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PARTIAL-BODY CRYOTHERAPY IN THE CONTEXT OF MEDICINE, HEALTH AND SPORTS: STUDIES ON PRACTICAL APPLICATIONS

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ABSTRACT

The purpose of this study was to examine isometric strength, pain threshold and perceived health responses to partial-body cryotherapy (PBC, 150s at a temperature range between -130 and -160°C).
Three different populations of subjects were enrolled for this study: 200 healthy people were exposed to a single PBC session in order to assess the maximum handgrip isometric strength responses by means of an hydraulic dynamometer, 30 healthy female carried out a cycle of ten consecutive PBC sessions for evaluate the objective pain threshold in the low back region via the pain-pressure test and finally 28 female fibromyalgic patients were exposed to repeated exposure to PBC (ten sessions in a row) with the purpose to examine perceived health and quality of life responses utilizing two self-reported questionnaires.
A single session of PBC lead to improve, immediately after the very-low temperatures exposure, muscle isometric strength in healthy people both in females and in males. Repeated exposures to PBC in healthy women was shown to be efficient in decreasing very rapidly the skin temperature in the low-back region, and thus to induce a significant increase in pain-pressure threshold values. The same protocol of repeated exposures to PBC induced significant improvements for all indexes and sub-indexes of the perceived health and quality of life questionnaires assessed in fibromyalgic patients.

KEYWORDS: partial-body cryotherapy, cryostimulation, cold exposure, isometric strength, pain threshold, fibromyalgia, quality of life.

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I would like to dedicate this work to my nieces Gaia and Greta: I hope it could be a motivation for the continuation of their school careers.

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ATP	adenosinetriphosphate
BMI	body mass index
CA^{2+}	calcium
CAT	catalase
Cd40L	CD40 ligand
CK	creatine kinase
CMJ	counter movement jump
CRP	c-reactive protein
CWI	cold water immersion
DHEA	dehydroepiandrosterone
DOMS	delayed onset of muscle soreness
DNA	deoxyribonucleic acid
EIMD	exercise induced muscle damage
EMG	electromyography
EPO	erythropoietin
FEV1	forced expiratory volume in 1s
FM	fibromyalgia
IL-1ra	interleukin 1 recepton antagonist
IL-1β	interleukin 1 beta
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IL-17	interleukin 17
LDL	low density lipoprotein
MVX	maximal voluntary contraction
NA	natrium (sodium)
NCV	nerve conduction velocity
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
OPG	osteoprotegerin
PAPP-A	pregnancy-associated plasma protein

PBC	partial-body cryotherapy
PEF	peak expiratory flow
PLGF	placental growth factor
PPT	pain-pressure threshold
SD	standard deviation
SE	standard error
sICAM-1	soluble intercellular adhesion molecule-1
SOD	superoxide dismutase
TAS	total antioxidative status
TBARS	thiobarbituric acid reactive substances
TNF-α	tumor necrosis factor alpha
VAS	visual analogue scale
VE	minute ventilation
VCO_2	carbon dioxide output
VO_2	oxygen uptake
VO_{2max}	maximal oxygen uptake
WBC	whole-body cryotherapy

LIST OF ORIGINAL PAPERS

The present thesis is based on the following papers:

i. De Nardi M., Pizzigalli L., Benis R., Caffaro F., Micheletti Cremasco M. (2017) Acute effects of partial-body cryotherapy on isometric strength: maximum handgrip strength evaluation. J Strength Cond Res; 31(12):3497-3502.

ii. De Nardi M., Bisio A., Faelli E., La Torre A., Ruggeri P. (2019) Effects of partial-body cryotherapy on pressure pain threshold in healthy women. Pain Medicine; in press.

iii. De Nardi M., Bisio A., La Torre A., Faelli E., Ruggeri P. (2019) Effects of partial-body cryotherapy on perceived health and quality of life of fibromyalgic patients. Complement Ther Med; in press.

SUMMARY

1. INTRODUCTION

Since I was studying for my master's degree I focused my attention on recovery strategies, more than on training methods, so that I decided to do an experimental thesis about cold water immersion after training in young soccer players. With great satisfaction it was published on The Journal of Sports Medicine and Physical Fitness in 2011 entitled "Effects of cold-water immersion and contrast-water therapy after training in young soccer players" (De Nardi et al, 2011). This allowed me to get interested on different applications of the cold therapy and I have experienced many devices based on cold therapy such as cold packs, cold immersion and cold garments. In that period I was particularly impressed by a relatively new type of cold therapy, the whole-body cryotherapy, which was getting a growing interest in the scientific community, clinicians and sports scientists worldwide. Whole-body cryotherapy has been developed in Japan since 1978 and arrived in Italy only in 2010, when in Orzinuovi a group of businessmen opened the BonGi polyclinic, the first one in our country to have used a cryochamber and a cryosauna. I was lucky to have got a job there, so I could deepen my knowledge on whole-body cryotherapy and collect data that I could use for my studies at the time. After working a year in that structure, I decided to open my own cryotherapy laboratory that I called Krioplanet: it was an opportunity to collect data daily and this led me to publish three articles over a period of two years. This is the path that induced me to request the admission to the PhD course, with the purpose of studying the topic of the whole-body cryotherapy in the field of sport recovery and in its different applications. In the last three years I worked on different research protocols and on different drafts of which I will talk about in the next chapters. My research activity also allowed me to participate as a speaker in different conferences and it also made me to join the working group on whole-body cryotherapy of the International Institute of Refrigeration.

2. REVIEW OF LITERATURE

2.1 PHYSIOLOGICAL PRINCIPLES RELATED TO CRYOTHERAPY

It is known there are six basic parameters that define human thermal environments: air temperature, radiant temperature, humidity and air movement, combined with the metabolic heat generated by human activity and clothing worn by individual. Thus, we are talking about a multifactorial approach: the interaction of the six factors to which humans respond (Fanger, 1970). Humans are homeotherms and are strictly subjected to maintain a constant core temperature (a narrow range around 37°C to avoid serious consequences in case of a marked difference from this value). The human body is usually surrounded by clothes and by air, but sometimes also with liquid surfaces as water, other fluids and space. The temperature of the human body will hence be greatly influenced by the temperature of air, fluids and solids surrounding it: core temperature remains almost constant despite varying ambient thermal conditions while skin temperature can vary considerably, in particular in cold environments. As already written the internal temperature should be maintained at around 37°C, so heat transfer into the body and heat generation within the body must be balanced by heat outputs from the body: we can summarize that for a constant temperature there will be a dynamic balance and not a steady state. It is important for the body to achieve a dynamic equilibrium. Indeed, external conditions continually change, so it has to respond regulating internal body temperature, not only maintaining internal temperature at around 37°C, but also maintaining every cell of the body at a temperature level which prevents damage. The temperature of the human body is an important indicator of its condition (comfort, heat or cold stress, health, sickness, performance, etc.). The primary control center for thermoregulation is the hypothalamus. In literature there are few proposed models of human thermoregulation and they all agree that when the body is exposed to hot it loses heat by vasodilatation and eventually sweating to allow cooling by evaporation; conversely if the body becomes cold the heat is maintained by vasoconstriction and, if needed, it is generated by shivering (Parsons, 2003). Shivering can increase metabolic heat production up to six times (Kenatinge et al, 1986) and when exposing to very low temperature, as for example in whole- and partial-body cryotherapy, it can arrests the fall in core temperature. There are two types of thermal sensitive receptors: warm or cold types, depending to the response to stimuli. They are located in the skin (over and within epidermis), in the hypothalamus and in other sites as blood vessels, midbrain, etc. Afferent signals from the peripheral receptors are transferred by nervous pathways to the hypothalamus: warm signals are mediated by anterior hypothalamus, while cold signals by posterior hypothalamus. The core temperature is maintained at a constant temperature while as a part the thermoregulation the mean skin temperature varies over the whole of the body. Humans could be subjected to both hot stress and cold stress. When the body is exposed to cold environments first of all vasoconstriction reduces blood flow to the skin and therefore heat loss, then non-shivering thermogenesis such as stiffness will increase metabolic rate, lastly shivery begins. Individuals respond to cold stress differently: body size, gender, age, fat percentage and fitness level are important factors which can induce to different thermal responses. For example subjects with a high fat percentage tend to have a core temperature higher than people with less fat % after cold exposure, especially regarding male subjects (Cuttel et al., 2017) as well as individuals with higher adiposity cooling more at the skin than thinner ones (Hammond et al., 2014). That is the reason why females, those usually have a greater body fat percentage than males, experienced a greater degree of skin cooling. Age difference could be a discriminant in the response to cold agents: elderly people are in fact more susceptible to the cold, the same goes for people with a low fitness level that is related with a lower metabolic rate than individuals which practice exercise with constancy (Castellani et al. 2006). Another individual factors to take in account is the body surface area-to-mass ratio: subjects with a greater values experience a more rapid fall in core temperature (Sloan & Keating, 1973).

Cryotherapy, i.e. the exposure for a certain time to cold environments or agents, can lead to acute and long term physiological responses such as circulatory, respiratory and neuromuscular ones. Physiological reactions after acute exposure to the cold are mediated by the sympathetic and parasympathetic nervous systems. During a cold exposure, cardiac workload, systolic and diastolic blood pressure significantly increase in healthy subjects (Li et al., 2009;

Korhonen, 2006). This might increase the risk of cardiovascular events in subjects with cardiac diseases (Hong et al., 2016; Ikaheimo T.M., 2018). Also respiratory system is subjected to significant changes during acute or chronic cold exposures: cold elicits an increase in pulmonary vascular resistance and chronic exposure induced morphological changes such as increased numbers of goblet cells and mucous glands, hypertrophy of airway muscular fascicles and increased muscle layers of terminal arteries and arterioles (Giesbrecht G.G., 1995). At the same time neuromuscular performance changes in the cold: dynamic exercise, which involves eccentric and concentric contraction, decreases because the co-ordination of muscles changes. In fact, while shivering occurs, antagonist muscles co-contract together, “fighting” against each other (Bawa et al. 1987). Isometric exercise is not subjected to this phenomenon (Burke D.G. et al., 2000). Another factor influenced by cold is the postural control: cold exposure impair postural sway in healthy males increasing the velocity of the center of pressure position in the medial-lateral position in the bipedal position while subjects stood on a force plate (Fukuchi C.A. et al, 2014).

2.2 WHOLE-BODY CRYOTHERAPY AND PARTIAL-BODY CRYOTHERAPY

Cryotherapy has ancient origins, in medicine it was used by several cultures: Egyptians first discovered the invigorating benefits of cold-water immersion, Romans were known to plunge in rivers after battles and Persians used cold remedies to treat a wide range of disease. Hippocrates, the father of western medicine prescribed ice-cold water as optimal treatment for relieving swelling, pain and inflammation. Nowadays the use of cold application has become very popular in medicine, health care and within sport therapists. Ice packs, winter swimming, cold-water immersion and cold garments are studied by scientific community and prescribed by the 80% of physicians as a symptomatic treatment following soft tissues injuries, distortions, contusions and overuse symptoms.

A peculiar form of exposure to extremely cold dry air in an environmentally controlled room for a short period of time was proposed in 1978 for the treatment of rheumatic diseases: this treatment was named whole-body cryotherapy

(WBC). The Japanese physician Prof. Toshiro Yamauchi built the first cryochamber, after observed his rheumatologic patients coming back from winter holidays spent in mountain feeling better. Originally it was developed to treat rheumatoid arthritis (Yamauchi, 1986) and other chronic pathologies as for example multiple sclerosis, fibromyalgia and ankylosing spondylitis. More recently it has gained greater acceptance in sports medicine as a method to improve recovery from intense exercise and injury (Banfi et al, 2010; Lombardi et al., 2017). This cold treatment is usually called “cryostimulation” if it is addressed to healthy people, that is without pathologies and “cryotherapy” if it is addressed to patients. Curiously, in a recent paper, Roszkowska and co-workers (2018) stated that it will be recommended also in animals such as horses, to shorten the recovery period after injury and to improve the muscular tiredness.

In the last decades besides local cryotherapy two extreme forms of cryotherapy were developed (Bouzigon et al, 2016): whole-body cryotherapy (WBC), as already mentioned and partial-body cryotherapy (PBC). Each form of cold therapy expose subject to very cold dry air for a short period of time and require a specialized cold controlled environment: a cryochamber (WBC) or a cryocabin (PBC). They differs each other for some aspects: the temperature range (-110°C for WBC and -160°C for PBC), the head exposed to the cold in WBC while is not exposed in PBC, the way to generate cold (compressor for WBC and nitrogen gas for PBC), mobility possibilities due to different device and nitrogen tank sizes (impossible to move for WBC unless in the case of the portable unit which can be pulled by a truck, possibility to move for PBC both for quite small size of the device and for the ease of movement of the nitrogen tank), in WBC enters a small group (generally from 2 to 4 people) and in PBC enters alone (Hausswirth et al, 2013). An interesting dissertation of actual technologies can be found in the paper written by Bouzigon and colleagues in 2016, where they summarize fourteen producers worldwide. To our best knowledge nowadays there are many others producers on the market (VacuActiv, CryoScience, CTN, Lifecube, MCryo, CryoPod, CryoInnovations, Aurora Concept and CryoNext). One of the main differences of these devices is the way to generate cold: cryochambers could be hybrid (nitrogen mixed to air) or electrical (electric energy) while cryocabins could be cooled only by nitrogen. One of the key points is that the actual temperature in the two types of devices is not precisely assessed: it is not known for example the site of assessment of chambers and cabins, so there is not standardization in assessing the temperature inside the device during the session. One study measured the actual temperature in a specific model of cryocabin (Criomed Space Cabin) with the empty cabin and with a manikin, and authors showed the temperature in the cabin was not homogeneous. The temperature in the cabin was different between the top of the cabin and the lowest part of it and between the front side (near the door) and the back side (near the nitrogen nozzle), showing that the actual temperature in the cryocabin was different compared to that reported by the manufacturer (Savic et al., 2013). This is a very huge problem for the validity and the reproducibility of scientific protocols, and comparing data from different research is quite difficult. In addition there is a lack of the literature on the comparative efficiency of the two methods, that is why, for example, the decision to switch from a former of device to the other is arbitrary and without any scientific criteria. There are only three studies, to our best knowledge which compared WBC and PBC: two of which evaluated the effects of a single session (Hausswirth et al., 2013; Polidori et al., 2018) and one the other one assessed the effects of a cycle of five consecutive sessions (Louis et al., 2015). In their research Hausswirth and colleagues compared the effects of a single session of whole- and partial-body cryotherapy on indices of parasympathetic activity, blood catecholamines and thermal comfort: they recorded changes in thermal variables such as skin temperature, for which they found an almost certainly decrease both after WBC (-110°C) and PBC (-160°C), and tympanic temperature (almost certainly decreased only after the WBC session). They evaluated cardiovascular parameters such as blood pressure (very liked increased after the WBC session), heart rate for which they found an almost certainly decreased after PBC and WBC, with a likely greater proportion for WBC compared to PBC (-15.2% vs -10.9%) and the resting vagal-related heart rate variability indices which very liked increased after PBC and WBC without significant differences between groups. As regards blood catecholamines plasma norepinephrine likely increased after PBC and very likely increase after WBC (+54.7% and +76.2%). Lastly thermal cold sensation was almost certainly altered after WBC and PBC, with thermal discomfort likely more pronounced after WBC than PBC. Summing up their outcomes showed that a WBC session induced a larger stimulation of the autonomic nervous system compared to a PBC session. In their second published research on this topic the French research group (Louis et al., 2015) studied the influence of exposing the head to cold during cryotherapy sessions on the thermal response and the autonomic nervous system. This study was conducted over five consecutive days with two groups of ten participants involved in the two different systems and another group of ten subjects constituting a control group not receiving any cryostimulation. WBC (-60°C) and PBC (-160°C) sessions, induced the same decrease in skin temperature for all body regions (-8.6°C for WBC and -8.3°C for PBC) which persisted up to 20-min after the sessions. They also found that tympanic temperature significantly decreased after WBC whilst decreased, but without any statistical significance, after PBC. As regards heart rate they found an almost certainly decrease both after PBC (-8.6%) and WBC (-12.3%) sessions. Resting vagal-related heart rate variability indices likely increased after PBC and almost certainly increased after WBC. Lastly plasma norepinephrine concentration was likely increased in similar proportion after each cryotherapy treatment, but only after the first session. Summing up their results they assumed that the head exposure to cold during cryotherapy session in a cryochamber may not be the main factor responsible for the effects of cryostimulation on the autonomic nervous system. Another French group (Polidori et al., 2018) recently focused their research on the difference between PBC and WBC based on the analysis of skin temperature distribution. They noticed that skin temperature difference between PBC (-140°C) and WBC (-140°C) varied according to the vertical location of the body regions, increasingly from 15% on the legs ($p=.171$) up to the 53% for the chest ($p<0.001$).

All authors agree that more experimental approaches are needed to improve the cryotherapy protocols used, especially when the choice can be made between WBC and PBC: just need to think that also in the three above mentioned publication they have set three different temperature levels (-110°C; -60°C; -140°C) for the whole-body cryotherapy sessions. The lack of standardization in protocols, understood as different temperatures, duration and numbers of the exposures makes it difficult to fix the difference between PBC and WBC, also taking account the individual different characteristics of each fellow.

