the increased risk of developing knee OA in the case of overweight people. Nevertheless, risk factors for the onset and progression of OA on the joints that don't bear any weight, as the hands, have also been linked to the BMI. In fact, recent studies show that obesity rather than just body mass excess affects the knee joint, and suggests that metabolic factors associated with obesity can contribute to the OA onset. Fat mass is associated to the loss of knee cartilage volume and adipose tissue reduction is more strongly connected to the symptomatic amelioration of knee OA rather than to the loss of body weight[9]. Recent studies propose leptin as a key-mediator that links obesity to OA (Ref. 10).

Table 2. Average values of leptin 10 and BMI classes

Lot	BMI classes		*N*	Minimum	Maximum	Average	Standard deviation
Control group	normal weight	leptin 10	4	5.90	13.80	9.3250	3.42479
	overweight	leptin 10	8	5.10	34.10	15.2750	9.93029
	obesity (class I)	leptin 10	9	5.20	36.80	18.1556	12.33563
	obesity (class II)	leptin 10	2	14.60	37.50	26.0500	16.19275
WMB group	normal weight	leptin 10	10	3.90	26.20	12.5500	8.63870
	overweight	leptin 10	8	6.40	40.40	19.7875	11.27785
	obesity (class I)	leptin 10	7	6.60	45.20	26.8143	15.99994
	obesity (class II)	leptin 10	5	24.60	43.40	34.6000	6.74129
	morbid obesity	leptin 10	2	28.80	48.30	38.5500	13.78858
CMB group	normal weight	leptin 10	7	3.80	23.50	10.7771	7.01197
	overweight	leptin 10	10	3.70	30.00	12.9700	9.37716
	obesity (class I)	leptin 10	5	5.20	18.20	12.3000	6.44864
	obesity (class II)	leptin 10	1	10.30	10.30	10.3000	.

Even if the average serum level of leptin has decreased after the 10 days of treatment, the directly proportional relation between the value of leptin and BMI classes (Table 2).

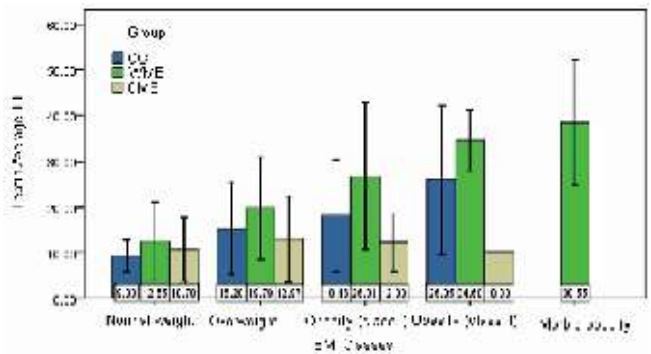

Fig. 2. Graphic representation of leptin 10 according to BMI classes

At the end of the treatment, we notice (Fig. 2) that there is a direct proportional relation between leptin levels and patients weight quantified in the BMI classes, the best example being the increased average value of leptin (38.55 ng/ml) registered in the case of patients suffering from morbid obesity.

More epidemiologic studies tried to advance the knowledge of Reijman and his collaborators[11] about the mechanism of BMI effect on osteoarthritis with specific articular localization. Previous studies showed few evidence of a metabolic connection between body weight and knee OA. With one exception[12], population studies showed a correlation of obesity (with all it suggests: serum lipids, glucose or glucose tolerance, distribution of body fat, blood pressure), with knee OA (Refs 13 and 14). Leptin can represent a systemic connection factor between body weight and osteoarthritis, even though this mechanism does not fully explain why the BMI effect should be any different in the case of the hip compared to the case of the knee. On the other hand, the special climatotherapy of the Techirghiol lake appears to inhibit the inflammation mediators[15] and the soil components (mineral and organic) must be part of the mechanism[16].

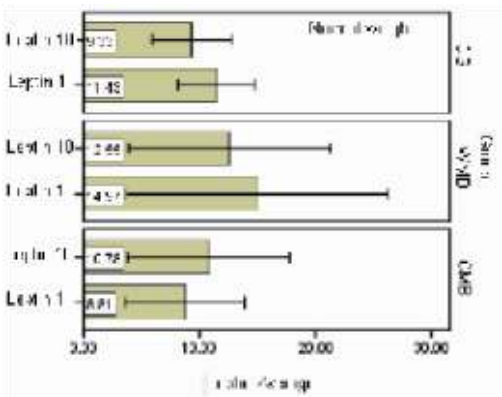

Fig. 3. Leptin variation in subjects from BMI class – normal weight

In the case of patients with normal weight, we notice a decrease of leptin level for CG, as well as for WMB, while in the case of CMB, we notice slight increase (Fig. 3).

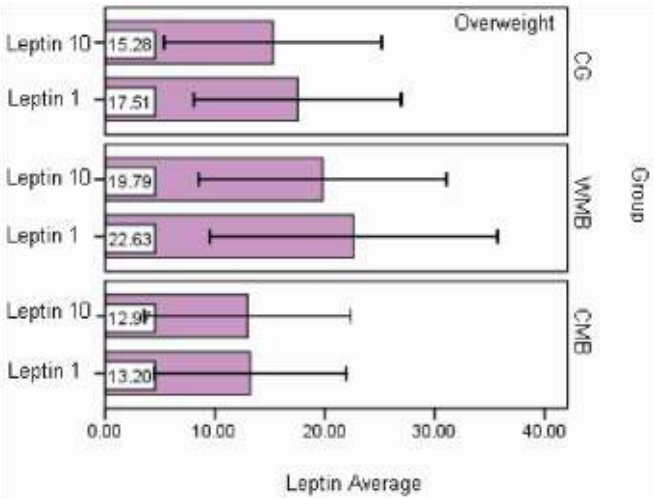

Fig. 4. Leptin variation in subjects from BMI class – overweight

In the case of overweight patients, we notice a decrease of leptin levels after 10 days of treatment for all 3 studied groups (Fig. 4). We believe that these results are generated by the fact that white adipose tissue has also the role of thermal insulation and is poorly vascularised.

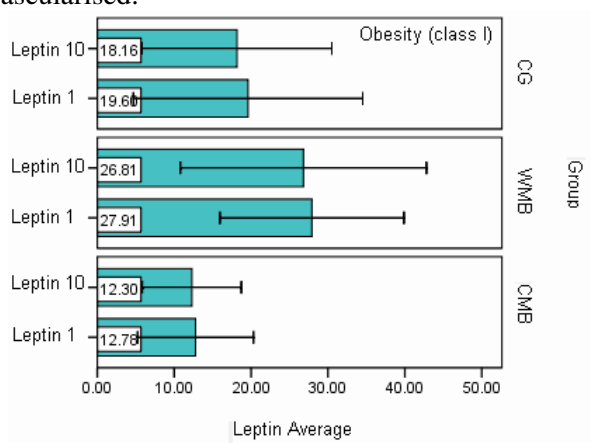

Fig. 5. Leptin variation in subjects from BMI class – obesity class I

In the case of patients suffering from class I obesity, we notice a decrease of leptin levels after 10 days of treatment for all 3 studied groups (Fig. 5).

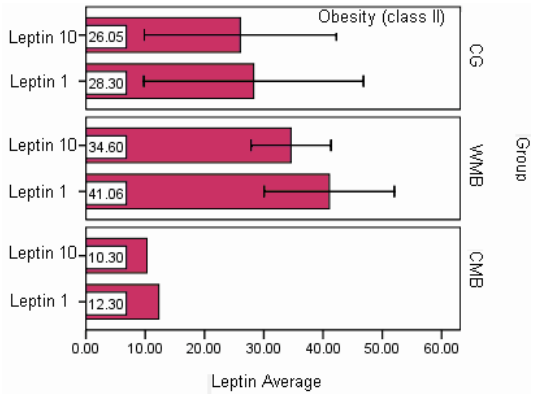

Fig. 6. Leptin variation in subjects from BMI class – obesity class II

In the case of patients suffering from class II obesity, we notice a decrease of leptin levels after 10 days of treatment for all 3 studied groups (Fig. 6). This shows the benefits of balneal treatment regarding the optimization of metabolic and inflammatory state.

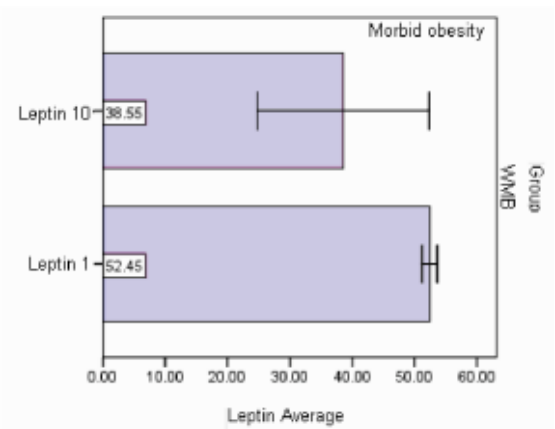

Fig. 7. Leptin variation in subjects from BMI class – morbid obesity in group WMB

Patients suffering from morbid obesity were registered only in the WMB group. In their case, we also notice a significant decrease of leptin values after 10 days of treatment, from 52.45 to 38.55 ng/ml (Fig. 7).

Variables leptin 1 and BMI are correlated, $r = 0.731$, $p < 0.01$ in WMB (Table 3). The value of Pearson correlation coefficient r being 0.731, we can state that in the case of group WMB, there is a strongly positive correlation between the leptin value registered on day 1 and the BMI. There are no significant correlations of the leptin value registered on day 1 and the BMI for the other two groups.

Table 3. Statistical correlations between leptin 1 and BMI

Lot			Leptin 1	BMI
Control group	Leptin 1	Pearson correlation	1	0.345
		Probability *p*		0.107
		N	23	23
	BMI	Pearson correlation	0.345	1
		Probability *p*	0.107	
		N	23	23
WMB group	Leptin 1	Pearson correlation	1	0.731**
		Probability *p*		0.000
		N	32	32
	BMI	Pearson correlation	0.731**	1
		Probability *p*	0.000	
		N	32	32
CMB group	Leptin 1	Pearson correlation	1	0.325
		Probability *p*		0.130
		N	23	23
	BMI	Pearson correlation	0.325	1
		Probability *p*	0.130	
		N	23	23

** Correlation is significant on level 0.01 (*p*).

On day 10, variables leptin 10 and BMI correlate (Table 4), $r = 0.430$, $p < 0.05$ in CG and variables leptin 10 and BMI correlate, $r = 0.668$, $p < 0.01$ in WMB group. Therefore, on day 10, there is a correlation also in CG, suggesting a positive effect of the balneal treatment on the leptin adjusting mechanisms, which at this moment are not fully clarified.

Table 4. Statistical correlations between leptin 10 and BMI

Lot			Leptin 10	BMI
Control group	Leptin 10	Pearson correlation	1	0.430*
		Probability *p*		0.040
		N	23	23
	BMI	Pearson correlation	0.430*	1
		Probability *p*	0.040	
		N	23	23
WMB group	Leptin 10	Pearson correlation	1	0.668**
		Probability *p*		0.000

		N	32	32
	BMI	Pearson correlation	0.668**	1
		Probability *p*	0.000	
		N	32	32
CMB group	Leptin 10	Pearson correlation	1	0.124
		Probability *p*		0.572
		N	23	23
	BMI	Pearson correlation	0.124	1
		Probability *p*	0.572	
		N	23	23

*. Correlation is significant on level 0.05 (*p*); ** correlation is significant on level 0.01 (*p*).

CONCLUSIONS

The plasmatic level variations of leptin show a different response depending on the balneal application type. Warm mud application leads to a significant decrease of the leptin level which translates into the remission of inflammation and rheumatic pain. In the case of the control group, we notice a major statistical remission that we could interpret as well as a remission of inflammation and rheumatic pain, even though these patients were not treated using natural methods. Cold mud application as a constant therapy leads to an increase of the leptin values at the end of the treatment, increase which is not statistically significant. We suppose that the lack of leptin variation in the case of the group that underwent constant therapy is due to the thermoregulation function that is highly strained during the cold application, while the thermal comfort during baths neutral from a thermal point of view stabilises the hypothalamus function and does not request major neuroendocrine answers. Recent data indicate that the hypothalamus is targeted by leptin actions, leptin which crosses the hematoencephalic barrier and interacts with the leptin receptor in the arcuate nucleus of the hypothalamus thereby controlling the thyroid-stimulating hormone, the melanocyte-stimulating hormone and gammaaminobutyric acid, which will eventually modulate the metabolic answers of the body. In this context, we can assume that not only leptin acts on the hypothalamus, but there's also a reverse relation: the hypothalamus could intervene in the adaption of the leptin level, this relation being linked to the hypothalamus mediated thermoregulation. In order to back this affirmation, additional studies are necessary.

There is a sure fact that there is a connection between the energy reserves of the organism (fat) and the control of the setting point (hypothalamus), in the way that the hypothalamus is targeted by leptin actions regarding food intake[17,18]. Following the

analysis of the results obtained for group CMB, where we notice a slight increase statistically insignificant of the leptin levels, we can assume that there is also a reverse relation: the hypothalamus could intervene in the adaption of the leptin level, this relation having a component from the area of the hypothalamus mediated thermoregulation. Backing this assumption, there is the reaction mode of CMB group which underwent the opposed therapy, stimulating the hypothalamus activity, but this hypothesis needs to be further proved by additional research.

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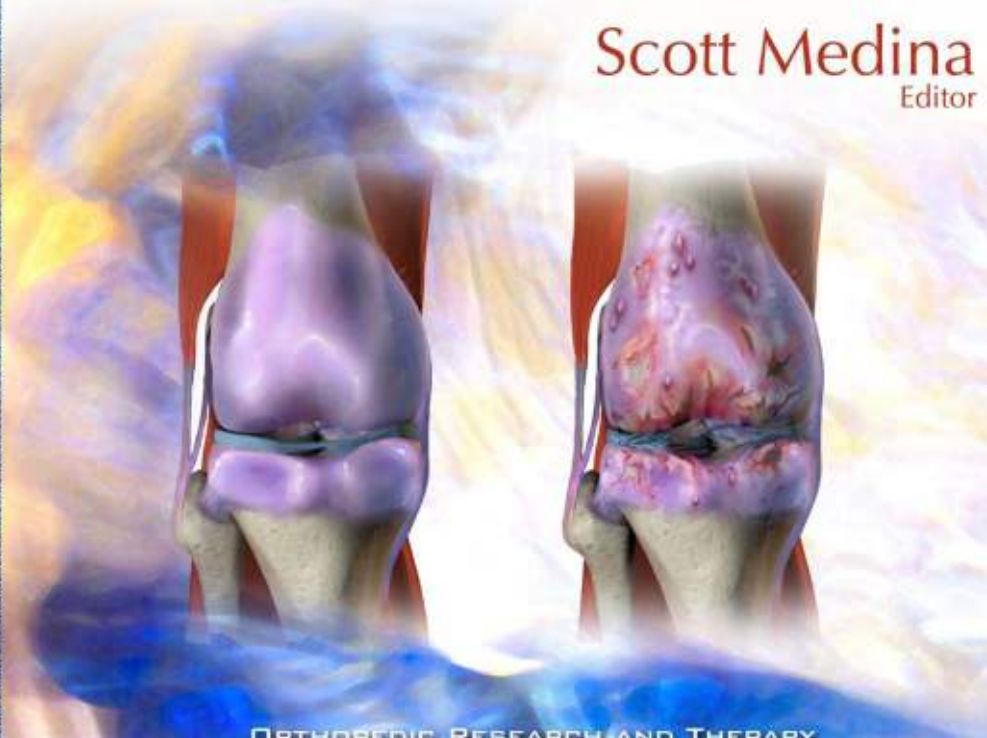
Knee Osteoarthritis

Diagnoses, Management and Health Effects

Scott Medina
Editor



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ORTHOPEDIC RESEARCH AND THERAPY

KNEE OSTEOARTHRITIS:

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A) SCOTT MEDINA
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B) CONTENTS

C) PREFACE

Osteoarthritis, the most common joint disease, causes appreciabledisability in a high percentage of older adults. Commonly attributed to progressive disruption of the articular cartilage and underlying bone structures constituting the joint, the condition can be extremely debilitating due to its widespread aversive consequences. Knee osteoarthritis (OA) is the most frequent localization of the arthrosis disease and the most common arthropathyof the knee, affecting over 37% of the population aged over 60 years and representing the main cause of pain and disability. In this book, Chapter One describes the most common less well documented psychological complicationsthat can prevail in knee osteoarthritis, their implications, and possible solutionsfor reducing the extent and severity of this disabling condition. Chapter Two reviews biological pathways in knee osteoarthritis. Chapter Three discusses ozone therapy for management of OA.

Chapter 1 - Knee osteoarthritis, a highly prevalent progressively disabling health condition affecting many older adults is often attributed to aging and biomechanical causes. This chapter describes the most common less well documented psychological complications that can prevail in knee osteoarthritis, their implications, and possible solutions for reducing the extent and severity of this disabling condition. To this end, the chapter describes the literature and research evidence concerning the prevalence of the psychological complications of depression and anxiety among this group, theirindependent and collective impact on the condition, and some possible solutions for minimizing the excessive burden attributable to psychological factors. Reported are data embedded in the relevant literature using PubMed, Scopus, Science Citation and Web of Science data bases over the last twenty

years, as well as data extracted from the records of 100 knee osteoarthritis patients that highlight the prevalence of depression and anxiety, and their effect on pain outcomes. Based on this evidence, the author highlights the importance of comprehensive efforts to identify and effectively treat people diagnosed as having knee osteoarthritis in a comprehensive manner. The author's insights and analysis should be of specific interest to clinicians working in this area who seek to apply their skills towards reducing the immense burden of knee osteoarthritis, while heightening their patients' life quality. The chapter specifically advocates for improved efforts to identify andtreat the psychological correlates of depression and anxiety that canaccompany knee osteoarthritis at all disease stages.

