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EFFECT OF PELOIDOTHERAPY ON THE PLASMA LEVELS OF LEPTIN AND ADIPONECTIN IN KNEE OSTEOARTHRITIS

SUMMARY OF THE PHD THESIS

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CONSTANŢA
2012

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Key words: leptin, adiponectin, osteoarthritis, balneal treatment, mud, peloidotherapy

I. THE KNOWLEDGE IN THE DOMAIN AND MOTIVATION OF CHOOSING THE TOPIC

Obesity is considered to be a risk factor for osteoarthritis and it is accepted that obesity contributes to the development and progression of osteoarthritis by increasing the mechanical loading of joints. Recent progress in the physiology of white adipose tissue showed that fat cells produce many factors, called adipokines, which play an important role in the development of osteoarthritis, besides their mechanical effects[1]. Osteoarthritis (OA) is a multi-factorial degenerative joint disease that profoundly affects articular cartilage, makes changes in sub-chondral bone, leads to osteophytes formation and synovial inflammation. Although it is considered the most common rheumatic disease, its etiology is largely unknown. Age, obesity, sex, and previous injuries are considered to be significant risk factors. In OA, although it is frequently described as a non-inflammatory disease, inflammation is known to contribute to its symptoms and evolution[2,3]. The theory according to which the white adipose tissue (WAT) could be an important factor with implications for the whole body homeostasis became plausible since 1994, when the first adipokine- the leptin[4] was discovered. Since then, there have been identified over 67 adipocitokins (adiponectin, resistin, visfatin, chemerin, lipocain, etc), but their roles are far from being unraveled as per evidence-based medicine[5].

Adipokines include a variety of pro-inflammatory peptides. These pro-inflammatory adipokines are increased in obesity and appear to contribute to the so-called "low-grade inflammatory condition" of obese subjects, creating a vicious circle of metabolic aberrations, including cardiovascular and autoimmune inflammatory complications. The present research focuses particularly on the balneal therapy effect on adiponectin and leptin plasma levels in patients with knee osteoarthritis[6].

Mud is a therapeutic agent used since ancient times to treat osteoarthritis. Although extensively used, the mechanisms by which balneal therapy acts are poorly studied, sometimes we can even say that they are "full of surprises". The complexity of its biochemical composition, the rich microbial flora, the inter-relationships between its components, slow down

the detection of the physiological and therapeutic effects and mechanisms by which they are produced[7].

The General direction of research in this field is moving towards scientific foundation of balneal therapy, to reveal its mechanisms of action as well as the effects of adipokines, having in mind the principles of evidence-based medicine, so that the empirical use of mud can be surpassed, in terms of the modern therapeutic approach of patients with osteoarthritis.

Research direction. Currently extensive research is being developed regarding the analysis of the effects of balneal therapy at the cellular level: variation of serum levels of cytokines, markers of chondrocytes, lymphocytes, etc. There are studies conducted abroad (Italy)[8-10] and in Romania on the dynamics of plasma cytokines under the action of balneal therapy both to healthy individuals and to patients with rheumatic and inflammatory conditions[11,12].

Current state of research regarding leptin and adiponectin plasma levels in patients with osteoarthritis. Until the present moment there has never been any **similar study conducted or any other study dealing with this specific topic in Romania**..

Internationally, at present time there are attemps to re-evaluate the already existing reseach, of meta-analysis, in order to scientifically prove that there is an strong association between OA and the plasma levels of adipokines. To sustain that, there have been published 139 studies, from which only 61 focused on the thoroughness of knowledge concerning the behavior of leptin in relation with OA. 45 studies focused on the thoroughness of knowledge concerning adiponectin levels in relation to OA and amongst all these research studies only 22 of them analyzed in parallel the evolution of leptin and adiponectin as"key" factors in the pathogenesis and evolution of OA. From the point of view of OA evolution in relation with balneal therapy, there are at this moment 241 finalized studies, but what is the most important is the fact that from all these studies, there is only 1, finalized in 2011 in Italy, which focuses on the effects of balneal therapy on serum levels of leptin and adiponectin in patients with knee OA[10].

For that purpose we investigated the standard medical data base: Medline, Embasse, Database, Pedro, PubMed, UpToDate and controlled trials Cochrane, analyzing the works and studies registered according to the requirements of medical codes of ethics and evidence-based medicine.

MOTIVATION OF CHOOSING THE TOPIC

The theme I approached is found at crossroads with several disciplines - physiology, immunology, genetics, rheumatology - medical recovery, physical medicine and balneology. In addition, the scientific progress in medicine has reached beyond the classical stage work that used mainly optical or electronic microscope. The study of cellular and molecular structures is, at this time, the appanage of cell biology, genetics and immunology, studying areas of biogenesis of cell organelles, macromolecular composition, genetic control for necessary synthesis of different structures. Despite all this progress, there has been no study conducted at national level on the influence of peloidotherapy on leptin and adiponectin levels in patients with osteoarthritis, in order to improve the therapeutic approach, that it is considered to be the most required structural and functional element during balneotherapy.

I started this research project due to the questions arising in clinical practice for which I was not able to find a response and because of the enthusiasm created by the challenge of discovering a new concept that will revolutionize the methods of therapeutic approach of knee osteoarthritis known until present date. I must specify the fact that I did not find any study in the national literature, whereas in the international literature I found only one study revealing the changes in serum levels of adipokines during mud therapy in patients with osteoarthritis.

By developing this theme I wanted to prove that balneotherapy represents a valuable therapeutic option when administered properly, the therapeutic effects products by mud are authentic not placebo, and that the metabolic status of patients with knee arthritis improves, which results from laboratory investigations before and after therapy.

I wanted to contribute to the scientific foundation of peloidotherapy in what concerns a new approach in treatment, with demonstrated beneficial effects on lipid metabolism in patients with osteoarthritis.

LEPTIN

Leptin is a 16 kDa non-glycosylated hormone produced mainly by adipocytes, and its circulating levels are correlated with TAA mass. This hormone decreases food intake and increases energy consumption by taking action on specific hypothalamic nuclei, inducing anorexigen factors by transcription related to cocaine of amphetamine and suppressing orexigenic neuropeptides such as neuropeptide Y[6,13]. Leptin level is largely dependent

on the amount of fat and its synthesis is also regulated by inflammatory mediators[14].

It is becoming more obvious the fact that this hormone plays a key role in the pathophysiology of OA.

The major effects of leptin on the immune and vascular system are:

1. pro-inflammatory action,
2. increases T cell activation,
3. increases proliferation of cytokines,
4. promotes lymphocyte responses (Th1),
5. increases NK cell activation,
6. releases cytokines (TNF, IL6, etc) and activates macrophages,
7. induces endothelial dysfunction by increasing blood pressure.

ADIPONECTIN

Adiponectin, known as GBP28, apM1, Acrp30, or AdipoQ, is a protein of 244 residues, which is produced mainly by WAT[6].

Adiponectin is a new three-dimensional protein specific to adipocytes, which has been suggested to play a role in the development of insulin resistance and atherosclerosis. Although circulating in high concentrations, levels of adiponectin are lower in obese subjects than in underweight subjects. Adiponectin is also involved in the pathogenesis of OA. Adiponectin has emerged as a regulator of immune and arthritic response of inflammatory nature.

Adiponectin is considered to be a "good" adipokine with the following effects:

1. inflammatory effect quite controversial,
2. decreases T cell activation, TNF-α, IL6,
3. increases IL10,
4. inhibits phagocytosis and oxidative combustion,
5. is vascular-protective,
6. prevents atherosclerosis,
7. lowers blood pressure.

It is now clear that adipokines have multiple relevant roles in the body and many research efforts are directed to elucidate the complex net between WAT, metabolic dysfunctions and inflammatory diseases. Although many aspects are still unclear, I summarized the current knowledge about adipokines role in the pathophysiology of OA.

The physiological role of adipokines has started to become clearer and more experimental and clinical studies have revealed the evidence of their contribution to inflammatory and rheumatic disorders. The complexity of adipokines network in the pathogenesis and progression of rheumatic

diseases raises, even from the beginning, an important question whether it may be possible to target the mechanism(s) by which adipokines contributes to disease selectively, without suppressing their physiological actions. The data presented in this paper suggest that adipokines and their signaling pathways may represent innovative therapeutic strategies for autoimmune and rheumatic disorders.

However, current knowledge is almost incomplete to allow translation of these approaches to an actual clinical practice, but several possible approaches are likely to be feasible. For example, controlling leptin levels by using antibodies in a manner similar to anti-TNF therapy could be an interesting strategy.

In light of the above mentioned, besides the pharmacological perspective, it is clear that the main source of all pro-inflammatory adipokines is the dysfunctional adipose tissue. Therefore, reducing fat mass, controling the nutrition and increasing physical activity remain the essential strategy in order to prevent the negative effects of the obesity pro-inflammatory state.