These two methodologies have in common that people enter in the cold environments minimally dressed, with socks, gloves, wooden clogs and, for as concerned the WBC, with headband and mask for the nose. For the entire duration of the treatment subjects have to walk on the spot and breath regularly. Visual and vocal contact between the subject and the operator or the physician in the PBC is made possible because the head of the subject is outside the cryocabin while in the WBC is allowed by a glass window put into the wall of the cryochamber and a microphones system. Typically the timescale of exposure is reported as between 2-4 minutes: in their research Selfe et al. (2014) established a duration time of 2 minutes of WBC exposure at -135°C as a safe protocol for future applications for male elite rugby players, while Fonda and colleagues (2014) concluded that the optimal duration for PBC is 150 seconds with a temperature range between -130°C and -170°C. Recently the topic of the standardization of the protocol has gained more interest because the same cold stimulus at the same temperature for the same time frame could be perceived in different ways by different subjects: in fact the key point is to assess individualized exposure protocols for the different anthropometric and gender characteristics in addition to their health status and fitness level. To the best of our knowledge only four studies compared the different response after a whole-body cryotherapy session between subjects with different BMI or gender. All of them were performed utilizing a cryochamber, so we can assume that there are no data in literature about this topic with PBC technology. The first to address this topic were Cholewka and colleagues in 2011: in their research they utilized a thermovision camera and a contact thermometer with thermocouples. The conclusions of this study were that the largest decrease in skin temperature was observed on the lower extremities with differences in the thermal response influenced by the BMI. In 2014 Hammond et colleagues explored whether anthropometric measures influenced skin temperature after a WBC session and observed that the lowering in skin temperature was more significant in females than in males and found also a significant relationship between body fat percentage and decrease of skin temperature. In 2017 Cuttel and colleagues investigated the effects of whole-body cryotherapy in temperature, heart rate, blood pressure, thermal comfort and sensation between males and females. They found sex differences and in mean arterial pressure, mean skin and mean body temperature following exposure to whole-body cryotherapy. Recently Polidori and colleagues investigated how body thermal resistance between sexes should be considered as a key parameter in WBC protocols: females presented a 37% higher inner thermal resistance than males when reaching an asymptotical thermal state at rest due to higher concentration of body fat percentage. The authors of these studies agree that, in order to optimize treatment protocols, effects of sex and anthropometrics should be considered: in particular Polidori et al. (2018) suggested that the duration of cryotherapy protocols should be shorter when considering females compared to males.

Despite the protocols utilized worldwide seems to be empirical and with a lack of scientific validation, WBC and PBC have demonstrated to be a safe procedure and to confer benefits in most domains and not to induce negative general and specific effects. There are accepted contraindications for WBC, including cryoglobulinaemia, cold intolerance, Raynaud disease, hypothyroidism, acute respiratory system disorders, cardiovascular system diseases, sympathetic nervous system neuropathies and local blood flow disorders (Lombardi et al., 2017). Whole- and partial-body cryotherapy are generally considered procedures with few side effects, but there are no large studies that have established its safety profile: in a mindful review of literature we found six articles reporting adverse events after a single or multiple WBC sessions. Selfe and colleagues in 2014 excluded one Samoan rugby player with intolerance to ice packs which not disclose his cold intolerance to the study team. After undergoing a three minute WBC exposure he suffered a mild superficial skin burn bilaterally on the mid portion of the anterior thigh consisting of erythema and minor blistering approximately 2 cm high and 10 cm wide the day following WBC. In 2017 Carrard and colleagues reported a case of a 63-year-old male who presented with transient global amnesia after undertaking a WBC session. As expected, the patient completely recovered in 24 hours but they firstly highlighted that WBC is potentially not as risk free as thought to be initially. In the same year Cámara-Lemarroy and colleagues in a case report referred of a 56-year-old patient who developed an abdominal aortic dissection after receiving a cycle of 15 sessions of WBC, suggesting that increase in blood pressure, heart rate and adrenergic response could have acted as a trigger for the onset of aortic dissections. Recently Greenwald and colleagues (2018) reported a 47-year-old man after received 8 WBC treatment in two weeks was diagnosed of cold panniculitis based by his clinical presentation and histology (eruption, pruritus, pink papules and plaques, and a lymphohistiocytic infiltrate with neutrophils in the reticular dermis and superficial fat). Rivera and colleagues in 2018 in one patients discontinued the therapy after two sessions of partial-body cryotherapy due to muscle stiffness and tremor. Finally Mackenzie O'Connor and colleagues in 2018 reported that a 71-year-old-man presented with a cold burn injury the day after his whole-body cryotherapy session maybe for a malfunctioned nozzle which sprayed liquid nitrogen directly on his back for a prolonged period (approximately 1 minute). These publications imply that further studies investigating the possible adverse events are required.

2.3 MECHANISM OF ACTION OF WHOLE- AND PARTIAL-BODY CRYOTHERAPY

The main effects of cryostimulation (Demoulin and Vanderthommen, 2012) are related to analgesia (gate control activation, lower nociceptor excitability thresholds and reduction in nerve conduction velocities), anti-inflammatory response (lower enzymatic activities, e.g., metalloproteinases, and decreas in production of pro-inflammatory mediators), lower levels of oxidative stress and decrease of edema and bleeding (lower vascular permeability and induced vasoconstriction).

In order to understand the mechanism of the effects of WBC and PBC, results related responses to pain, stress and inflammation have been reported. Localized analgesia requires a skin temperature that is below 13.6°C, this is made possible by vasoconstriction and direct skin cooling by very cold air, and the explanation for this analgesic effect is the reduction in nerve conduction velocity and acetylcholine formation (Bugaj, 1975). As can be seen in the figure 1 (Guillot et al., 2014), cryostimulation activates the autonomic nervous system (Mourot at al., 2007) and the release from efferent sympathetic neuron of acetylcholine that links a7nAchR receptor and noradrenaline that binds β2-adrenoceptor. Cold exposure has an immunostimulating effect caused by an increase noradrenaline response which is dependent on the relationship between core temperature decrease and duration of exposure (Bettoni et al.,

2013). ACh anThe d NA may inhibit NF-kB pathway (nuclear factor kappa-light-chain-enhancer of activated B cells, a protein complex that controls transcription of DNA, cytokine production and cell survival) and its downstream effectors (pro-inflammatory cytokines, ROS and adhesion molecule gene transcription)(Pournot et al., 2011). NA induces vasoconstriction through α-adrenoceptor (Shepherd et al., 1983) which could limits inflammation and cold stimulus down-regulates and inhibits the enzymatic pathways involved in joint inflammation and destruction (Banfi et al., 2009). Cold application decreases the requests oxygen and ATP with an improvement in cell survival and diminished secondary hypoxic lesions (Guillot et al., 2014).
WBC is comparable, to physical activity, at least in the pulsed, and temporarily limited, induction of pro-inflammatory cytokines and the induction of irisin expression, a myokine induced by exercise which activates oxygen consumption in fat cells and stimulating thermogenesis at the adipose tissue level (Dulian et al., 2015). This founding indicate that the sympathetic activation induced by the cryostimulation can be able to mimic physical activity (Lombardi et al., 2017).

In conclusion, mechanisms of action of partial- and whole-body cryotherapy are not completely clear: further studies are needed to establish the causes of the main effects of these cryostimulation techniques.

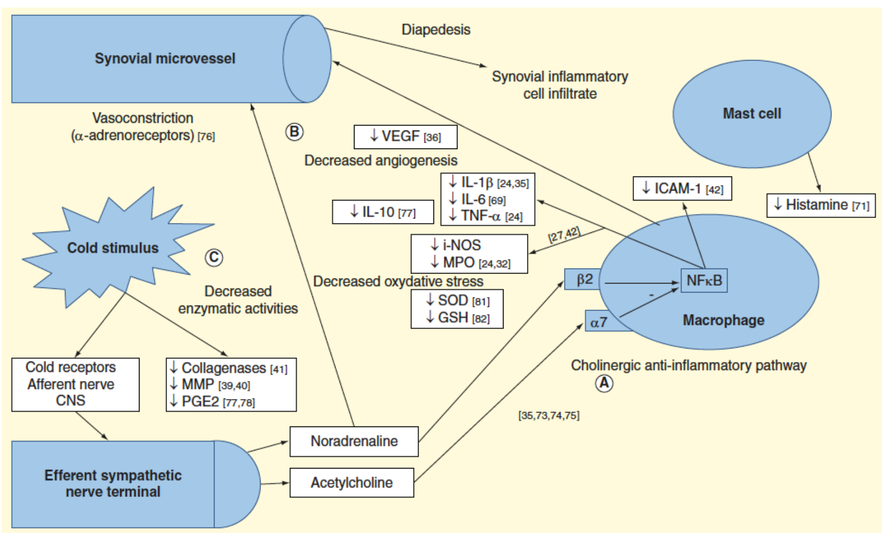

Figure 1: Proposed model of molecular pathways involved in cryotherapy (from Guillot et al., 2014).

2.4 CIRCULATORY AND RESPIRATORY RESPONSES TO WHOLE- AND PARTIAL-BODY CRYOTHERAPY

In accordance with scientific literature, it can be say that for a healthy individual, cryotherapy is to be considered as a safe method as regards the cardiorespiratory system, even if Càmara-Lemarroy and colleagues published in 2017 a paper reporting a cardiovascular complication associated with WBC.

Many studies investigated the effects of whole- and partial body cryotherapy on heart rate, heart rate variability and blood pressure. For example Lubkowska and Szygula in 2010 evaluated changes in blood circulation and also aerobic capacity in healthy young men exposed to fifteen sessions of whole-body cryotherapy lasted three minutes at a temperature of -130°C, preceded by 30 seconds of adaptation in a vestibule at -60°C. Authors tested blood pressure and heart rate before, immediately after and ten minutes after each WBC session. They calculated also pulse pressure and the mean arterial blood pressure, finding a significand increase in systolic blood pressure after each cryostimulation and a significant increase in diastolic blood pressure only after the first session. They also found a significant decrease in heart rate, without any adaptation changes after the cycle of ten consecutive sessions. This led them to consider whole-body cryotherapy a safe technique, but not recommended for those with advanced or not pharmacologically controlled hypertension. For their part Zalewski and colleagues (2013) in their research evaluated the complex hemodynamic physiological reactions in response to a three-minutes long session of whole-body cryotherapy with temperature set at -120°C in thirty healthy subjects. They collected data at four time points: before cryostimulation, immediately after, three hours later and finally six hours later. They found a significant decrease of heart rate after whole-body cryotherapy exposure, but after three hours it returned to basal values and remained at the very similar level after six hours. This decrease could depend from two different mechanisms: the decrease in the sinoatrial node rate resulting from the inflow of cooled blood and for the enhanced parasympathetic stimulation, due to activation of baroreceptors and vagus nerve. Conversely beat-to-beat stroke volume and stroke index showed a significant increase immediately after cold-exposure and returned to rest values after three and six hours respectively.

Regarding blood pressure parameters (systolic, diastolic and mean values) they found a slight increase after the whole-body cryotherapy session, although they not found significant changes from baseline. The scores of blood pressure parameters remained higher than baseline also after three and six hours respectively, however without reaching a significant statistical level. After one whole-body cryotherapy session they noticed also a significant increase of the baroceptor sensitivity, even if it decreased significantly both after three and six hours respectively: it could be a possible reason of the significant reduction of the heart rate and a non-significant increase of blood pressure, due to intense vasoconstriction which affect baroceptor sensitivity after several hours. They concluded that whole-body cryotherapy appears to be safe for healthy people, despite it strongly stimulates the baroreceptor cardiac reflex in response to body fluid changes which sequentially modulate heart rate and blood pression control. The same Polish group studied in 2014 (Zalewski et al., 2014) analyzed the heart rate variability and blood pressure variability, confirming the strong modulation of autonomic activity induced by a whole-body cryotherapy session. They found an increase in high-frequency component of the heart rate variability spectrum, corresponding to enhanced parasympathetic response, and a less pronounced increase of the low-frequency component. These changes were observed up to six hours after the cold exposure and were considered safety for healthy people. In another research, Cuttel and co-workers (Cuttel et al., 2017) investigated the effects of a single bout of whole-body cryotherapy (30 seconds at -60°C and 150 seconds at -110°C) on heart rate and blood pressure, examining the influence of sex and body composition. They found significant differences in mean arterial pressure between sexes both immediately after and after 35 minutes, with men showing higher values and no differences in heart rate between sexes after the WBC session. Regarding partial-body cryotherapy Louis and colleagues in 2015 verified the influence of exposing the head to the cold on the autonomic nervous system with a protocol including five consecutive cryotherapy sessions performed in two different systems: one exposing the whole-body to cold (cryochamber) and the other without exposing the head (cryocabin). Authors did not found significant changes in systolic and diastolic blood pressure after both cryostimulation techniques. They found that heart rate almost certainly decreased in both techniques with an higher bradycardia recorded after WBC than PBC, maybe explained by the additional stimulation of trigeminal cold receptors located in the face, and resting vagal-related heart rate variability indices (the root-mean square difference of successive normal R-R intervals and high frequency band) were increased after partial-body cryotherapy (+49.1% and +123.3% respectively) and whole-body cryotherapy (+38.8% and +70.3% respectively): suggesting that the magnitude of parasympathetic response to very low temperatures would be related to the magnitude of the cold stimulus and not to the head exposure to cold. Heart rate variability during the night was also assessed in a recent study (Douzi et al., 2018) after an evening session of whole-body cryotherapy utilizing a recently validated (Bouzigon et al. 2017) cryochamber with technology based on forced convection (30 seconds at -25°C followed by 180 seconds at -40°C, with an average wind speed of 2.3 m s^{-1}). They did not find significant differences for any parameter of heart rate variability recorded during the entire night sleep and the first 4-hour period. They found high-frequency bands significantly higher in the WBC group than in control group after the first ten-minutes long slow-wave sleep episode, and at the same time they found low frequency and the power ratio significant lower in WBC group than in control group, showing a greater parasympathetic and lower sympathetic activity during the slow-wave sleep episode following WBC. Finally, in 2014 I studied in person the effects of one single session of partial-body cryotherapy on cardio-vascular response (Fonda et al., 2014). The protocol consisted of four exposures of different duration (90, 120, 150 and 180 seconds) in a cryocabin with temperature maintained between -130°C and -170°C. We found that different durations did not significantly affect the heart rate nor diastolic and systolic blood pressure responses.

As can be seen, many authors found contradictory findings. These discrepancies could result from different methodologies of measurement, namely determining the cardiovascular parameters immediately after the exposure, when the response of participants was predominated by stress. Despite this lack in the standardization of measurements and of cold exposure parameters it can be assumed that whole- and partial-body cryotherapy are safe for healthy individuals and don’t induce long term adaptations in cardiovascular parameters.

Whole- and partial-body cryotherapy have been shown not to be deleterious also to lung function (Smolander et al., 2006): authors investigated the effects of repeated cold exposures (three times per week for twelve weeks) for two minutes in a temperature controlled unit set to -110°C. They measured the peak expiratory flow rate (PEF) and the forced expiratory volume in 1 second (FEV-1) before and after two and thirty minutes respectively to the first whole-body cryotherapy session. They repeated the same measurements after 4, 8 and 12 weeks. A sudden exposure to very cold environments may elicits several effects on the respiratory system, such as a gasp response, increase in ventilation and bronchoconstriction, but with repeated exposures they found that the peak expiratory flow rate and the forced expiratory volume in 1 second did not changed statistically significantly. In fact the PEF values did not changed between pre-exposure and after two minutes at any time of the study period, while after thirty minutes of the whole-body cryotherapy session they were slightly lower compared with pre-exposure values with a significant p value after one and three months respectively. No significant changes were reported at any time of the study period for the FEV-1 values: these results may depend from an airway acclimatization through habituation. Their findings indicated that whole-body cryotherapy is not harmful for lung function, inducing only minor bronchoconstriction in healthy individuals. In a recent study (Kojima et al., 2018) authors collected respiratory gas samples in young male athletes after an high-intensity intermittent exercise, followed by a three-minutes long whole-body cryotherapy session (-140°C), determining oxygen uptake($V0_2$), carbon dioxide output ($VC0_2$) and minute ventilation (VE) with an automatic gas analyzer. $VC0_2$ and VE values were significantly lower in the whole-body cryotherapy group than in the control group approximately ten minutes after the cold exposure, reflecting a lower parasympathetic activity in the WBC group than in the control group. Finally in the above mentioned study, Lubkowska and Szygula in 2010, measured the VO_{2max} in a population of normotensive, young and physically active men, involved in a protocol consisting of fifty sessions of whole-body cryotherapy, lasted three minutes each, with the temperature set to -130°C. Authors did not find changes in the level of aerobic capacity, in fact VO_{2max} values showed a slight decrease after the treatment period, confirming the founding of Klimek and co-workers (Klimek et al., 2010) which determined the influence of whole-body cryotherapy on aerobic and anaerobic capacities after ten sessions of cryogenic chamber treatment (180 seconds at -130°C). They did not find changes in aerobic capacity in the whole population after completing the ten sessions cycle and at the same time they found increase in maximal anaerobic power in males, but not in females, concluding that whole-body cryostimulation could be beneficial for increasing anaerobic capacity in sportsmen involving in sport disciplines characterized by speed and strength.

Summarizing a single or a cycle of whole-body cryotherapy sessions does not be detrimental for the lung function or for aerobic or anaerobic capacity in healthy population, but should be applied with caution in susceptible individuals, such as asthmatics.