Chapter 2 - Based on the high prevalence of obesity worldwide and on the recognition of its pathogenic role on the induction of degenerative osteo-articular diseases, the authors propose a review chapter on the interrelation between them, and especially on the biochemical mechanisms by which obesity impacts, non-mechanically, on the development of the knee osteoarthritis (OA).

The last 10 years' research indicates that human osteoarthritis should be reconsidered as a systemic musculoskeletal disease rather than a focal articularone, while the adipokines may be the biomarkers responsible for the initiation and progression of osteoarthritis (OA).

Knee OA represents the most frequent location of osteoarthritis and the most common knee arthropathy, affecting more than 37% of the population over 60 years of age. It is characterized by the degradation of the articular cartilage, subchondral osteosclerosis, osteophytes formation, and synovial inflammation.

Obesity is considered a risk factor for OA, and it is accepted that it contributes to the development and progression of OA by increasing the mechanical loading of the joints. Aside from the mechanical role, the white adipose tissue (WAT), considered now as a huge endocrine organ, produces cytokines and other molecules, collectively known as adipokines, which appear to be responsible for the cartilage inflammation and degradation in OA associated with obesity. The adipokines are pleiotropic molecules that contribute to the so-called low-grade inflammatory status in obesity, forming acluster of metabolic disorders which lead to autoimmune and inflammatory diseases affecting the bone and the joint.

Recent studies have highlighted the role of adipokines in inflammatory diseases, autoimmune and rheumatic, illustrating the WAT role in the pathophysiology of obesity-related diseases. Advances have been made in

research on these molecules, focusing in particular on the role of leptin, adiponectin, resistin, visfatin, and chemerin, with more than 67 adipokines having so far been identified, and research continues. Even in 2015 were described other possible intra-articular adipokines: Serpin peptidase inhibitor, clade E member 2 (SERPINE2), WNT1 inducible signaling pathway protein 2 (WISP2), glycoprotein (transmembrane) nmb (GPNMB) and inter-alpha- trypsin inhibitor heavy chain family member 5 (ITIH5).

There is enough evidence that adipokines have relevant, multiple roles within the body, and a great deal of research effort has been dedicated to elucidating the complex network between WAT, metabolic disorders and inflammatory diseases. Although many issues are still unclear, the authors summarize the existing knowledge about the role of adipokines in OA pathophysiology.

Considering all of the abovementioned, it is clear that the main source of the pro-inflammatory adipokines is a dysfunctional WAT. Therefore, reducing the fat mass through diet control and increased physical activity remain an essential strategy to prevent and counteract the negative effects of obesity pro- inflammatory status.

Only further, additional research to clarify the pathophysiologic mechanisms of the adipokines and their concrete roles within the pathology of OA may propose new pharmacological approaches for this disease.

Chapter 3 - The approach to knee osteoarthritis here suggested the use of agaseous mixture of oxygen and ozone, aiming to integrate the spread therapy using hyaluronic acid, and possibly replacing the analgesic drugs (NSAIDs).

The purpose is improving symptoms and autonomy, in order to avoid or delay more invasive procedures.

Thanks to the various biochemical actions, if the therapy is used with proper concentrations of ozone in oxygen, this gas is applied following a therapeutic cycle of both intra-articular injections, to obtain analgesic and anti-inflammatory effects, and extra-articular, to promote the tropism of tendons and ligaments.

It is also recommended, more often in women, to treat the stasis in lymphatic and capillary vessels with local oxygen-ozone therapy and/or systemic therapy.

The therapy, if it is applied using a correct technique and with the right doses and concentrations, doesn’t present contraindications or side effects, andit can’t cause intolerance or allergies.

It actually aims to improve and, if possible, to solve the pain symptoms, making easier the rehabilitation exercises, and giving a better quality of life, with lower costs for the Health Care System.

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Chapter 1

D) KNEE OSTEOARTHRITIS AND ITS PSYCHOLOGICAL COMPLICATIONS: CONSEQUENCES, AND INTERVENTION

Ray Marks*

Department of Health and Behavior Studies, Program in Health Education, Columbia University, Teachers College, and School of Health and Professional Studies, Department of Health, Physical Education and Gerontological Studies and Services, City University of New York, York College, New York, US

ABSTRACT

Knee osteoarthritis, a highly prevalent progressively disabling health condition affecting many older adults is often attributed to aging and biomechanical causes. This chapter describes the most common less well documented psychological complications that can prevail in knee osteoarthritis, their implications, and possible solutions for reducing the extent and severity of this disabling condition. To this end, the chapter describes the literature and research evidence concerning the prevalence of the psychological complications of depression and anxiety among this group, their independent and collective impact on the condition, and somepossible solutions for minimizing the excessive burden attributable to

* Ray Marks: Department of Health and Behavior Studies, Box 114, 525W 120 St., New York NY 10027. E-mail: rm226@columbia.edu.

psychological factors. Reported are data embedded in the relevant literature using PubMed, Scopus, Science Citation and Web of Science data bases over the last twenty years, as well as data extracted from the records of 100 knee osteoarthritis patients that highlight the prevalence ofdepression and anxiety, and their effect on pain outcomes. Based on this evidence, the author highlights the importance of comprehensive effortsto identify and effectively treat people diagnosed as having knee osteoarthritis in a comprehensive manner. The author's insights and analysis should be of specific interest to clinicians working in this area who seek to apply their skills towards reducing the immense burden of knee osteoarthritis, while heightening their patients' life quality. The chapter specifically advocates for improved efforts to identify and treat the psychological correlates of depression and anxiety that can accompany knee osteoarthritis at all disease stages.

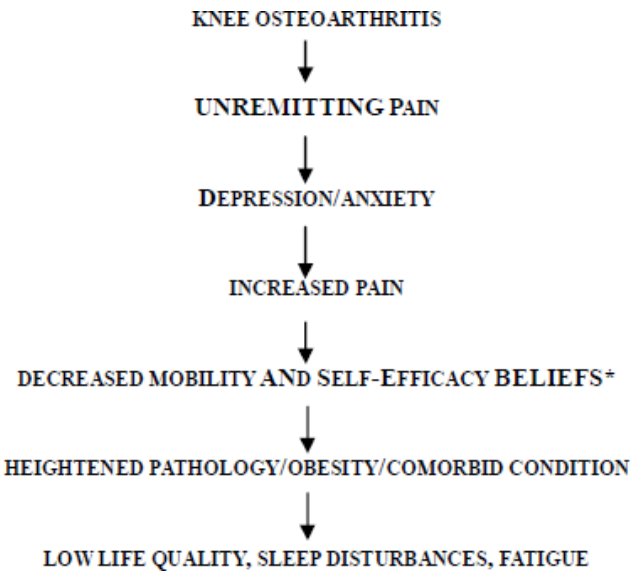
Keywords: anxiety, depression, disability, knee osteoarthritis, pain, treatment

INTRODUCTION

Osteoarthritis, the most common joint disease, causes appreciabledisability in a high percentage of older adults [1-5]. Commonly attributed to progressive disruption of the articular cartilage and underlying bone structures constituting the joint [6-8], the condition can be extremely debilitating due to its widespread aversive consequences [9, 10]. These include, but are not limited to varying degrees of unrelenting pain, joint stiffness and swelling, physical deconditioning, as well as multiple functional, social, occupational challenges and restrictions. Moreover, the disease is frequently accompanied by the presence of one or more debilitating chronic health conditions, such as cardiovascular diseases and obesity, as well as the presence of negative personal perceptions of low self-esteem and self-efficacy, plus heightened fear states, disfigurement, a decreased earnings capacity, and a low life quality or diminished ability to cope.

Unfortunately, despite its prevalence, and significant physical, economic, psychological, and social impact, there is presently no known cure for osteoarthritis. As well, treatments that do exist are also not always efficacious in restoring function and ameliorating pain, or may be contra indicated for a particular patient [5] as well as harmful to the joint tissues [7, 8], and may foster toxic side effects [11], and/or excess mortality due to their gastrointestinal effects. There is thus an urgent need to minimize the extent of the disability, and to seek interventions that can offset or delay predictable

consequences of excess morbidity and possibly of excess or premature mortality.
To this end, this chapter outlines some recent findings concerning knee osteoarthritis and the extent to which it may be detrimentally impacted by psychological factors, such as depression and anxiety. Some suggestions for treatment based on this information are included as well. The impact of depression and anxiety is specifically discussed due to their potential prevalence alone or in combination among patients with chronically painful disabling knee joint osteoarthritis and their potential for detection, prevention and/or remediation.
In addition, emerging evidence suggests it is increasingly important to assess psychological correlates of osteoarthritis because although the radiographs of most individuals seeking medical help for painful symptomatic osteoarthritis consistently exhibit some degree of bone damage and joint space narrowing consequent to progressive degeneration of the articular cartilage lining the joint, the degree of observable joint damage alone may be very minor and may implicate psychological factors [12]. In addition, age, and the presence of one or more chronic comorbid conditions makes it likely depression and/or anxiety will prevail in people with osteoarthritis as thedisease progresses, and will contribute negatively to the cycle of pain and disability, if untreated.
Indeed, based on many years of research in the realm of chronic pain and other chronically painful health conditions, it is proposed that where present, depression and/or anxiety can be expected to arise as a result of experiencing unrelenting pain, and this in turn, can significantly heighten the overall pain experience and resulting disability of the individual [13, 14].
In this respect, evidence from the chronic pain literature strongly suggestscentral nervous system influences of emotions and cognitions includingindividual behavioral characteristics [15] along with psychosocial factors [16],and increased sensitivity to pain signals by the brain [17] should not beignored as potent factors that can mediate or moderate osteoarthritis outcomes.In turn, it appears the experience of perpetual pain can provoke underlying tendencies towards depression, plus feelings of helplessness, poor copingability, and sleep disturbances commonly associated with depression [18].This negative series of feedback responses where depression, anxiety andcoping ability are significantly correlated with the osteoarthritic patient's painand disability levels [19-21] potentially produces a vicious cycle of excesspain and disability, plus varying degrees of negative affect [22], regardless of the prevailing degree of osteoarthritic damage [23] as outlined in Figure 1.



* Self efficacy issues may include a decreased degree of confidence:

1. To decrease pain substantively
2. To continue activity
3. To control pain to be able to sleep
4. To use of methods other than medication to relieve pain
5. To make large pain reduction with methods other than medication
6. To control fatigue
7. To be active without aggravating condition
8. To do something to help oneself feel better if feeling blue
9. To manage pain compared to others with arthritis
10. To manage symptoms so as to enjoy things of value
11. To deal with frustration of arthritis

Figure 1. Hypothetical pathway linking knee osteoarthritis pain and depression or anxiety to excess disease burden.

METHODS

To obtain relevant information on this topic, the Academic Search Complete Data Base and PubMed was used to examine articles of relevance to knee osteoarthritis and depression. Only those articles that addressed the current themes and referred to one of these topics was read and analyzed. No synthesis of the data was undertaken due to the variation in topics and

approaches used in the various studies. After the relevant articles or information on this topic were downloaded and reviewed, basic information pertaining to the role of depression and anxiety in knee osteoarthritis was extracted, and then the implications of the findings were examined. Emergent issues and themes of clinical significance were sought and are specifically discussed in this chapter. Finally some recommendations for clinicians are presented based on the results of the literature review.

RESULTS

The key words *depression and knee osteoarthritis* when entered into the Academic Search Complete data base yielded 83 contributions publishedbetween 2002 and 2016; there were 306 articles in PubMed from 1980-2016.When the terms *anxiety and knee osteoarthritis* were entered into PubMed 165articles listed. Only 27 articles were located using these key words inAcademic Search Complete. In both cases it was decided to examine these twokey correlates relative to pain, the complaint of most relevance to patients.This focus was also chosen because in the case of both data bases, so manydiverse topics were forthcoming when using the key words listed above asoutlined below, but few, other than pain, were pertinent to the presentdiscussion. See Box 1 and 2 for the themes embedded in the relevant literature.As depicted, when comparing Box 1 and Box 2, fewer topics prevail overall with respect to anxiety, compared to depression, and the depressionand anxiety topics that are discussed in the literature in both cases are verydiverse. Articles that discussed both depression and anxiety are less numerousand where reported, commonly refer to those health conditions in the contextof total knee joint arthroplasty surgery, mental health in general, sleep health,and quality of life issues. In the next section, the discussion focuses on theextent to which depression and anxiety may prevail in the context of kneeosteoarthritis, followed by the findings of research examining the relationshipof pain and these psychological symptoms. The implications of this information are also discussed.

Box 1. Key Topics Concerning Depression and Knee Osteoarthritis Covered in Major Data Bases

Association of body-mass index and depressed mood* Depression as assessed prior to joint replacement surgery*Depression as an explanatory factor for fatigue Depression and health services utilization
Depression as a predictor of gait speed Depression as a predictor of weight gain Depression as a predictor of weight loss Depression as a predictor of surgical outcomes
Gender differences in depression in knee osteoarthritis casesImpact of depression on function and perceived health Impact of depression on life quality

* Areas for treatment opportunities.

Box 2. Key Topics Concerning Anxiety and Knee Osteoarthritis Coveredin Major Data Bases

Anxiety and pain*
Anxiety and functional disabilityAnxiety and gait speed
Anxiety and quality of lifeImpact of anxiety
Improvements in anxiety after interventionMeasurement of anxiety

* Most common topic.

Prevalence of Depression

In comparison to the thousands of publications on knee osteoarthritis and functional disability and pain, the literature revealed very few studies have examined the possible presence of depression in this patient population specifically, even though depression is likely to be a common reactive symptom to a disabling condition. That is, the precise occurrence of depression among people suffering from knee osteoarthritis, while theoretically important, has been very sparsely documented. In addition, no method of assessment has been consistently applied, even where the condition has been sought, and even when the same instrument is used in different studies, or the same study, the published results vary considerably. For example, Gandhi et al. [24] estimated the occurrence of depression to be12.2% among individuals undergoing either hip or knee surgery forosteoarthritis, but that those with more affected joints had a 19% increased odds of reporting depression than those with fewer symptomatic joints. Moreover, those with six or more painful joints had a 2.5 fold odds of depression when compared to surgical patients with the same diagnosis, but only one affected joint. Although Ghandi et al. reviewed a cohort of both knee and hip osteoarthritis, Wong et al. [25] found 39% of a group of 115 knee osteoarthritis cases to have a psychiatric disorder, suggesting this is a serious condition with considerable potential for patients to exhibit depressive symptoms. The study was a valid one because not only was a recognized instrument employed, but interviews were held to supplement the survey data.

In other reports however, for example, that by Smith et al. [26], this group found only 8% of cases undergoing hip or knee arthroplasty to have a diagnosis of depression, even though das Nair et al. stated one third of cases with osteoarthritis have mood problems. More recently, Khatib et al. [27] who assessed psychological traits in patients waiting for total knee arthroplasty, found 26.8% reported psychological distress, but whether these were all related to depression or not was unclear. Data by Castano Carou et al. [28] do similarly support the idea that depression may be an important comorbidcondition to examine in the osteoarthritis patient since this group found 24.5% of patients with osteoarthritis in primary care had comorbid depression or anxiety. But the specific prevalence of depression among cases with knee osteoarthritis alone was not documented.

The presence of depression may also vary dependent on the sample studied. For example among cases of osteoarthritis with severe disease, including knee osteoarthritis, Howard et al. [29] found 38% of indigent

patients were distressed psychologically before surgery. Psychological distress, possibly including depression can also occur following kneereplacement surgery as outlined by Creamens-Smith et al. [30] even though the diseased joint has been removed and pain would be expected to have abated. This group found up to 20% of 110 patients reported noteworthy levelsof distress, although whether this was specifically related to being in a depressed state was not documented. Tonelli et al. [31] did find depression rates of approximately 15% in men and women prior to total knee replacement, and for a mixed group of knee and hip osteoarthritis post-surgicalcases, and mild-to moderate depression was observed by Smith et al. to accompany the knee osteoarthritis condition [26]. A 22% prevalence ratebefore surgery was identified by Duivenvoorden et al. [34] suggesting depression may be an important correlate to examine both in day to day primary care practices as well as before and after knee joint replacement surgery.

Other data show high rates of depressive symptoms in cases of knee osteoarthritis of moderate severity [33], where 52 of 100 cases met criteria for depression, and of these 52 cases, 27 had moderate or severe signs of depression. Individuals younger than 60 years of age who had a higher prevalence of unilateral rather than bilateral knee osteoarthritis, reported higher mean pain scores as well as depression scores than those over age 60. Males with knee osteoarthritis reported higher levels of depression than females. Based on the observed correlations between the depressive symptom scores and key selected variables, there was a significant relationship between the pain, function, and self-efficacy scores and the depressive scores, suggesting a link between these psychological constructs that has implications for intervention as well as for understanding the nature of this health condition. The rates of depression may also be higher than those reported because often the symptoms go unexamined. For example, rates of 65% were reported to occur in patients with knee osteoarthritis examined by Aflaki et al. [61].