Only additional perspectives to clarify adipokines control mechanisms and their specific roles in the pathology of OA, may propose new pharmacological approaches for this disease.

II. BALNEAL THERAPY

Techirghiol benefits from the existence of special natural conditions, resulting in the presence of healthy natural factors, namely: climate, sapropelic mud, salty water of the lake. The climate is temperate continental, steppe, with marine influence, the average annual temperature is around 11 degrees Celsius, the humidity is around 80% and the wind is almost always present. The water from the lake is chlorinated, sulphatated, brominated, soda, magnesian, hypertonic. The sapropelic mud, alkaline pH, is extracted with our own boat from the bottom of lake Techirghiol[15].

The mud, used as therapeutic remedies since ancient times are, is in its essence earth, rocks of pasty consistency. Some beneficial effects of mud are empirically known since antiquity, while other effects were described and studied recently, but their investigation is still in an incomplete stage. Mud was and remains "a trial stone" for many pathologies and field yet to be explored, with many paths still unknown to researchers.

In clinical practice a simple classification of peloid is used: sapropelic mud, peat and mud from mineral sources[16].

1. Sapropelic muds are black sulphide deposits, very unctuous that have great plasticity, which recommends them for therapy.
2. Mud peat are brown organic deposits .
3. Mineral mud consist of salts sedimentation of certain carbonated, calcium, ferruginous and sulphurous sources

The deposit from the bottom of Techirghiol lake is composed of sapropelic mud[17].

The formation of sapropelic mud, therapeutic or peloidogenesis represents a complex process that features geological, physic-chemical, climatic and biological factors.

PHYSIOLOGICAL AND THERAPEUTIC EFFECTS OF MUD

The active chemical components of sapropelic mud and its natural extracts dispel in the body, either by crossing the skin barrier or entering directly into circulation, in relation with its administration, and trigger local functional, tissular and general responses, inhibiting or activating some enzymatic systems and intermediate metabolites. The bio- and histo-chemical changes, that occur in the body by using the mud or its extracts,

are nonspecific, and they are categorized as being overall reactions of the body functioning. The general reactivity of the organism is modulated under the action of mud is and pointed towards optimal parameters.

A. Skin reactivity reveals increase in skin excitability, improvement of hyaluronidase diffusion capacity and stimulation of the reticulo-endothelial system. At skin level were also found metabolic and enzymatic tissular changes, that took place under the influence of hot mud and natural extracts, which were parenteral administered. These changes are: increased alkaline phosphatase and oxidase, decreased PAS reaction for highlighting neutral muco-polysaccharides of skin and an increase of Hale reaction for acid muco-polysaccharides (hyaluronic acid). The general mud bath at 43 C changes colloidal state of the fundamental substance in the skin and activates the oxide- reducing enzyme functions and liberating energy with glycogen consumption[18].

B. Metabolico-neuro-endocrine reactivity. A fairly long period of time there were recognized only the estrogenic effects, then St. Milcu has revealed also progesteronic[19] properties, and later on it is shown the tendency to restore the balance of the thyroidal function (also by the intervention and elective setting in thyroid of the iodine present in the composition of the mud). Under the action of mud and / or mud extract a harmonic stimulation occurs in all glands meaning the increase of enzyme activity and synthesis, while keeping the specificity of each.

The stimulation of hypothalamic-pituitary-adrenal axis, which is clinically translated by improving symptoms[20] persist for at least three months after finishing the cure[21]. The reactivity to chemical mediators (acetylcholine and adrenaline) as well as the one of correlated ions with autonomic activity (Ca, Mg, K) is increased after external treatment with mud and the vegetative tone has a regulary / normalization tendency, which leads to vegetative stigmas remedying[16].

C. Immunological reactivity shows an increase of antimicrobial defense capacity through the increased phagocytic power of leukocytes.

In addition, it was also revealed an anti-inflammatory effect of about 50% of the diclofenac effect, for both peat extract and sapropelic mud[18]. So, studies have shown the involvement of mud in specific and nonspecific immune response and in combating inflammation.

Other studies had demonstrated that mud thermotherapy influence serum levels of several cytokines involved in the chondrocyte metabolism and pathogenesis of arthrosis by modulating their production: IL1, TNFα (whose growth is correlated with the presence of inflammatory phenomenon), IGF1 (whose growth has protective influence on cartilage)[22].

The decrease of prostaglandin PGE2 and leukotriene LTB4 levels (compounds with potential pro-inflammatory and painkiller properties) in the blood of arthritic patients undergoing treatment with 12 packs of mud leads to the conclusion that the application of mud exerts a protective effect on cartilage and is able to induce less pain by decreasing inflammation[20]. The anti-inflammatory effect of mud appears to be pretty obvious. The endocrine mechanism is also involved in the induction of anti-inflammatory effects of fango-therapy by stimulating the hypothalamic-pituitary-adrenal axis and by equilibrating overall balance of endocrine state persistent and post-cure, and the thermal factor also intervenes by stimulating the defense processes, as discussed in thermal factor action.

Balneotherapy has proved its efficiency through multiple studies conducted on the principle of evidence-based medicine in many other conditions: OA, fibromyalgia, spondylitis, low back pain[23-26], and the effects are remanent while there is evidence of their effectiveness for up to 6 months[23,27].

III. PURPOSES AND OBJECTIVES

The purpose of this paper is to assess the dynamics of plasma cytokines - leptin and adiponectin - under the action of mud therapy in patients with knee OA in order to bring new arguments regarding the pathogenesis of this disease and to identify possible predictive factors involved in further development.

The main objective of this study is:

- **The assessment of the impact of mud therapy on leptin and adiponectin plasma levels**. For this purpose we evaluated in the first stage the leptin and adiponectin using ELISA Technique on the first day and after 10 days of treatment, on all 3 groups, and compared adipokines variation between the groups.

The secondary objectives of this research were:

- **The clinical and functional assessment of the effectiveness of mud therapy and the identification of the interrelations between adipokines and clinical - functional parameters**. To achieve this objective, I evaluated the WOMAC index, Lequesne algo- functional index and pain scores using visual analog scale (VAS), their evaluation was done dynamically, in the first and last day of treatment. All three parameters were analyzed in correlation with the serum levels of leptin and adiponectin.
- **The assessment of the impact of leptin and adiponectin variation on the main pathogenic links in OA**. For this purpose, we determined blood biochemical parameters: erythrocytes sedimentation rate, blood glucose, uric acid, total cholesterol, triglycerides, HDL cholesterol, total lipids. With the same purpose, we analyzed demographic and bio-psychosocial characteristics of the subjects included in the study: gender, age, educational level, age of disease, frequency of treatments at Balneal Sanatorium and Rehabilitation Techirghiol, intensity of physical activity made during life, body mass index and the existence of metabolic syndrome.

IV. MATERIALS AND METHODS

METHOD OF ACHIEVING THE RESEARCH PROJECT

This paper is based on the study of three groups of patients, 78 subjects in total, who have had a complex balneal and physical treatment during 2010-2011 at Balneal Sanatorium and Rehabilitation Techirghiol. All subjects signed an informed consent form for giving their consent to participate in this study.

The groups were divided as follows:

- control group (CG) consisting of 23 patients who were undertaking the following treatment: a herbal bath per day day, three adjuvant procedures per day, a massage therapy session per day and daily physical therapy sessions;
- warm mud bath group (WMB), consisting of 32 patients, was subject to the following treatment: daily mud bath alternated with salty bath (the pool or tub), three adjuvant procedures per day, a massage therapy session per day and daily physical therapy sessions;
- cold mud bath group (CMB), consisting of 23 patients, performed daily cold packing of mud (general progressive heliotherapy, mud packing, immersion in the lake), three adjuvant procedures per day, a massage therapy session per day and daily physical therapy sessions.

Thus, the difference between the three groups is the application method of the main hydro-thermo-therapeutical procedure, meaning that the control group did not perform treatment with specific natural factors from Techirghiol area, while the other two groups were exposed to natural therapies, the difference between them consisting of how these therapies where applied.

Patients from all 3 groups were evaluated at admission and at the end of the treatment at the same moment of the day, knowing that some constants bio humoral have a certain circadian variation in plasma levels[28,29]. The admission evaluation, before the starting the treatment, included: completing the informed consent, completing the demographic questionnaire - age, sex, education, duration of disease, frequency of balneal treatment at Balneal and Rehabilitation Sanatorium Techirghiol, BMI, metabolic syndrome X, physical activity done during life, algo-functional Lequesne index, WOMAC index, VAS. In para-clinical terms there were

dosed at admission: leptin, adiponectin, ESR, CRP, RF (latex), CBC, uric acid, glucose, HDL-cholesterol, total cholesterol, triglycerides and total lipids.

At discharge, patients were reassessed both clinically – algo-functional Lequesne index, WOMAC index, VAS and para-clinically: leptin, adiponectin, ESR, uric acid and glucose.