2.5 ADVANTAGES AND LIMITS

Whole- and partial-body cryotherapy suit for the treatment of almost all known diseases connected with the immune system or metabolism disturbance, having a great positive remedial and normalization influence on them (Lubkowska et al., 2010a; Ziemann et al., 2013). Organism’s positive response to cryotherapy supports treatment of basic diseases and facilitates kinesitherapy. Cryotherapy represents a large wide of advantages: it takes short time for applying, being well tolerated by individuals and patients, and sportsmen status improves quickly. Athletes testimony is generally high and this contributes to develop cold-treatments sometimes regardless of the underlying evidence. A wide range of positive cryotherapy effects is documented by many published researches and many of them are already unknown, or are not confirmed by the scientific community. For example in rehabilitative medicine whole- and partial-body cryotherapy are indicated for articular pain due to degenerative diseases (Hirvonen et al., 2006; Ma et al., 2013), low-back pain due to different etiology (Sliwinszki et al., 2017; Nugraha et al., 2015; Giemza et al., 2014; Giemza et al., 2015), neck pain (Thomas et al., 2016), inflammatory pathologies of the muscle-skeletal apparatus (Rose et al. 2017), psoriatic arthritis (Lange et al., 2008), rheumatoid arthritis (Gizinska et al. 2015); osteoarthritis (Chruściak, 2016), prevention of primary and secondary osteoporosis (Ksiezopolska-Pietrzak, 1998; Galliera et al., 2013), fibromyalgia (Rivera et al., 2018; Vitenet et al., 2018; Bettoni et al., 2013), multiple sclerosis (Miller et al., 2011a; Miller et al., 2013; Miller et al. 2016; Pawik et al., 2019), anxious-depressive syndrome (Rymaszewska et al., 2008) and even mild cognitive impairments (Rymaszewska et al., 2018). However these studies are affected by several biases such as for example, as already discussed in this thesis, the lack of standardization of single- and multi-sessions cryostimulation protocols, no randomized controlled trial experiments and heterogeneous gender and anthropometrical characteristics within the study designs. These methodological shortcomings, in a scientific point of view, are the main limitation of cryotherapy techniques and it is required a scientific cooperation for building up standardized and safety protocols validated by wide and controlled studies. Bouzigon and co-workers (2016) indicated as the first limit of these technologies the lacking in available data concerning the actual temperature of exposure inside WBC chambers and PBC cryocabins: there is only one study (Savic et al., 2013) that showed a temperature of approximately -60°C rather than the expected -150°C inside a CryoMed cryocabin and no one about cryochambers. There are also other limits such as the practicality in terms of cost of each and multiple sessions (in Italy the range for a single PBC session varies from 30 to 70 euros) and in terms of access because these facilities are not located in all districts and during sport events, for example, the possibility of having one of these available is still too rare. In fact, with the exception of some models of PBC, these technologies are not mobile and even if they can be moved from place to place it must take into account that it is not possible to store nitrogen everywhere, because of the restrictive regulations for nitrogen storage. Another important limit of these technologies is the safety, first of all because of the direct contact with nitrogen inside cryocabins, and then because of the adverse events reported in literature. Another aspect linked with the safety limits is that the operators of cryotherapy facilities have not a standardized preparation (university career, specific training course, knowledge of scientific literature, etc.) so the practitioners tend to follow the crowd without examining the merits of these modalities: there are consistent difference between social and scientific proofs, that is why more researches are required in order to fully understand the potential benefits of whole- and partial-body cryotherapy and, of course, every practitioners have to attempt to the scientific findings.

2.6 STANDARD PROTOCOLS OF WHOLE- AND PARTIAL-BODY CRYOTHERAPY

The number and the frequency of sessions that should be applied in order to obtain the best results for each individual are crucial topics which have been investigated by the scientific community in the last two decades. Right now there are not standardized protocols in the field of cryostimulation, neither for athletes, nor for patients and nor for healthy people. The dosage, in terms of frequency, temperature and time of exposure, are almost always decreed by the staff members of the cryotherapy facilities without any scientific support. In the last five years the growth of the cryotherapy market increased exponentially worldwide, becoming a business trend: localized, whole- and partial-body cryotherapy are shifting from a medical treatment to a wellness routine, so the frequency of the cold exposures are more often decided by the possibilities, in terms of money and time, of the individuals instead of the protocols indicated in the scientific literature and this makes difficult to follow the indication of the literature slavishly. For example in their paper published in 2016, Giemza and colleagues stated that the common standard of ten sessions is insufficient, while at least twenty sessions of whole-body cryotherapy may efficiently enhance neuromuscular performance with an ability to sustain the improvements for at least three days. One year later, in the conclusions of their review also Lombardi and colleagues (2017) suggested that in the sports field a minimum of twenty consecutive sessions should be done for effectiveness evaluation and thirty sessions should be the optimum in the perspective of a complete hematological recovery after the initial response and Ksiezopolska-Pietrzak in 1998 suggested that to get the most benefits cryotherapy treatments need to be applied twice a day, with at least three hours interval and a kinesitherapy session after each cold exposure.

Precisely, in literature can be found many researches with a specific proposed protocol based on different pathologies and purposes.

In ankylosing spondylitis the effectiveness of whole-body cryotherapy has gained attention in the last year, with three published articles about this topic (Straburzynska-Lupa A., 2018; Stanek et al., 2018a; Stanek et al., 2018b). In 2015 a study of Stanek and colleagues already showed the positive effects of ten consecutive whole-body cryotherapy sessions in patients suffering from ankylosing spondylitis. In this study the experimental group was exposed to ten three-minutes long whole body cryotherapy sessions once a day, followed by one hour of kinesiotherapy session. The researchers evaluated the answers of two questionnaires related to the pain intensity, stiffness degree, disease activity and functional scores. In addition they measured also spine mobility parameters both in the experimental group and in control group (which were exposed only to the one hour of kinesiotherapy). After the study period all measured parameters improved in each group, but changes in whole-body cryotherapy group were significantly higher than in control group, demonstrating that a protocol of ten sessions in a row of WBC (duration of three minutes each and temperature ranging for -60°C seconds for the first 30 seconds and -120°C for the last 3 minutes) have a positive effects on the parameters of pain, stiffness, disease, functional scores indexes and spinal mobility parameters. The same author recently (Stanek et al., 2018a) estimated the impact of whole-body cryotherapy on cardiovascular risk factors in male patients with ankylosing spondylitis evaluating markers of inflammation, oxidative stress, lipid profile and atherosclerosis plaque. They compared the results of a control group (kinesiotherapy sessions only) with those of the WBC group (ten consecutive WBC sessions, which consists of 30 seconds in the antechamber with a temperature of -60°C and 3 minutes in the chamber where the temperature reached -120°C, followed by one hour of kinesiotherapy. They demonstrated that in the experimental group there was a decrease in oxidative stress markers and an higher differences in the total cholesterol, LDL cholesterol, triglycerides, s CD40L, PAPP-A and PLGF levels, suggesting that WBC could be a useful method of atherosclerosis prevention in patients with ankylosing spondylitis. The same authors (Stanek et al., 2018b) evaluated the routine parameters of oxidative stress in patients with ankylosing spondylitis utilizing the same standardized protocol of research in terms of randomization in the experimental and control group and in terms of cold exposure. They found a significant decrease of oxidative stress markers (total oxidative status and oxidative stress index) and a significant increase of total antioxidant status suggesting that a cycle of ten sessions of WBC performed in a closed cryochamber could be useful to decrease oxidative stress, in addition to pain, disease and stiffness indexes, in males suffering of an active phase of ankylosing spondylitis. Another polish group (Straburzynska-Lupa et al., 2018) evaluated the effects of three study procedures in 65 patients with ankylosing spondylitis: WBC at -110°C, WBC at -60°C and exercise therapy (control group). Two indexes of diseases activity (BASDAI and ASDAS-CRP) and biochemical parameters such as concentration of C-reactive protein (CRP) and the concentration of IL-8 and IL-17 were measured at the beginning and at the end of the study period. They also studied the trend of the total antioxidant capacity and the thiobarbituric acid reactive substances (TBARS). In comparison to other groups, the group which was exposed to the chamber set at -100°C had a significant increase in the concentration of TBARS. They also found a significantly lower BASDAI scores after ten consecutive sessions in the WBC at -110°C compared to the non-WBC group. The results of their study indicated that eight sessions of WBC at -110°C had a positive effect on lowering ankylosing spondylitis clinical activity as measured by the BASDAI.

To the best of our knowledge, on the basis of the literature we can assume that an appropriate protocol for patients suffering from ankylosing spondylitis should be structured at least from eight to ten consecutive sessions of WBC with a temperature range at least from -110°C to -120°C. It's however important to underline that neither of these studies considered a follow-up, so is not possible to know how the benefits last over time.

Another kind of chronic pain disorder which gained the attention of the scientific community working on cryostimulation techniques is fibromyalgia. In the last year (2018) two studies documented the effects of whole- and partial-body cryotherapy in fibromyalgic patients. Ferrera and colleagues evaluated the efficacy of WBC for the control of pain and impact of disease by two indexes (Fibromyalgia Impact Questionnaire and Combined Index of Severity of Fibromyalgia). They enrolled 60 patients in a crossover study, treated on alternate days during three weeks with a three-minutes long partial-body cryotherapy session. After ten sessions, patients underwent a 1-week washout and subsequently they were inverted. They found positive effects of PBC after a cycle of ten sessions on ΔVAS pain, ΔFIQ and ΔICAF scores, significantly higher than in control group. Recently also Vitenet and co-workers (2018) investigated about the effectiveness of whole-body cryotherapy on health-reported quality of life assessment in fibromyalgic patients. They enrolled 24 patients with fibromyalgia diagnosis randomizing them in two groups: the whole-body cryotherapy group performed ten three-minutes long sessions at a temperature exposure of -110°C in a cryoair chamber (produced in Germany by Mecotec and cooled down electrically) whilst control group did not change anything in their everyday activities. Compared with the control group they found significant changes in WBC group in health-reported quality of life scores and they repeated the survey after a month showing that the positive effects lasted for at least a month. Another study (Bettoni et al., 2013) demonstrated that fibromyalgic patients, after a cycle of 15 consecutive sessions (30 seconds at -60°C and 3 minutes at -140°C), obtained significant improvement in health-reported quality of life indexes compared to control group. For this study they used a cryo-chamber utilizing liquid nitrogen (they did not revealed the model).

In conclusion we can summarize that a cycle of a minimum of ten consecutive sessions in PBC or in WBC should be recommended to fibromyalgic patients in order to improve quality of life parameters. The temperature of exposure should be lower than -100°C: this approach should guarantee maintenance of benefits for at least one month.

Since its introduction whole-body cryotherapy was associated with the treatment of rheumatoid arthritis (Yamauchi, 1986). In the last years were published few researches about the effectiveness of the whole-body cryotherapy in patients with rheumatoid arthritis. In 2017 Hirvonen and colleagues measured the total radical-trapping antioxidant parameter TRAP, which reflects the global combined antioxidant capacity of all individual antioxidants in blood, in 60 patients which was randomized I three groups: whole-body cryotherapy at -110°C, whole-body cryotherapy at -60°C and local cryotherapy (cold air -30°C applied to five swollen joints at a time for 10-30 minutes). Each treatment was given three times a day for seven consecutive days. The results of this study indicated that whole-body cryotherapy at -110°C induced a short-term increase in TRAP during the first treatment session but not during other type of cold exposures, but this effect was short and the cold treatments did not cause significant oxidative stress or adaptations during one week. Another Finnish group of research (Hirvonen et al., 2006) compared two cold-exposure protocols in forty patients diagnosed with rheumatic arthritis: -60°C and -110°C cryotherapy sessions performed three times a day for a week for a total amount of twenty sessions. They found a more remarkable decrease in pain sensation and morning stiffness in the whole-body cryotherapy at -110°C than during other cryotherapies, but there were not significant differences in the disease activity between the groups.

In 2015 Gizinska and colleagues showed that after a cycle of ten three-minutes long cold exposures in a row at a temperature of -110°C, after passing through pre-chambers (-10°C and -60°C) there was a positive effect on patients with rheumatoid arthritis proven by a reduction in IL-6 and TNF-α. Moreover subjects involved in the experimental group improved pain, disease activity and fatigue scores, in addition to the time of walking and the number of steps over a distance of 50m. Similarly Jastrzabek and colleagues (2013) found consistent results on functional parameters and on decrease of TNF-α after a cycle of twenty three-minutes long sessions performed in ten consecutive days of PBC (-160°C) and local cryotherapy (-30°C) with 20 patients diagnosed with rheumatoid arthritis randomized in each group. However, they did not find differences in the effectiveness of either cryotherapy.

According to the literature whole- and partial-body cryotherapy have a positive effects on quality of life, physiological and performance scores in patients with rheumatoid arthritis. It seems not to be discriminating a lower value of temperature: a wide range such as from -60°C to -160°C should be effective for rheumatoid arthritis patients. It appeared that for this condition constancy is the key to obtain significant results in terms of pain (almost one week of twice daily sessions). As for ankylosing spondylitis neither of these studies considered a follow-up, so is not possible to know how the benefits last over time.

In the last decade a Polish group driven by Elzbieta Miller has studied, with different research protocols, the effectiveness of whole-body cryotherapy in patients affected by multiple sclerosis (Miller et al., 2011; Miller et al., 2013; Miller et al., 2016). In their most recent study (Miller et al., 2016) they monitored the effects of whole-body cryotherapy on fatigue and functional status in multiple sclerosis patients with different levels of fatigue in two groups of subjects, the first one (n= 24) with a low fatigue score (measured with the Fatigue Severity Scale) and the second one with an high fatigue score. All multiple sclerosis patients involved in this study were exposed to ten consecutive whole-body cryotherapy sessions in a period of two weeks. Self-reported functional status indexes were assessed before and after the cycle of cryotherapy. They found significant improvements in both group after completing the treatment in the functional status and in the feeling of fatigue, especially in the group with an high fatigue score. In another study (Miller et al., 2013) they investigated in a population of secondary progressive clinical form of multiple sclerosis patients the uric acid concentration and their functional state by means of the expanded disability status scales. They enrolled 22 patients and 22 healthy control individuals participating in a protocol of ten three-minutes long exposures of daily whole-body cryotherapy. They tested the subjects before the study period, immediately after and after one month and then after three months. They noticed that in the group of multiple sclerosis patients significantly increased the uric acid concentration in plasma both after ten sessions and after three months by the end of the study period. Their functional status improves after the study period and the positive changes in the expanded disability status scale maintained a significant level also after one also after three months. In conclusion, they indicate that whole-body cryotherapy may be used as adjuvant therapy in secondary progressive multiple sclerosis patients. Miller and colleagues in 2011 compared the effects of ten consecutive exposures in a cryo-chamber (with a temperature range of the main chamber between -110°C and -160°C) on the total oxidative status (TAS) of plasma and activities of antioxidative enzymes in erythrocytes from depressive and non-depressive multiple sclerosis patients. They measured TAS, superoxide dismutase (SOD) and catalase (CAT) levels before and after the study period, founding an increase of the TAS level in depressive patients more than in non-depressive patients after a cycle of ten consecutive sessions of WBC. In their conclusions authors stated that a protocol of ten consecutive cold exposures in a cryochamber induced a suppression of oxidative stress in secondary progressive disease multiple sclerosis patients, especially in depressive ones. Recently Pawik and co-workers (2019) has studied the effectiveness of whole-body cryotherapy and physical exercises on the psychological well-being of patients with multiple sclerosis. They enrolled 60 patients, who were divided into three groups: cryotherapy, physical exercise training and cryotherapy with physical exercise training. Participants who had WBC performed 10 sessions in a 2-week period. The temperature was maintained at -110°C during the first session and reached -160°C on the last day of the study. The physical exercise training lasted 60 min per session: after an initial warm-up, it consisted by 40 min of resistance exercises using the Thera-Band in closed kinetic chains. They measured well-being indexes by means of validated questionnaires such as The Psychological General Well-Being Index (PGWBI), Hospital Anxiety and Depression Scale /HADS) and the Rivermead Mobility Index (RMI). Authors find that the use of WBC reduced anxiety and depressive symptoms in studied patients with multiple sclerosis, particularly when combined with physical exercise training, concluding that the introduction of WBC into the standard physiotherapy protocol for patients with multiple sclerosis in fully justified.

According to the literature, whole-body cryotherapy have a positive effect on oxidative stress, functional status and uric acid concentration in patients with multiple sclerosis. A minimum of ten consecutive sessions with a temperature of exposure between a range within -110°C and -160°C seems to ensure positive effects in multiple sclerosis patients. Benefits of the functional status can last up to three months, supportive even more the effectiveness of whole-body cryotherapy in multiple sclerosis patients.