In sum, the rate of depressive symptoms that can accompany knee osteoarthritis may vary from 8-65% based on current literature. The lack of uniformity in the results of a search to discern the expected prevalence of depression in this cohort is likely to reflect:

1. Testing approaches
2. Population studied
3. Disease severity of cohort

4. Disease duration
5. Age of sample
6. Gender of sample
7. Social factors
8. Overall health status

Prevalence of Anxiety

Among reports where the attribute of anxiety has been examined in the context of knee osteoarthritis, Axford et al. [36] found that among 54 patients with osteoarthritis, the majority of whom were women, the prevalence of clinically significant anxiety and/or depression was 40.7%. Duivenvoorden et al. [34] found an anxiety prevalence of 20.3% for those awaiting 133 knee osteoarthritis replacement surgery. High rates of anxiety were also noted by Ferreira et al. [35] among patients with knee osteoarthritis in a comparative study. Tonelli et al. [31] found that women had similar degrees of anxiety before total knee joint replacement, regardless of whether trait or state anxiety was assessed. This was slightly higher than that recorded for both measures by men undergoing similar surgery. As well, pain during movement was predicted in part by state anxiety. Ellis [37] who examined 154 indigent knee arthroplasty surgery cases found 11% has presurgical anxiety, and anxious patients reported lower patient perceived outcome scores at 1 year. Jacobs et al. [38] who followed 1020 knee arthroplasty surgery cases for 2 years found 41.9% of less severe cases, reported anxiety, and were more dissatisfied than more severe cases.

Thus, as with depression, anxiety may be quite common among knee osteoarthritis cases, regardless of the disease status, and severity. These rates that vary from 11-40% are important to acknowledge in their own right given the linkage between anxiety and pain, function, and surgical outcomes.

Depression, Anxiety, and Pain

Pain, the symptom of most concern to knee osteoarthritis patients, can both mask depression, exacerbate depression, or be heightened by depression and/or anxiety, thus intensifying the pain experience [39]. This is an important point to note, given that Ghandi et al. [24] found those subjects reporting 6 or more painful joints including the hips and knees had a 2.5 fold or greater odds

of depression when compared to those whose symptoms were limited to a single surgical joint. Moreover, Holla et al. [40] found that among patientswith knee osteoarthritis, depression was independently associated with knee pain and activity limitations.

Axford et al. [36] too found that greater pain was associated with greater depression in cases with knee osteoarthritis and advocated for the treatment of both of these conditions among this patient group. This recommendation appears salient given that Hirshmann et al. [41] found individuals with higher levels of psychological distress to be at higher risk for experiencing more pain and more disabling disease status before and after knee surgery than those withno such problems.

Depression also appears to interfere with sleep as well as contributing to the osteoarthritis pain cycle [42], while Parmalee et al. [43] found depressed mood rather than anxiety, or somatic symptoms correlated with both pain and disability, and for a mixed group of Veterans with either hip or knee osteoarthritis Zullig et al. [44] found depressive symptoms were associated with worse pain.

Valdes et al. [45] found one risk factor for pain post surgery among cases undergoing total knee joint replacement was the presence of depression, among other factors. Similarly, Riddle et al. [46] found depressive symptoms were highly predictive of worsening function over a 2-year period in thosewith knee pain. Murphy et al. [47] suggested there are subgroups or clusters of differing comorbid symptoms in older adults with osteoarthritis, and that Cluster 1, in their research included patients with high pain levels, and mood disturbances. Cluster 3 had low levels of pain, and depression. Although van der Esch et al. [48] found depressive mood was only one of five cluster types among cases with knee osteoarthritis, Wylde et al. [49] found that the presenceof severe persistent pain at 3 or 4 years after total knee replacement was significantly related to major depression, as well as number of pain problemsat other body sites. Bierke et al. [2016] similarly found depressive symptoms negatively affected the course of pain one year after uncomplicated total knee replacement surgery, whereby more depressed patients had higher pain scores pre and post operatively, as did anxious patients, but this latter association wasnot significant [41]. Salaffi et al. [51] who examined the relationship between disability and psychological variables of anxiety and depression among 61 women with symptomatic knee osteoarthritis using an anxiety inventory, and apain index found pain correlated with the degree of psychological involvement.

Likewise, Smith and Zautra [52] who examined the effects of anxiety on pain in women with osteoarthritis and rheumatoid arthritis found anxiety was related to elevations in current and forthcoming pain estimates, suggesting anxiety is important in explaining the progression of the disease. In particular, when studying the differential effects of depression and anxiety on pain, it wasnoted that the effects were nearly twice as large for anxiety compared to depression, suggesting the mechanism of action is unique.

These data were further substantiated by Axelrod et al. [36] who examinedthe interrelationships between the severity of osteoarthritis, pain, disability, anxiety and depression, using the Hospital Anxiety and Depression Scale(HADS), along with a structured clinical interview. Among the 54 patients studied, this group found pain correlated with the prevailing anxiety and depression scores and disability was greater in patients with a combined depression and anxiety history. More recently Sinikallio et al. [53], who examined cases with knee osteoarthritis found elevated anxiety was associated with pain, and that the osteoarthritis cases reported high levels of anxiety. Wong et al. [25] also found a high prevalence of psychiatric morbidity, including anxiety among cases with knee osteoarthritis visiting an orthopedic clinic. Earlier, Somers et al. [54] noted osteoarthritis cases with higher levels of depression tend to be more prone to report increased osteoarthritis pain.

Most of the above mentioned data concur with findings of Perrot et al.

[55] who observed many patients with either hip or knee osteoarthritis hadmild anxiety as recorded using the HADS, and Zautra and Smith who found elevations in depression correlated with pain elevations among cases with osteoarthritis [56], as did Dekker et al. [57] who found pain and anxiety were correlated among people with osteoarthritis, and Ozcetin et al. [58] who found life quality of cases with osteoarthritis was highly impacted by depression as well as anxiety. In addition, the presence of depressive symptoms can impair the ability to detect the origin of knee pain complaints [59], as well as effortsto optimize the status of the osteoarthritis patient and to do this effectively [60]. Moreover, once can expect worse knee osteoarthritis outcomes if depression is untreated given the finding that depression is associated with elevations in current, as well as anticipated pain in the next week [62].

Unsurprisingly, Van Baar et al. [63] found that regardless of the importance of systemic or mechanical mechanisms affecting osteoarthritic pain, psychosocial factors, including depression predicted the extent of the disease impacts more strongly than the apparent extent of visible joint damage.In addition, Summers et al. [19] found that even after controlling for disease severity, psychological variables including depression, remained a strong

predictor of individual differences in pain among adults with knee osteoarthritis, a finding supported by Salaffi et al. [51], Okma-Keulen and Hopman-Rock [64] and Bookwala et al. [65]. Studies examining the influence of anxiety on the pain experienced by adults with knee osteoarthritis have similarly found a positive correlation, regardless of study design, sample, or anxiety instrument used, for example Summers et al. [19] and Salaffi et al. [51].
Consequently the literature reveals:

- A high percentage of adults with mild to moderate knee osteoarthritis, as well as those undergoing knee replacement joint surgery, are likely to present with depressive symptoms, as well as anxiety, or both, if examined carefully.
- The magnitude of the depressive symptom scores and/or anxiety scores appears to be associated with pain, functional mobility, and self-efficacy for managing pain and other symptoms in a dose dependent manner, regardless of disease status or sample studied.
- Failure to treat depression and/or comorbid anxiety effectively may heighten the disability experienced by the knee osteoarthritis patient.

Treatment Approaches

There are two basic treatment categories employed to ameliorate knee osteoarthritis pain, and improve functional ability, including work capacity. These are non-surgical approaches including pharmaceutical interventions, physical and occupational therapy, and counseling, and when one or more of these fail, surgery or intra articular injection or both may be used. In this regard, assessment of the knee osteoarthritis patient's psychological status is essential, regardless of disease status in our view. After that, appropriately designed and thoughtful tailored strategies designed to minimize psychologicaldistress among patients with knee osteoarthritis are advocated. These may include, but are not limited to:

- Acceptance therapy
- Cognitive behavioral therapy
- Coping skills training
- Counseling

- Education
- Fostering pain skills
- Group therapy
- Heightening self-efficacy for pain control
- Medications or pharmacotherapy to control pain and depression
- Meditation
- Mindfulness training
- Music therapy
- Pre-operative counseling
- Psychotherapy
- Social and emotional support
- Stress management and control
- Yoga

CONCLUSION

As outlined above, knee osteoarthritis, a chronic degenerative joint disease of unknown cause produces high degrees of pain and disability among older adults. Among the specific disease consequences evidence reveals:

- Psychological correlates of depression and anxiety are highly implicated in the pain disability cycle [28], yet are often overlooked as potent predictors of prevailing and future symptoms and outcomes in patients with knee osteoarthritis [25].
- Conversely, treatment of depression and anxiety is predicted to improve treatment outcomes, especially pain.
- Treatment may also improve adherence to recommended intervention strategies, such as exercise.
- Failure to acknowledge the presence of psychological distress among cases with knee osteoarthritis is likely however, to produce a less than optimal outcome, regardless of intervention and disease status.
- More work that examines both these correlates and how they contribute to the pain cycle in well-defined knee osteoarthritis samples using consistent validated measures and approaches is likely to impact favorably on the burden of the disease.

In the interim, it is the author's view that the psychological wellbeing of the knee osteoarthritis patient should be assessed regularly using a consistent approach as this is likely to have a strong bearing on the pain experience, as well as on the disease process, including its management, and outcomes.

Providing carefully construed tailored treatments based on careful periodic examination of the individual patient in line with disease presentation, is hence strongly advocated. This is especially so because even though depression and anxiety are frequently neglected in the realm of clinical preventive strategies against osteoarthritis disease progression, those who are treated appropriately are likely to experience significantly lower degrees of pain and disability than those with undiagnosed depressive or anxiety symptoms. Advocated are appropriately designed and targeted cognitive and behavioural therapies, including self-efficacy enhancing strategies, social support, and pain reduction therapies.

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Chapter 2

E) ADIPOKINES: BIOLOGICAL PATHWAYS IN KNEE OSTEOARTHRITIS?

Elena Valentina Ionescu[1,2], Săndica Albină[3] and Mădălina Gabriela Iliescu[2,3,*]

[1]Balneal and Rehabilitation Sanatorium Techirghiol, Romania
[2]Faculty of Medicine, "Ovidius" University of Constanţa, Romania
[3]Rehabilitation Clinical Hospital Eforie Nord, Romania

Motto: "We do not receive wisdom. We must discover it for ourselves,after a journey which no one else can make for us."

Marcel Proust

ABBREVIATIONS LIST

apM1: adiponectin Acrp30:
adiponectin AdipoQ:
adiponectin
AdipoR1: adiponectin receptor 1
AdipoR2: adiponectin receptor 2
ADAMTS: a disintegrin and metalloproteinase with thrombospondin-like motifs

* Corresponding author: Mădălina Gabriela Iliescu, iliescumadalina@gmail.com.

ADSF: adipocyte-secreted factorALP:
alkaline phosphatase
AMPK: AMP-activated protein kinaseARC:
hypothalamic arcuate nucleus BMI: body mass
index
BMR: basal metabolic rateCRP:
C-reactive protein
eNOS: endothelial NO synthase
ERK: extracellular signal-regulated kinaseFDA:
Food and Drug Administration FIZZ3: found in
inflammatory zone 3 FGF: fibroblast growth
factor
GBP28: adiponectin
GPNMB: glycoprotein (transmembrane) nmbGRO:
growth-related oncogene
IFP: infrapatellar fatIL:
interleukin
ITIH5: inter-alpha-trypsin inhibitor heavy chain family, member 5IGF-1:
insulin-like growth factor I
iNOS: inducible nitric oxide synthase
ITIH5: inter-alpha-trypsin inhibitor heavy chain family, member 5JAK:
janus kinase
JNK: C-Jun N-terminal kinase
LRb: long form of the leptin receptor
MAPK p38: the p38 mitogen-activated protein kinaseMCP-1:
monocyte chemotactic protein-1
MEK1: mitogen-activated protein kinase 1MetS:
metabolic syndrome
MMP: matrix metalloproteinasesmRNA:
messenger RNA
MAPK: mitogen-activated protein kinases NAMPT:
nicotinamide phosphoribosyl transferaseNF-kβ: nuclear
factor-κB
NO: nitric oxide
NOS: inducible nitric oxide synthaseOA:
osteoarthritis
Ob: obesity geneOC:
osteocalcin
OPG: osteoprotegerin

Ox-LDL: oxidized low-density lipoproteinPBEF:
pre-B cell colony enhancing factor PI3K:
phosphatidylinositol 3-Kinases
PPAR: peroxisome proliferator activated receptor
RANKL: receptor activator of nuclear factor kappa-B ligandRBP4:
retinol binding protein 4
RELMs: resistin-like molecules
REMS: Risk Evaluation and Mitigation StrategyRF:
rheumatoid factor
RNA: ribonucleic acid
ROS: reactive oxygen species
sCOMP: serum cartilage oligomeric proteinSAA3:
serum amyloid A 3
SERPINE2: serpin peptidase inhibitorSF:
synovial fluid
SNS: sympathetic nervous system SOCS:
suppressor of cytokine signaling
STAT: signal transducer and activator of transcriptionTYK2:
tyrosine kinase 2
TGF – β1: transforming growth factor β1TIMP:
tissue inhibitors of MMPs
TNF: tumor necrosis factor
TRH: thyrotropin-releasing hormone
uCTX-II: urinary C-telopeptide of type II collagenVCAM:
vascular cell adhesion molecule
VAS: visual analogue scale
WISP2: WNT-inducible secreted protein 2

ABSTRACT

Based on the high prevalence of obesity worldwide and on the recognition of its pathogenic role on the induction of degenerative osteo- articular diseases, the authors propose a review chapter on the interrelation between them, and especially on the biochemical mechanisms by which obesity impacts, non-mechanically, on thedevelopment of the knee osteoarthritis (OA).

The last 10 years' research indicates that human osteoarthritis should be reconsidered as a systemic musculoskeletal disease rather than a focal

articular one, while the adipokines may be the biomarkers responsible for the initiation and progression of osteoarthritis (OA).

Knee OA represents the most frequent location of osteoarthritis and the most common knee arthropathy, affecting more than 37% of the population over 60 years of age. It is characterized by the degradation of the articular cartilage, subchondral osteosclerosis, osteophytes formation, and synovial inflammation.

Obesity is considered a risk factor for OA, and it is accepted that it contributes to the development and progression of OA by increasing the mechanical loading of the joints. Aside from the mechanical role, the white adipose tissue (WAT), considered now as a huge endocrine organ, produces cytokines and other molecules, collectively known as adipokines, which appear to be responsible for the cartilage inflammation and degradation in OA associated with obesity. The adipokines are pleiotropic molecules that contribute to the so-called low-grade inflammatory status in obesity, forming a cluster of metabolic disorders which lead to autoimmune and inflammatory diseases affecting the bone and the joint.

Recent studies have highlighted the role of adipokines in inflammatory diseases, autoimmune and rheumatic, illustrating the WAT role in the pathophysiology of obesity-related diseases. Advances have been made in research on these molecules, focusing in particular on the role of leptin, adiponectin, resistin, visfatin, and chemerin, with morethan 67 adipokines having so far been identified, and research continues. Even in 2015 were described other possible intra-articular adipokines: Serpin peptidase inhibitor, clade E member 2 (SERPINE2), WNT1 inducible signaling pathway protein 2 (WISP2), glycoprotein (transmembrane) nmb (GPNMB) and inter-alpha-trypsin inhibitor heavy chain family member 5 (ITIH5). There is enough evidence that adipokines have relevant, multiple roles within the body, and a great deal of research effort has been dedicated to elucidating the complex network between WAT, metabolic disorders and inflammatory diseases. Although many issues are still unclear, we summarize the existing knowledge about the role of adipokines in OA pathophysiology.

Considering all of the abovementioned, it is clear that the mainsource of the pro-inflammatory adipokines is a dysfunctional WAT. Therefore, reducing the fat mass through diet control and increased physical activity remain an essential strategy to prevent and counteract the negative effects of obesity pro-inflammatory status.

Only further, additional research to clarify the pathophysiologic mechanisms of the adipokines and their concrete roles within the pathology of OA may propose new pharmacological approaches for this disease.

Keywords: adipokines, osteoarthritis, white adipose tissue, pathophysiology

INTRODUCTION

The introduction of a new concept of white adipose tissue as an extremely dynamic organ with complex metabolic role in the human organism poses newchallenges for scientific progress in medicine regarding understanding of osteoarthritis pathogenic mechanisms.

The subject approached in the present paper is situated at the intersection of the study objects of various disciplines: physiology, immunology, genetics, and rheumatology. Scientific progress in medicine grew out of the classicwork phase in which one used mostly the optical and electron microscope. Thestudy of molecular and cellular structure is now the prerequisite of cellular biology, genetics and immunology, fields that study the biogenesis of cellular organelles, their macromolecular composition, and the genetic control of syntheses that various structures need.

Obesity is considered a risk factor for osteoarthritis, and the fact that obesity contributes to the development and progress of osteoarthritis, through the increase of mechanical strain on joints, is now accepted. Recent progress inthe physiology of white adipose tissue has shown that fat cells produce a seriesof factors, called adipokines, which have a critical role in the development of osteoarthritis, besides the mechanical effects. Osteoarthritis is a multifactorial degenerative joint disease which affects to a great extent the joint cartilage, modifies the subchondral bone, forms osteophytes, and leads to inflammation of the synovium. Despite being the most common rheumatic disease, its etiology remains mostly unknown. Age, obesity, sex, and anterior lesions are considered to be significant risk factors. Although osteoarthritis is frequently described as noninflammatory, inflammation contributes to its symptoms and evolution. The theory according to which white adipose tissue is an important factor with implications in the homeostasis of the whole organism has gained credibility since 1994, when the first adipokine, leptin, was discovered. Since then, over 70 adipocytokines have been identified (adiponectin, resistin, visfatin, chemerin, lipocalin, etc.), but their role is far from being understood according to evidence-based medicine, recent research evolving from the pointof view of their implication on osteoarthritis pathogenesis.