Blood samples were collected at both times respecting a digestive rest for 12 hours and were transported in optimal cold conditions at 2-8^{0}C, and processed in first 24 hours in Medgidia SC Micro Medical Ltd. laboratory, which holds a RENAR accreditation. Specific reagents were used to determine human leptin and adiponectin using Eliza technique and specific reagents for other biological dosages. ELISA (Enzyme Linked Immunoabsorbent Assay) is a immunological technique for detection of circulating antibodies and antigens. The method exploits the property of antibodies to specifically bind with certain chemical compounds called antigens. In the direct method the primary antibody for recognition of the antigen is bounded with the signal generating enzyme. In the indirect method the recognition antibody titrates first and then the conjugated antibody with enzyme.

Normal values of specific analyzer ELISA kit used are:

- Leptin: in women: 3.63 to 11.09 ng / mL,
  in men: 2.05 to 5.63 ng / mL
- Adiponectin: 1.56 to 100 ng / mL.

Inclusion criteria in the study for the subjects were: patients diagnosed with primitive osteoarthritis according to ACR criteria, regardless of the stage of disease, age between 30 and 80 years old. We chose as a criteria for inclusion knee osteoarthritis, because it is the most common location of osteoarthritis, hoping I will have no problems in building up the study groups.

Exclusion criteria of the research were: ESR>40mm/h; people with skin disorders (chronic or acute), including continuity solutions; serious cardiovascular, respiratory, digestive, renal, neurological; cancer; presence of inflammatory rheumatic conditions; necessary non-steroids and steroids for at least 4 weeks before study entry; surgery (arthroplasty, arthroscopy, abrasion, etc) performed at least 3 months before the study; managing intra/peri-articular corticosteroids for at least 6 weeks before study 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, anticonvulsants or muscle relaxants at least 2 weeks before study entry.

Out of the 92 patients who were initially evaluated according to the protocol described above, 14 patients were excluded because they had one or more exclusion criteria (significant abnormalities ESR, CRP, RF, CBC), the present thesis comprising a total of 78 patients distributed in three groups previously characterized.

The statistical analysis of the present study used SPSS 18.0[30].

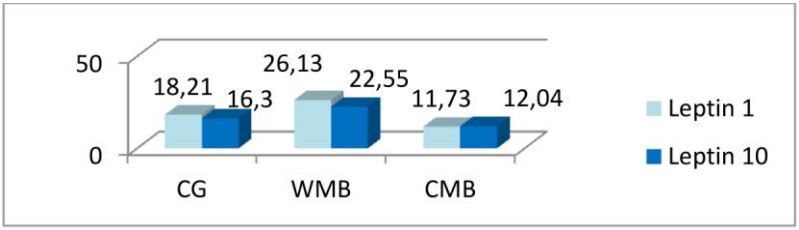
V. RESULTS AND DISCUSSION

ASSESSMENT OF THE IMPACT OF BALNEAL THERAPY IMPACT ON LEPTIN AND ADIPENCTIN PLASMA LEVELS

LEPTIN

Leptin is a 16 kDa non-glycosylated hormone, secreted mainly by adipocytes.

I dosed the leptin both in the moment of inclusion in the project-day 1 and after 10 days of treatment (**Graphic No. 1.).** Blood samples were collected at both times respecting a digestive rest of 12 hours, at certain moment of the day, knowing the circadian variation of leptin[31-33].



Graphic No.1. Comparative graphical representation of the average values of leptin.

Comparative analysis of the groups revealed that leptin registered a statistically significant decrease in the control group (**p=0.019<0.05**) and WMB (**p=0.010<0.05**), while in the CMB group (**p=0.773>0.05**) was increased, but not statistically significant. We suppose that the lack of leptin variation in CMB group is due to the fact that cold application is thermal contrasting, stressful load (thermal contrast is done 4-5 times during the 2 hours that the application lasts) and it is added to other stress factors of balneal cure: climate, physiotherapy, electrotherapy, massage. Under the action of thermal contrasting compound, the thermoregulatory function is optimized in the “hypothalamic thermostat“ and it is secretor adapted with prompt responses. The thermoregulatory response depends on the peripheral "error" of hypothalamic set point. When the "error" value is reduced or the number of "errors" is low, then the adaptive response is reduced as well.

On the other hand, the application at neutral temperature does not require significant thermoregulation (groups CG and WMB) does not

generate stress by thermal differences, on the contrary, it is is gentle, soothing, because during bath application the average temperature remains almost constant. The thermal comfort during neutral temperature bath stabilizes hypothalamic function, does not require major neuro-endocrine responses, but is addressed to that area where physiological responses are minimal.

There are at least five forms of leptin receptors, the most distributed is its short form, which may serve to transport leptin into the brain. The long form of the receptor is located in areas where it is believed that leptin acts, including hypothalamic nuclei[34]. Recent data indicate that the hypothalamus is a direct target of leptin action. Leptin produced in fat cells cross the blood brain barrier and interact with leptin receptors in the hypothalamic arcuate nucleus, and thus controls the expression of Thyroid Stimulating Hormone, melano-stimulating hormone and gamma-aminobutyric acid, among other mediators, which ultimately will modulate the metabolic response, behavior and autonomic nervous system[35].

It is clear that there is a link between body energy reserves (fat) and the set point control (hypothalamus), meaning that the hypothalamus is a target of leptin action in respect of food intake[36,37]. After analyzing the results obtained from CMB group, where there is a slight increase of leptin values, statistically insignificant, we hypothesize that there is an inverse relationship as well, meaning that the hypothalamus could be involved in the adaptation of leptin levels, this relationship having a component related to the thermoregulation mediated by the hypothalamus. In support of this hypothesis comes the method of reaction of CMB group, which has undergone contrasting therapy, stimulating the hypothalamus activity, but this hypothesis requires further research.

ADIPONECTIN

Adiponectin is a protein of 244 kDa that is produced mainly by adipose tissue[6], which is part of the adipokines family, but unlike leptin, it has been studied much less, at present time, its roles in the body are extremely controversial.

Also as the main objective of this research I set the dosage of adiponectin at the population studied, both at the moment of inclusion in the project- day 1 and after 10 days of treatment (**Graphic No. 2.**). Blood samples were collected at both times respecting a digestive rest of 12 hours.

Graphic No. 2. Comparative graphical representation of the average values of adiponectin.

After 10 days of treatment there are no statistically significant differences in the variation of this biohumoral parameters in CG (**p=0.852>0.05**) and the variation of adiponectin levels in WMB group (**p=0.013<0.05**), which supports the hypothesis that warm mud is a real benefit in addressing the pathological conditions, because it decreases inflammation.

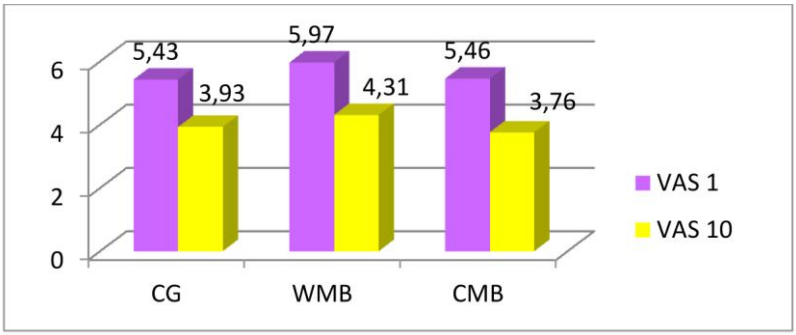
Adiponectin acts via two receptors, the first (AdipoR1), which is found predominantly in skeletal muscle and the second (AdipoR2) which is found in the liver[38,39]. The biological effects of adiponectin are mediated primarily through these two receptors, that are believed to activate different signaling pathways[40,41]. In human cartilage are expressed both AdipoR1 and AdipoR2, but it seems they have a heterogeneous distribution in chondrocytes[42], which could explain the different reactivity of the 3 groups in terms of variation in adiponectin values.

Another subject very little studied until now, is the involvement of the hypothalamus in the expression of adiponectin effects. Adiponectin may act on the brain by increasing energy expenditure and reduce body weight by mechanisms that are not entirely known. Is considered that both adiponectin receptors are expressed in warm sensory neurons from the preoptic hypothalamic area, having a critical role in regulating basal body temperature and energy balance[39]. The way how they are regulated by the hypothalamus is not yet understood and requires further studies . Values obtained in the present study may plead to the hypothesis that there is an interrelation hypothalamus-adiponectin, by the pathway of thermoregulation because the two neutral thermal applications (CG and WMB groups) results are different from CMB group where it was registered an increase in the values of the 21.47 ng/mL to 23.76 ng/mL, this group being under the

complex contrasting thermal action. Although the role of adiponectin in OA is controversial, recent studies[43] support the pro-inflammatory role of adiponectin in this pathological condition. In this regard I hypothesize that in terms of inflammation decrease, warm mud bath act on this pathogenic link, unlike the cold mud bath.