Whole-body cryotherapy is often used as a symptomatic therapy against acute and chronic back pain: facilities working with cryotherapy chambers or cabins often promote the benefits of very cold exposures on pain relief in the low back. Few studies demonstrated the effectiveness of whole body cryotherapy on this musculoskeletal disease. For example Śliwinski and colleagues in 2017 examined the differences of gender in two groups with low back pain about different etiology after a cycle of thirty interventions of whole-body cryotherapy followed by thirty minutes of kinesitherapy exercises. They utilized the VAS scale and the Schober test to evaluate the stepping of pain ailments and the mobility of lumbar spine respectively. They found significant improvements of the VAS score in men group, associated with an improvement in column mobility vertebral spine. However these improvements were not statistical significant in women group. In 2016 Nugraha and colleagues in a prospective randomized double blind study design evaluated the effects of ten consecutive sessions of whole-body cryotherapy at different temperatures: in fact one group was exposed to a temperature of -67°C and the other one was exposed to a temperature of -5°C. Authors evaluated the degree of low back pain by means of self-related questionnaires such as Mainz Paing Staging System (MPSS), Pain Visual Analogue Scale (VAS), Pain Perception Scale (PPS), Pain Disability Index (PDI) and a self-designed patient questionnaire about subjective pain perception and general comfort. Their results showed that cold exposures to both temperatures could improve pain-related scores at the end of a cycle of ten consecutive cryochamber sessions. Their main founding was that was found the same effect from ten three-minutes long sessions at -67°C and at -5°C in a cryochamber for low back pain. Accordingly to these results they concluded that there is no need to treat patients with low back pain with very low temperatures (below -60°C). Concerning cold exposures and low back pain I was pleased to participate as co-author of a study (Giemza et al., 2015) in which we examined the differences in two groups of patients with low back pain of 40 subjects each. The first group performed two whole-body cryotherapy sessions a week, while the second group was exposed to WBC daily (from Monday to Friday), for a period of three week. The temperature of exposure was the same for each group and it was set to -60°C for the pre-chamber, where they spent a period of thirty seconds and then they moved in the main chamber, where they spent another three minutes, at a temperature of -120°C. We measured lumbar spine mobility with the assessment of active flexion and extension, rotation to the right and left, lateral flexion to both sides and the EMG measurement of erector spinae in the lumbar part of the spine performed at rest and in flexion, in adjunct to subjective parameters such as a pain scale (VAS) and functional limitations determined by the Activities of Daily Living survey (ADL). We found significantly lower values of active potentials of erector spinae muscles in the lumbar part of the spine and a significant increase in the range of the lumbar spine mobility only in the patients who was exposed to cryogenic temperatures five times a week for a total of fifteen sessions, concluding that only the regular application of WBC treatments is effective in pain reduction and mobility improvement in patients with low back pain. In a similar research, Giemza and colleagues (2014) studied the influence of WBC treatments on the improvement of spine activity in elderly men (age range 65-75 years). The protocol of cold exposure was the same to the study above mentioned in terms of number of sessions, temperature and time of exposure. Consequently, one group was exposed to fifteen WBC sessions and one group, as control, did not use whole-body cryotherapy. Mean increase in the range of spine mobility was 69.06% for the WBC group while was only 21.26% in control group. The same results were found for muscle tension: a decrease of 59,25% for the experimental group while a lower increase (32.25%) in the control group. This induced the authors to state that, first of all whole-body cryotherapy is a safe procedure even to elderly people, and then is also effective in association with physiotherapy on people suffering from low back pain.

WBC could be a useful treatment for low back pain, to improve range of motion and to decrease pain. The optimal protocol to achieve best results is debatable: it appears that a cold exposure at a temperature of -5°C could be enough to decrease pain. At the same time it is important that only the regular application of WBC treatments is effective because when the period between treatments is too long, positive results disappear and the procedures which occur next do not have a positive influence on a patient because they do not add up: that is why that ten consecutive daily sessions is the minimum gold standard to achieve best results.

The areas of whole- and partial-body cryotherapy are varied, in fact in literature we can found also researches who have dealt the correlation with the exposure to very low temperatures to mental disorders, anxiety and depression. In a recent article (Rymaszewska et al., 2018) the authors evaluated the effects on cognitive functioning and blood parameters in 21 patients with mild cognitive impairments of a cycle of ten two-minutes long sessions of whole-body cryotherapy in a cryo-chamber cooled at a temperature between -110°C and -160°C. Immediately after the last cryotherapy session and two weeks later, patients had improved significantly from baseline on two-word recall subscales of the DemTec, a psychometric screening tool, on the semantic and anterograde subscales of the 5-minute Test Your Memory (TYM) scale and on the logical memory subscale of the Saint Louis University Mental Status (SLUMS) exam. A cycle of ten WBC sessions induced also a significant increase of nitric oxide (NO) plasma level, brain-derived neurotrophic factor (BDNF) concentration and reduction of interleukin-6 (IL-6). Their preliminary results suggested that a cycle of two minutes of daily whole-body cryotherapy sessions could be indicated as a supportive therapy in patients with mild cognitive disorders. Another Polish group (Szcepanska-Gieracha et al., 2014) evaluated the impact of a cycle of ten whole-body cryotherapy exposures (from 1 to 2 minutes in the first two sessions to 3 minutes during the last eight sessions; they did not declared the temperature of exposure) on various parameters of mental state of the patients divided in two diagnostic groups (spinal pain syndromes and peripheral joint diseases). The patients completed a survey before the first treatment and after the last one. Results showed a significant improvement of the score of the administered questionnaires after the last session of WBC, having a significant impact on the improvement of patient's well-being, mood and quality of life indices. No difference of age was noted, but a difference in gender was reported, with females having a more appreciable improvement than males. Better improvements were found also in the group with spinal pain and in which started the treatment period with more severe values of depressive symptoms. Finally in another paper (Rymaszewska et al., 2008) authors assessed the efficacy of whole-body cryotherapy in patients with anxiety disorders receiving standard psycho-pharmacotherapy. The experimental group received also fifteen consecutive whole-body cryotherapy sessions (duration from 2 minutes for the first visit to three minutes for the following and temperature -110°C for the first visited and then lowered to -160°C over successive visits). Depressive and anxiety levels was evaluated by the Hamilton's depression rating scale (HDRS) and Hamilton's anxiety rating scale (HARS). Authors found positive effects already after the first week of treatment and after three weeks these results were more consistent (34.6% of the study group decreased the HDRS scores more than 50% of the basal value and the 46.2% of the study group decreased of at least 50% from the baseline the HARS score). In the materials and methods section they stated to have measured these values also after three and six months respectively, but in this article they considered only the first three weeks of treatment. The findings of this study suggested that WBC could be an adjuvant treatment for mood and anxiety disorders in the short-term period.

The mechanism of action of whole- and partial-body cryotherapy on mental state, anxiety, depression and mild cognitive disorders is not completely clear but its effectiveness is supported by few data. For sure, further and strongest researches are needed to comprehend the mechanism of action, the benefits and their duration in the long-term of a cycle of whole-body cryotherapy in mental disorders. Protocols are vague and different from each study. A minimum of ten sessions is required with a duration lasting from 2 to 3 minutes and a temperature set from -110°C to -160°C: further researches are required to standardize the protocol in order to assist practitioners with patients with mental disorders.

2.7 WHOLE- AND PARTIAL-BODY CRYOTHERAPY IN SPORT MEDICINE

In the last decades whole- and partial-body cryotherapy modalities have been often utilized by athletes, trainers and sport physicians to promote enhanced athletic performance and recovery. It is thought that cryostimulation dampens the inflammatory response and reduces edema formation following strenuous exercise and the consequent exercise-induced muscle damage (EIMD). Current evidence supporting its utilization is limited and contradictory: athletes testimony is generally high, but it could be maybe induced by a possible placebo effect. Generally in literature, when authors tried to confirm the eventual benefits of whole- and partial-body cryotherapy on recovery or on athletic performance, they investigated the trend over time of the athletic performance by means of physical tests and the trend of indirect markers of muscle damage (creatine kinase, CK) and systemic markers of inflammatory response such as cortisol or cytokines (pro-inflammatory ones: interleukin-8 and -1β and tumor necrosis factor-α, TNF-α; anti-inflammatory ones: interleukin-10 and -1ra; and the inflammation regulator interleukin-6)

In 2017 two review articles summarized the findings of literature about the utilization of whole-body cryotherapy as a recovery technique after exercise and its utilization in athletes population (Rose et al., 2017; Lombardi et al., 2017).

In their interesting work, Rose and colleagues conducted a systematic search of the literature without restriction on the device utilized in the studies (WBC or PBC), on the duration, on the temperature of exposure, on the number and the frequency of the sessions and finally on the timing in relation to the exercise protocol administered. They evaluated only studies including a form of physical exercise to induce muscle damage and an outcome measure associated with muscle damage including at least one measure between inflammatory markers, pain scores, muscle damage markers and sport performance or muscle function indicators. They included in their review 16 articles (Hausswirth et al.,2011; Pournot et al.,2011; Costello et al., 2012; Fonda et al., 2013; Mila-Kierzenkowska et al., 2013; Ferreira-Junior et al., 2014a; Ziemann et al., 2014; Ferreira-Junior et al., 2015; Vieira et al. 2015; Krüger et al., 2015; Wozniak et al., 2007; Banfi et al.; 2009; Mila-Kierzenkowska et al., 2011; Ziemann et al. 2012; Wozniak et al., 2013; Schaal et al., 2015), which were divided in two groups: the first one included researches with controlled damage protocol (Laboratory controlled studies, N=10) and the second one included researches with treatment proposed by means of routine training cycles, not standardized in terms of application and intensity of the stimulus, for all participants (Applied studies, N=6).

In terms of standardization there were some differences between each proposed protocol: first of all they noticed that were recruited highly trained athletes in ten studies and in the remaining six articles were recruited healthy and physically active people. There were also numerous gender differences: two studies included only female participants, thirteen studies included only male participants and in the remaining one article were recruited both genders. In all studies participants were exposed to very low temperature for at least two minutes and for a maximum of three minutes. The number of cryostimulation sessions varied from one to thirty, performed in a range of one to ten days.

In laboratory controlled studies, all four studies using multiple cryotherapy sessions found a significant decrease in muscle pain, associated with improvements in muscle function. Costello and colleagues (2012) did not find influence of a single WBC session on maximum voluntary contraction (MVC), but he administered cryostimulation only once and moreover 24 hours after the exercise inducing muscle damage. Also Vieira and colleagues (2015) did not found improvements in a performance test such as vertical jump: in this case they repeated the test very close to the cold exposure (30 minutes later) with a possible limited muscle functionality due to reduced muscle temperature. These two articles imply that the timing of whole-body cryostimulation could be a key point in the validation and in the standardization of protocols aiming to enhance recovery and restore physical performance.

Another crucial factor could be the total number of exposures to very-low temperatures during the recovery phase. This notion is supported by the analysis of circulating CK, a biomarkers of muscle damage. In one study (Ziemann et al., 2014) CK level in the experimental group (10 sessions in a period of five days) decreased significantly compared to the control group while in other two studies where researchers utilized a protocol of three (Hausswirth et al., 2011) and six (Fonda et al., 2013) cryostimulation sessions respectively no significant changes in CK scores were found. Finally, concerning the inflammatory response after very-low temperature exposures, the three study with standardized damage protocol that investigated levels of cytokines found similar results (Pournot et al., 2011; Mila-Kierzenkowska et al., 2013; Ziemann et al., 2014) with an increase of concentration of the anti-inflammatory cytokine IL-10, a drop of pro-inflammatory IL-6 concentration and a limited increase in IL-1β concentration when participants were exposed to whole-body cryotherapy treatments.

The authors of the review concluded that these findings suggest that whole-body cryotherapy may influence the pathways of inflammatory modulation, supporting the theory that WBC may prevent detrimental overexpression of inflammatory proteins.

In applied studies, every single research used a protocol with more than a single WBC session: only one of these six studies found no significant results in inflammatory and damage markers (Mila-Kierzenkowska et al., 2011).

Athletic performance, in terms of skill recovery after exercise, was investigated in two studies only and both of them found an improvement in performance in subjects involved in the experimental group: Ziemann and colleagues (2012) found improvements in tennis drills after a cycle of ten whole-body cryotherapy sessions (3 minutes; -120°C) performed in five consecutive days (twice a day) and Schaal and colleagues (2015) found a less remarkable reduction of the swim speed in a 400m trial in experimental group (ten sessions of WBC lasting three minutes at a temperature of -110°C) compared to control group.

Concerning the applied studies, four of them founded a remarkable reduction in circulating CK levels and only one of them (Mila-Kierzenkowska et al., 2011) found no significant difference in circulating CK concentration after a cycle of twenty WBC sessions (according to the original paper: two exposures a day for a period of ten days with a temperature range from -120°C to -140°C, while in the review authors wrote three exposures each day). In another study Banfi (Banfi et al., 2009) found an increase of the anti-inflammatory cytokine IL-10 after a week of typical training combined with daily WBC exposure (2 minutes at -110°C) in the Italian national rugby players. Finally in another study (Ziemann et al., 2012) authors found a significant decrease in TNF-α and an increase in IL-6 concentration after 10 WBC sessions after a study period of five days where international level tennis players combined low intensity training and cold exposures (twice a day). According to the 16 studies analyzed in this review, authors indicate that whole-body cryotherapy may be successful in enhancing MVC and returning athletes to pre-exercise strength at a faster rate than control conditions. WBC showed an analgesic response after damaging exercise, lowering pain scores on average 31% than control.

Summarizing, Rose and colleagues suggested the utilization of WBC after exercise, taking in account a possible dose-response relationship between exposures with the recovery of muscle damage indicators. A minimum of three sessions, lasting three minutes are required. They have to be performed immediately after the strenuous exercise and in the two to three days post training. Cold exposure seems have no differences in term of efficacy if the temperature is colder than the average of -140°C.

For their part, Lombardi and colleagues (2017) did not declare what kind of keywords they utilized for their review and neither the sources where they search for eligible papers, but they wrote an update of literature since the publication of their first review article concerning the topic of whole-body cryotherapy in athletes (Banfi et al., 2010). They analyzed the literature in order to discuss of the effects of whole-body cryotherapy (they did not take in account studied published on partial-body cryotherapy) on physiological and functional parameters.

Concerning the hematological response to whole-body cryotherapy they analyzed articles which have studied erythrocytes and hemoglobin changes, iron metabolism, hemolysis, leukocytes and platelets levels. In one of the articles included in their review a group of 27 elite rugby players, which performed two daily sessions of whole-body cryotherapy in a whole week, showed a significant decrease of erythrocytes, hematocrit and hemoglobin (Lombardi et al., 2013) with a similar decrease of hemoglobinization reported by a previous article (Banfi et al., 2008). Also Szygula and colleagues (2014) reported a significant decrease in hemoglobin in a study performed of physically active subjects which underwent thirty whole-body cryotherapy sessions (3 minutes at -130°C) in a row. In this study hemoglobin remained lower than baseline until the twentieth session and it recovered during continued exposures after thirty sessions. A couple of studies (Ziemann et al., 2012; Sutkowy et al., 2014) did not find changes in hemoglobin and red blood cells in group of elite tennis players after a cycle of ten sessions of whole-body cryotherapy (twice a day for five days lasting three minutes at a temperature of -120°C) and in a group of kayakers after a cycle of twenty whole-body cryotherapy sessions (twice a day for ten days lasting three minutes at a temperature range of -120°C to -145°C) respectively. Lombardi and colleagues speculated that the shifts in hemoglobin and red blood cells counts induced by whole-body cryotherapy could depend on the sport discipline and individual baseline hematological profile. The phenomenon of the reduction of hemoglobin allows the whole-body cryotherapy to distance itself to any form of doping because the OFF-score, a parameter used to calculate the probability of blood doping in athletes, depends on hemoglobin concentration and Ret count (Lombardi et al., 2017). Moreover whole-body cryotherapy enhances hemolysis: a decrease of haptoglobin was described by Szygula and colleagues (2014). These findings are in contrast with those reported by Banfi and co-workers (2010) in their review analyzing data collected on rugby players after a whole week of whole-body cryotherapy treatments. Hemolysis stimulates the release of erythropoietin (EPO), as also documented by Szygula and colleagues (2014), with the aim to recover to baseline levels of red blood cells count and hemoglobin. The changes of EPO concentrations after thirty sessions of whole-body cryotherapy (temperature: -130°C; treatment duration: 3 minutes) had a small, but statistically significant increase, stimulating erythropoiesis, which could facilitate a return of erythrocytic system indices to initial levels after the cold exposure cycle.

The above mentioned papers (Lombardi et al., 2013 and Sutkowy et al., 2014) demonstrated also that levels of leukocytes did not show any changes after a cycle of very-low temperature exposures (fourteen and twenty WBC sessions respectively), whilst Szygula and co-workers (2014) shown an increase of leukocytes after ten and twenty whole-body cryotherapy sessions in students of military academy, but recorded a return to baseline after thirty sessions: however these variations remained within the physiological range.

Finally, platelet levels did not change after a cycle of whole-body cryotherapy sessions as reported by several articles (Lombardi et al., 2013; Ziemann et al., 2014; Szygula et al., 2014).

Concerning the lipids concentrations and the energy metabolism whole-body cryotherapy showed a dose-dependent response improving the lipid profile as already reported by Lubkowska and colleagues (2010c) in their study involving a group of professional rugby players and healthy individuals which performed from five to twenty WBC sessions daily. They found that five sessions are not enough to modify subjects' lipid profile, while ten sessions induced a significant decrease in triglycerides and finally twenty sessions induced a decrease of triglycerides and low density lipoprotein cholesterol with an increase of high density lipoprotein cholesterol. These findings are consistent to those reported by Ziemann and colleagues (2014) after a cycle of ten whole-body cryotherapy sessions in a period of five days, where they reported a significant decrease of both total cholesterol and low density lipoprotein cholesterol values.

In their studies on sportsmen the research group lead by Ziemann analyzed the trend of resting metabolic rate after very-low temperature exposures (Ziemann et al., 2012; Ziemann et al., 2014), in particular in professional tennis players exposed to ten WBC sessions, values of resting metabolic rate and of the percentage of fat used as a metabolic substrate did not change, as well for healthy, physically active men.

Also bone metabolism was studied in professional athletes, in particular rugby players (Galliera et al., 2013), which underwent five consecutive two-minutes long whole-body cryotherapy sessions at a temperature of -120°C: the major findings of this research was an increase both of the osteoprotegerin (OPG) and consequently of the OPG/RANKL ratio, an index of reabsorption-to-formation balance, that could be have a beneficial role in the prevention of stress fractures.