In order to write the present paper and outline the present stage of knowledge in the field, we studied, up to the beginning of April 2016, all

scientific references accessible to us from the international databases, usingspecific keywords: adipokines, leptin, adiponectin, osteoarthritis, obesity, etc.

OSTEOARTHRITIS AND OBESITY

Knee osteoarthritis (OA) is the most frequent localization of the arthrosis disease and the most common arthropathy of the knee, affecting over 37% of the population aged over 60 years and representing the main cause of pain and disability. Prevalence is higher in women than men (42.1% vs 31.2%) [1]. We can say that OA is a real public health problem because it is associated with a significant decrease of productivity and generates increased expenses for medical care, representing 97% of the total knee replacements and 83% of the total hip replacements in 2004 [2].

Obesity has long been known as a risk factor for OA, especially OA of theknee, even though the definition of obesity based on a body mass index (BMI) higher than 30 kg/m2, was not adopted on a large scale until 1990. 1988 saw the publication of the results of the Framingam study, a cohort study on 1420 subjects which whether obesity had preceded OA of the knee, and whether it had been a possible cause. The results showed that obesity, either alone or in combination with other hitherto unknown factors, can cause OA of the knee [3].

Obesity is associated with high risks of developing a series of chronic diseases, including OA. The biomechanical overstrain effect on the cartilage can partially explain the high risk of knee OA in the case of overweight people. Nevertheless, risk factors for the onset and progression of OA on jointsthat don't bear any weight, like the hands, have been proven to be linked to BMI. In fact, recent studies show that obesity, rather than simply excess body mass, affects the knee joint, and suggest that metabolic factors associated with obesity can contribute to OA. Fat mass is associated with the loss of knee cartilage volume, and adipose tissue reduction is more strongly connected to the symptomatic amelioration of knee OA rather than to the loss of body weight [4-6].

Moreover, Wang and et al. proved that the risk of primary prosthesis ofthe knee and hip for OA increases in direct proportion to the adipose mass, as well as central adiposity [7]. The actual belief is that obesity is an important risk factor not only because of the mechanical effect of the fat excess, but also because of the variety of substances that white adipose tissue (WAT) secretes [8]. The discovery of leptin in 1994 by Zhang introduces a new concept of

WAT as a highly dynamic organ which releases a multitude of immune and inflammatory mediators, like adipokines and cytokines, which are involved in many pathologies [9].

In addition to the central role of depositing lipids, WAT is now known asa multifactorial organ. It has an important endocrine role of secreting various hormones, including adipokines, together with a vast range of signal-proteins and other factors. These adipose-derived peptides are collectively known as "adipokines." It is important to underline the fact that these factors could also be synthesized in other tissues aside from WAT, and they have the capacity to participate in other pertinent functions correlated with the homeostasis ofenergy and of metabolism [10].

Adipokines perform potent modulatory actions on target tissues affected by rheumatic disease: cartilage, synovium, bone, and immune system cells.

Obesity generates a disorder of both immune system and adipose tissue- derived factors. WAT secretes more adipokines, including leptin, resistin,vaspin, visfatin, hepcidin, adiponectin, RBP4 (retinol binding protein 4), and inflammatory cytokines, which act on a local level (autocrine / paracrine) and systemically (endocrine) in order to regulate sensitivity to insulin, immune response, cardio vascular function, and many physiological processes [11].

Functionally, adipokines are integrated in a communication network with other tissues and organs, like the cerebral cortex and autonomic nervous system, the sympathetic component, the somatic structures of the body, with participation in increasing appetite and energy balance, immunity, insulin sensitivity, angiogenesis, modulation of blood pressure, lipid metabolism and hemostasis.

Research in the last decade shows that human OA should be reconsidered as a systemic musculoskeletal disorder rather than a focal joint disease, and adipokines would be the biomarkers responsible for the inflammation and degradation of joint cartilage in OA associated to obesity [12], contributing to the so-called obesity low-grade inflammatory state, generating a series of metabolic disorders which lead to autoimmune diseases and inflammatorydisorders that affect the bone and joint [13].

The low-grade inflammatory state in OA is characterized by the release of inflammatory mediators in the blood in the case of metabolic syndrome (Mets) (obesity, insulin resistance, anomalies of lipids, hypertension) or in the case of ageing (secretory senescence).

These inflammatory mediators are harmful to joint tissues, therefore initiating and/or contributing to perpetuation of OA process. Once activated, the cells of OA joints release inflammatory mediators in the joint, and

eventually in the blood (Figure 1). Mediators amplify the low-grade inflammatory state which could cause or accelerate chronic diseases sensitive to this kind of state [14].

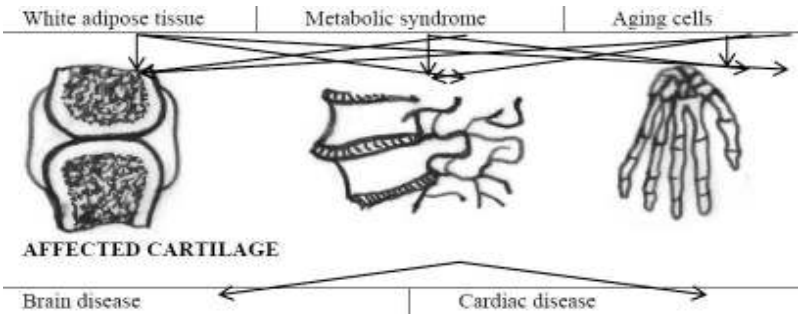

Figure 1. Potential systemic consequences of OA-derived inflammatory mediators.

Moreover, studies have shown the association of OA with somecomponents of MetS, such as hypertension or type 2 diabetes, independentlyof obesity and other known risk factors of OA. Evidence shows a harmful effect of lipid and carbohydrates anomalies on joint cartilage homeostasis. Low-grade inflammation is a common trait of OA and metabolic disorders,and can contribute to both geneses. Therefore, OA is a disease that has various phenotypes, including a metabolic phenotype, in addition to those linked toage [15].

OSTEOARTHRITIS AND ADIPOKINES

Adipokines, a variety of proinflammatory peptides, are a constantly growing family of factors produced by WAT which carry out pleiotropic functions in various ways and are involved in a broad range of activities, not only in the glucose and lipid metabolism but also in the modulation of inflammatory and immune response [16]. Therefore, some adipokines can be considered potential basic factors regarding obesity, inflammation and OA, a link between these three entities.

Initially limited to metabolic activities, adipokines represent a new family of compounds that can now be considered “key-actors” in the complex network of soluble mediators involved in the physiopathology of rheumatic diseases. It has been reported that obesity increases OA incidence, especially

on the level of weight bearing joints such as, for instance, the knees, while weight loss is correlated with the slow progression of OA. One notable hypothesis is that obesity increases mechanical strain in the joint cartilage, which leads to its degradation. Nevertheless, obesity is also associated with OA, in non weight-bearing joints like finger joints, which suggests the non- mechanical link between obesity and OA [17].

Recent studies underline the role of adipokines in inflammatory, autoimmune and rheumatic diseases, proving the role of WAT in the physiopathology of diseases associated with obesity. Progress has been madein researching these molecules, based mostly on the role of leptin, adiponectin,resistin, visfatin, and chemerin, with over 70 adipokines having hitherto been identified, and research still ongoing. In 2015, other possible adipokines were described as intra-articulary: Serpin peptidase inhibitor, clade E member 2 (SERPINE2), WNT1 inducible signaling pathway protein 2 (WISP2), glycoprotein (transmembrane) nmb (GPNMB) and inter-alpha-trypsin inhibitor heavy chain family member 5 (ITIH5) [18].

Most adipokines' production is high in the case of obesity, with the exception of adiponectin. Plasmatic levels of adipokines depend on sex, being higher in women than men, even after the adjustment of BMI. This could contribute to the high OA prevalence in women. In knee OA, adipokines are produced by infrapatellar fat, synovial fat, chondrocytes, osteoblasts, and osteoclasts. [19].

OA adipokines can be directly involved in the degradation of the joint orin the control of the inflammation. The four important adipokines (leptin, adiponectin, resistin and visfatin) have been detected in the synovial fluid (SF)of the knee OA. Therefore, the high level of adipokines both systemically (plasma) and locally (SF) is correlated with the degeneration of joint cartilage and synovial inflammation [19]. The information regarding the relation between the four adipokines and OA can be found in Table 1.

In OA, even though frequently described as noninflammatory, inflammation contributes to its symptoms and evolution [20]. Supporting this assertion is a study carried out on 845 women suffering from early knee OA, with slightly increased, but significant values of C-reactive protein (CRP) who were radiologically reassessed in four years when it was discovered that high values of CRP can predict the evolution of knee OA, suggesting that low- grade inflammation plays a major role in the evolution of the disease [21].

Table 1. Relationship of adipokines with OA

Adipokines	Leptin	Adiponectin	Visfatin	Resistin
Association with BMI	Positive	negative	positive	unclear
Plasma levels between genders	women > men	women > men	unclear	women > men
Plasma levels between groups	OA > control	control > OA	OA > control	OA > control
Levels in OA patients	SF > plasma	plasma > SF	SF > plasma	plasma > SF

BMI: Body mass index; OA: Osteoarthritis; SF: Synovial fluid. (Thitiya Poonpet and Sittisak Honsawek. *Adipokines: Biomarkers for osteoarthritis?* World J Orthop. 2014 Jul 18; 5(3): 319-327)

Inflammation can be the main event of OA or a secondary one in the case of other aspects of the disease, like, for instance, the biochemical modifications inside the cartilage. Obesity is also considered as a "weight" risk in OApathogenesis and its contribution can be due to different convergentmechanisms that ultimately lead to cartilage destruction, studies showing that obesity is positively correlated with OA, irrespective of its localization [3, 22].In addition, studies have shown that obesity is also a risk factor for non weight-bearing joints [17, 23]. Obesity is strongly linked to the release by WAT of a series of factors, calling into question whether OA can be considered a metabolic disease [15].

Many of the pro-inflammatory mediators and proteases attributed to a senescence-associated secretory phenotype in fibroblasts, such as Interluekin- 1β (IL-1β), IL-6, IL-8, matrix metalloproteinase 3 (MMP-3), and MMP-13, arecommonly increased in osteoarthritic tissues. These pro-inflammatory mediators, along with nitric oxide (NO) and prostaglandin E2, promote an imbalance in the synthesis and degradation of the cartilage extracellular matrixwhich eventually leads to cartilage degradation [24].

It is certain that adipokines act in knee OA on the infrapatellar fat (IFP), synovium, chondrocytes, osteoblasts, and osteoclasts. A recent study conducted on bovines launches the hypothesis that the meniscus is more susceptible to the catabolism determined by adipokines compared to the joint cartilage.

LEPTIN

Leptin is a 16 kDa non-glycolized hormone, which acts biologically through the activation of the isoenzyme of the long form of the receptor or (LRb) and falls under Class 1 of the superfamily of cytokines receptors [20]. Itis mainly produced by adipocytes, but also by the placenta, where secretion is modulated by estradiol [26] and by the stomach, where it is first released and then absorbed [27, 28]. Big adipose cells produce more leptin than small ones; serum concentrations of leptin are strongly correlated with the content of body fat in the case of new born children and adults, and this predicts the weight at birth [29].

Leptin Production in Humans

We can say that leptin regulates weight on a central level and represents a link between nutrition status, neuroendocrine system, immune function and OA.

Leptin was initially known for its role as a hypothalamic modulator of food intake and body weight, crossing the hematoencephalic barrier. Its circulating levels are correlated with WAT mass and act on the level of the hypothalamus, inducing a reduction of food intake and an increase of energy metabolism [30].

There is a diurnal rhythm of leptin serum concentration, the values being 20-40 percent higher in the middle of the night than during the daytime [31], and the maximum shifts parallel to the changes in meal synchronization. Leptin RNA messenger and its secretion by the adipocytes rapidly decrease during hunger. These processes are stimulated by insulin, glucocorticoids and tumor necrosis factor (TNF-α), another product of adipocytes. These observations suggest that leptin sends signals to the brain about the quantity of fat [30].

Leptin serum concentration indicates the quantity of adipose tissue in the body. Even though fat deposits on the fetus rise significantly during the third trimester, leptin's role during pregnancy has not been yet clarified, but pregnant women have higher leptin serum concentration than non-pregnant women [32]. Leptin levels change in the first days after birth [33, 34]. Leptin serum concentrations are similar in the case of white children and children of color, with a similar organism composition [35]. Lactation can reduce the risk

of obesity of the child [36] and leptin could have a possible role in this, in the way it is produced in the breast and present in the milk [37].

Leptin serum concentrations increase during childhood, with the higher concentrations in the case of children who gain the most weight; high leptin serum concentrations are associated with premature puberty [38]. The possibleimportance of leptin in this context is shown by the observation that mice and rats with leptin deficit fail to enter puberty, while administration of leptin induces the onset of puberty [39].

In adults, leptin serum concentrations increase with progressive obesity. Concentrations are higher in women than men, for any degree of obesity [40, 41] and they decrease with age, both in women and men [42]. Concentrations are similar in healthy subjects and type 2 diabetes patients with the same weight [43].

Leptin serum level is associated with metabolic syndrome. Moreover,leptin strongly correlates with the hip circumference and insulin sensitivity [44].

Plasma leptin is correlated with blood pressure level both in the case of patients with normal blood pressure [45] and hypertensive patients [46, 47]. Quarcioli et al. observed that weight loss, and therefore leptin serum level decrease, do not correlate with the improvement of coronary circulation [48].

LEPTIN AND OSTEOARTHRITIS

Leptin has the ability to intervene in inflammation development,especially the one responsible for OA, in the context of obesity co-existence. Obesity is almost constantly associated with modifications marked in the secretory function of adipocytes and macrophages, recognizing through them the presence of inflammation like atherosclerosis, OA, etc. Inflammation onsetin obese patients takes place on the adipose tissue level, especially the visceral one. The involvement of the two types of cells in the biology of inflammation is based on the following observations: macrophages are more frequent in adipose tissue from obese subjects than in adipose tissue from thin subjects, and their number decreases together with weight loss; both macrophages and adipocytes are capable of accumulating lipids, but also of synthesizing and secreting cytokines that can be a cause of inflammation. Leptin is classified as an adipocytokine because it has joint structural properties with cytokine, and it plays an important autoimmune role [49]. Actions developed by leptin, including inflammatory immune actions, reflect

the coupling at the level of target-cells membrane between leptin and itsspecific receptors, especially LRb, which induce through stimulation the activation of cytoplasmic signaling pathways, especially dependent JAK, associated with receptors. Leptin receptors belong to the superfamily of receptors from class 1 of cytokines, and more precisely to the class of receptors from the family IL-6 [50]. As a matter of fact, intracellular actions ofleptin intensify together with those developed by IL-I, increasing the activityof proteins involved in signal transduction such as JAK2, PI3K, MEK1 and MAPK p38 [51]. From a structural point of view, class 1 receptors for cytokines lack enzyme activity, containing three layers: an extracellular layer, where the leptin binding site is located, the cross-membrane middle layer, and the intracellular layer which represents the cytoplasmic signaling layer. The denomination of the receptor subtype LRb is given by the sequence of the first
29 amino acids from the COOH-terminal end, variable according to the subtype of leptin receptor [52]. The amino acids sequence conditions thedevelopment of the effects transmitted through the signals of leptin. Followingthe coupling of leptin at the extracellular layer site, tyrosine residues areactivated through phosphorylation; the residues belonging to the critical regionformed by amino acids 31-36 from the structure of the intracytoplasmic layer of the receptors, which, modifying itself conformationally, becomes able to associate with one of the activated JAK [52]. The process includes a double phosphorylation reaction: leptin coupled to LRb activates throughphosphorylation the tyrosine from the JAK2 structure, which induces the subsequent phosphorylation of tyrosine residues kept at the tail of the intracellular layer of LRb isoform. From the four identified members of the family JAK – tyrosine kinase (Jak-1, Jak-2; Jak-3 and Tyk 2) – leptin seems totransduce its intracellular signals mostly by stimulating isoform Jak-2-kynase [53]. The activation of this isoform includes, from its structure, the sequenceof amino acids 31-48, from which sequence 31-36 is compulsory. The critical role of this sequence, formed of seven amino acids which condition the development of intracellular signal transmission, arises from its compulsory presence in all systems of signal transmission which use Jak kinases. Moreover, every phosphorylate tyrosine residue is involved in the transmissionof distinct signals.
According to Bjorbak et al., at the intracellular level, signals are transmitted through three different mechanisms:

F) activation of tyrosine phosphatase pathway containing SH2, (SHp-2 = SH2-containing tyrosine phosphatase), via Tyr985 of LRb; the pathway is

important because it represents the way in which most extracellular signals transduced via ERK are regulated;

G) stimulation by Tyr(1138) from LRb structure of the transduction of signals by means of increasing the action of transcription 3 activator (STAT3 = signal transducer and activator of transcription); for this purpose, phosphorylated Tyr (1138) recruits STAT3 responsible after phosphorylation for the modulation of the transcription of proteins type cytokines that suppress signaling (SOCS3) or other positive effectors of leptin.