CLINICAL AND FUNCTIONAL ASSESSMENT OF BALNEAL TREATMENTS AND IDENTIFYING INTERRELATIONS BETWEEN ADIPOKINES AND CLINICAL AND FUNCTIONAL PARAMETERS

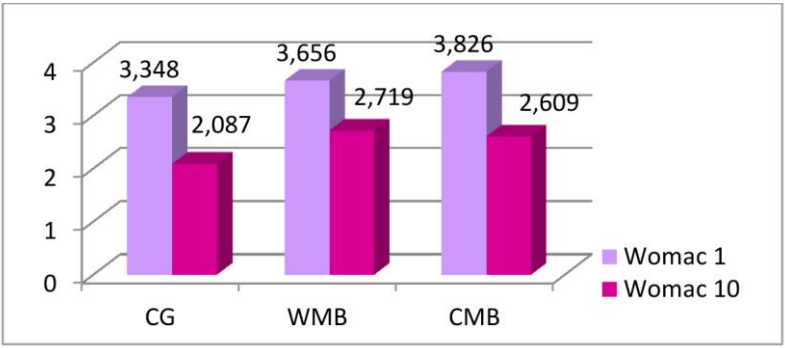
1. VISUAL ANALOGUE SCALE (VAS)



Graphic No. 3. Comparative graphical representation VAS values on day 1 and day 10.

In all 3 groups analyzed I observed a high, statistically significant decrease in pain level, subjectively appreciated by patients (**p <0.0001**), which brings strong evidence for the clinical effectiveness of complex the physical and balneal treatment, regardless of the method of application. The results obtained (**Graphic No. 3.**) are consistent with studies in international databases showing statistically significant pain reduction measured by VAS, at application of non-pharmacological complementary therapies of OA[44-46].

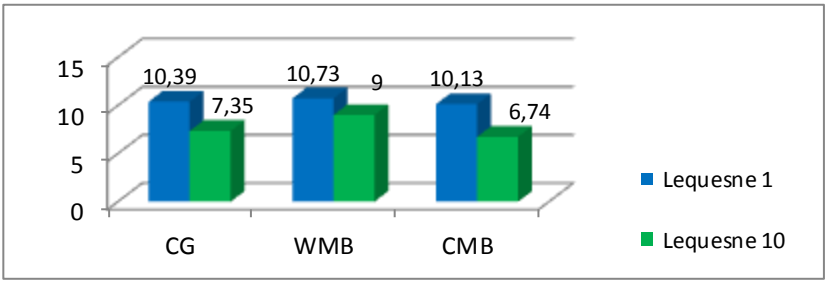
2. WOMAC INDEX



Graphic No.4. Comparative graphical representation WOMAC index on day 1 and day 10.

In all 3 groups analyzed (**Graphic no. 4**) I observed a high, statistically significant decrease joint stiffness , subjectively appreciated by patients (**p <0.0001**), which brings strong evidence for clinical effectiveness of the complex physical and balneal treatment, regardless of its method of application. Analyzing data from the literature, I found that there are numerous studies that certify balneal therapy in knee OA, having strong evidence of improving the patients' quality of life [47,48].

3. LEQUESNE INDEX



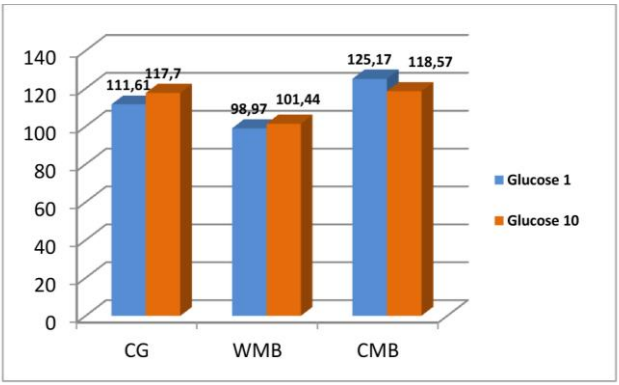
Graphic No. 5. Comparative graphical representation Lequesne index on day 1 and day 10.

In all 3 groups analyzed, we observed a high, statistically significant decrease in Lequesne index value, subjectively appreciated by patients (**p <0.0001** in CG and CMB groups , **p<0.05** in group WMB),

which brings strong evidence for the clinical effectiveness of the complex physical and balneal treatment, regardless of the method of its application. The results obtained (**Graphic No.5.**) are consistent with studies in international databases that reveal statistically significant improvement in the Lequesne index at application of non-pharmacological complementary therapies of OA[44,46-48].

ASSESSMENT OF THE IMPACT OF LEPTIN AND ADIPONECTIN VARIATION ON MAIN PATHOGENIC LINKS OF OSTEOARTHRITIS

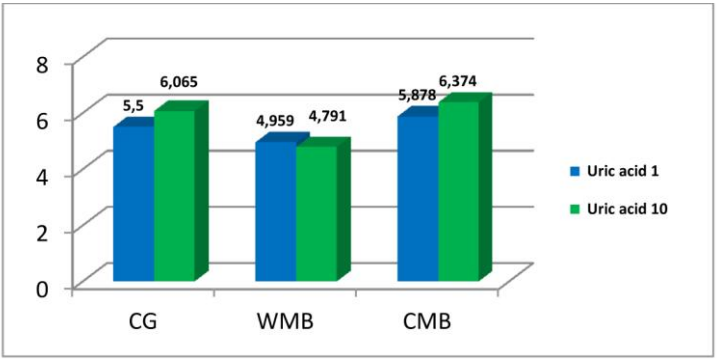
1. GLUCOSE



Graphic No. 6. Comparative graphical representation of the average blood glucose.

As a result, balneal therapy application does not change the glycemic status in any of the analyzed groups (**Graphic No.6.**), which highlights the positive aspect of balneal therapy, the protective role of glucose in oxidative stress being well known.

2. URIC ACID

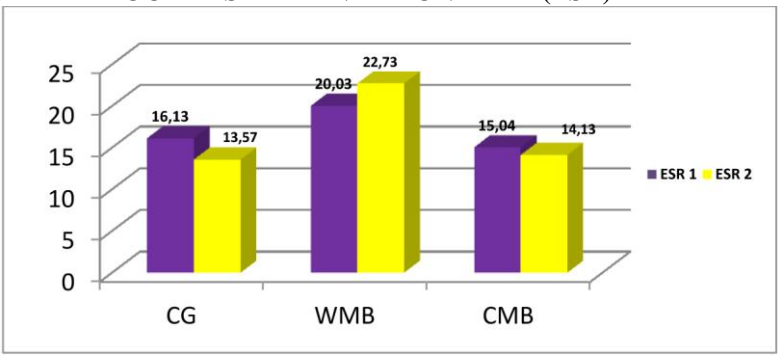


Graphic No.7. Comparative graphical representation of the average uric acid registred.

Also the role of uric acid is highly controversial at this time. Many epidemiological studies have revealed an interrelation between uric acid and various disorders like obesity, metabolic syndrome, hypertension and coronary artery disease. What is certain is that uric acid is a marker of oxidative stress, because it can inhibit endothelial function by inhibiting NO function[49].

Uric acid level is kept constant at WMB and CMB groups (**Graphic No.7**), but has a statistically significant decrease in group CG (**p=0.046<α=0.05**). These results strongly support the hypothesis that the peloid is anti-inflammatory and condroprotector, given the protective role of uric acid in oxidative stress.

3. ERYTHROCYTE SEDIMENTATION RATE (ESR)

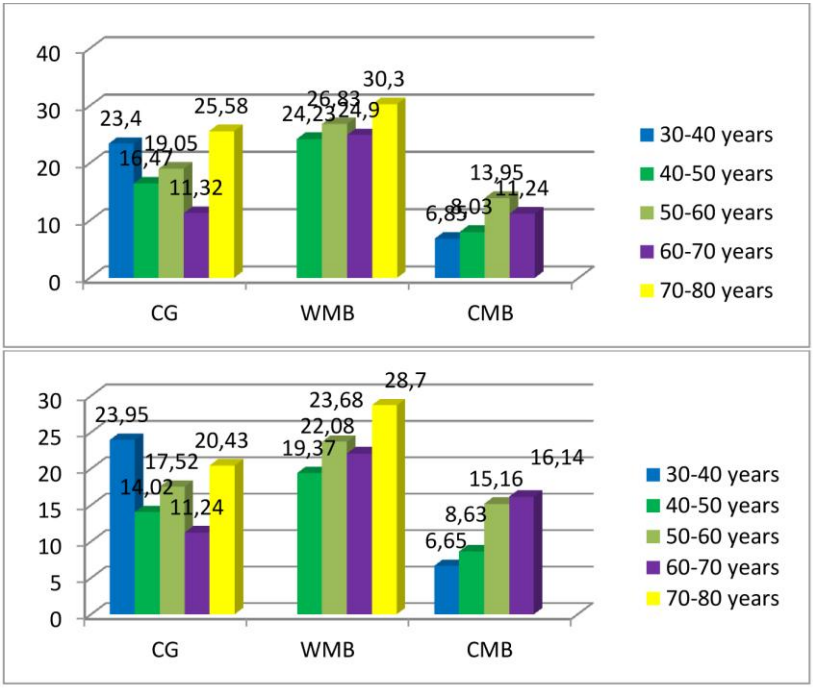


Graphic No. 8. Comparative graphical representation of the average values of ESR.