In the field of sport recovery the anti-inflammatory effect of whole-body cryotherapy is the key factor of most of the studies. Lombardi and co-workers (2017) confirmed the results reported by Pournot et al., (2011), on the anti-inflammatory markers such as IL-6 and IL-10 (which not changed after a three-minutes long session whole-body cryotherapy at a temperature of -100°C), whilst it was noted a lower increase of the pro-inflammatory markers TNFα and IL-1β compared to the control group and a parallel inducing effect on the anti-inflammatory IL-1ra one hour post-treatment, concluding that one session of WBC enhanced muscular recovery. Also the above mentioned paper, concerning the utilization of WBC in professional tennis players (Ziemann et al., 2012), reported a more remarkable decrease of TNFα serum concentration in the experimental group compared to the untreated group. Also Mila-Kierzenkowska and colleagues (2013) reported an anti-inflammatory response following a three-minutes long cold exposure at a temperature of -130°C with a prevention of the increase of IL-1β and IL-6 values. Selfe and colleagues (2014), for their part, reported that a single session of WBC performed at a temperature of -130°C did not affect IL-6 values in elite rugby players, no matter the duration of the treatment (lasted one, two or three minutes respectively). A single session of whole-body cryotherapy performed in a cryo-chamber for three minutes at a temperature of -110°C after an eccentric workout inducing delay onset muscle soreness (DOMS) in physically active young men increasing levels of IL-6, IL-β and IL-10 and thus mimicking the effects induced by strenuous exercise, while after ten sessions of whole-body cryotherapy performed in five consecutive days, a group of tennis players reported no significant changes for the values of IL-6 and IL-β and a significant increase of IL-10 compared to baseline (Ziemann et al., 2014). In conclusion, despite the fact that there are conflicting evidences in literature, it can be assumed that whole-body cryotherapy has an anti-inflammatory response in athletes, with a similar trend of IL-6 compared to no cold exposure treatment after one session and a faster recovery to baseline after several consecutive sessions.

Concerning hormones profile an interesting article written by Grasso and colleagues (Grasso et al., 2014) evaluated the trend of elite rugby players during a whole week of training in which subject were exposed twice a day to a cryochamber sessions lasting three minutes each with a temperature of -140°C. They found a decrease of cortisol and dehydroepiandrosterone (DHEA) both after one and seven days. At the end of the study also estradiol decreased with a concomitant increase of testosterone level: this allowed to the testosterone-to-cortisol ratio to increase, concluding that cryostimulation could potentially enhance the athletic performance. Nevertheless, it seems that the above mentioned article is the only one which reported a decrease of cortisol level after a cycle of whole-body cryotherapy. In fact, other studies found different results in the scores of this hormone: Ziemann and colleagues (2012) found an increase of cortisol after ten cryotherapy sessions (three minutes at -120°C) performed in a period of five days in professional tennis players, while testosterone was unchanged. Also in sixteen kayakers (Sutkowy et al., 2014), who underwent twenty consecutive whole body cryotherapy sessions in a period of ten days, levels of cortisol and testosterone remained unchanged. In a small group of elite rowers subjected to twelve whole-body cryotherapy sessions, lasting three minutes at a temperature range between -125°C and -150°C in a period of training of six days, cortisol levels registered an increase on the third day of study until it reduced its increase at the end of the study period (Wozniak et al., 2013). Cortisol levels was unchanged also in a group of elite synchronized swimmers studied by Schaal and colleagues (2015) exposed to daily whole-body cryotherapy sessions (3 minutes at -110°C) for a period of two weeks. Finally in a more recent study (Russel et al. 2017) there was no changes in cortisol levels after a single two minutes lasting very low-temperature exposure in a cryo-chamber set at a temperature of -135°C performed immediately after a repeated sprint exercise.

In literature the effect on prooxidant-oxidant balance of whole-body cryotherapy was already studied in elite kayakers and in untrained men (Wozniak et al., 2007) and it was confirmed by a more recent study from the same Polish research group (Wozniak et al., 2013) on elite rowers subjected to a period of training of six days combined with two daily whole-body cryotherapy sessions (three minutes at a temperature between -125°C and -150°C), where they found a lower activity of superoxide dismutase and glutathione peroxidase after the third day of training with WBC than without WBC. Also in another study performed in elite kayakers (Sutkowy et al., 2014) was assessed the efficacy of the WBC on an oxidant-antioxidant balance, in which the efficiency of TBARS elimination increased after the end of a training period of ten days, combined with two daily cryostimulation treatments performed in a cryo-chamber at a temperature range from -120°C to -145°C. Summarizing the use of whole-body cryotherapy may reduce the risk of oxidative stress and the extent of muscle fiber injuries provoked by intense exercise, but it seems to have a dose-dependent improving effect.

Whole-body cryotherapy enhanced physiological and physichological recovery in nine endurance runners after performing a simulated trial run on a motorized treadmill in a study comparing three different recovery modalities (Hausswirth et al. 2011) in which the best results were observed after one hour, 24 h and 48h post-exercise in the WBC group. A possible explanation of the anti-inflammatory action of whole-body cryostimulation was also proposed by Ferreira-Junior and colleagues in 2014 (Ferreira-Junior et al., 2014b), suggesting the influence of WBC in inducing lower values of serum soluble intercellular adhesion molecule-1 (sICAM-1) immediately after exercise induced muscular damage: this fact could be limit the migration of leukocytes from blood circulation to the damaged tissues. An interesting commentary on the above mentioned article was written by Benoit Dugué in 2015 explained that cold could induce an improvement of release of sICAM-1, leading a decrease of muscle inflammation: that is why Benoit stated there is for the time being no information available on the changes of sICAM-1 due the combine effects of EIMD and whole-body cryostimulation.

Performance recovery, assessed with the time-to-exhaustion difference between a ramp-test protocol, was studied in endurance athletes in the research performed by Krüger and co-workers (2015) after a single session of whole-body cryotherapy (three minutes at a temperature of -110°C) compared to a control group involving for the same time (three minutes) on a low intensity walk. Acute recovery was improved in the experimental group after high-intensity intermittent exercise, might be induced by enhanced oxygenation of the working muscles, as well as a reduction in cardiovascular strain and increased work economy at submaximal intensities.

For its part, Bleakley and colleagues, reported only little benefit towards functional recovery in their meta-analysis (Bleakley et al., 2014). They speculated that less expensive cooling modalities should be used in order to obtain the same physiological effects of whole-body cryotherapy.

Synthesizing the review of Lombardi and colleagues (2017), the WBC effectiveness of post-exercise recovery in athletes is evidenced by many articles even if, however, a small number of studies did not reported any positive effects.

Analyzing the above mentioned reviews (Rose et al., 2017; Lombardi et al., 2017), it is clear that WBC is an useful method for enhancing recovery, but is still to investigate if and how this treatment is better than other ones (also because the studies evaluating long-term WBC treatment were proposed during training and summer camps, and not during the season). Further researches are required for confirm the benefits of whole-body cryotherapy in population practicing sports, with wide and accurate studies.

Whole- and partial-cryotherapy in the sport domains are utilized both by professional and recreational athletes. We cited in the above mentioned reviews only articles referred by the utilization of very-low temperature exposure by elite or professional athletes published before 2017. Noteworthy, there are many articles, which have investigated the utilization of whole- and partial-body cryotherapy also in recreational athletes or in healthy people aiming to evaluate their recovery after some bouts of proposed exercise.

For example, recently a French group (Douzi et al., 2018) evaluated the effect of whole-body cryotherapy exposure in a cryo-chamber based on forced convection (three minutes at -40°C and a wind speed of 2.3 m s^{-1}) after training in the evening on a group of physically active men. They examined the heart rate variability, as already reported in a previous paragraph, and the sleep quality, as a tool for measure the level of recovery. They proposed to the subjects involved in their study an evening training consisting of 25 minutes of continuous running at 65% of the maximal aerobic speed followed by intermittent running at 85% of the maximal aerobic speed and assigned them to very-low temperature exposure or to passive recovery in a random order. They found a significant decrease in the number of movements during the night following the

WBC exposure compared with the control condition. Also the subjective quality of sleep, measured with the Spiegel questionnaire was significantly better in the experimental condition than in the control condition. Finally the pain scores were significantly decreased following WBC compared to the control condition: that is why authors concluded that a three-minute single session of whole-body cryotherapy performed in a new kind of cryo-chamber based on forced convection (Bouzigon et al., 2017) in the evening improved the subjective and objective sleep quality in physically active men, enhancing the level of recovery. Interestingly few weeks before the publication of the above mentioned paper, another research was published about the use of cryostimulation as a recovery intervention in athletes (Wilson et al., 2018). Curiously also in this paper the protocol of exposure, in terms of time and temperature, was different compared to most of the articles (three minutes at a temperature range close to -130°C). In this protocol the experimental group was exposed to two cold treatments in a cryotherapy chamber, spending three minutes in the chamber set to -85°C and then having a fifteen minutes warming period in a temperature controlled room before entering for a further four minutes in the cryo-chamber at the temperature close to -85°C. Authors evaluated the perception of soreness and training stress, the markers of muscle function and inflammation and finally the efflux of intracellular proteins. These evaluation were done before and up to 72 hours post exercise. WBC at -85°C attenuated soreness after 24 hours and influenced positively peak force after 48 hours compared to cold water immersion (CWI, ten minutes in a mobile ice bath cooled to 10°C) and placebo (a cornstarch pill instead of branched chain amino acids), but many of the remaining outcomes were trivial even favorable to the placebo group. These findings let authors conclude that whole-body cryotherapy was not more effective than placebo treatment at accelerating recovery. Surely this study feeds the debate on the optimal protocol of exposure and also what are the optimal dosage and frequency of the cryogenic sessions. A plus point of this study was the insertion of the placebo group: in future studies about whole- and partial-body cryotherapy must have to take in account a placebo intervention.

Another recent study (Krueger et al., 2018) investigated the acute effects of a single session of whole-body cryotherapy following severe intermittent running exercise on biomarkers of inflammation, muscle damage and stress involving in

a cross-over study eleven endurance-trained males. After an exhaustive intermittent running exercise subjects were exposed in a cryochamber for three minutes at a temperature of -110°C or to a passive control condition. Before and after one hour from the strenuous exercise a ramp test was completed and for seven time points up to 24 hours post exercise venous blood samples were analyzed for serum inflammation biomarkers evaluation. In their research they found that compared to the control group the whole-body cryotherapy did not attenuate exercise-induced changes in IL-6, IL-10, sICAM-1, myoglobin, cortisol, testosterone and their ratio. Accordingly, they stated that physiological mechanisms by which WBC is proposed to improve recovery may be inaccurate.
In several paragraph of this thesis has been underlined that one single session should not be proposed as a recovery or anti-inflammatory protocol to achieve best results, that is why the aim of this above mentioned article seems not to provide a significant contribution to this topic and to the standardization of whole-body cryostimulation protocols.

Athletes were involved also in a recent study of a Japanese group (Kojima et al., 2018) in which the authors investigated the effect of a three-minute long whole-body cryotherapy treatment (-140°C) after an high-intensity treadmill exercise. After ten minutes following the completion of the exercise subjects were exposed to WBC or underwent a rest period (control group). Authors collected blood samples, respiratory gas parameters, skin temperature and ratings of subjective variables. Thirty minutes after the strenuous exercise ad libitum energy and macronutrient intake were evaluated during the buffet meal test, in which participants were instructed to "eat until they felt comfortable satiety". Energy intake was calculated by measuring the weight of energy intake consumed. At the end of the study authors found a significant increase of energy intake level during the buffet meal test in WBC group than the Control group, concluding that a single very-low temperature exposure could increase energy intake in male athletes. Authors stated that increased energy intake following WBC may assist physical recovery, since insufficient energy intake during post-exercise periods may delay recovery of muscle energy substance (e.g., muscle glycogen).

Last year, another article about whole-body cryotherapy in endurance runners was published (Szymura et al., 2018), in which the effect of repeated whole-body cryotherapy sessions on the erythrocyte system in elder marathon runners, was studied and compared to untrained subjects. Master marathon runners and untrained subjects were exposed to twenty-four whole-body cryotherapy sessions (three minutes at a temperature to -130°C) performed every other day. Blood samples were taken before, at the half and at the end of the study, as well as one week after its conclusion. They showed that repeated cryostimulation sessions did not cause significant hemolytic blood changes in elderly men, despite of their fitness level. The protocol proposed did not increase the level of erythrocytes or their hemoglobinization: it could safely utilized also by elderly because it did not induced hemolytic anemia and, in case of athletes such as marathon runners, it can be assumed that WBC is not a form of doping.

The topic of the utilization of WBC as a recovery technique in the sport field has been addressed also by Abaïdia and co-workers (2017) which compared the effects of cold-water immersion (ten minutes at 10°C) and whole-body cryotherapy (three minutes at -110°C) on recovery kinetics after exercise-induced muscle damage. In a randomized crossover design they enrolled ten physically active men which performed 5 sets of 15 eccentric knee flexors contraction on one leg, after that they performed either a CWI or a WBC intervention. Authors evaluated the recovery level immediately after and 24, 48 and 72 hours after the EIMD: they found a very like moderate effect in favor of CWI to improve recovery kinetics of single leg and 2-leg countermovement jump at the time point of 72 hours after the EIMD. Moreover CWI did lower ratings of muscle soreness and increase perceived recovery across 24-48 hours post-exercise, these findings lead authors to conclude that CWI was more effective than WBC in accelerating recovery kinetics for CMJ performance at 72 hours post-exercise. In this study there was not a control group, so it is unknown if after an EIMD on a single leg WBC is useful to improve recovery in comparison with passive recovery.

Another article published in the same year similarly evaluated the effects of a single whole-body cryotherapy (30 seconds at -60C followed by 120 seconds at -135°C) exposure on recovery levels after repeated sprint exercise in professional soccer players (Russel et al., 2017). Blood and saliva samples, as well as counter movement jump performance and perceived muscle soreness and recovery were assessed immediately, 2 hours and 24 hours after the strenuous exercise. Authors found a greater testosterone response at 2 hours and 24 hours post exercise in WBC group than in control group, while other variables such as peak power, CK, cortisol, testosterone/cortisol ratio, blood lactate, perceived soreness and recovery were not influenced by trial, but timing effects were significant with a subsequently returned to pre-exercise values within 2 hours. That is why they concluded that a single session of whole-body cryotherapy elicited greater testosterone concentrations for 24 hours after repeated sprint exercise when compared to rest and it could be linked to an attenuated inflammatory response to exercise, with possible implication for subsequent training motivation, indicating that WBC could be useful for soccer players' recovery involved in close agonistic commitments.

Finally in a Cochrane review Costello and colleagues (2016) examined the effects and safety of whole-body cryotherapy in alleviating DOMS after exercise in order to improve recovery. They found four eligible studies, but all of them had design features that carried a high risk of bias, thus limiting the reliability of the finding. Two studies used whole-body cryotherapy devices (specialized cryochamber set to a temperature of -110°C) and the other two used partial-body cryotherapy in a special cryo-cabins (temperature between -140 and -195°C). As the quality of the evidence was graded as a very low quality, authors assumed that a clinically meaningful was absent. That is why they did not supported the effectiveness in the reduction of muscles soreness and in the improvement of subjective recovery after exercise in physically active young men. Accordingly to their conclusions it is desirable to have high-quality and well-reported research on this topic.

Most of the articles concerning the utilization of cryostimulation in the field of sport recovery were published utilizing whole-body cryotherapy devices and the minor part of them utilized partial-body cryotherapy devices. A study conducted with a partial-body cryotherapy technology was has been articulated by a Brazilian group (Ferreira-Junior et al., 2014c), which evaluated the effects of a single session of whole-body cryotherapy on neuromuscular performance, particularly on elbow flexor site. This study involved thirteen participants which were subjected to a partial-body cryotherapy session (3 minutes at -110°C, that is the most warm temperature ever published regarding PBC) or to a control group. They were tested before and 10 minutes after each condition for their maximal isokinetic elbow flexion at 60°C. Authors did not find significant differences in peak torque, average power, total work or muscle activity between conditions. Thus it seemed that PBC exposure did not affect EMG signals of the elbow flexor, so authors concluded that partial-body cryotherapy could be utilized also before training or rehabilitation without compromising neuromuscular performance of the elbow flexors.

Exposure to very-low temperature in cryo-cabin was recently studied also by another research group (Hohenauer et al., 2018) which compared the physiological responses and the effects on recovery following a EIMD on healthy males both after cold-water immersion and partial-body cryotherapy. Interestingly authors cited two works of mine when explaining the protocol utilized for very-low temperature (Fonda et al., 2014; De Nardi et al., 2015), but at the same time they utilized a protocol of cold exposure probably not reproducible with the device utilized for this study (Cryomed s.r.o., Cryosauna Space Cabin, Nové Zámky, Slowakia), that is 30 seconds of pre-cooling at -60°C and then 2-minutes cooling at -135°C: for our best knowledge, and for my personal expertise in 7 years of utilization of the same device, is not possible to obtain a pre-cooling treatment at -60°C with the Space Cabin cryosauna model, and this is the only one study published with a pre-cooling exposure in a cryo-cabin. Despite of this methodological issue, they analyzed the effects on physiological variables of the above mentioned two cooling modalities prior and up to 60 minutes post treatment, with an interval of 10 minutes between each measurement, and the effects on recovery variables prior and up to 72 hours post treatment, with an interval of 24 hours between each measurement. Authors did not find significant differences in DOMS between groups, also maximum voluntary contraction of the right knee extensor and vertical jump performance returned to baseline in both groups after 24 hours within no significant differences between groups. Concerning physiological response such as thigh muscle oxygen saturation and cutaneous vascular conductance CWI showed a greater impact than PBC, however both cold techniques showed similar recovery profiles during a 72-hours follow-up period.