H) this allows the action of feedback inhibition of SOCS3 proteins or other positive effectors of leptin action [54].

Leptin's Effect on Joint Structures in OA

At the level of joint cartilage, leptin plays a key role in the etiology of arthrosis in obese patients [55]. As opposed to adiponectin, in which case pro- inflammatory effect is limited solely to the level of chondrocytes and degradation of cartilage interstitial matrix, in the case of leptin, the effects of cytokines and inflammation mediators through which it acts occur on allstructural levels of the joint:

1) affecting the function of various types of cells that form the joint structures through TNFα, IL-1, IL-6, etc.;

2) inducing the remodeling of cartilage interstitial matrix through mechanisms similar to the actions developed by MMP, MMP/TIMP, and TNFα;

3) affecting the endothelial function through the action of TNFα and activation of iNOS, indirectly responsible for vasodilation, by means of modulating the synthesis of nitrogen monoxide [56].

Most studies from the last decade admit that leptin is involved in the control of bone mass and joint cartilage volume, controlling the functionality of these levels through direct local action on the joint structure, or through the initial activation of the hypothalamic structure and, later on, of theneurohormonal pathways represented by the sympathetic component of the autonomic nervous system and the renin-angiotensin II system [57-59].

Globally, at joint level, leptin acts in developing the osteoarthritis process, including in terms of remodeling modifications at the level of the subchondral

bone, developed due to the matrix [60] and TGF-β 1 cytokine, responsible for stimulating the forming and chemotaxis of osteoblasts.

The onset of knee OA in obese patients takes place primarily at thecartilage level, and almost at the same time as the degradation of the subchondral bone [61, 58].

Some initial findings suggested an anabolic role of this hormone in the cartilage. Leptin expression is much higher in the human cartilage affected by OA than in the normal cartilage. Leptin intra-articular injection can strongly stimulate the synthesis of IGF-1 and TGF-β, and also messenger RNA and protein synthesis, which can undertake anabolic actions in the cartilage metabolism. Leptin concentrations in the synovial fluid were also significantly correlated with BMI in people suffering from severe OA. These findingssuggest that high levels of circulating leptin in obese people can protect the cartilage against osteoarthritis degeneration [62].

Nevertheless, in a study conducted on rodents, it was shown that NOS2 is synergically activated by the combination of leptin and interferon-γ, and that the activation of Nitric oxide synthase 2 (NOS2) by IL-1β is increased byleptin through a mechanism that involves PI3Ks, JAK2 and mitogen-activated kinases (MEK1 and MAPK) [63-64]. NO, which is induced by a wide range of pro-inflammatory cytokines, is a well-known pro-inflammatory mediator ofthe joint cartilage [20].

Increased leptin expression at the cartilage level in osteoarthritis diseases, compared to the normal level, even in the absence of obesity, seems to be responsible for its initial deterioration. Sequentially, following the excessive expression of leptin at the level of chondrocytes, at least two effects occur: the short term increase of chondrocyte proliferation and constant IL-1 hyperproduction [65-55]. Because they act synergically together with leptin, they determine the local increase of NO synthesis. The local excessive bioavailability of NO interferes with the functions of chondrocytes affected directly by leptin, turning the adipokine into a factor responsible for the priority development of destructive processes due to: the inflammatory immune mechanism and the involvement in the expression of genes responsible for protein synthesis at chondrocyte level and activation of MMP-9 and MMP-13 [67-69].

The intensity of enzyme activation depends directly on the leptin's bioavailability degree and indirectly on NO's bioavailability degree at joint level [66]. Excessive synthesis of the latter at the chondrocyte level is theresult of leptin's synergic action with other pro-inflammatory cytokines (IL 1- interleukin 1 and IFN γ-interferon γ), of the stimulation of the activity of NOS

type I via JAK-2, or of the signaling pathway which involves PI-3-kinase MEK-1 and p38 kinase. NO formed this way determines the destruction of the cartilage due to the activation of MMP and the induction of the apoptosis [70]. The catalytic action of leptin on the cartilage is induced also by stimulating theexpression of genes ADAMTS -4 and-5 [71-73].

These activated genes are responsible for the alteration of proteoglycans comprised in the fundamental substance of the interstitial matrix, inhibiting thecollagen II synthesis [62]. Previous actions of the type of joint-deteriorating lesions caused by the local excess of leptin seem to belong to a complex of actions induced by it, such as the reduction of chondrocytes proliferation and the increase of IL-8 and IL-1β synthesis.

At a later stage, IL-1β facilitates the upregulation of MMP-9 and MMP-13 expression, via the activation of NF-kB, protein kinase C, and the pathway of MAP-kinase [73]. The increase of the expression of these matrix metalloproteinases means the increase of their activity responsible for thedeterioration of the joint cartilage matrix. Together with MMP-13 and MMP-3 (with the activation increased also by leptin), it develops strong local catalytic action that has a pro-inflammatory effect [20, 73]. The result of the pathogenicmechanisms complex is the loss of cartilage structure, in fact an expression of the affection of chondrocytes functionality, which, on the one hand, affects the balance between synthesis and the degradation of extracellular matrix, and on the other, increases the production of inflammatory mediators toward these cells. Physiologically, both on the level of tissue and extra-tissular fluids, matrix metalloproteinases are inactive due to four types of proteins, members of the family of tissue inhibitors of MMPs (TIMPs). The transcription of these inhibitors is under the control of cytokines: IL-1, IL-6, TGF-β, TNFα, whose synthesis is modulated by leptin. In the evolution of the joint inflammatory process, the ratio of MMPs activity/TIMPs activity varies; the first stages are characterized by the increase of the ratio and, as the chronic state progresses, the level of TIMP increases (irrespective of the duration), which leads to the reduction of the ratio of MMPs activity/TIMPs activity and to the increase of fibrillary collagen content. This moment marks the passage from the degradingmechanism of the extracellular matrix to the remodeling mechanism of the matrix, singularized by a type II collagen deficit.

Recently, it has been proven that the expression of leptin is higher at the level of IFP and synovial tissue obtained from patients suffering from OA thanin healthy patients [74]. Moreover, the chondrocytes of human cartilage affected by OA produce more leptin than normal cartilage [62]. In fact, the leptin expression is linked to the degree of destruction of the joint cartilage,

and the highest levels of leptin appear in the advanced stages of the disease. [73, 75].

Leptin can maintain the process of degradation of the joint cartilage,inducing the expression of VCAM-1, adhesion molecules responsible for the leukocyte and monocyte infiltration in the inflamed joints [76]. In addition, leptin induces the secretion of IL-8 in human chondrocytes [77]. The presence of leptin is needed for the development and progression of obesity-associated OA. Leptin pathological signals associated with severe obesity induce alteration at the level of the subchondral bone, but without increasing the incidence of OA [78], which suggests that obesity, per se, is not enough to cause OA.

The studies' results provide compelling evidence of a possible role of leptin in the pathogenesis of OA. However, some authors have not managed toestablish a direct association between serum leptin (and other adipokines) and OA of the hand. In a study conducted on 44 patients, Massengale et al. were unable to prove the existence of a correlation between the degree of hand OA and the level of serum adipokines [79]. In another study conducted on over 1000 patients, the authors could not prove a significant difference between the levels of serum leptin concentration in patients with OA (symptomatic and asymptomatic) [80]. Nevertheless, in many studies, serum level of leptin was correlated with the visual analogue scale (VAS) for pain, thus proving its inflammatory role in OA [81]. Contradictory results of recent clinical and laboratory studies cast a shadow over the exact role of leptin in OA pathogenesis; however, the lack of correlation between leptin serum levels andthe severity of OA could be explained by means of the hypothesis according towhich leptin is not responsible for the onset of OA, but only for the secondary inflammatory response that follows the joint cartilage degradation [82].

At the bone level, leptin reduces the spongy bone and increases the compact cortical one. The admission that leptin is involved in the bone metabolism through dichotomous action is supported by the hypothesis that there is a necessity for a synchronism between weight gain and the support role of the bones in order to cope with overstrain [61].

Adipocytes which are excessively present in the case of obesity produce leptin at a high rate. This leptin:

- acting directly, excites the membrane pressoreceptors, being therefore able to induce a dysfunction of osteoblast bone cells;
- through indirect synergic action with the one induced by other adipokines (adiponectin, visfatin, resistin, etc.) and through the

secretion of pro-inflammatory cytokines, acts not only on osteoblasts but also on other types of local cells like synoviocytes, chondrocytes, and fibroblasts [83].

Leptin could also contribute to the abnormal function of osteoblasts inOA. In fact, high production of leptin in the abnormal subchondral osteoblasts in OA is correlated with high levels of alkaline phosphatase of (ALP), OC, type I collagen and TGF-β1, which induce a dysregulation of osteoblasts' function [62].
In knee OA, the deterioration of osteoblasts leptin at subchondral level is the expression of the dysfunctionalities of the effects physiologically induced by it in:

- the process of stimulating osteoblasts clones, via the activation of the pathways of signals transmitted through Erk 1/2 and p. 38 [72].
- phenotypic differentiation of osteoblasts [51, 72].

The arguments that support the pathogenic role of leptin in the development of lesions that deteriorate the subchondral bone are:

- compared to the normal levels, the expression of leptin is approximately five times higher, and the expression of proteins with anabolic role is approximately two times higher in the osteoblasts sampled from the osteoarthritis lesions;
- the correlation of the level of leptin produced endogenously in the osteoblasts with the exacerbation of the synthesis and, implicitly, of the activity of ALP, OC, type I collagen and TGF-β1, which can be seen and used as biomarkers of OA [72].

In the case of extreme obesity caused by a signaling deficiency of leptin, the alteration of subchondral bone morphology takes place without increasing the incidence of knee OA [78]. This finding has recently suggested that obesityper se is not enough to induce knee OA, but that leptin is necessary for the physiopathology of OA development and for the progression of the disease associated with obesity.
In patients with clinical knee OA, Berry et al. proved that leptin was significantly associated with high levels of bone turnover biomarkers, such as OC. At the same time, the basic expression of leptin receptors has been associated with low levels of turnover of cartilage and OC biomarkers, with

increased score of cartilage defects, and with the increase of cartilage volume loss. All of these results are irrespective of age, sex, and body mass index [70]. Among synovial fluid modifications in obese patients there is also the presence of leptin in levels of concentration that can be linked to body massindex, turning its level determination in synovial fluid into a diagnosis and riskmarker [73]. Ku et al. have proven a relation between leptin concentration inSF and radiographic severity in patients with OA, suggesting a role of leptin as an efficient marker for the severity of OA [75].

ADIPONECTIN

Adiponectin, also known as GBP28, apM1, Acrp30, or AdipoQ, is aprotein made out of 244 residues, and it is produced mainly by WAT [9]. Adiponectin has a homologous structure with type VIII and X collagen and with the factor of complement C1q; it can be found in the blood in relatively high quantities, under different molecular shapes (trimers, hexamers and also 12-18-mers) [85]. The gene that encodes human adiponectin is localized on chromosome 3q27, a region linked to susceptibility to diabetes and cardiovascular diseases [86, 87]. Adiponectin acts by means of two receptors, AdipoR1 and AdipoR2, the former found in skeletal muscles, cartilage, bone, and synovium, and the latter mainly in the liver [88, 89].

Adiponectin Production in Humans

The main properties of adiponectin are the increase of insulin sensitivity, the improvement of carbohydrates' metabolism, and the increase of fatty acids oxidation and of anti-atherosclerotic activities [90, 91].
Even though it circulates in high concentrations, the levels of adiponectin are lower in obese patients than in underweight patients [85], and weight loss leads to the increase of adiponectin serum level [87, 92]. Circulating levels of adiponectin tend to be low in patients suffering from morbid obesity and increase when taking thiazolidinediones which intensify insulin sensitivity [85, 87]. Serum level of adiponectin is correlated with BMI and waist-hip ratio, especially in women [93].
Besides the negative correlations with the degree of adiposity, adiponectinlevels are also low in association with insulin resistance and type 2 diabetes. Visceral adiposity has been proven to be a negative predictor independent of

adiponectin, and in this way most characteristics of negative associations between metabolic syndrome and adiponectin were shown. Adiponectin levels seem to be low before the onset of type 2 diabetes, and the administration of adiponectin was accompanied by a lower level of plasma glucose, as well asby the increase of insulin sensitivity. Moreover, the reduced expression of adiponectin has been associated with a certain degree of insulin resistance in animal studies, which indicates a role of hypoadiponectinemia in relation with insulin resistance. The main mechanisms through which adiponectin intensifies insulin sensitivity seem to be the increased oxidation of fatty acids and the inhibition of liver glucose production. Adiponectin levels are increased through thiazolidinediones treatment, and this effect could be important in the increase of insulin sensitivity induced by thiazolidinediones. Instead, adiponectin levels are reduced proportionally to the pro-inflammatory cytokines, especially TNF-α [85].

In women, the plasma level of adiponectin is significantly higher than in men [94], and the level of circulating adiponectin is not significantly linked to age [95]. In a study conducted on 58 women and 67 men, aged between 20-90 years, analyzed in age groups (< 50 years old, 50 up to 70 years old and > 70 years old), plasma concentrations of adiponectin did not change significantly with age in women, but were higher in men over the age of 70 than in younger men [96]. High levels of adiponectin in older men could reflect a longitudinal change that takes place together with ageing or simply the consolidation of survival in men. In order to find an answer to this question, additional studies are required.

Adiponectin is especially expressed in adipose tissue, and it directly sensitizes the organism to insulin. Hypoadiponectinemia caused by the interaction of genetic factors and environmental factors which lead to obesity seems to have an important causal role in insulin resistance, type 2 diabetes, MetS, and OA. Adiponectin receptors, AdipoR1 and AdipoR2, which mediate the antidiabetic metabolic actions of adiponectin, are regulated at low level in the case of insulin resistance linked to obesity. High-level regulation of adiponectin is a partial cause of insulin sensitivity and is influenced by the antidiabetic actions of thiazolidinediones. Therefore, adiponectin receptors andadiponectin itself represent possible versatile therapeutic targets for combatingobesity and other diseases characterized by insulin resistance [85].

Adiponectin can link insulin sensitivity to energy metabolism, and is also useful in correlating obesity and insulin resistance. Obesity is the basis of different disorders, including metabolic syndrome, diabetes, atherosclerosis, hypertension, coronary disease, and OA, and these pathological conditions are

associated with low plasma concentration of adiponectin, insulin resistanceand endothelial dysfunction. Adiponectin sensitizes the organism to insulin, and also has antiatherogenic properties. Lifestyle changes and some pharmacological therapies for atherosclerosis, hypertension, coronary disease, and OA have important effects regarding the simultaneous increase of adiponectin levels, decrease of insulin resistance, and improvement of endothelial function. The effects of lifestyle changes and cardiovascular drugs on adiponectin levels and insulin resistance suggest plausible mechanisms that can be important in treating atherosclerosis, coronary diseases, hypertension, and OA [97].

High levels of adiponectin are associated with improved lipid profile, better glycemic control and the reduction of inflammation in women with diabetes mellitus [98]. Adiponectin has also been found to be inversely associated with diabetes mellitus risk in the non-diabetic population [99, 100].

Higher levels of adiponectin are associated with a lower risk of diabetes inthe elderly, both men and women [101], but the extent to which this hormoneis a marker of low content of visceral and abdominal fat or a mediator of insulin sensitivity when visceral fat is eliminated remains unclear. Lower levels of adiponectin are more strongly linked to the degree of insulin resistance and hyperinsulinism than to the degree of glucose intolerance and adiposity [192].

In addition to the strong association with the risk for type 2 diabetes, preliminary data suggest that adiponectin can be moderately associated to cardiovascular morbidity and mortality. Higher adiponectin concentrations are associated with a favorable cardiovascular risk profile [103-105]. However,the relation is much more complex. High concentrations of adiponectin have been associated with the increase of all types of cardiovascular mortality [106, 107]. The discrepancy can be linked to the studied patients (men vs women, older people vs young people, cardiovascular diseases' prevalence). Additionally, adiponectin cannot directly affect the cardiovascular risk, but it can be a marker of other risks. Additional studies are necessary in order to clarify the relation between adiponectin and cardiovascular diseases.

Adiponectin acts by means of the two previously described receptors, AdipoR1 andAdipoR2. Transduction of adiponectin signal by AdipoR1 and AdipoR2 involves the activation of AMPK, PPAR-α, PPAR-γ, and of other signaling molecules [108, 109]. It should be noted that targeted perturbation ofAdipoR1 and AdipoR2 causes the cancellation of all metabolic actions of adiponectin [110].

Therefore, current studies suggest that adiponectin deficit plays an important role in the development of insulin resistance and subsequently in the onset of type 2 diabetes, and in the onset of other pathological conditions related to obesity [85].
To sum up, adiponectin, aside from its possible anti-inflammatory and antiatherogenic effects, seems to be an insulin enhancer that could become a new type of pharmacological treatment of metabolic syndrome and type 2 diabetes. In fact, agonists of adiponectin receptors and its sensitizers should represent treatment strategies for obesity linked to various pathologies like, for instance, diabetes mellitus, MetS, and OA [85].