Sedimentation hemoglobin level is not modified by the therapeutic application with cold or hot mud in any of the patients, nor to the patients in the control batch, who did have any therapeutic application of mud (**Graphic No.8**). I must mention that the ESR value was an exclusion criterion in the present study, the maximum was 40 mm/h. The results obtained in this thesis are consistent with the international literature studies that show a weak correlation between OA and the ESR value[50].

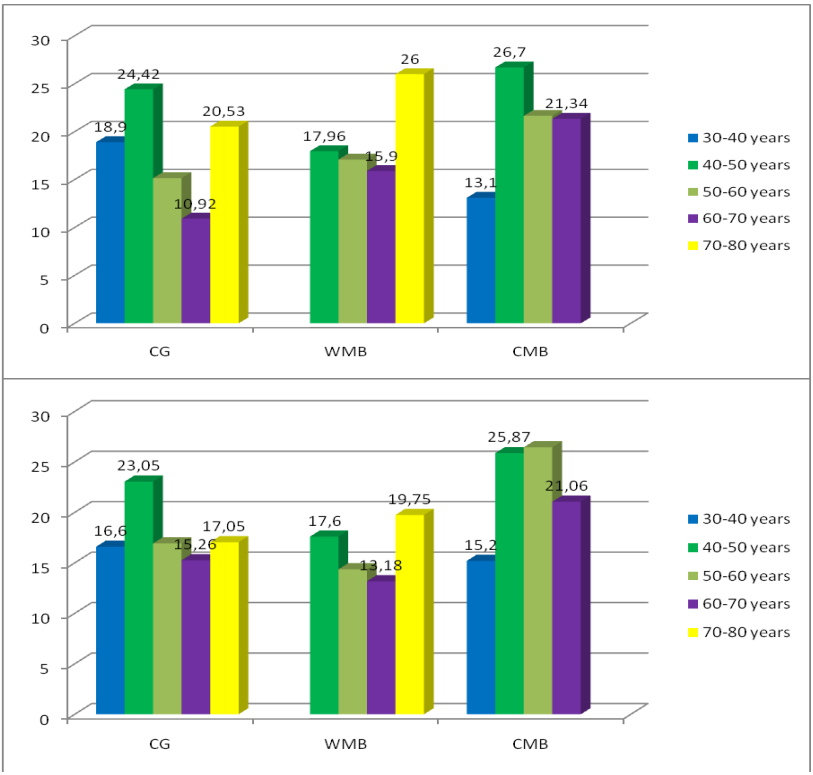
4. CORRELATION BETWEEN VARIATION IN SERUM LEPTIN / ADIPONECTIN AND AGE

The highest values of leptin are found in the highest age groups.



Graphic No. 9 Graphical representation of average values of leptin 1 and 10 by age.

I noticed that also in day 10 the same distribution of leptin is maintained, according to age ranges, but having lower values than the first day for CG group and WMB group. In CMB group there is a slight increase in leptin values (**Graphic No.9.**).

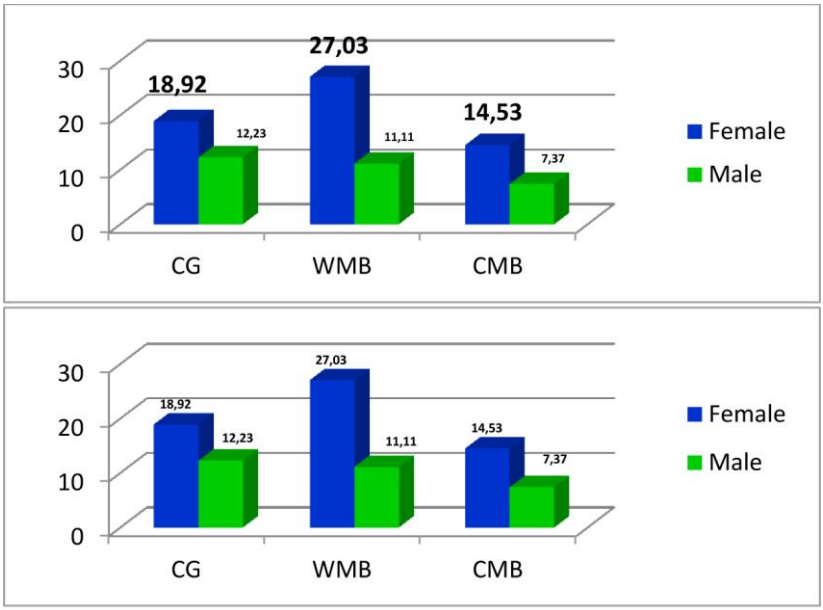


Graphic No.10. Graphic representation of adiponectin values 1 and 10 by age.

I did not find any correlations between adiponectin value on the first day, depending on age intervals, probably because in all 3 groups studied predominate females, while men over 70 years old category is under-represented (**Graphic No.10.**).

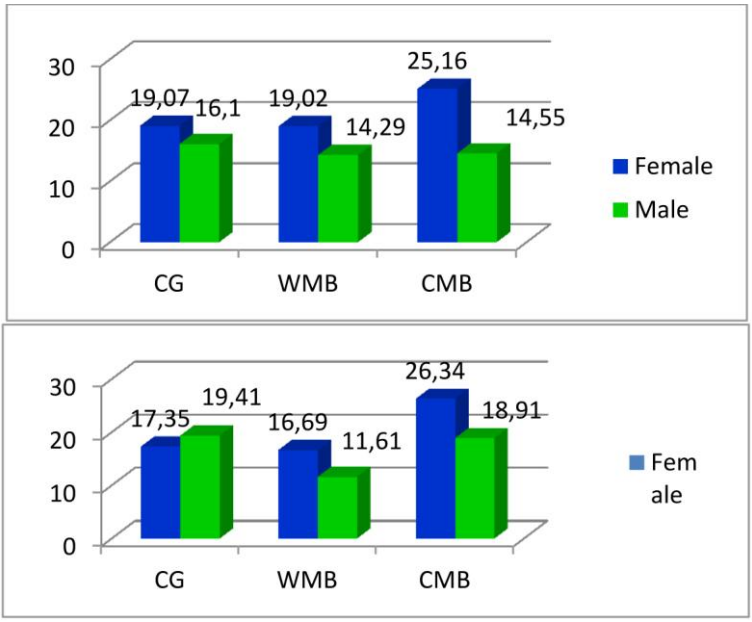
I can also state that, after 10 days of treatment,the adiponectin does not comply with age groups, but an increase in values can be noticed in CMB group. This observation comes to argue that contrast therapy performed for CMB group , has a protective role for cardiovascular disease, the proof being in the increase of average values of adiponectin.

5. CORRELATION BETWEEN VARIATION IN SERUM LEPTIN / ADIPONECTIN AND SEX.



Graphic No.11. Graphical representation of average values of leptin 1 and 10 by gender.

Average values of leptin were found to be higher in females than in males, this distribution being maintained also after 10 days of treatment (**Graphic No. 11**).



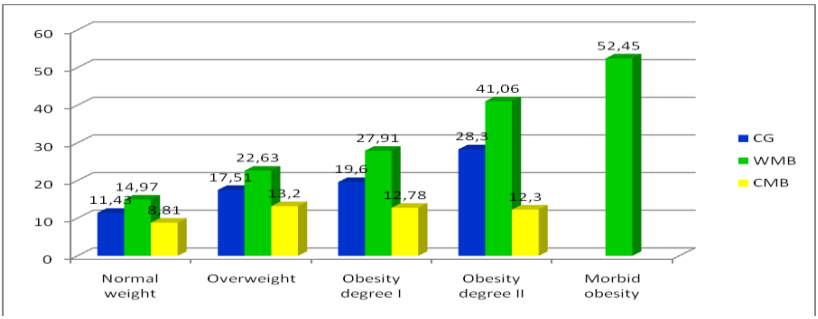
Graphic No. 12 Graphical representation of average values of adiponectin 1 and 10 by gender.

Also, adiponectin on day 1 is higher in women than in men (**Graphic No. 12.**), but at the end of the balneal cure, the same distribution is not mentained in CG group, suggesting that peloid is involved in the pathogenesis of OA.