One of the most cited papers in the topic of recovery following PBC practice is that written by the Slovenian research team (Fonda and Sarabon, 2013), which cooperated with me in the drafting of a couple of paper (Fonda et al., 2014; De Nardi et al., 2015), examining the effects of a five cryostimulation sessions lasting three minutes in a cryo-cabin at a temperature between -140 and -195°C in a 5-days period. They subjected participants to a bout of damaging exercise for the hamstring muscles on two occasions separated by 10 weeks (control and cold-exposure condition). Authors tested biochemical markers, perceived pain sensation and physical performance by means of squat jump test, counter movement jump test and maximal and explosive isometric torque production. Values of pain sensation tests differed statistically significantly between the control and PBC group 1-72 hours after exercise, with PBC condition showing lower pain values compared with control condition. Other interactions were not statistically significant, that is why authors did not provide conclusive support of the use of PBC for recovery enhancement after strenuous training.

Finally, one of the few articles published utilizing PBC technology on the topic of the sport field, was that one I wrote in 2015 on the effects of PBC on flexibility (De Nardi et al., 2015). In a group of healthy people we evaluated the effects of a single partial-body cryotherapy session (150 seconds at a temperature between -130 and -160°C) by means of the sit-and-reach test. We evaluated the acute effect of a single very-low temperature exposure on the range of motion in men and women and we found a greater improvement of the sit-and-reach scores in the experimental groups compared to control groups, supporting the hypothesis that, after the decrease of neural activity due to cryostimulation which allows more elongation of the muscle at a given load, the range of motion is increased immediately after a single session performed in a cryo-cabin. These findings should encourage practitioners working with clientele aiming to increase range of motion to include PBC in their training periodization.

3. THE PURPOSE OF THE STUDY

The general purpose of this thesis was to study possible practical applications of partial-body cryotherapy in the context of medicine, health and sport.
More specifically, the aims of this thesis can be expressed as follows:

a. To evaluate the acute effect of a single PBC session on maximum isometric strength, in healthy subjects;

b. To evaluate the effects of repeated PBC sessions on pain threshold in healthy subjects;

c. To evaluate the effects of repeated PBC sessions on quality of life in fibromyalgic patients.

4. ACUTE EFFECTS OF PARTIAL-BODY CRYOTHERAPY ON ISOMETRIC STRENGTH: MAXIMUM HANDGRIP STRENGTH EVALUATION.

4.1 INTRODUCTION

Muscle contraction is defined by the changes in length of the muscle during contraction: it can be classified into isotonic or isometric. Isometric contraction occurs when the muscle tenses while not changing its length. It is required in some sports (for example climbing and gymnastics) and physical and handwork activities. Many daily functions and sporting events require high activity levels of the flexor musculature of the forearms and hands. The most common method of assessment for grip strength is the use of a handheld dynamometer. This measurement may provide over all body strength and muscles performance, individual nutritional status and well-being, by a simple and non-invasive evaluation (Shea, 2007). It is known that temperature is a relevant determinant of muscles performance (Oksa et al. 1997; Ranatunga, 2010). Isotonic and isometric strength respond differently due a decrease in temperature (Sargeant, 1987; Nodehi Moghadami et al, 2012). Some studies reported that cold application provides an effective way to increase isometric strength (Nodehi Moghadami et al, 2012; Burke et al., 2000). Human body responds to cold application with a vasoconstriction to maintain the core temperature. After this first response usually occurs the opposite phenomenon, a vasodilatation, which increase muscle blood flow with increased oxygen supply, therefore potentially improving muscle performance (De Ruiter et al. 1999).

In the last decades, despite the increasing popularity of WBC in sports and exercise medicine, few studies have investigated the effectiveness of WBC on muscle-performance recovery after exercise in adults (Akehi et al., 2016; Costello et al., 2016), but till now no studies have examined the acute effects of cold on the maximum isometric strength.

There are limited data about the effects of the cryogenic temperatures on isometric strength. Consequently, to evaluate the effects of a single PBC session performed before a maximum hand grip strength test, one hundred healthy men and one hundred healthy women were required to be present for one time at the testing venue. Both men and women populations were divided in experimental and control group to assess the differences in maximum isometric strength after 150 seconds of standing rotations carried out in a cryo-cabin or in a thermoneutral room.

4.2 SUBJECTS

The subjects were recruited to the study through announcements. All of them underwent a medical examination by a physician to avoid contraindications with exposure of extreme low temperature of partial-body cryotherapy. All of them had not practiced partial-body cryotherapy before. They was informed about all procedures and every step of the experimental protocol was explained before starting to collect data. A informed written consent form was fulfilled by the subjects before participating. All the procedures used complied with the principles of the Declaration of Helsinki. Table 1 shows the anthropometric characteristics of the subjects in this study.

	Age (years)	**Height (cm)**	**Weight (Kg)**	**BMI (Kg/m^2)**
(PBC-male, n=50)	42.42±14.17	178.02±7.1	79.41±9.52	25.21±3.14
(PBC-female,n=50)	41.26±11.73	165.0±5.1	62.0±7.95	22.79±2.79
(CON-male, n=50)	37.12±9.32	177.2±5.3	76.12±12.01	24.18±3.61
(CON-female, n=50)	39.56±11.38	167.12±6.2	64.94±7.79	23.38±3.03

Table 1. Anthropometric characteristics of the subjects. Values are means ± SD.

4.3 EXPERIMENTAL PROCEDURES

Partial-body cryotherapy took place in a specially built, temperature-controlled cabin (Space Cabin, Criomed Ltd, Kherson, Ukraine), a single-user open tank equipped with a mobile lift, which allows to adjust the height of every subject. Thus each participant was exposed to the very cold dry air up to the shoulders,

with the neck and the head out of cabin. Every single session consisted of a 150 s-long exposure at a temperature range between -130 and -160 °C (Fonda et al., 2014). During the session subjects wore swimwear, a pair of gloves, woolen socks and wooden clogs. Well instructed members of the cryotherapy studio was always present during the PBC sessions and instructed participants to turn around continuously in the cabin for each 150 s-long exposure session.
Each subject enrolled in the experimental group was exposed once to PBC. On the other hand, each subject of the control group was asked to perform standing rotations for 150 seconds in the turned off cryocabin (22.0±0.5°C).

4.4 MAXIMAL HANDGRIP STRENGTH

Maximal handgrip strength was recorded at two time points (T0 and T1) using a portable JAMAR Hydraulic Hand dynamometer (Sammons Preston Rolyan Nottinghamshire, United Kingdom). It was regulated for each subject fitting the hand and allowing flexion at the metacarpophalangeal joints. After completing individual adjustment operations each subject performed 3 submaximal voluntary isometric contractions maintained for 5 seconds as familiarization to the testing protocol. The scale of the dynamometer indicated handgrip strength in kilograms (kg). The testing protocol consisted of 3 maximal voluntary isometric contractions maintained for 5 seconds with rest period of at least 60 seconds; the highest values was used for the determination of the maximal grip strength. Specific verbal instructions were given to subjects before the evaluations and the experiments were performed with verbal encouragement. Each group performed the baseline measurement (T0) and after the cryocabin treatment (T1 PBC) or control duty (T1 Control) repeated the maximal handgrip strength test.

4.5 STATISTICAL METHODS

The Statistical Package for the Social Sciences (SPSS) for windows (SPSS 22.0, Inc., Chicago, IL, USA) was utilized.

A series of independent t-tests was used to control for any possible differences in the anthropometric characteristics and differences among handgrip strength values in both female and male PBC and control groups.
A mixed-design repeated measures ANOVA was used to analyze handgrip strength values. Time (T0,T1) was the within-subjects factor, whereas Group (PBC and control) and Gender were the between-subjects factors. The probability level taken to indicate significance was $p<0.05$. Data are presented as means±SD.

4.6 RESULTS

PBC induced a significant increase of the maximum isometric strength both in males (T0: 50.75±8.77 kg; T1: 52.27±7.76 kg; $p<0.01$) (Fig.2) and in females (T0: 28.47±4.45 kg; T1: 30.43±4.06 kg; $p<0.0001$) (Fig.3), whereas control group did not show significant changes. A high intraclass correlation emerged (ICC 0.987), (95% CI .975, .992) The results of the ANOVA showed a significant main effect of exercise (continuously standing rotations) in both groups ($F_{1.196}=45.59$, $P<0.05$, $\eta 2=.189$) on handgrip strength increase and a significant Exercise x Group interaction ($F_{1.196}=12.77$, $P<0.05$, $\eta 2=.061$). Both PBC and control groups showed an increase in handgrip strength values compared with T0 (T0=39.55 kg, T1=40.68 kg), in the experimental group (PBC: T0=39.61 kg, T1=41.34 kg) (Figure 4). The analysis reported also a significant effect of Gender ($F_{1.196}=491.99$, $P<0.05$, $\eta^2=.715$), with female participants showing lower handgrip strength values compared with male participants (females=30.43 kg, males=52.27 kg).

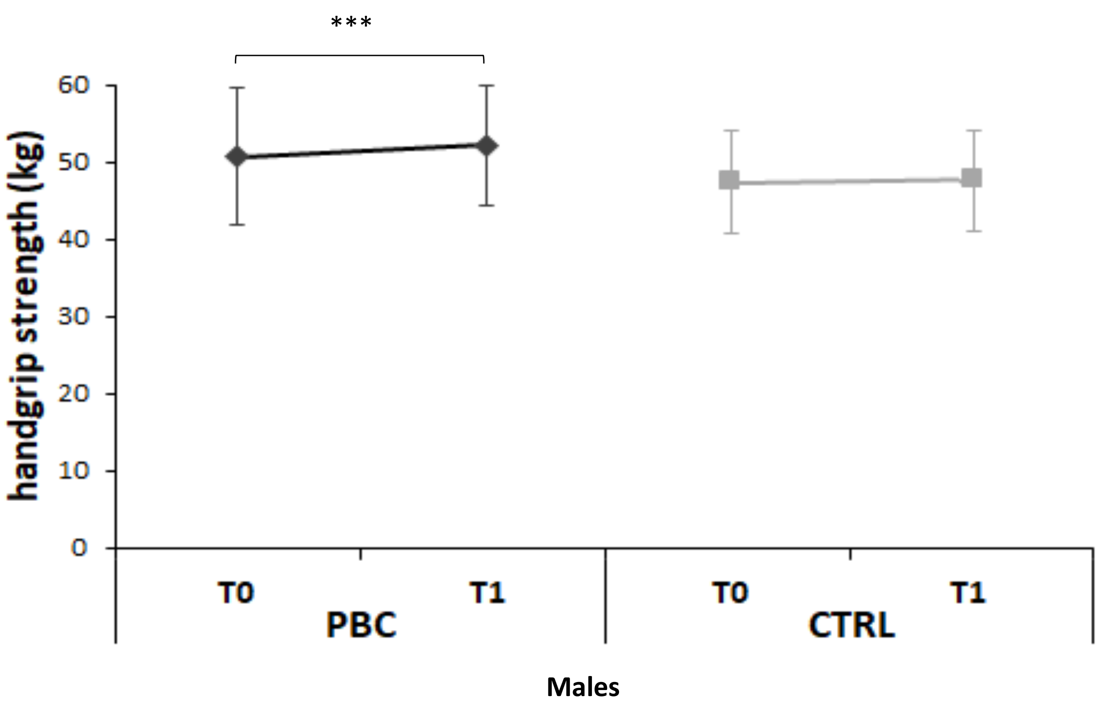

Figure 2. Handgrip strength in male partial-body cryotherapy group (PBC) before (T0) and after (T1) cryotherapy session and in the control group (CTRL). **** p<0.01

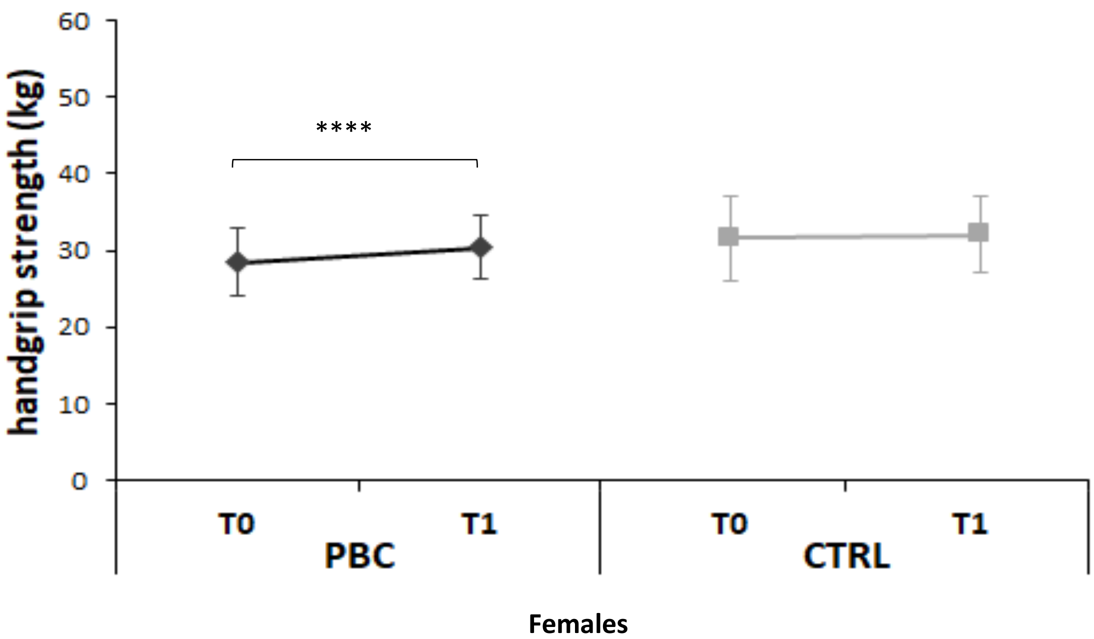

Figure 3. Handgrip strength in female partial-body cryotherapy group (PBC) before (T0) and after (T1) cryotherapy session and in the control group (CTRL). **** p<0.0001

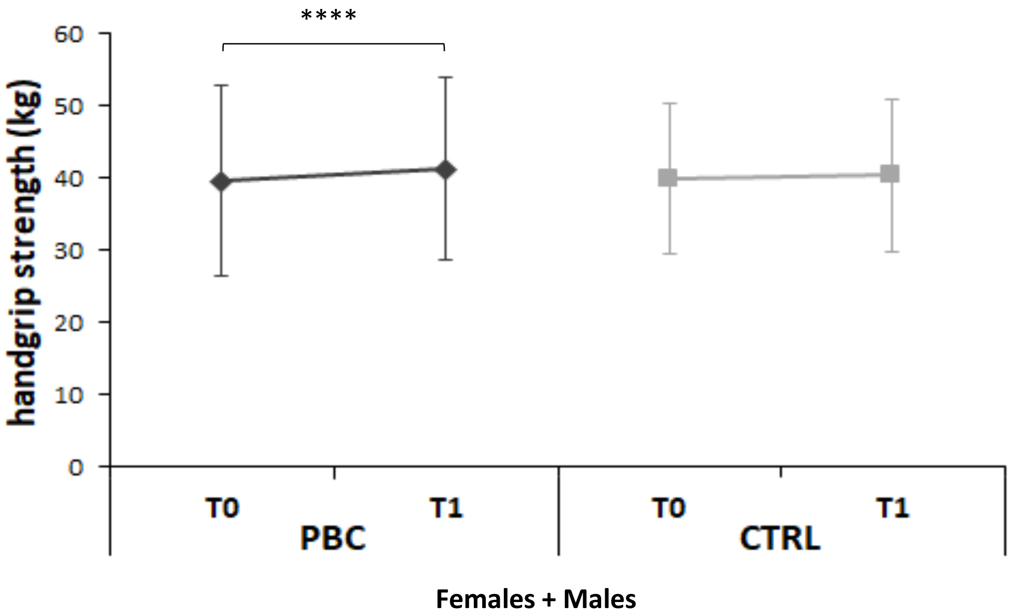

Figure 4. Handgrip strength in female and male partial-body cryotherapy group (PBC) before (T0) and after (T1) cryotherapy session and in the control group (CTRL). **** $p<0.0001$

4.7 DISCUSSION

In this study, the main finding was that maximal handgrip strength increased after a single session of partial-body cryotherapy lasting 150s. Because literature contains no reported data on isometric strength after a single PBC treatment, the findings of the present study can be compared with studies on cold therapy (cold water baths, ice packs). Our results confirmed that immediately after a PBC session there was a more remarkable increase in handgrip strength compared to baseline and to the control group. The results reported in the present study are in line with others that evaluated the effect of cold agents on the maximal isometric force (Burke et al., 2000; Nodehi Moghadami et al., 2012). In fact, 10 minutes of cold water bath provided an increase of the maximum isometric force production of the hip extensor significantly greater than that of the control and hot (43°C hot water bath) groups (Burke et al., 2000). Furthermore Burke and colleagues found a sex difference in the cold group, with men experiencing greater increases. Additionally, a previous study verified that after placing ice on the arm for 15 minutes significantly increased muscle force and, analogous to our findings, even for the control group there was a minimal increase in strength compared to baseline (Nodehi Moghadami et al., 2012). In our study we did not measure neither the muscle and skin temperature during or after a PBC session, but is known that skin temperature of the forearm is near to 23°C (Fonda et al., 2014). Therefore, it has been documented that a reduction on muscle temperature below the threshold of 27°C could decrease the maximal isometric force level (Clarke et al., 1962). Hence it would make sense, according to Westerlund and colleagues (2009), to assume that a single exposure in PBC does not lead to reach the threshold of 27°C for the muscle temperature, under which occur a decrease of the maximal isometric force level. To confirm this, immediately after a PBC exposure we noted an increase of the maximal hand grip strength, that may could be induced by the vasodilatation occurred after the PBC session, which determines an increased blood flow to muscles and, according with Nodehi Moghadami et Dehghane (2012), this may have a beneficial effect on muscle function.