Adiponectin and Osteoarthritis

Plasma levels of adiponectin are significantly lower in patients with OA than in healthy individuals [111]. Moreover, plasma level of adiponectin was almost 100 times higher than that of SF, being negatively correlated [89].
Recent studies have tried to identify correlations between OA and women aged over 50, but the results have been inconclusive, perhaps because the relationship is too complex [112].
Adiponectin also seems to be involved in the pathogenesis of OA, but its role in OA and cartilage degradation is controversial, and, in many aspects, insufficiently understood [113], with adiponectin also seeming to have both catabolic and anabolic effects on the pathological changes that appear during the onset and progression of OA. The pro-inflammatory role is supported by the fact that treating chondrocytes and synovial fibroblasts with adiponectin and IL-6 leads to the generation of NO by inducing NOS2 expression. Furthermore, these adipokines increase the production of key-mediators for thedegradation of the joint cartilage, such as IL-6, IL-8, TNF-α, MMP-3, MMP-9,MCP-1 and growth-related oncogene (GRO) in chondrocytes [110, 114, 115]. Chen's study also supports the pro-inflammatory effect, having demonstrated that down-regulated IL-1β induced MMP-13 production and up-regulated its associated inhibitor, TIMP-2 (tissue inhibitor of metalloproteinase-2) production in primary chondrocytes at both mRNA and protein levels, suggesting a protective role against joint cartilage degradation [89]. As further proof of its pro-inflammatory role, Filkova et al. have discovered higher serumlevels of adiponectin in women with erosive OA, compared to patients suffering from non-erosive OA [94], their results suggesting that adiponectin

can be considered a molecule potentially involved in joint disorders and matrix degradation.

Therefore, the role of adiponectin in OA is controversial. There is clear evidence showing an inhibition of the expression of IL-1β induced by MMP- 13 and a high-level regulation of TIMP-2 mediated by adiponectin in the chondrocytes [89], suggesting a protective role of this adipokine in the development of the disease. It should be noted that clinical data also support the notion that adiponectin could be a protective molecule against OA. A very recent study conducted by Honsawek and Chayanupatkul showed a reverse correlation between adiponectin and the degree of severity of the disease[116]. Additionally, it has been reported that patients with high levels of adiponectin had a low risk of hand OA progression, suggesting that this adipokine can be a protective hormone of the affected cartilage [117].

Generally, adiponectin can be found both in plasma and SF, but with differences in distribution [118]. In the blood, it circulates in high concentrations (0.01% from the total of serum proteins), higher than in the case of SF [113].

This adipokine has been detected in synovial fluids OA correlated with theseverity of OA [119], but the serum level of adiponectin isn't correlated with its level in SF [118].

Recent studies show a potential source of adipokines on a joint level: IFP. In fact, recent evidence indicates an inflammatory phenotype of this adipose compartment in the case of patients suffering from OA, showing that IFP could contribute to the physiopathological modifications in OA by means of the local production of cytokines and adipokines [120-122].

Adiponectin Effect on Joint Structures in OA

At the level of joint cartilage, biological effects of adiponectin are mediated mainly by means of the two receptors AdipoR1 and AdipoR2, which are believed to activate different signaling pathways: AdipoR1 activates the AMPK pathway, while AdipoR2 is more strongly connected to the PPAR-α pathway from the liver [123, 124].

Chen et al. [89] showed that human cartilage expresses only AdipoR1. However, Kang's study [123] showed that both AdipoR1 and AdipoR2 are expressed in the human cartilage, in concordance with the results of Lago et al. [115]. A heterogeneous distribution of AdipoR1 and AdipoR2 on the chondrocytes could be a factor that explains the difference between the results

obtained by the abovementioned studies. In Kang's study [123], AdipoR2 expression was higher from the point of view of immunostaining intensity and percentage of stained cells, but the increased rate of AdipoR1 was twice ashigh as that of AdipoR2 when the areas of lesion and non-lesion cartilage werecompared. This finding suggests that the change of AdipoR1 expression could better reflect the status of catabolic cartilage than that of AdipoR2, and that theAdipoR1 AMPK pathway could be associated with the cartilage catabolism [123].

In 2010, Parul Singla et al. [125] brought evidence that adiponectin has a protective anti-inflammatory role in OA, but the same year, the study by Kang et al. [123] showed that adiponectin increased the production of NO and of the three MMPs in human OA chondrocytes, mainly on the AMPK-JNK pathway in vitro, and that NO and MPPs induced by adiponectin led to the acceleration of the degradation of the matrix of OA cartilage ex vivo, therefore contributingto the destruction of cartilage in OA.

Plasma levels of adiponectin show positive associations with the joint cartilage degradation markers, such as IL-1 β, uCTX-II and sCOMP, butnegative associations with the serum level of CRP, and these associations become stronger after adjusting the BMI. On the other hand, there are studies that suggest a positive association between CRP and adiponectin level in SF inpatients with advanced knee OA [95]. Other authors note that, in the chondrocytes, this adipokine induces pro-inflammatory mediators, such asNO, IL-6, MCP-1, MMP-3 and MMP-9, and also IL-8 [77, 115, 123].

It has been scientifically proven that adiponectin activates AMPK [85]. Lago et al. [115] reported that AMPK/Akt pathway is involved in the iNOS and MMP-3 induction by adiponectin. In Kang's study [123], by contrast, AMPK / JNK are the major signaling pathways involved in adiponectin-meditated induction of iNOS and MMPs in human OA chondrocytes, whilethe AMPK / Akt or AMPK/p38 pathway is partially involved in the induction of MMP-13 or, respectively, MMP-3. JNK pathway is one of the signaling intermediates activated by adiponectin [126] and JNK activation induced by adiponectin has been proven following the activation of AMPK [127, 128]. Moreover, JNK is involved in the expression of MMPs and iNOS in human joint chondrocytes [129, 130]. Therefore, it is to be expected that adiponectin induces expression of iNOS and MMP by means of JNK in AMPK at the levelof human chondrocytes, and also the fact that the AMPK / JNK axis is a major signaling system responsible for the adiponectin-induced degradation of cartilage matrix [131, 132].

Because NO can regulate at high level the expression or activity of MMPs [133, 134], the attempt has been made to establish whether NO mediated the adiponectin-induced synthesis of MMPs. Unexpectedly, MMP expression was increased even more by adiponectin after a pre-treatment with a non-specific NOS and a specific iNOS inhibitor. In this context, one may be tempted to speculate that NO is a negative feedback regulator of the adiponectin action in the cartilage, and that this negative feedback can lead to delayed effects of adiponectin on the OA cartilage catabolism, compared to the IL-1β [123]. NO's role as catabolic mediator has been controversial. Various studies have proven the protective effect of NO against the degradation of the cartilage, both in human subjects and animals [135-137], in which treatment with NOS inhibitors accelerated the particularization of proteoglycans by means of increasing the levels of MMP in cultures [135]. Thus, the role of NO in the homeostasis of the cartilage seems to be complex. Additional studies regarding the effect of NO on the activation of AMPK or JNK in the chondrocytes will elucidate the mechanisms through which NO influences the adiponectin- induced production of MMP [123].

At bone level, adiponectin seems to have a clear negative effect on bone mass, and it also seems to independently predict bone mass loss, although scientific data regarding its action on cartilage are extremely controversial, showing both pro-inflammatory and anti-inflammatory effects [138].

Adiponectin stimulates the proliferation and mineralization of human osteoblasts [126] through the p38 MAPK pathway, in a paracrine and/or autocrine manner [126, 139]. By contrast, adiponectin indirectly activates the osteoclasts by stimulating the receptor activator of nuclear factor Kappa-B ligand (RANKL) and inhibiting the production of osteoprotegerin (OPG) inthe osteoblasts [140]. Undoubtedly, the effects of adiponectin on bone metabolism were inconsistent when comparing in vivo and ex vivo studies [141, 142]

Osteoblasts stimulation by adiponectin increases the production of inflammation mediators IL-6, IL-8, and MCP-1. In the first-grade osteophytes (non-ossified), adiponectin is detected in the fibroblasts from the connective tissue, which suggests the involvement of adiponectin in the early formation ofosteophytes [143].

LEPTIN AND ADIPONECTIN AS MEDICAMENTARY THERAPEUTIC TARGET

Taken individually, therapeutic intervention in obesity has had limited success, with the exception of bariatric surgery, a procedure that presents a high risk of mortality. It has been proven that adipokines have an important role in the regulation of food appetite and satiety sensation, regulation of inflammation, fat distribution, insulin sensitivity, and energy expenditure. In short, it has a role in all diseases associated with obesity. Nowadays, adipokines are considered promising candidates for developing new treatmentsthat could combat obesity [144].

Leptin as Therapeutic Target

Leptin has been approved as a medicine in the United States of America in2014 and is used for treating congenital leptin deficiency and generalized lipodystrophy [145]. An analogue of human leptin, metreleptin, was approved as a medicine in Japan in 2013 and in the US the following year, and is being used in treating complications of leptin deficiency and type 1 diabetes mellitus associated with hypertriglyceridemia in congenital or generalized lipodystrophy. It is to be expected that metreleptin improves insulin sensitivity, hypertriglyceridemia and hyperglycemia in patients suffering from rare forms of lipodystrophy that do not respond to conventional treatment. The use of metreleptin as a treatment for amenorrhea has also been assessed, but it seems that all progress in this direction has been interrupted [146].

Metreleptin was approved in 2014 by the US Food and Drug Administration (FDA) under the trade name of Myalept, for subcutaneous injection administration, as a substitute therapy to treat complications arising from leptin deficiency, in addition to a diet plan, in patients suffering from generalized, acquired or congenital lipodystrophy.

The safety and efficiency of Myalept were assessed in a study conducted on 48 patients suffering from congenital or acquired lipodystrophy and diabetes, hypertriglyceridemia and high levels of rapid-acting insulin. The trialshowed the decrease of HbA1c, glucose and triglycerides.

Myalept administration can stimulate the production of anti-drug antibodies, which can lead to severe infection or the loss of treatment

efficiency. T-cells lymphoma has been reported in patients with acquired generalized lipodystrophy, irrespective of being treated or not using Myalept, therefore professionals in the health industry should take into careful consideration the benefits and risks of the Myalept treatment in patients with significant hematological abnormalities and/or acquired generalized lipodystrophy. Because of the risk of developing lymphoma and neutralizing antibodies, Myalept is prescribed only in the Myalept Risk Evaluation and Mitigation Strategy (REMS) Program. It is contraindicated in patients with general obesity.

Myalept is not approved for use in patients with lipodystrophy associated with HIV or in patients with metabolic diseases, including diabetes mellitus and hypertriglyceridemia, with no simultaneous proofs of generalized lipodystrophy. The FDA requested additional studies for Myalept, after it was launched on the market, including long-term studies in order to assess and analyze the reports concerning potential severe risks linked to its use. Themost common adverse effects observed during the clinical trials were hypoglycemia, migraines, weight loss, and abdominal pain.

"Myalept is the first approved therapy indicated for treating the complications associated with congenital or acquired generalized lipodystrophy and provides a needed treatment option for patients with this orphan disease", said Mary Parks, M.D., deputy director of the Office of Drug Evaluation II in the FDA's Center for Drug Evaluation and Research [147].

Adiponectin as Therapeutic Target

Adiponectin is secreted by adipocytes, and binds itself to AdipoR1 and AdipoR2 receptors, having antidiabetic effects by means of activating AMPK and PPAR-alfa pathways. Adiponectin plasma levels are low in obesity, which causes insulin resistance and type 2 diabetes. A study conducted on mice reports the identification of small synthesis molecules, orally active, with agonist AdipoR effect, bound to both AdipoR1 and AdipoR2 in vitro. AdipoR agonists showed very similar effects to those of adiponectin in muscles and liver, such as, for instance, the activation of AMPK and PPAR-a pathways. Moreover, AdipoR agonists have improved diabetes mellitus in genetically obese mice. Therefore, orally active AdipoR agonist represents a promising therapeutic approach for the treatment of diseases linked to obesity, such as type 2 diabetes [148].

It has been reported that sweet potato extract (Caiapo) could increase adiponectin levels and could improve glycemic control in humans. The sweet potato can be considered a natural insulin sensitizer, and it seems to have a potential antiatherogenic role [149].

Another randomized controlled study comparing sweet potatoes (Ipomoea batatas) with placebo, conducted on 140 patients for five months, showed a statistically significant decrease of glycolized hemoglobin A1c. However, there is not sufficient evidence to support the hypothesis that sweet potato could treat type 2 diabetes [150].

Scientific evidence from this review-chapter indicates substantial and fast progress in understanding the interaction between inflammation and elements of the immune/metabolic system in cartilage and bone diseases. Having this information, it could become possible to develop new treatment strategies or interventions with successful results for many people affected by disorders of the cartilage and bone. Undoubtedly, the in-depth study of internal mechanisms that regulate the activity of adipokines could be a great advantagefor future pharmacotherapeutic approaches of joint and bone diseases.

VISFATIN

According to the relevant published literature, there are fewer studies on other adipokines than on leptin and adiponectin, even though sufficient indications that suggest their involvement in OA physiopathology alreadyexist. New research is needed in order to understand their complex role and also the non-mechanical link between adipokines and obesity.

Visfatin is a protein made out of approximately 471 amino acids, of about 52 kDa in weight. It is also called PBEF (pre-B cell colony enhancing factor)or NAMPT (nicotinamide phosphoribosyl transferase). No visfatin receptor has yet been identified [13]. Visfatin has been discovered in the liver, bone marrow, and muscles, but it can also be secreted by macrophages such as visceral adipose tissue [95, 151]. Visfatin serum level is increased in obese patients, and it correlates with inactivity, high level of serum glucose, amino acids, insulin, and triglycerides. There are no significant differences between sexes, but it seems to be higher in women than in men [152].

Some research data underline the role of visfatin in producing IL-1α, TNF- α, and IL-6, therefore proving its possible involvement in the inflammatory state associated with obesity [13, 153]. The results of a recently published study show increased levels of visfatin and leptin inside the bone

affected by osteoporosis [154]. The expression of visfatin is higher on the IFP level than on the level of the corresponding subcutaneous adipose tissue [120]. Studies have shown that the level of visfatin is high in obese patients and that it can be reduced through weight loss and regular physical exercise [19,95]. A recently published article shows that losing 5 kg causes the visfatinlevel to decrease and induces the improvement of the clinical state of obese patients suffering from knee OA [155].

Since 2008 there has been acknowledgement of the important role that visfatin plays in the degrading of OA-affected cartilage by means of a direct catabolic effect, a hypothesis supported by subsequent research [95, 156, 157].Visfatin is produced by the osteophytes and chondrocytes of patients suffering from OA [156, 157]. Visfatin secretion is caused by osteoblasts and osteoclasts, suggesting its role in forming osteophytes [95]

RESISTIN

Resistin is a circulating hormone, first identified in 2001 in mice. Its namewas given because it causes insulin resistance, and it has been seen as the link between obesity and diabetes mellitus [158, 159].

Resistin is a 12.5 kDa protein belonging to the resistin-like molecules (RELMs) family [20]. Resistin is also known as "ADSF (adipocyte-secreted factor) or FIZZ3 (found in inflammatory zone 3)" [13].

It has been initially identified in the abdominal area adipose tissue and hasbeen categorized as an adipokine, playing a role in the pathogenesis of obesity caused by insulin resistance [158]. Subsequent research has shown that it is also a pro-inflammatory mediator secreted by other cells beside adipocytes, such as macrophage, monocytes, and neutrophil [13, 20]. The resistin receptor has as yet not been discovered [160].

By synthetizing the available data, one can state that resistin plasma levels are significantly increased in: obese patients, in women more than in men, in the synovium of the inflamed joints in the case of rheumatoid arthritis and OA,and also in the plasma and SF of joints that have been subject to trauma [19, 95, 161].

Since the discovery of resistin, its pleiotropic role has been researched. Even though it is classified as an adipokine, it also has an important role in the inflammatory process, being identified in the macrophage. The connection between a number of diseases, such as diabetes, obesity, metabolic syndrome,

rheumatoid arthritis, OA, and resistin has been intensely studied [81, 120, 162,163, 164].

Specialty data show that resistin stimulates the release of other pro- inflammatory cytokines (TNF- a, IL-1b and IL-6) and also the prostaglandin 2 synthesis [13, 95, 160, 165].

The link between resistin and OA, especially knee OA, has been intensely studied [19, 81, 95, 120, 160, 162, 163, 164, 165, 166, 167]. This association has as yet not been clarified, the research conducted showing contradictory data. The data of a study published in 2012 suggested the involvement of adipokines in OA through the association of high levels compared to thecontrol group that didn't suffer from arthrosis [81]. As for the interdependence between resistin, obesity and knee arthrosis, some research has hypothesized that this adipokine determines the emergence of osteophytes, stimulates the degradation of proteoglycans and inhibits their production, also stimulating the emergence of radiological modification, despite the body mass index; its level can increase with age [81, 95, 160]. Increased resistin level is associated with cartilage modifications and bone abnormalities, proving its catabolic role in patients suffering from knee OA [19, 163, 164, 166]. Resistin has a direct effect on cartilage recovery and cytokines production in local trauma; it can also lead to secondary arthrosis onset [20, 165]. More recent studies have shown that increased levels of resistin in obesity and knee trauma determine the alteration of the mechanical integrity of the joint, by means of a metabolic effect on the meniscus and joint cartilage [25, 160, 168]. Resistin level in SF can be a marker that reflects the severity of the disease and also the degradation level of the joint cartilage in knee OA [167].

CONCLUSION

With respect to the risk factors, most recent studies underline the role of adipokines in inflammatory, autoimmune and rheumatic diseases. Thedescription of the product of an obesity gene proved the role of adipose tissue in the physiopathology of diseases associated with obesity, and has also helped to identify many other adipokines, most of them inflammatory. Progress has been made in studying these molecules, focusing particularly on the role of leptin, adiponectin, resistin, visfatin and other recently identified adipokines in inflammatory, autoimmune, and rheumatic diseases, taking into account the fact that WAT produces over 70 cytokines and other molecules.