6. CORRELATION BETWEEN VARIATION IN SERUM LEPTIN / ADIPONECTIN AND BODY MASS INDEX (BMI)

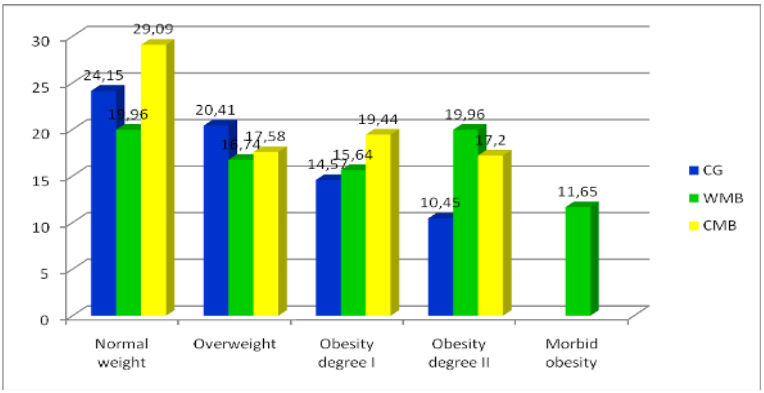
It has been debated whether arthritis by the hypomobility and sedentary lifestyle that it induces, favorizes obesity, or if, on the contrary, it is reversed, that obesity favors the appearance of osteoarthritis. Studies seem to tip the balance in favor of the last hypothesis, showing that weight reduction slows down the osteoarthritis process. The association between osteoarthritis and obesity is more evident in women and is related mainly to its knee location (where it causes bilateral damage), less than that of the hip and not at all at hands. The mechanism is not fully elucidated. The first and easiest explanation would be that of mechanical overload, but this only, does not explain the differences between osteoarthritis (closely associated with obesity) and cox-arthrosis (for which there is no obvious association).

Lately, there are more and more discussions about the predominant involvement of metabolic factors and not of mechanical factors, aspect that I intend to investigate by trying to identify if there is a relationship of interdependence between leptin, adiponectin and index body mass.



Graphic No. 13. Graphical representation of leptin on admission according to BMI classes.

On addmission is has been noticed that (as shown in **Graphic no. 13.**) there is a linear relationship between leptin levels and weight patients measured in BMI classes, the most significant example is the increased average leptin level (52.45 ng/mL) registered in subjects with morbid obesity.



Graphic no. 14. Graphical representation of adiponectin on admission according to BMI classes.

On addmission is has been noticed that (as shown in **Graphic No. 14.**) that there is an inverse relationship between adiponectin levels and

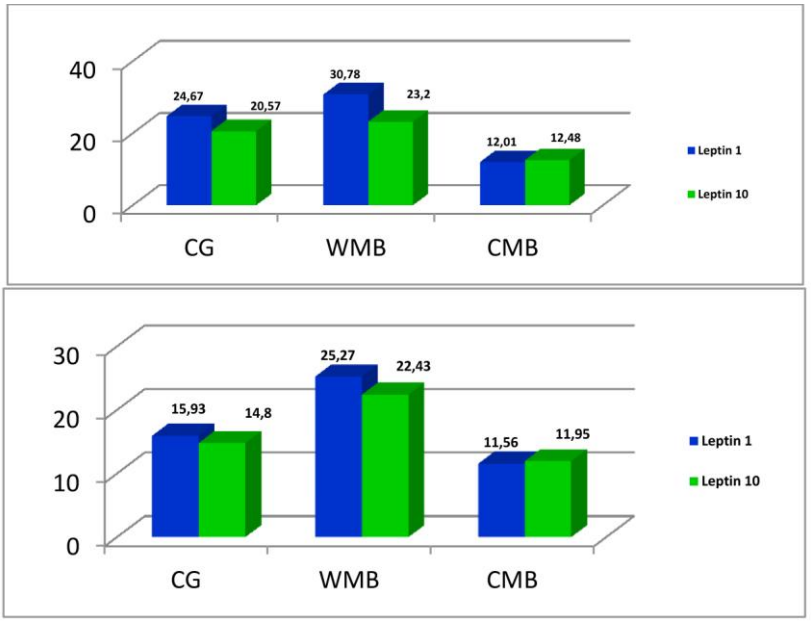
overweight patients measure in BMI classes, the most obvious example being the low average adiponectin level (11.65 ng/mL) registered at subjects with morbid obesity.

7. ANALYSIS OF LIPID PROFILE IN CORRELATION WITH LEPTIN AND ADIPONECTIN VARIATIONS

Obesity is a multifactorial disease resulting from the combination of multiple factors: genetic, environmental, psychological, social and cultural. It is considered a major public health problem that is associated with insulin resistance, diabetes, hyper-tension, dyslipidemia and coronary artery disease. At this moment, white adipose tissue is considered to be a secreting endocrine organ.

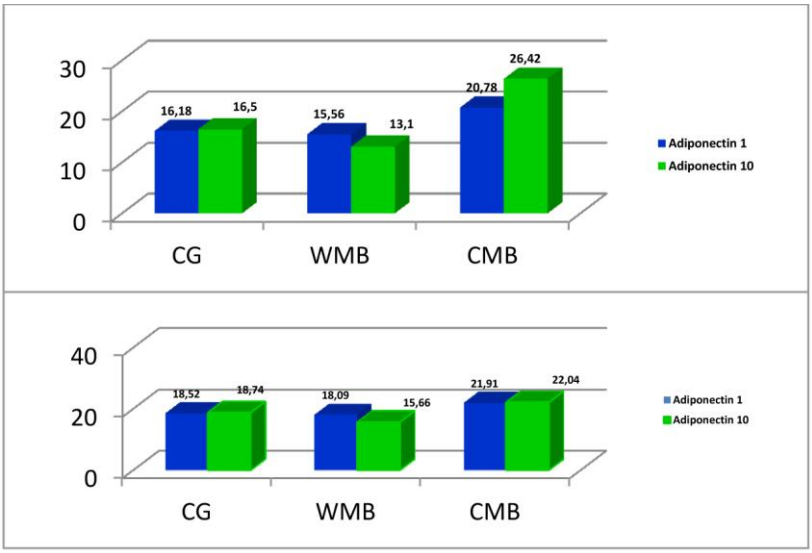
I wanted to analyze the lipid profile in relation to the leptin and adiponectin variations in balneal treatment because there is no study in the literature on this topic. Analyzing the results of my study I found that there is no obvious correlation between the variations of leptin and adiponectin levels and lipid profile of the subjects examined, except for the adiponectin in relation to HDL-cholesterol, which is consistent with data from the literature, providing an additional argument for the protective role of adiponectin in cardiovascular disease. In the literature, data related to this aspect are poor.

8. CORRELATION BETWEEN VARIATION OF SERUM LEPTIN AND ADIPONECTIN LEVELS AND METABOLIC SYNDROME



Graphic no. 15. Comparative graphic representation of leptin variation depending on metabolic syndrome.

It is clear that in patients with metabolic syndrome, the average level of leptin are significantly higher than in patients without metabolic syndrome, both in day 1 and day 10. Also, in patients with metabolic syndrome present, there is a greater decrease in leptin values in all 3 groups compared with subjects without metabolic syndrome (**Graphic no. 15.**).



Graphic no. 16. Comparative graphic representation of the variation in adiponectin levels according to metabolic syndrome.

It is clear that in patients with metabolic syndrome, average values of adiponectin, are significantly lower than in patients without metabolic syndrome, both on day 1 and day 10, excluding the values registered in CMB group, where it is observed that after 10 days treatment adiponectin value 10 is higher in patients with metabolic syndrome present (**Graphic no. 16.**). I must mention that these results are consistent with the results presented in international databases.

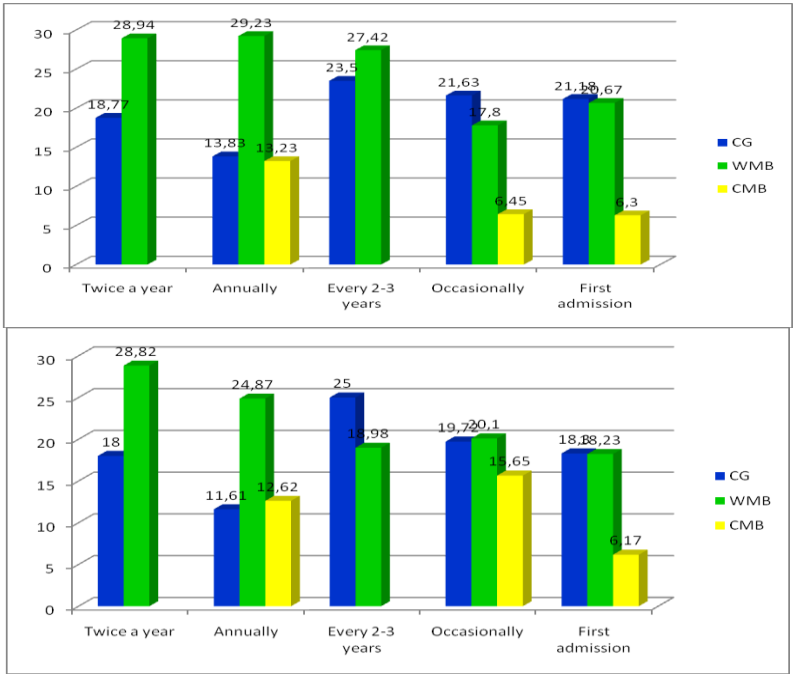
9. CORRELATION BETWEEN VARIATION SERUM LEPTIN AND ADIPONECTIN LEVELS AND PHYSICAL ACTIVITY THROUGHOUT LIFE

According to the analysis that I concluded, the average values of leptin on day 1 and after 10 days of treatment in CG are declining once the physical activity done during life increases. There is no such correlation in the other two groups. There is no data in the literature dealing with this aspect.