5. EFFECTS OF PARTIAL–BODY CRYOTHERAPY ON PRESSURE PAIN THRESHOLD IN HEALTHY WOMEN

5.1 INTRODUCTION

In the last years pain management with cryostimulation technologies has been studied both in patients and in healthy subjects (Cholewka et al., 2006; Giemza et al., 2015; Costello et al., 2012). The most common approach to pain measurement in WBC and PBC studies is a participant self-assessment survey, using visual analog scale or other similar scales. Self-reported pain intensity is a combination of physiological and psychological aspects that can lead to a difficult interpretation of the results (Alonso-Perez et al., 2012). Indeed, it should be important to have pain measures that are not self-reported, in order to improve the assessment of the efficacy of the analgesic effects induced by cold agents. Pressure Pain Threshold (PPT) is defined as the least mechanical stimulus intensity at which a subject perceives pain (Vanderween et al., 1996). In literature Pressure Algometry (PA) performed with a handled algometer is a method extensively used to assess mechanical pain sensitivity in different body regions and to compare pain over time (Kardouni et al., 2015; Walton et al., 2011; O'Neill et al., 2007).

There are no peer-reviewed studies published on the evaluation of the effects of the PBC on PPT assessed by pressure algometry. Since PBC may play an important role in the pain management, the aim of this study was to investigate in a group of healthy women the effects induced on PPT by both one single session and a cycle of ten consecutive sessions of partial-body cryotherapy (PBC).

5.2 SUBJECTS

Thirty healthy women, naïve to the purpose of the experiment and in good health, were recruited for this study (Table 2). Inclusion criteria were the following: absence of pathology, injury, illness, chronic lower back pain and menstruation during the two-week trial. The exclusion criteria were

contraindications to cryotherapy and refusal to participate in the study. All participants never underwent to a PBC session before. They were instructed to refrain from consuming alcohol, caffeine, theine and hot drinks 24 hours before testing started, and to not take supplements or drugs during the study.
They did not receive any incentive to participate in the study and they were free to leave the study before the end. All subjects signed an informed, written consent approved by the University's ethic committee (Università degli Studi di Milano, n.9/15) and were examined by a qualified physician to exclude any contradictions to PBC. Subjects were randomly divided by use of computer-based allocation into two age-matched groups: Partial-Body Cryotherapy group (PBC group, n=15, mean age±SD=46.07±11.40 years) and Control group (CTRL group, n=15, mean age±SD=45.79±7.83 years). All of them completed the entire protocol of the research.

	Age (years)	Height (cm)	Weight (Kg)	BMI (Kg/m^2)
(PBC, n=15)	46.07±11.4	160.60±6.82	63.15±9.42	23.49±4.43
(Con, n=15)	45.79±7.83	162.87±5.38	60.73±12.72	23.82±3.6

Table 2 The anthropometric characteristics of the subjects involved in this study. Values are means ± SD.

5.3 EXPERIMENTAL PROCEDURES

In this study the subjects of the experimental group had 10 PBC exposures in a period of two weeks (performed every day from Monday to Friday). Partial-body cryotherapy took place in a specially built, temperature-controlled cabin (Space Cabin, Criomed Ltd, Kherson, Ukraine), a single-user open tank equipped with a mobile lift, which allows to adjust the height of every subject. Thus each participant was exposed to the very cold dry air up to the shoulders, with the neck and the head out of cabin. Every single session consisted of a 150 s-long exposure at a temperature range between -130 and -160 °C (Fonda et al., 2014). During the session subjects wore swimwear, a pair of gloves, woolen socks and wooden clogs. Well instructed members of the cryotherapy studio was always present during the PBC sessions and instructed participants to turn around continuously in the cabin for each 150 s-long exposure session. Each subject of the control group was asked to perform standing rotations for 150 seconds in the turned off cryocabin (22.0±0.5°C). The experimental protocol is summarized in Figure 5.

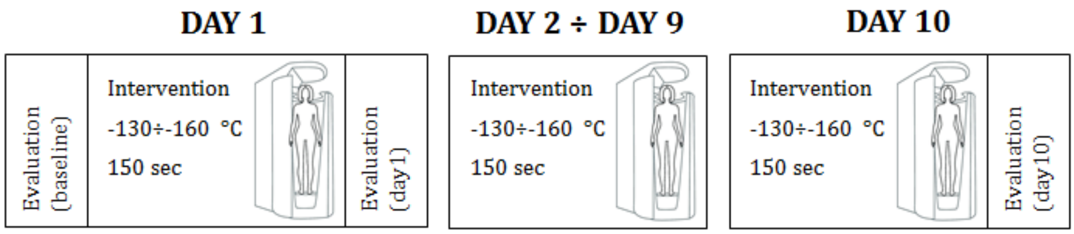

Figure 5 Experimental protocol administered to the partial-body cryotherapy (PBC) group. In DAY 1, during the evaluation phase, the skin temperature and the pressure pain threshold were evaluated (Evaluation phase) before and after 1 cryotherapy session (Intervention phase), which lasted 150 s and during which the temperature ranged between -130 and -160 °C. From DAY 2 to DAY 9 subjects participated to the Intervention phase. In DAY 10 they took part to the last cryotherapy session and the last Evaluation phase.

5.4 SKIN TEMPERATURE

Skin temperature was measured with noncontact, digital, infrared thermal camera (Flir C2, Flir System, Danderyd, Sweden) with an emissivity set to 0.98. Thermal images were analyzed with an appropriate software (Flir Tools+, Flir System, Danderyd, Sweden). Skin temperature was evaluated in the Control group in day1 and in the PBC group before and after the first and the tenth PBC sessions (baseline, pre1, post1, post10).

5.5 PRESSURE-PAIN THRESHOLD

A pressure algometer, with 1cm diameter hard rubber disk (Wagner pain test, Wagner Instruments, Greenwich, USA), was used to evaluate pressure-pain threshold (PPT). Before starting the experiment, in order to familiarize with the algometer, the experimenter applied the instrument on the tibialis anterior of the subject. After this familiarization procedure, subjects laid down comfortably in a prone position, in a controlled temperature and soundproof room and were instructed to say “stop” when a sensation of pain occurred. Pressure was applied perpendicularly to the skin at constant speed (5 N/s) by the examiner, who performed one single measurement for subject. All measurements were taken by one examiner. In the PBC group measurements of PPT were conducted before (baseline) and immediately after the first PBC session (day1), and after the tenth PBC session (day10). In the Control group PPT was evaluated at baseline and at day10 (De Nardi et al., 2017).

5.6 STATISTICAL METHODS

The probability level taken to indicate significance was $p<0.05$, values were expressed as means ± SE. Variables were normally distributed according to the Shapiro-Wilk test. A t-test was use to compare skin temperature of the two groups at baseline in order to check for possible a difference between the two groups. Furthermore, in the PBC group skin temperature variations were evaluated before and after the first and the tenth sessions by means of a repeated measure ANOVA with SESSION (2 levels, day1 and day10) and TIME (baseline/pre10 vs. post1/post10), as within subjects factor.

In order to test for possible modifications of the PPT values after the PBC sessions with respect to no intervention, a repeated measure ANOVA with TIME (2 levels, baseline and day10), as within subject factor, and GROUP (2 levels, PBC group and CTRL group), as between subject factor, was applied on PPT data. In order to evaluate the short- and long-term effect of PBC protocol administration, in the PBC group PPT values acquired before (baseline), immediately after the first PBC session (day1) and after the tenth PBC session (day10) were statistically evaluated by means of a one-way ANOVA. Newman-Keuls post hoc comparisons were applied to assess significant interactions. All statistical analyses were performed using Statistica 13.0.

5.7 RESULTS

The skin temperature of the two groups at baseline was statistically comparable, as shown by the result of the t-test (PBC: 34.06±0.29 °C; CTRL: 34.78±0.29 °C; $t=-1.79$, $p=0.09$). The statistical analysis on skin temperature of the low-back region measured on subjects of the PBC group showed a significant effect of the factor TIME ($F(1,14)=519.47$, $p<0.001$ $\eta^2=0.97$), and a significant decrease of skin temperature occurred after both PBC sessions (post1: 25.06±0.51 °C; pre1: 34.32±0.19 °C; post 10: 25.65±0.47 °C) (Figure 6). No frostbite was found during and after PBC.

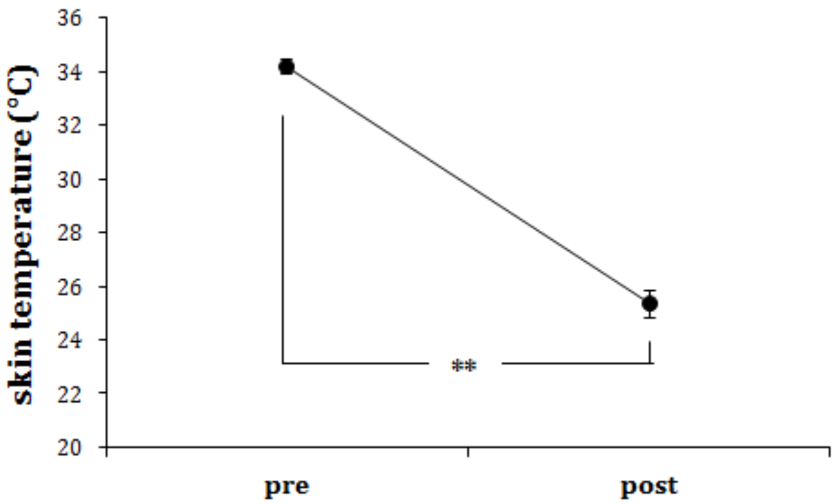

Figure 6. Mean skin temperature on the low-back region in subjects of the PBC group before partial-body cryotherapy (pre) and after partial-body-cryotherapy session (post) p<0.001.

The values of the pain threshold assessed in the PBC group and in the CTRL group during the evaluations at baseline and at day10 are represented in Figure 7. The results of the statistical analysis showed a significant interaction between TIME and GROUP ($F(1,28)=11.82$, $p=0.002$ $\eta^2=0.30$). No differences were found between the two groups during the baseline evaluation (PBC group: 84.53±7.89 N; CTRL group: 104.89±7.89 N; $p=0.27$). In the PBC group post hoc tests revealed a significant increase of the pain threshold from baseline to day10 (day10: 110.01±6.39 N; baseline vs. day10: $p=0.002$), whilst no differences between baseline and day10 appeared in the CTRL group (day10: 99.47±6.39 N; baseline vs. day10: $p=0.39$).

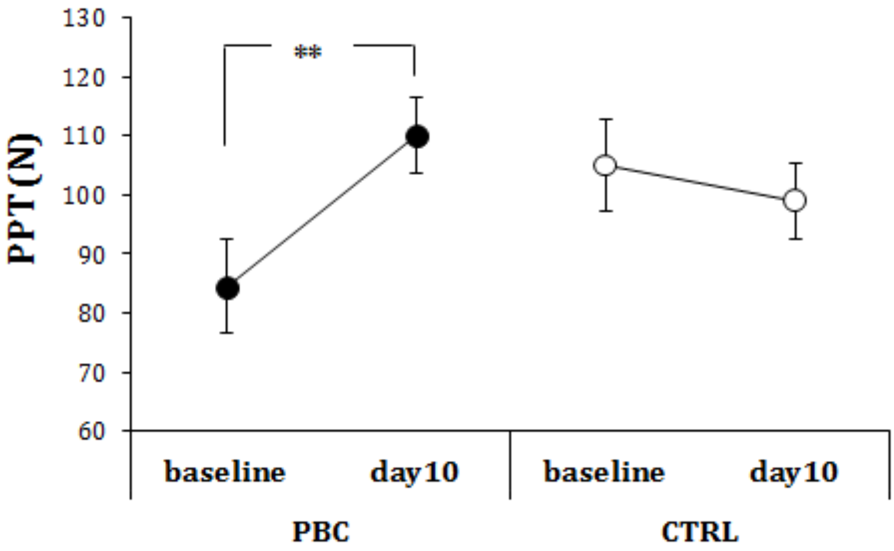

Figure 7 Values of pain-pressure threshold (N) for the two groups (PBC: partial-body cryotherapy; CTRL: control group) in different evaluation epochs (baseline: before intervention; day 10: after the last PBC session).**p=0.002.

Figure 8 shows pain threshold values of the PBC group at different time points: baseline, after the first (day1) and the last (day10) cryotherapy session. The results of the one-way ANOVA showed a significant effect of the factor TIME ($F(2,28)=6.63$, $p=0.004$ $\eta^2=0.32$). Newman-Keuls post hoc comparisons showed a significant increase of the pain threshold in day10 with respect to baseline (day10 vs. baseline: $p=0.003$) and day1 (day1: 96.06±9.68 N; day10 vs. day1: $p=0.04$).

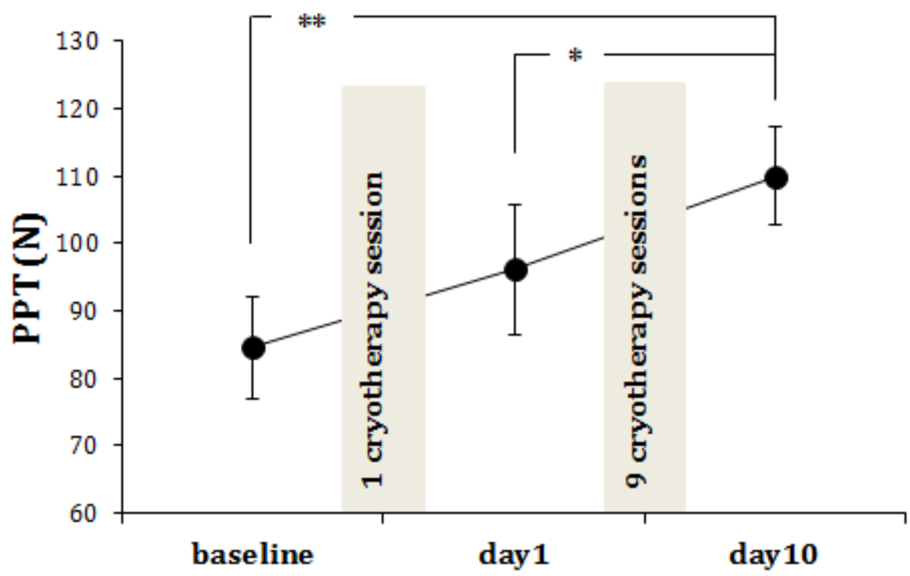

Figure 8 Mean pressure pain threshold (PPT, N) values evaluated in the partial-body cryotherapy group. Measurements were obtained at baseline, after the first cryotherapy session in DAY 1 and after the last cryotherapy session in DAY 10. Error bars represent the standard error. * and ** refer to $p<0.05$ and $p<0.01$, respectively.

5.8 DISCUSSION

In this study, we assessed the effects of our protocol of partial-body cryotherapy (duration: 150s; temperature range: -130 ÷ -160°C) on mean skin temperature, pre- and post-PBC exposure, measured by thermal imaging. Indeed, under our experimental conditions, the calculated variations in mean skin temperature on the low-back region was -8.84±1.8°C, with a statistically significant difference between pre- and post-PBC value. These values are in agreement with those reported by Bouzigon and co-workers (2016). For our study we involved only female participants. For its part, one study have investigated the role of sex difference on mean skin temperature following whole-body cryotherapy, and, even if the reported findings must be interpreted with caution as also suggested by the Authors, this study showed that female participants experienced a greater reduction in skin temperature than males (Cuttel et al. 2017). An objective method to calculate the cooling rate of the skin such as thermal imaging (Costello et al., 2012) is a key index in the evaluating the analgesic effects of

whole- and partial-body cryotherapy, that is why it should be included in every further research on this topic.
In this study we also tested the effects induced in healthy women on PPT via pressure algometry by both one single session and a cycle of ten consecutive sessions of partial-body cryotherapy. We found that healthy women involved in a cycle of 10 sessions of PBC showed significantly higher PPT scores after the tenth PBC session compared to those obtained either after one single session or in Control group. Consequently one single session of partial-body cryotherapy did not demonstrate to be enough to increase significantly pain threshold in healthy women.
Pressure algometry has been shown to be a non-invasive, efficient and reliable method in the study of pathophysiological mechanisms involved in pain and pressure-pain algometric measurements have been demonstrated to provide reliable data on PPT measures obtained in healthy subjects also on consecutive days (Nussbaum and Downes, 1998). Reliability is enhanced when all measurements are taken, as in our study, by one examiner.
To the best of our knowledge there are no papers in literature that have examined directly the pain threshold by means of a direct measurement. However our results are in line with those of the Authors who have investigate the pain trend after a single session of whole- or partial-body cryotherapy utilizing self-related questionnaires. For example Hohenauer and colleagues (2018) did not found reduction in pain scores after a muscle-damaging protocol followed by a single session of partial-body cryotherapy.
On the other hand, after a cycle of ten sessions of PBC we found that healthy women showed significantly higher PPT scores after the tenth PBC session compared to those obtained either after one single session or in Control group. These results are in agreement with previous reports on the analgesic effects of systemic cryotherapy (Cholewka et al., 2006 ,Giemza et al., 2015, Bettoni et al., 2013).
The analgesic effect of cooling therapies could be due to many mechanisms, including reduced nervous conduction velocity, inhibition of nociceptors, reduction of metabolic enzyme activity levels. Moreover, cryotherapy could also be effective in pain management via diffused noxious inhibitory controls, pain gait theory, decreased nociceptive receptors sensitivity, or via analgesic

descending pathways from central nervous system, with release of endogenous opioids, such as endorphins (Saeki, 2002).