It is now clear that adipokines have multiple relevant roles in the organisms, with a great deal of research efforts being put into the clarification of the complex network formed by WAT, metabolic disorders, and inflammatory diseases, even though many aspects are still unclear.

The role of adipokines becomes even less clear, and many clinical and experimental studies have shown proof of their contribution to rheumatic and inflammatory disorders. The complexity of adipokines' network in the pathogenesis and progression of rheumatic diseases has questioned from the outset whether it is possible to target the mechanism(s) through which adipokines contribute selectively to the disease, without repressing their physiological actions. The data presented in this paper suggests thatadipokines and their signaling pathways can represent innovative therapeutic strategies for autoimmune and rheumatic disorders.

However, actual knowledge is not sufficiently comprehensive as to enable the application of this kind of approach in clinical practice; more types of possible approaches are perhaps feasible. For instance, the control of leptin level by means of using antibodies in a similar way as anti-TNF therapy could be an interesting strategy.

In the light of the above, besides the pharmacological perspectives, it is clear that the main source of all pro-inflammatory adipokines is dysfunctional adipose tissue. Therefore, reduction of fat mass, control of food intake and increase of physical activity remain the essential prevention strategies that helpcounteract the negative effects of the pro-inflammatory obesity state.

Undeniably, adipokines have an important role in the physiopathology of OA, and show an association with the progression of this disease. Thereby, it can be possible to use adipokines as biomarkers for monitoring the disease progression and for tracking the efficiency of therapeutic interventions.

Only additional perspectives that would clarify the regulation mechanisms of adipokines and their actual roles in OA pathology could suggest new types of approach for this disease.

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Chapter 3

I) OZONE THERAPY IN KNEE OSTEOARTHRITIS

Gabriele Tabaracci, MD and Viviana Covi*, MD
San Rocco Poliambulatori, Montichiari (BS), Italy

ABSTRACT

The approach to knee osteoarthritis here suggested the use of a gaseous mixture of oxygen and ozone, aiming to integrate the spread therapy using hyaluronic acid, and possibly replacing the analgesic drugs (NSAIDs).
The purpose is improving symptoms and autonomy, in order to avoidor delay more invasive procedures.
Thanks to the various biochemical actions, if the therapy is used with proper concentrations of ozone in oxygen, this gas is applied following a therapeutic cycle of both intra-articular injections, to obtain analgesic andanti-inflammatory effects, and extra-articular, to promote the tropism of tendons and ligaments.
It is also recommended, more often in women, to treat the stasis in lymphatic and capillary vessels with local oxygen-ozone therapy and/or systemic therapy.
The therapy, if it is applied using a correct technique and with the right doses and concentrations, doesn't present contraindications or side effects, and it can't cause intolerance or allergies.

* Corresponding author: Email: v.covi@sanrocco.net.

It actually aims to improve and, if possible, to solve the pain symptoms, making easier the rehabilitation exercises, and giving a better quality of life, with lower costs for the Health Care System.

Keywords: ROS, ozone, therapy, protocol, arthritis

INTRODUCTION

Knee osteoarthritis is a common, progressive condition, which isassociated with severe pain, functional disability and impairment of health related quality of life, causing a significant social and economic burden.

It is a chronic, degenerative joint disorder affecting millions of people worldwide [1], and it is considered as one of the most common causes of disability, affecting more than 50% of the people over the age of 65 and is more prevalent in women after menopause (18%) than in men (9.6%) [2].

It can be classified into two different forms: primary and secondary osteoarthritis.

Primary or idiopathic osteoarthritis is influenced by and depends on genes,while secondary or post traumatic osteoarthritis occurs mainly after a traumatic event. Although primary and secondary types are caused by differentfactors, they both result in the same degenerative phenomenon complicated by inflammatory reactions [3].

It is a complex disease causing a change in the tissue homeostasis of articular cartilage and the subcondral bone, inflammation of the synovial membrane, narrowing of joint spaces, hypertrophic bone, geodes andosteophytes.

The main symptoms are pain, which is initially sporadic, then intense and continuous, swelling, stiffness and functional limitation of movement. The stiffening of the subcondral bone causes the bone to be less able to absorb impact loads and thereby leads to increased stress in the cartilage [4].

Pain is often both in the anterior and posterior compartment, grows during the motion, and regress with the rest.

It has been strongly associated with overweight, gender and previous knee injury. Diabetes is also associated with its progression.

Osteophyte, joint space narrowing, and subcondral sclerosis are the main radiologic findings. Radiological disease progression can be measured by Kellgren-Lawrance score (KL).

Sometimes a TC can be useful to evaluate quality of bone, or a MRI to assess the quality of ligaments and meniscus or tibial and/or femoral distress.
This disease has many bad consequences, such as a decrease in quality of life, inability to work and to perform daily activities, need for constant care and economic damage.

TREATMENT

The main goals of treatment are to relieve pain, to achieve optimal joint function and mobility, to educate the patients regarding avoidance of precipitating and aggravating factors [5].
The conventional treatment can use a non-pharmacological therapy: incase of overweight, the reduction of obesity and physiotherapy (for example, quadriceps strengthening exercises).
Other non-pharmacological therapy are heat therapies (SWD, UST, TENS) and a useful tip could be the use of plantar support, helpful for symptomatic and functional improvement [6].
Moreover, it frequently employes the viscosupplementation with hyaluronic acid, in order to improve the quality of the synovial fluid.
Pharmacological therapy uses NSAIDs and cortisone.
There are several techniques that have been developed in the past to treat damaged articular cartilage, bone marrow stimulation technique, osteochondral autografting (OCG), autologous chondrocyte implantation (ACI) and more recently mesenchymal stem cells (MSCs).
Other techniques, more invasive, are arthroscopic lavage and debridement or a total joint replacement, that usually constitutes the final option, but there is a risk for complications. Thus, when applying this treatment, possible side- effects, age, weight and general physical condition of the patient must be takeninto consideration [7].
So, conservative treatment constitutes the first choice and many suggestions have been made to alleviate symptoms, expecting at the same timean improvement in the histological features.
Ozone therapy is a non-pharmacological treatment, is minimally invasive, safe and applies to everything. It can be used in association with hyaluronic acid and rehabilitative physiotherapy, in order to ease suffering, to improve mobility and possibly to postpone or avoid more invasive and expensive treatments.

With regards to the results, there are numerous scientific papers and case reports related to the use of ozone therapy in case of knee arthritis.

In recent times, a multicentric, comparative, randomized and double- blinded clinical trial concerning a comparison between intra articular ozoneand placebo in the treatment of knee osteoarthritis has been presented at the American College of Rheumatology Annual Meeting in San Francisco, in order to assess if knee osteoarthritis treatment with intra articular ozone is more effective than knee osteoarthritis treatment with intra articular placebo inrelation to pain reduction, joint functional improvement and quality of life [9].

By employing many rating scales (VAS, Lequesne's Index, TUG test, SF- 36 questionnaire, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Geriatric Pain Measure (GPM), after allocation, afterthe 4^{th} and the 8^{th} injection and 8 weeks after the last injection, it has been shown that injecting ozone gas into the knee reduces pain and improves functionality and quality of life in people with knee osteoarthritis.

The group on ozone therapy had significantly better results in tests that measured pain, function, and overall health, which showed significant improvements in the ozone group throughout the course of the study.

Lastly, an evaluation of quality of life (obtained using the Short Form-36 Health Survey) showed participants in the ozone group reported improvement in all areas that pertain to quality of life after their fourth injection.

WHAT IS OZONE: ITS CHARACTERISTICS AND ITS HISTORY

Ozone is a metastable gas, faintly blue, with pungent odor, which isknown for the layer existing at a distance of 22 km from the Earth, necessary to protect us from the most part of the ultraviolet radiations and for its toxicity on the airways, as a result of air pollution.

It is detectable at concentrations between 98.16 $\mu g/m^3$-19,63 $\mu g/m^3$ (0.002 ppm); it is composed by three atoms of oxygen (allotropic form) and it is formed with an endothermic process: $3O_2$ + 68,400 cal è $2O_3$; it has a PM of48 and a rate of decomposition of 105-106 mol/s. It owes its name to the characteristic pungent odor (from the Greek *ozein*: smell).

In water, ozone is 1.6 times denser and 10 times more soluble than oxygen(49 ml/100 ml of water at 0°C). Although it's not a radical, it is the thirdlargest oxidant, after fluoride and persulfate.

Soon the dogma of ozone's toxicity was passed, thereby encouraging its use as a therapeutic agent: there are many other gaseous and non-gaseous molecules, which are harmful if used in excessive doses and concentrations,but if they are used in a correct range of values, and by means of appropriate routes of administration they have a useful and important therapeutic effect in many fields of medicine.

Ozone was used for therapeutic purposes since the end of XVIII century,in several branches of medicine.

Martin Van Marum, a Dutch physician, first discovered the distinctiveodor of O_3 in 1785. In 1840, a German chemist, C.F. Schonbein observed the changes of properties of the oxygen, when working with a voltaic pile in the presence of this gas.

During the study of slow oxidation of white phosphorus and water electrolysis, he noticed a distinctive smell, similar to what it is perceived after lightning during a storm, and from there he coined the term "ozone" from the greek *ozein* (smelling) [10].

In 1860, Jacques-Louis Soret, a Swiss chemist then proved that the ozone molecule was composed by three atoms of oxygen. The disinfecting and oxidating properties of O_3 were first proved in the laboratory of an Irish chemist, Thomas Andrews.

In 1857 Werner von Siemens did a practical demonstration of the fact that O_3 derives from oxygen, when an electric discharge is generated by a voltaic arc: he built the super induction tube (Siemens' tube), consisting of two interposed electrode plates set at high voltage, which could generate O_3 in the presence of oxygen.

O_3 was used for the first time to disinfect operating rooms in hospitals in 1856, and in 1860, the first ozone water treatment plant was built in Monaco todisinfect water.

The first scientific publications on papers date back to the 1800, in scientific journals such as Lancet and the British Medical Journal.

In September 1896 Nikola Tesla patented the first ozone generator, and in 1900 the marketing of ozone generators and ozonated oil began.

In 1950 Joachim Hansler built the first medical ozone generator that had the ability to dose the ozone concentration in relation to oxygen.

The first medical applications of O_3 has been described by C. J. Kenworthy: in 1885 he published the book "Ozone," for the Florida Medical Association, in which he explained the uses of local ozone therapy in medicine.

In 1897, Major G. Stoker established in London the Oxygen Hospital for the treatment of ulcers and wounds through oxygen.
The idea took life during the Anglo-Boer War at the end of 800, by observing how the injuries of the wounded, if left in the nearby mountains for some days, healed more quickly.
In 1911 the hospital was closed, but Stoker continued to use oxygen-ozoneduring the First World War at the Queen Alexandra's Military Hospital in London.
This therapy was also applied in the treatment of post traumatic gangrene in German soldiers [11].
In the 50s, J. Hansler and H. Wolff introduced the use of systemic ozone therapy.
Since its discovery O_3 has been used as a therapeutic agent to treat different diseases.
The current applications of ozone therapy include many fields of medicine, such as orthopedics (herniated discs, arthritis, spinal stenosis, tendinitis, peripheral neuropathy from compression, etc.), internal medicine (hepatitis, diabetes), cardiology (outcomes of ischemia and infarction, atherosclerosis), bronchopneumology (COPD, pulmonary fibrosis, emphysema), rheumatology (rheumatoid arthritis, SLE, Sjogren's syndrome), neurology (vasomotor and cluster headaches, multiple sclerosis, dementias of various causes), gastroenterology (Crohn's disease, ulcerative colitis), ophthalmology (maculopathy sicca, retinal arterial disease, dry eye syndrome),gynecology (vaginitis, dysmenorrhea), surgery (vascular ulcers, diabetic foot, infected wounds), aesthetic medicine (cellulitis, visible capillaries, wrinkles, dystrophic scars).

PRODUCTION OF MEDICAL OZONE

Medical ozone is produced from three sources of energy: chemical electrolysis, electric discharge and UV radiation, through a reversible reaction; its half-life depends in inverse proportional to the ambient temperature and the salinity of the water, and in direct proportional to the pressure, the capacity of the syringe and the concentration of the mixture.
Therefore, it can't be stored for medical purposes, but it must be prepared at the moment of use.

The mixture should be prepared using an apparatus equipped with a photometer, which allows the control of the produced concentration in order toexactly administer what is needed for the therapeutic purpose.
You can obtain different effects by changing the method of administration and the concentration of the mixture: we can obtain modulation of oxidative stress, reactivation of innate defense system and immunomodulation, activation of the pentose phosphate pathway with increased production of 2,3- DPG and ATP, and increased release of oxygen to tissues, bactericidal, virus static and antifungal action, anti inflammatory and analgesic effect, action on endothelium (synthesis of NO and remodelling of endothelial cells), detoxifying action on liver, sensation of well-being [12].
If it is done correctly, using adequate doses and therapeutic concentrations, both the local and systemic oxygen - ozone therapy are extremely safe: it is without side effects, it has very few contraindications (for systemic therapy: favism, patent ductus arteriosus Botalli) and can not cause allergies. The therapy can be applied locally, by injections, bagging or insufflation, or systemic, depending on the therapeutic indication. Often the methods are associated, in order to obtain a better result.

BIOCHEMISTRY AND OZONE THERAPY

Ozone is the third strongest oxidant after fluorine and persulphate and in presence of organic compounds reacts immediately and generates a large number of oxidized molecules, that disappear in a few seconds.
Ozone promptly dissolves in pure water and in biological water (physiological saline, plasma, lymph, urine) according Henry's law in relation to temperature, pressure and ozone concentration, but unlike oxygen, does not establish an equilibrium with the remaining ozone in gaseous phase, becauseof the high ozone affinity to carbon-carbon double bonds.
Ozone has the ability to react with the majority of organic and inorganic substances, up to the complete oxidation. It presents a preferable selectivity forthe double and triple bonds present in the cells, fluids or tissues, such as aminoacids and unsaturated fatty acids, part of lipoprotein complexes of the plasma and of the double layer of cell membranes, DNA molecules and cysteine residues of the protein. When the gas mixture gets in touch with biological fluids, it will dissolve within a few seconds in the present water. The effectiveness of administration is closely linked to the reaction with phospholipid membranes, determining, after a partial initial consumption, the

formation of ozonides, aldehydes, ROS (Reactive Oxygen Species), including hydrogen peroxide (H_2O_2), and lipoperoxides (LOP), in controlled amounts [13].

ROS formation in plasma is very rapid (less than one minute), and it is accompanied by a transient decrease of antioxidant capacity (from 5% to 25%), returning it to a normal value within 15-20 minutes. Hydrogen peroxide and other mediators spread within cells, triggering more pathways into eritrocytes, leukocytes and platelets.

Considering the small amount of ozone used in blood and in tissues,compared to the different systemic actions, a direct action of such products on all the membranes is not possible, but there is a mechanism of induction of the synthesis and activation of various biologically active components. The obtainable effects on the organism determine an improvement of many metabolic processes.

During the systemic therapy, the ozone being a powerful oxidizing agent,it reacts immediately with biomolecules present in biologic fluids, preferably polyunsaturated fatty acids (PUFAs), bound to albumin and present in the great majority of lipids and phospholipids, as well as with antioxidants, proteins and carbohydrates, etc. Indeed, phospholipids and cholesterol present in cell membranes and/or lipid-proteins are protected by antioxidants andalbumin molecules rich in PUFAs [14, 15].

Ozone's reaction with so many molecules implies several basic processes occurring at the same time. The most part of it is consumed in a reaction of "unsaturated fatty acid addition to carbon-carbon double bonds", known as "Criegee Reaction", being one of nature's most rapid molecular chemicalreactions (millions of moles/l per second) and very well depicted. This reaction, whose first product is denominated "primary ozonide", in hydrophilicphysiologic conditions, produces the breakage and immediate reorganizationof its fragments, producing as final result in the presence of abundant water (asin the case of physiologic environment), aldehydes and α-hydroxy- hydroperoxide of different structures, depending on the initial fatty acids.

These ozone reactions, completed in seconds, with a "specific" ozone dosage, only generate a "specific" quantity of Reactive oxygen species (ROS), lipid oxidation products (LOP) and oxidized antioxidants producing immediately two crucial messengers: hydrogen peroxide, one of the reactive oxygen species, and aldehydes such as 4-hydroxy-2-nonenal (4-HNE) as the result of PUFA peroxidation.

ROS are only produced over a short period of time, a few seconds, and they produce early biologic effects in blood, while LOPs, produced

simultaneously, have a longer life and reach the vascular system as well as allorgans where the late effects are produced.

$R—CH=CH=R' + O_3 + H_2O \rightarrow R—CH=O + R'—CH=O + H_2O_2$

Low levels of ROS act as signaling molecules, modulating cell proliferation and apoptosis, gene expression through activation of transcriptionfactors like nuclear factor kappa beta (Nfkβ) and hypoxia inducible factor (HIF). Excessive amounts of H_2O_2 (early and short acting messenger) can be detrimental to cells while at physiological amounts H_2O_2 acts as a regulator of signal transduction and is an important mediator of the host defense andimmune responses [16].

Paradoxically the best method of defense and repair during chronic oxidative stress may be oxidative stress itself with controlled exposure to ROS, since the expression of many DNA repair enzymes is upregulated duringthe oxidative stress. The terms "hormesis" describes beneficial effects after an exposure to a low-intensity stressor. Studies reported the life prolonging effects of aldehydes, hydrogen peroxide H_2O_2, physical exercise, caloric restriction. Whether ROS will act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper time and side.