It is revealed that the average values of adiponectin both at the start of this research project as well as after 10 days of treatment have no correlation with the degree of physical activity made throughout life in any

of the 3 study groups. I did not find references in the literature related to this issue.

10. LEPTIN AND ADIPONECTIN VARIATION BY FREQUENCY OF TREATMENT AT BALNEAL SANATORIUM AND REHABILITATION TECHIRGHIOL (SBRT)



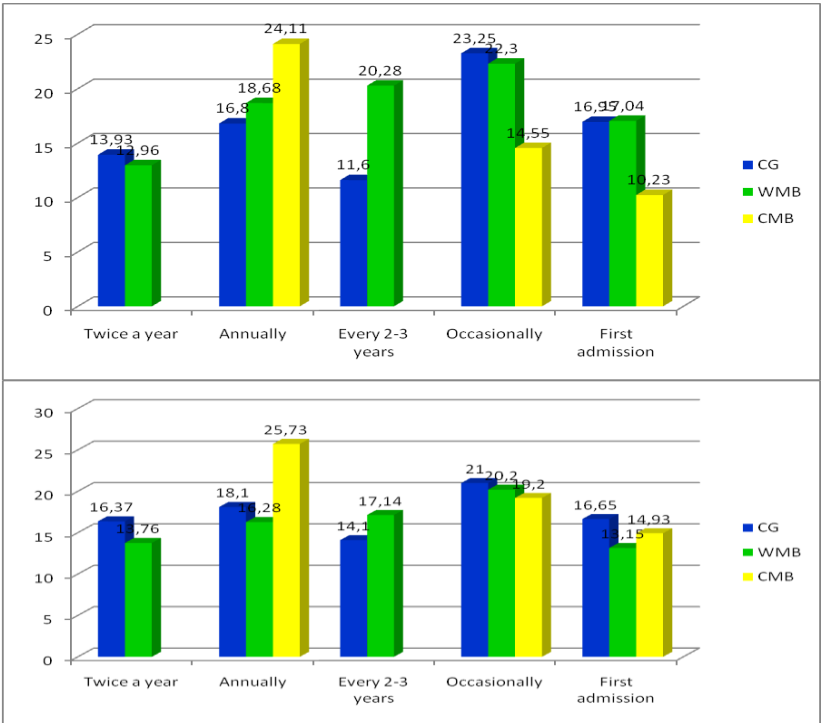
Graphic no. 17. Graphical representation of leptin 1 and 10 by treatment frequency in the SBRT

Analyzing leptin values on day 1 according to the frequency of treatment in SBRT, I noticed that they do not suit with any model of statistical correlation for none of the 3 groups studied. I also analyzed the leptin values in relation to the frequency of treatment after 10 days in SBRT (**Graphic no. 17.**). There is a general trend of decrease in leptin levels after 10 days of treatment as frequency of treatment decreases in this health unit.

Obviously leptin values after 10 days of balneal treatment tend to decrease proportionally with the decreasing frequency SBRT treatment, the best example being the group WMB: 28.82 ng/mL, 24.87 ng/mL,18.98

ng/mL these characterizing the leptin levels as per the research protocol on day 10 for subjects who received treatment twice a year, every year and every 2-3 years.

In patients who received treatment twice a year , leptin levels discretely decreased at the end of cure, compared to leptin levels at the time of admission for groups CG and WMB. (**Graphic no. 17.**). This discrete decrease of leptin values, can be explained by the fact that patients undergoing bi-annual balneal therapy (according balneal therapy protocols) have generally low values of leptin levels, leptin secretion is controlled by peloidtherapy, this way underlining the therapeutic chondroprotection value and the inflammation decrease of balneal therapy.

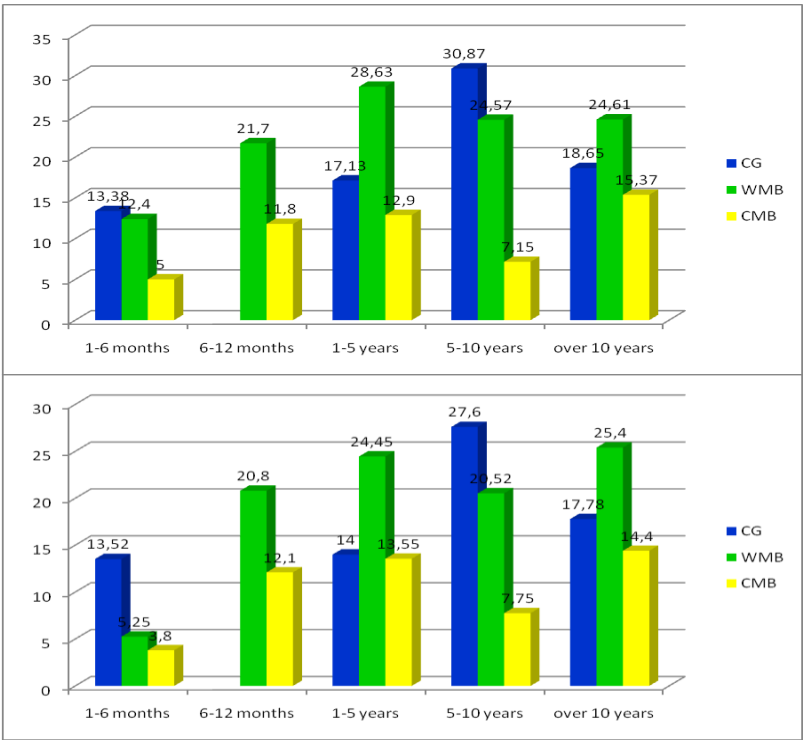


Graphic no. 18. Graphic representation of the variation in adiponectin 1 and 10 by SBRT treatment frequency.

Analyzing adiponectin values on day 1 according to the frequency of treatment in SBRT, I noticed that they do not suit with any model of statistical correlation for none of the 3 groups studied. (**Graphic no. 18.**).

After 10 days of treatment, there is no clear correlation between these two variables, except in CMB group, where average adiponectin on day 10 decreases as balneal treatments become more rare.

11. CORRELATION BETWEEN VARIATION IN SERUM LEPTIN AND ADIPONECTIN ACCORDING TO THE AGE OF DISEASE



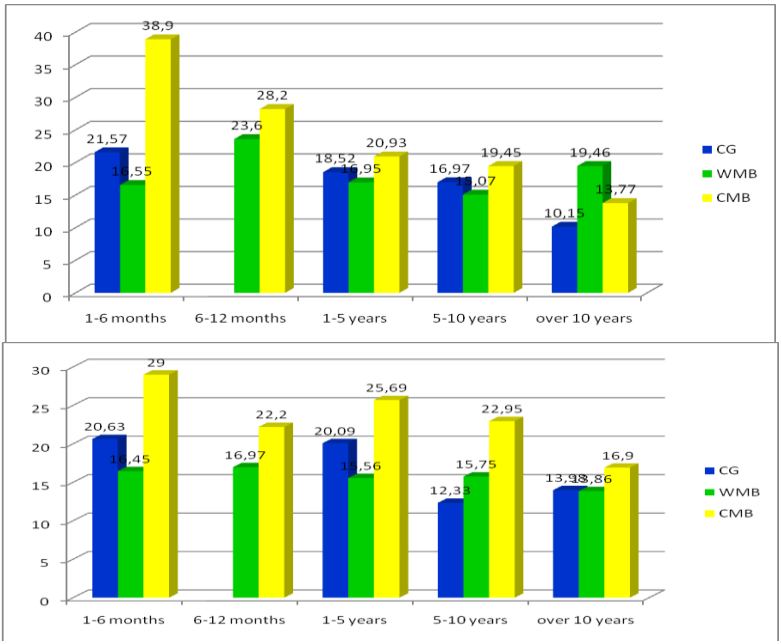
Graphic no. 19. Graphical representation of leptin 1 and 10 depending on the age of disease.

The highest average values of leptin were present in pain appearance within 5-10 years, we can probably hypothesize that the disease becomes more aggressive during this period (**Graphic No. 19.**) and inflammation is evident. After 10 days of treatment, I noticed a decrease in average level of leptin in the 3 groups, which reveals the fact that all

patients are responsive to balneal treatment regardless of duration of disease.