H_2O_2, by entering into the mass of erythrocytes, activates glycolysis witha transient increase of ATP and of 2-3-diphosphoglycerate (2,3-DPG). This compound causes a shift to the right of oxyhemoglobin and this very useful process improves the release of oxygen into ischemic tissues, an important step in ischemic diseases.

4-HNE produced during PUFA peroxidation circulates all over the body and particularly in chronically inflamed organs and can enter into cells undergoing chronic oxidative stress. 4-HNE, by its binding to either Cys 273 or Cys 288 of Keap 1, allows the release of the transcription factor nuclear releasing factor 2 (Nrf2), normally inactive because bound to a large protein called Keap1 (Kelch-like ECH associated protein), which then transfers into the cell nucleus and binds to the antioxidant response element (ARE) of gene promoters. This induces the expression of over 230 genes able to provide the synthesis of strong cellular antioxidant and detoxification systems.

The induced genes include the synthesis of glutathione (GSH), GSH reductase, GSH transferase, GSH peroxidases, thioredoxin, thioredoxin reductase, nicotinamide adenin dinucleotide phosphate, reduced form

(NADPH), NAD(P)-quinone oxidoreductase 1 (NQO1), uridine diphosphate glucose (UDP) syaliltransferase and heme-oxygenase.
Nrf2 also plays a role in the negative regulation of insulin and growth hormone which increases stress resistance [17].
O_3 therapy activates the enzymes that are responsible for protecting the body against processes linked to the overproduction of superoxides: there is a stimulation of transmembranous flow of oxygen and the induction of enzymes such as superoxide dismutase, peroxidases or catalases and the oxygen utilization in the mitochondrial respiratory chain becomes more effective [18].
Superoxide is the most crucial radical, playing a key role in biological regulation [19].
Repeated exposure to O_3 therapy may cause resistance against oxidative stress via inducing the antioxidative system.
As a consequence, the use of local and systemic ozone should be encouraged as treatment for all chronic oxidative diseases, as in presence of knee arthritis.

KNEE OSTEOARTHRITIS AND OZONE THERAPY

In the presence of knee osteoarthritis, there are many alterations, such as inflammation, sufferance of chondrocytes, release of enzymes (proteases, hyaluronidases) and reduced resistance of the articular cartilage.
Moreover, there are damages in cartilage fibrils, changes in synovial fluid and an inadequate cell turnover.
From the point of view of biochemistry, there is a close correlation between reactive oxygen species (ROS) and osteoarthritis.
ROS are the most abundant free radicals in the biological system and are continuously formed as normal by products of cellular metabolism [20]. When the oxidant level does not exceed the reducing capabilities of cells, ROS are vital for physiological processes such as cell differentiation, protein phosphorylation, transcription factor activation, apop- tosis, cell immunity, steroidogenesis and cell defense against microorganisms [21].
Under normal conditions, a delicate balance exists between free radical production and their scavenging mechanism [22].
In pathological conditions, there is an excessive generation of ROS which can harm cell functionality because it damages cellular lipids, proteins and DNA. ROS modify proteins by the oxidation of amino acids resulting in impaired biological activity, altered protein structure and the accumulation of

damaged proteins in tissue. Oxidized molecules can react with non-oxidized molecules resulting in the formation of more oxidized molecules which may further damage the tissue [23].

In the joint disease, proinflammatory factors such as cytokines and prostaglandins are released at sites of inflammation, together with ROS and NO. These factors are associated with very low Superoxide Dismutase concentrations in joint fluids and an increase of ROS. In the articular cartilage advanced glycation end products (AGEs) was found and there is a correlation between increased osteoarthritis severity and cartilage AGE levels.

There are growing evidences for an involvement of the oxidative damage in the pathophysiological changes of the knee osteoarthritis. ROS are produced by chondrocytes in response to stimulation by numerous cytokines and growth factors, including Interleukin -1 (IL-1) Tumor Necrosis Factor alpha (TNF alpha) and Transforming Growth Factor beta (TGF beta). IL-1 dependentproduction of ROS is involved in causing DNA damage in chondrocytes. Recently the dysfunction of chondrocyte mitochondria was involved in the establishment and progression of osteoarthritis.

The development and progression of osteoarthritis, as other degenerative diseases, is associated with the continuous exposure to oxidants [24].

In normal physiological conditions, chondrocytes live in low oxygen supply conditions and some of their metabolic functions require oxygen which is supplied by the synovial fluid. Chondrocytes have a metabolism adapted to anaerobic conditions, and in response to changes in partial oxygen pressure, mechanical stress, inflammatory and immunomodulatory mediators, chondrocytes produce increased levels of ROS.

The main ROS produced by chondrocytes are nitric oxide (NO) and superoxide anion that can result in the formation of derivative radicals such as H_2O_2 and peroxynitrite. The ROS produced by chondrocytes can damage the cartilage and synovial fluid by reducing its viscosity [25]. The production of NO is stimulated by tumor necrosis factor (TNF)-b, interleukin (IL)-b, interferon (IFN)-c and lipopolysaccharides and inhibited by IL-4, IL-10, IL-13 and transforming growth factors (TGF)-b [26]. Excessive production of NO contributes to the pathogenesis of chronic arthritis. NO can also interfere with chondrocyte function resulting in the loss of cartilage matrix by apoptosis induction, type 2 collagen synthesis inhibition and matrix metalloproteinase activation [27]. Besides ROS itself, lipid peroxidation products may also result in the formation of reactive aldehydes and lead to inflammations [28]. These lipid peroxidation products have longer biological lives than free radicals and

can diffuse from the site of formation to reach a distant target and induce cellular injury.

O_3 therapy acts on inflammation, tissue remodeling and improvement of the arthritis-related condition.

It acts on inflammation by the reducing TNF-alfa concentrations, which is a proinflammatory cytokine, and by activating the nuclear factor-kB (NFkB) pathway, leading to a downstream cascade of other proinflammatory cytokines, giving rise to a vicious cycle which perpetuates the chronic inflammatory process. TNF-alfa is strongly associated with joint and bone injury [29].

This cytokine is also known to increase the mitochondrial ROS production and inhibition of TNF-alfa may decrease ROS production in osteoarthritis, thereby causing the improvement and prevention of joint erosion. The inhibition of TNF-alfa may break the noxious NFkB pathway causing a reduction of the inflammation. ROS can act as a second messenger to activate NFkB, which co-ordinates the expression of a wide range of genes involved in the inflammatory response.

The efficacy of O_3 therapy is not only through the actions of cytokines, O_3 also has the ability to re-establish the cellular redox homeostasis. O_3 can protect against overproduction of NO and this is likely because of ozone's action on NFkB, since inducible nitric oxide synthase (iNOS) is regulated at the transcriptional level by NFkB.

NFkB is a major regulator of inflammations and regulates inflammatory and immune responses, cell proliferation or cell death [30]. Activation ofNFkB seems to be a positive regulator of cell growth and an inhibitor of apoptosis in fibroblasts like synoviocytes. On the contrary, some studies have shown that NFkB activation may promote apoptosis of neural cells [31].

The functions of NFkB in whether a cell should undergo cell proliferation or apoptosis seem to be cell type dependent.

O_3 inhibition of NFkB activation may cause a reduction in inflammation and apoptotic cell death. ROS can activate NFkB directly or through other stimuli such as TNF-alfa [32].

In osteoarthritis, ROS activates NFkB pathways by increasing its translocation into the nuclei and this causes the release of proinflammatory cytokines such as IL-1b, IL-6, TNF-a and COX-2. These cytokines inhibit the synthesis of aggrecan and collagen type II, which are the major components ofthe cartilage matrix.

These cytokines can also increase the release of matrix metalloproteinases and aggrecanase, enzymes that degrade the matrices, causing cartilage damage, disturbance of the metabolic balance of the cartilage matrix and eventually apoptosis.
IL-1b and TNF-alfa can induce chondrocytes apoptosis by promoting the production of other proinflammatory factors such as prosta- glandin E2 (PGE2) and NO [33].
O_3 therapy can stop the activation of NFkB and therefore inhibiting the release of proinflammatory cytokines and can change the levels of cytokines such as interleukin 8, TNF-alfa, transforming factor beta1 (TGF beta1) and platelet derived growth factor (PDGF) [34]. This inhibits the degradation ofthe cartilage matrix and initiation of the apoptotic pathway, thereby causing cell survival and proliferation.
Moreover, O_3 acts as a bioregulator by releasing factors from endothelial cells and normalizing the cellular redox balance when it comes into contact with a biological fluid: the controlled administration of O_3 may promote an oxidative preconditioning (adaptation to oxidative stress) that stimulates the antioxidant endogenous systems resulting in the protection against tissue damage.
O_3 can also stimulate the production of adenosine triphosphate (ATP) through the glycolysis enzymatic pathway and increase oxygenation to injured cells, thus preventing cell death [35].
O_3 therapy stimulates the production of interferon and interleukins in the body and initiates the production of antioxidant enzymes.
The immune system can also be stimulated by activating neutrophils and stimulating the release of cytokines.
O_3 increases the levels of TGFb which is important in tissue remodeling, and increases the production of VEFG and NO, to improve the hematic flow and the drainage of the phlogogenic agents.

THERAPEUTIC PROTOCOL

O_3 therapy has been widely used in the treatment of osteoarthritis [36] in the last decades a number of orthopedics in Europe have used ozone to treat acute and chronic knee arthritis and the results show rapid pain relief, disappearance of edema, decongestion and increased mobility.
Treatable osteoarthritis cases are both mild and severe.

During the medical examination and during the treatment cycle is very important to explain the characteristics of the treatments, the way in whichthey are conducted, what's expected, the absolute security and the absence of contraindications and adverse effects.

Knee osteoarthritis needs to be treated with local therapy, both with intra and articular injections.

The intra articular injection of O_3 has been suggested for the treatment of osteoarthritis and has yielded positive results [37].

In the last decades a number of orthopedics in Europe have begun to treat acute and chronic knee arthritis with intra articular or peri articular injectionsof small volume of ozone (5-10 ml with ozone concentration from 5 to 15 micrograms/ml of gas) with a rapid pain relief, decongestion, disappearance of edema, and increased mobility.

Ozone, when injected in the knee, dissolves in the synovial fluid that contains antioxidants like SOD, GSH, vitamin E, PUFA and proteins and generates ROS and LOP which in order are responsible for:

1) A possible inactivation and inhibition of the release of proteolytic enzymes and proinflammatory cytokines;
2) An induction of the proliferation of chondrocytes and fibroblasts;
3) A release of the synthesis of antioxidant enzymes (SOD, GSH-Px and catalase) as adaptive response of chronic oxidative stress;
4) A release of immunosuppressive cytokines such as TGF beta1 and Interleukin 10 (IL-10) that modulate the synthesis of integrins and stimulate the synthesis of matrix protein such as collagen and proteoglycans [38].

In case of conservative treatment, hyaluronic acid is of great importance:it is one of the main constituents of articular cartilage, it is present in high concentration in the synovial fluid, and determines the viscoelastic and functional characteristics.

In joint diseases, the rationale for its use is its ability to increase joint lubrication (viscosupplementation), slowing the degeneration of the cartilage matrix and reduction of local inflammation. Its use presents safety and long- term efficacy. In our experience, the positive effect of hyaluronic acid and ozone is amplified if it is used for intra articular injection, in combination during the therapeutic cycle, in alternated sessions.

Moreover, knee should also be treated in periarticular level: cartilagedamage causes the approaching of capi articolari, resulting in an overstress in tendons and ligaments.
Periarticular structures have poor vascularization and elasticity, and because of this overload and over-distension of the patella ligament, pes anserine bursitis, collateral ligaments, patellar area, medial compartment, patellar tendon can lead to inflammation, and fibrillar mucoid degeneration.
The therapeutic cycle comprises almost always the treatment of both knees.
Sessions are bi-weekly.
During the first session, intra articular and peri articular injections are performed, using ozone and treating only the most painful knee; during the second session both knees are treated using ozone.
As of the third session, in the intra-articular area you have to use ozoneand hyaluroniuc acid alternatively, for 6 or 10 sessions, according to the kindof needed acid, right for that pathology.
In case of acute pain, or severe chondral lesions (type 2 or 3) and indication of surgical replacement, it is preferable to choose a viscosupplementation with high molecular weight, using 3 vials, alternating them with ozone sessions.
Every two months a vial of acid is needed.

THERAPY MAINTENANCE

The usual protocol of hyaluronic acid with low molecular weight provides for the use of 1 vial weekly for 5 weeks, each 6 months.
In this way, there will be many months without therapy.
Therefore, after the initial treatment cycle it would be preferable to use 1 vial every 2 months, and one ozone session every month, thus avoiding possible pain or functional impotence.
Thus the curative effect will be better, at a lower cost also.
The Patient should be placed in a supine position, with a pillow under his knees, to maintain them in a slight flexion. It is preferable that the Patient shaves his knees, to avoid every possibility of infection.
To disinfect, it is better to use gauze balls, and disinfectant like povidone iodine.

Intra-articular injection has to be performed using a superolateral or medial access. Some physicians prefer to use an anterior access (Figure 1, Figure 2).

Figure 1. Reference marks, intra and peri-articular injections.

Figure 2. Reference marks, intra and peri-articular injections.

After the injection move several times in successive flexion/extension motions for a better distribution of the gas at the level of articulation and alsoat the level of recurrences.
In this way you can generate a characteristic noise, due to the mixing of synovial fluid with gas.
The extra-articular treatment is applied to pes anserin tendon, collateral ligaments, medial compartment, patellar ligaments, prepatellar bursa (Figure 3,Figure 4).

Figure 3. Extra articular therapy: reference marks.

Figure 4. Extra articular therapy: reference marks.

Concentrations and doses:

- 20 cc syringe
- 25G x 25 mm needle (intra-articular injection), 27G x 20 mm needle (peri-articular injections)
- Concentration: 16 $\mu gO_3/ccO_2$ intra-articular, 18 $\mu gO_3/ccO_2$ peri-articular
- Intra-articular injection dose: 5-10 cc
- Peri-articular injection dose: 1-2 cc for each injection

The absence of pain over time has also been evaluated, after thetherapeutic cycle. Following this protocol, in most cases positive results can beobtained, with the disappearance of pain and recovery of joint function at 90%.

In the event of a clinical and radiographic severe situation, there is an absolute indication for surgery (Figure 5, Figure 6).

The patient refused surgery and opted for a conservative therapy. The treatment started 2 years ago, following the protocol described, maintaining a monthly session of local and systemic ozone, and a bimonthly session with hyaluronic acid. Currently, the patient can walk without pain and can ride a bike.

Figure 5. RX with clear signs of knee osteoarthritis.

Figure 6. RX with clear signs of knee osteoarthritis.

SYSTEMIC OZONE THERAPY (SO_3T)

It would be useful to associate local and systemic therapy, executable in the same session, in accordance with the biochemical principles previously explained in order to improve the inflammatory and degenerative conditions ofjoint tissues.
The therapeutic cycle is made of 10 sessions, twice a week.
The frequency of therapies provides for a session after 10 days from the end of the therapeutic cycle, then another session after 20 days, and then a session every month, as previously explained in case of local therapy.
A monthly session, both local and systemic, is recommended.
The needed concentration ranges from 20 $\mu gO_3/O_2$ for the first session, then to 25 $\mu gO_3/O_2$ for the second session, up to 30 $\mu gO_3/O_2$ from the following therapies.
The method uses a specific kit, dedicated to systemic ozone therapy,typically with one-way system (one way in and out) and it is totally safe and simple to apply.

VASCULAR DISEASE

For each Patient, it has to be assessed the presence of disorders of arteriovenous and lymphatic system, joint limitations (knee flexion) with muscle hypotrophy of the lower limbs (especially quadriceps), correct plantar support, osteoporosis and other intercurrent diseases. Vascular disease can be an obstacle to rehabilitation in arthritic diseases, in women particularly, with heaviness of limbs. It can be therefore associated a infiltrative pericapillaryand paravenous treatment, useful also in case of need of replacement.
The protocol consists in:

- 50 cc syringe (number depending on the area to be treated, 3-4 on average)
- Needle: 27G x 20 mm or 30G x 13 mm
- Concentration: 10 $\mu g O_3/cc O_2$

Treated tissues are treated with more tonic, edema is reduced, with a sensation of lightness of lower limbs.
You can treat at the same time both evident capillaries and teleangiectasies.
It would be very important to associate the rehabilitation physiotherapy, inorder to improve the muscle tone of lower limbs. In this way, it would be possible to obtain a correct flexion/extension.
The recovery of muscle tone is very important, even if surgery should be needed.
Foot pathologies have often implications for knee, hip and spine
Thus, it is helpful to evaluate the plantar support, using insoles when appropriate.

CONCLUSION

Knee osteoarthritis is a wide spread disease, that deserves to be treated in the best way possible, in order to limit the disease progression, and the consequences on the quality of life.
It may be preferable to initiate a treatment with non-pharmacological or minimally invasive methods, and for this purpose ozone therapy can be very useful, both local and systemic. If applied accordingly to the correct protocol it

ensures safety and in most cases yeilds very valid results, both from the symptomatic and functional point of view, especially associated with rehabilitation therapy. This means the achievement of a state of well-being andsatisfaction for the person, both from a personal and working perspective, and an important economic advantage for the health system, due to a lower need of pharmacological or more invasive therapies.

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