As illustrated in **Graphic No.19**, the most obvious decrease in leptin levels occurred in patients with occurance of pain in the last six months, and as the duration of disease increases, leptin levels decrese discretely.

In addition to other parameters studied, I aimed to determine whether there is any correlation between knee OA age (age is measured by time of occurrence of pain) and adiponectin variation in the balneal treatment. Analyzing adiponectin values at admission correlated with the time of occurrence of pain, there is a decrease tendency in relation to the duration of disease in CG and CMB groups, except in group WMB, which does not reveal any obvious statistical correlation.



Graphic no. 20. Graphical representation of adiponectin 1 and 10 depending on the age of disease.

By Analyzing **Graphic No. 20.,** I found that the highest adiponectin values registered at the beginning of balneal cure, are found in the group of patients with an occurrence of pain in the last six months,

which argues for its pro-inflammatory effects. I also analyzed adiponectin values after 10 days of treatment according to the protocol research in all 3 groups and I concluded that there is no clear statistical correlation between the behavior of this biohumoral parameter and the duration of disease.

VI. CONCLUSIONS

Introducing a new concept about white adipose tissue, as being a very dynamic organ with complex metabolic roles in the human body launches new challenges to scientific progress in medicine for the understanding of the pathogenesis of osteoarthritis. Recent progress in the study of the physiology of white adipose tissue revealed that adipocytes produce a variety of biologically active factors involved in the genesis and progression of osteoarthritis generically called adipokines. On the other hand, it is known that diseases have appeared in the same time with the man who instinctively learned to treat his suffering with elements that surrounded him: water, heat, plants. Nature was the first to offer help the sick man while being in the same time doctor and therapeutic remedy. Balneotherapy, dating "since the world and earth" has archaeological evidence ever since the Bronze Age, being for many people in ancient times therapeutic method with spiritual purifying and healing role of the body.

The originality of this research paper results from key factors that appear to play a major role in the pathogenesis of osteoarthritis, leptin and adiponectin, latest generation adipokines, and their behavior under the effect of balneal therapy, treatment method with a consecrated age, that improved over time, in order to achieve maximum benefit for the management of osteoarthritis patients.

For drawing conclusions about the variations in leptin and adiponectin in peloidtherapy, I grouped the results obtained according to the goals that I have set. Where data was found in the literature in Romania or abroad, I have compared the results obtained in this study with the already existing results.

1. The assessment of the impact of peloidotherapy on leptin and adiponectin plasma levels.

Leptin: Variations in plasma levels of leptin show a differential response depending on the type of balneal application. Warm mud application significantly decreases leptin level **p=0.010**, this decrease can be interpreted by reducing inflammation and rheumatic pain. I found a statistically significant decrease in the control group **p=0.019**, which I interpret also in this context of decreasing inflammation and pain, although these patients were not submitted to natural factors. Cold mud application as

a contrasting form of therapy increases leptin values at the end of the cure, not statistically significant, **p=0.773**. I assume that the lack of leptin variation to the group who did contrasting therapy is due to the intervention of the thermoregulatory function which is significantly required at cold application, while the thermal comfort during thermo-neutral bath stabilizes hypothalamic function and do not require major neuroendocrine responses. Recent evidence indicates that the hypothalamus is a target of leptin action, which crosses the blood brain barrier and interacts by its receptor in the arcuate nucleus of the hypothalamus, controlling in this way the expression of thyroid hormone, melanostimulatinghormone and gamma-aminobutyric acid, which will eventually modulate the metabolic responses of the body. In this context we hypothesize that not only leptin acts on the hypothalamus, but also that there is an inverse relationship meaning that the hypothalamus could be involved in adjusting the leptin levels, this relationship being linked to thermoregulation mediated by the hypothalamus. Further research study is needed to support this hypothesis. In the only similar study of literature exists till now, leptin has registered a statistically insignificant increase.

Adiponectin: Similarly with variation leptin, the adiponectin has a different behavior as well, depending on the type of balneal application used. Following thermo-neutral application of mud, we registered a statistically significant decrease in the average level of adiponectin **p=0.013**, which supports the hypothesis that warm mud is a real benefit in approaching these pathological conditions. Plasma levels of adiponectin at the end of therapy with cold mud and herbal baths is statistically insignificant increased after 10 days of treatment. Different reactivity of the three groups can be explained through the heterogeneous distribution of adiponectin receptors on chondrocytes or the complexity of therapeutic agent used, because the cure takes place in a seaside resort with a climate different from that of the place of residence of the patient, changing their daily activities and diet. It is difficult to quantify the effect of balneal cure. We do not exclude the involvement of the hypothalamus also in expression of adiponectin effects. It is known that there is a relationship between the hypothalamus and adiponectin, but how to define this relationship is far from being elucidated.

Although at this moment it cannot be stated with certainty whether adiponectin has a pro-inflammatory or anti-inflammatory effect, most of the evidence is supporting its anti-inflammatory role. In the only similar study in the literature so far, the same statistically significant decrease is found in applying warm mud baths which argues for its pro-inflammatory effects.

2. Clinical and functional assessment of the effectiveness of balneal therapy and identifying the interrelations between adipokines and clinical and functional parameters.

Unlike leptin and adiponectin variations, clinical and functional evaluation parameters that I used in this thesis do not vary differently depending on the type of application used. For VAS and WOMAC index there is a highly significant decrease (**p<0.001**) in all three groups, regardless the method of treatment application. Lequesne algo-functional index decreased statistically significant (**p<0.001**) for groups CG and for CMB, but for WMB group p value is lower than 0.05 but is also statistically significant.

All these represent strong evidence for the clinical and functional effectiveness of complex balneal-physical therapy, regardless of its method of application.

to determine whether there is correlation between leptin and adiponectin values at both time frames and variations of these three clinical-functional parameters, I concluded that there are no strong correlations between them. Analyzing the report adiponectin/leptin correlated with the three clinical parameters (this has been proposed as a predictor of pain) I found a strong correlation between this ratio and the clinical parameters than the two adipokines considered individually.

3. Assessment of the impact of leptin and adiponectin variation on the main pathogenic links of OA

- Balneal therapy does not alter glycemic status and there is no correlation between the variations in leptin and adiponectin with and the variation in glucose in blood during the 10 days of treatment.
- Uric acid level is kept constant during balneal therapy, beneficial effect, given its protective role in oxidative stress. Also there is no obvious correlation between leptin and adiponectin variations in relation to variations in uric acid level.
- Erythrocyte sedimentation rate does not change significantly during balneal therapy, and there cannot be identified any statistically significant correlations between variations in leptin and adiponectin with the variations in erythrocyte sedimentation rate.
- The leptin level is directly proportional to age. Adiponectin does not correlate with age groups.
- Average leptin levels are higher in women than in men. There is no interrelation between the average values of adiponectin and sex of patients.

- Average leptin level increases in the same direction as the body mass index. Adiponectin varies inversely with patient weight measured by body mass classes. Analyzing dynamically the average values of leptin in obesity according to classes classes, I noticed that as body mass index class is greater leptin decreases more and more after 10 days of balneal treatment . Variations in adiponectin after 10 days of treatment are independent of body mass index classes.
- There is no correlation between leptin and adiponectin values and the parameters of lipid status of human body (total cholesterol, HDL-cholesterol, triglycerides and total serum lipids), except for adiponectin in relation to HDL-cholesterol, which comes in line with data literature, providing an additional argument for the protective role of adiponectin in cardiovascular disease.
- Average leptin values are higher in patients with metabolic syndrome compared to those without metabolic syndrome. Average adiponectin values are lower in patients with metabolic syndrome.
- I found no correlations between variations in leptin and adiponectin and physical activity done during life.
- Analysing the variation of leptin according to the frequency of treatment frequency at Balneal Sanatorium and Rehabilitation Techirghiol I concluded that patients who are treated bi-annually and annually are most responsive to balneary therapy, emphasizing in this way the chondroprotection therapeutic value and decrease inflammation after balneal therapy. Adiponectin does not behave like leptin in relation to frequency of treatment at Balneal Sanatorium and Rehabilitation Techirghiol, but one thing I revealed is its elevated values in the group that made contrasting therapy, which comes as a pro-argument of the beneficial action of adiponectin in cardiovascular diseases, the stimulant effect of cold baths on the cardiovascular system being already well known.
- Analyzing the interrelationship between the average values of leptin and the duration of disease.I found the highest values in patients with disease of 5-10 years old, and the decrease in leptin values after 10 days of treatment is more obvious in patients with an age category disease of 1-5 years and 5-10 years. About adiponectin I can say that there is a decrease tendency in relation to duration of disease.

In light of the findings mentioned above, I support the hypothesis that adipokines are pathogenic links of osteoarthritis and carrying out research in this direction will help in developing new therapeutic approaches against osteoarthritis and its components.

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