Any specific hypothesis on mechanisms leading to the increase in PPT observed in our study with cryotherapy is purely speculative. However, this study was limited to subjects without established pain syndromes and the participants were submitted to a pain-induction procedure. Therefore, we can focus only on some of the above mentioned putative mechanisms to explain the analgesic effects we observed. On this basis, one plausible hypothesis is that in our study cryotherapy could have exerted its effect on pain through a decrease in nerve conduction velocity (NCV). NCV can be affected by gender, age, and, more pertinently, skin temperature (Binnie et al., 1995). A previous study showed that cryotherapy, performed by ice application, increased PPT in adult male sports players and this effect was associated with a significant decrease in NCV (Algafly and George, 2007). On the basis of the literature, two possible mechanisms could be take in account for the effect of cryotherapy on NCV. A previous research has suggested that temperature can affects the exchange between Ca^{2+} and Na^{+} in neural cells (Reid et al., 2002). Indeed, these Authors reported that low temperature could increase the friction between Ca^{2+} and its cellular gates during ionic exchange, that could result in the delay of action potential generation. A second mechanism refers to the increased sympathetic adrenergic activity, induced by the exposure to extreme cold, causing constriction of arterioles and veins and, as a consequence, reducing nerve conduction velocity (Herrera et al, 2010).

Indeed, the demonstrated significant reduction in mean skin temperature could have led to a strong stimulation of cutaneous thermoreceptors and, as a consequence, to activation of thermoregulation hypothalamic center, with excitation of sympathetic adrenergic fibers. The stimulation of the sympathetic system, the release of noradrenaline and the vasoconstriction may also have induced other significant effects on pain management. It has been demonstrated that noradrenaline spinal administration in animals as well as epidural injections of adrenergic agonists in humans alleviate pain (Pertovaara and Kalmari, 2003; Gordh, 1988). Moreover, an increased release in noradrenaline reaching, via the posterior spinal arteries, the substantia gelatinosa in the spinal cord, where pain afferents from skin terminate, may be involved in mechanisms, that reduce pain transmission at the spinal level (Bouzigon et al., 2016).

In physical exercise, the exposure of skin and muscles to extreme cold temperatures may also lower enzyme activities and metabolism by inducing the peripheral vasoconstriction that reduces peripheral blood flow by attenuating inflammatory response and limit protein degradation, which can be important features of recovery improvement (Banfi et al, 2009; Bouzigon et al., 2016).

6. EFFECTS OF PARTIAL-BODY CRYOTHERAPY ON PERCEIVED HEALTH AND QUALITY OF LIFE OF FIBROMYALGIC PATIENTS

6.1 INTRODUCTION

Fibromyalgia (FM) is a widespread pain disorder, characterized by neurological pain, fatigue, arthralgias, stiffness, sleep disturbances, and other frequent physical and mental disorders (Borchers et Gershwin, 2015). The neurologic origin of pain has been attributed to an imbalance in the levels of pro- and anti-inflammatory mediators and to an impaired central processing of painful stimuli (Sluka et Clauw, 2016). FM patients often report additional somatic and psychological symptoms to different degrees, which may change over time and with treatment (Clauw et al., 2003). FM is associated with central nervous system aberrance, such as alteration of sensory processing in the brain, reduced reactivity of the hypotalamus-pituitary-adrenal axis to stress (Wingenfeld et al, 2010), increased pro-inflammatory and reduced anti-inflammatory cytokine profile (Rodriguez-Pintó et al., 2014) and disturbances in neurotransmitters, such as dopamine and serotonin. Females are affected at least 6 times more often than males (Branco et al., 2009).

Research has not found effective therapies for FM, and long-term follow-ups demonstrate that FM remains chronic, with scarce probability of full recovery (Isomeri et al, 2018). Recently, accepted treatment modalities of FM have shifted significantly from a pharmacologic therapy alone to a systematic multimodal approach. Therapies that focus on restoring sleep quality and reducing anxiety and stress have shown encouraging results as well as low intensity aerobic exercise (Ellingson et al., 2016) seems to prove an effective alternative to drugs. Recent studies have shown that whole body cryotherapy (WBC) can improve health-reported quality of life on fibromyalgic patients (Bettoni et al., 2013, Vitenet et al., 2018). However, only few studies were published on the effectiveness of WBC on parameters of wellbeing and quality of life in fibromyalgic patients (Bettoni et al., 2013, Vitenet et al., 2018). In the above mentioned studies the authors found beneficial effects on health-reported quality of life in patients with FM utilizing WBC technologies. However there are

no data in literature about the effect of PBC on perceived health and quality of life indexes in patients affected by FM.
The aim of the present study was to investigate the effects of PBC on the perceived health and quality of life in fibromyalgic patients.

6.2 SUBJECTS

Twenty-eight female participants were recruited for this study. The inclusion criteria were to be adult and clinically diagnosed with Fibromyalgia using the American College of Rheumatology' standard criteria (Wolfe et al., 2010). All volunteers underwent an initial physical examination by a qualified physician to avoid contradictions to PBC. They did not receive any incentive to participate in the study and they were free to leave the study before the end. The research was undertaken in compliance with the Helsinki Declaration and each participant signed an informed consent in which risks and procedures of the study were exhaustively explained. During the two week observation they were asked to not modify pharmacologic treatments they were subjected to prior to the study. Subjects were all not accustomed to PBC treatment. Subject were randomly divided into a partial body cryotherapy group (n=14; age: 46,31±10,02; PBC group) and control group (n=14; age: 47,36±7,53). All of them completed the entire protocol of the research and also fulfilled the final survey. Socio-demographic information for each group is shown in Table 3.

	Age (years)	**Height (cm)**	**Weight (Kg)**	**BMI (Kg/m^2)**
(PBC, n=14)	46.31±10.02	164.46±7.03	66.08±13.91	24.51±4.47
(Con, n=14)	47.36±7.53	161.53±6.12	62.29±11.21	23.99±3.79

Table 3. The anthropometric characteristics of the subjects involved in this study. Values are represented as means ± SD. All participants were female.

6.3 EXPERIMENTAL PROCEDURES

The subjects in the partial body cryotherapy group completed ten consecutive treatments, performed in two weeks, in a cryocabin (Space Cabin, Criomed Ltd, Kherson, Ukraine). Every single session consisted of a 150 s-long exposure at a temperature range between -130 and -160 °C (Fonda et al., 2014). During the session subjects wore swimwear, a pair of gloves, woollen socks and wooden clogs. Well instructed members of the cryotherapy studio was always present during the PBC sessions and instructed participants to turn around continuously in the cabin for each 150 s-long exposure session. All subjects kept their prescribed pharmacological therapy and their everyday activities.

6.4 SELF REPORTED QUALITY OF LIFE

All subjects filled out a questionnaire at two time points – before the start of the study (T0) and at the end of the treatment (T1). The Psychological General Well-Being Index (PGWBI) and the VASFIQ were utilized in this study. The PGWBI questionnaire consists of 22 items and it is designed to assess the subjective representations of intrapersonal affective or emotional states reflecting the level of well-being or distress. This survey evaluates six dimensions: anxiety, depression, well-being, self-control, general health, and vitality. The maximum score of each element is 5 points. The VASFIQ is a seven-item scale composed of Fibromyalgia Impact Questionnaire (FIQ) and Visual Analog Scales (VASs) designed to quantify global fibromyalgia severity and identify seven symptoms widely regarded as clinically relevant to patients with FM such as pain, fatigue, poor sleep, work-related pain, stifness, anxiety and depression. Each item is rated on a scale ranging from 0 to 10. A high score is indicative of a high degree of patients' disease.

6.5 STATISTICAL METHODS

Statistical analysis were performed on the results of PGWBI and VASFIQ as a whole and on each domain. In particular, a repeated-measure analysis of variance (RM-ANOVA) was applied on PGWBI, with TIME (2 levels, PRE and

POST) as within subject factor, and GROUP (2 levels, PBC and Control) as between subject factor. The same RM-ANOVAs were applied also on the PGWBI domains whose data resulted to be normally distributed, namely anxiety, well-being, self-control, general health, and vitality. Significant interactions were interpreted with Newman-Keuls post hoc comparison. Since the scores of the depression domain were not normally distributed the comparison within each group (PRE vs. POST) was done by means of Wilcoxon test, and the comparison between each group in each evaluation epoch was done by means of Mann-Whitney tests. VASFIQ scores were not normally distributed, both as a whole and in each domain. For this reason the comparison within each group (PRE vs. POST) was done by means of Wilcoxon test, and the comparison between each group in each evaluation epoch was done by means of Mann-Whitney tests. All statistical analyses were performed using Statistica 13.0. Data are means±SE.

6.6 RESULTS

The results of ANOVA on PGWBI (fig. 9) showed a significant GROUP*TIME interaction ($F(1,26)=59.29$, $p<0.001$). Post hoc analyses revealed that the two groups did not differed at baseline, whilst PGWBI at POST of PBC group was significantly higher than that of the CONTROL group ($p<0.01$). Furthermore, a significant increase of the score from PRE to POST evaluation epoch was found in the PBC group ($p<0.001$).
The same results were found on PGWBI domains; namely, no differences were found between groups at PRE, whilst significant improvements were found in POST evaluation epoch in PBC group with respect to CONTROL group. In particular, significant interactions between TIME and GROUP were found in anxiety ($F(1,26)=24.08$, $p<0.001$), well-being ($F(1,26)=27.52$, $p<0.001$), self-control ($F(1,26)=17.35$, $p<0.001$), general health ($F(1,26)=27.67$, $p<0.001$), and vitality ($F(1,26)=50.19$, $p<0.001$). In all these domains post hoc analyses revealed that PBC group at POST significantly improved with respect to CONTROL group (always $p<0.05$). From PRE to POST evaluation epochs a significant increase of the scores in all these domains were found in the PBC

group (always *p<0.001*). Concerning the depression domain, Mann-Whitney comparisons showed no differences between groups at PRE and a significantly higher value in PBC group than in CONTROL group in POST (*p<0.001*). Wilcoxon tests revealed a significant increase from PRE to POST in PBC group (*p<0.01*) and no difference in the CONTROL group.

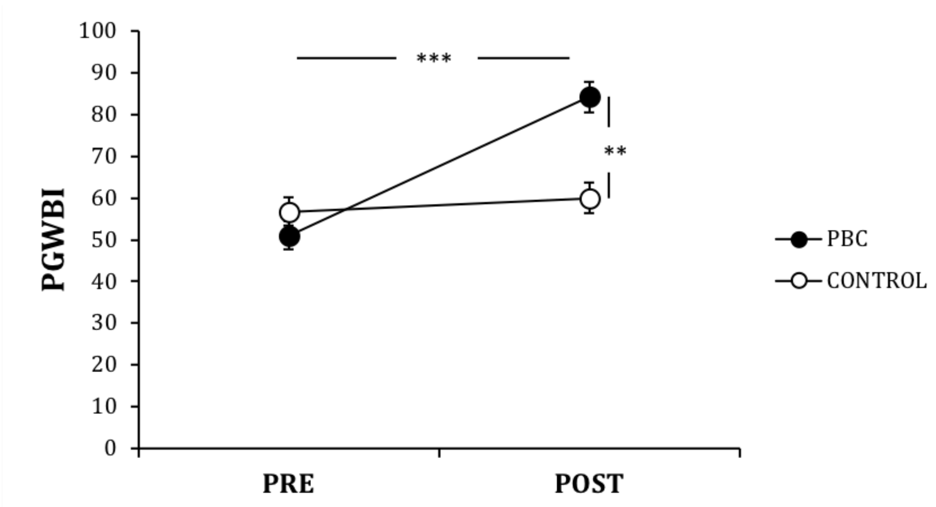

Figure 9 Global scores of the Psychological General Well-Being Index (PGWBI) obtained in the two groups (PBC: partial-body cryotherapy; Control: Control group) before (PRE) and after (POST) the intervention period. **p<0.01; ***p<0.001.

Regarding VASFIQ global score (fig. 10), Mann-Whitney comparisons showed no differences between groups at PRE and a significantly lower value in PBC group than in CONTROL group in POST (*p<0.001*). Furthermore, Wilcoxon tests revealed a significant increase from PRE to POST in PBC group (*p<0.01*).The same pattern of results was found also for the VASFIQ domains work, pain, fatigue, sleep, stiffness, anxiety, depression; namely, no differences appeared between groups at PRE, whilst significant improvements (lower values) were obtained in PBC group with respect to CONTROL group at POST as shown by the results of the Mann-Whitney comparisons (*always p<0.01*). Wilcoxon tests for all the domains showed significant lower values in PBC group (*always p<0.01*) and no differences in CONTROL group.

In particular, the mean value of VASFIQ global score did not differ at the baseline between PBC group and Control group (*p*=0.29). After 10 PBC sessions PBC group showed a highly significant (p<0.00001) decrease in the

VASFIQ global score. Similarly, the mean value of the PGWBI global score did not differ at the baseline between the PBC group and the Control group (p=0.39). After 10 PBC sessions there was a remarkable improvement in the PBC group in all items (Depression p<0.01, Anxiety, Well-Being, Self-Discipline, General Health and Vitality p<0.001) with no significant changes in any of these items for the Control group (p>0.05).

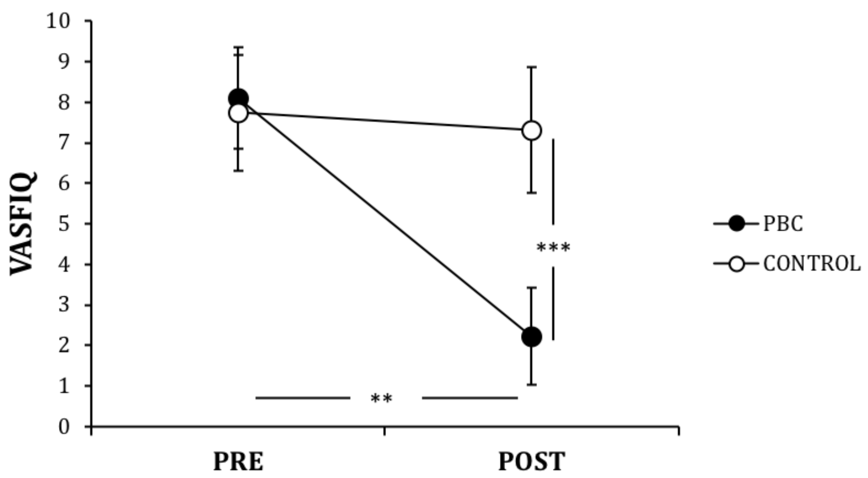

Figure 10: Global scores of the VASFIQ scale (composed of Fibromyalgia Impact Questionnaire -FIQ- and Visual Analog Scales -VAS-) obtained in the two groups (PBC: partial-body cryotherapy; Control: Control group) before (PRE) and after (POST) the intervention period. **p<0.01; ***p<0.001.

6.7 DISCUSSION

Data obtained in previous studies using health-related self-questionnaire indicated that after cycles lasted from 10 to 15 WBC sessions there was a significant improvement in the group of fibromyalgic patients, compared to baseline condition and to control groups. Particularly, Bettoni and colleagues (2013) and to Vitenet and co-workers (2018) demonstrated that the mean values of the responses to the quality of life questionnaire for each variable increased significantly after completed the WBC cycle compared with the baseline (p<0.01). In our study, cryotherapy was performed in a cryocabin, and we showed that partial-body cryotherapy induced significant improvements in all variables both of the PGWBI and of the VASFIQ scale compared with pre-treatment condition and with control group. Our findings are in line with those of

Rivera and colleagues (2018) who evaluated the efficacy of multiple partial-body cryotherapy exposure on patients with fibromyalgia measuring pain by a visual analogue scale (VAS), burden of disease by the Fibromyalgia Impact Questionnaire (FIQ) and severity of Fibromyalgia with the Combined Index of Severity of Fibromyalgia (ICAF) with a significant improvement in all scores after the cryostimulation period. To our best knowledge their research is the only one carried out in a cryosauna cabin involving patients with a diagnosis of fibromyalgia.
The improvements on self-reported quality of life indixes might originate from the balance between pro-inflammatory and anti-inflammatory mediators, which have been found to influence pain modulation and, consequently, quality of life (Vitenent et al., 2018) and from the above mentioned analgesic effect of the cooling agents. According to our explanation of pain reduction sensitivity after very-low temperatures exposures Bettoni and colleagues (2013) attributed the positive effects of cryostimulation on the referred pain sensation to the changes in nerve conduction induced by cryogenic temperatures. In their manuscript Rivera and colleagues (2018) indicated as a key point for explain the mechanisms of action of cryotherapy in fibromyalgia patients the relieve of stress by the activation of neuroendocrine and metabolic functions, specially in fibromyalgia patients which constantly deal with stress. Further studies are needed to demonstrate the effects of the cryostimulation exposures over time, because only in the research conducted by Vitenent and colleagues there was a follow-up period (one month).

7. METHODOLOGICAL CONSIDERATIONS

In all studies the same cold exposure protocol was utilized, in order to standardize the results and to bridge the gap in the standardization of procedures in the field of cryostimulation research.

In study I we recruited participants of both genders, while in study II and III we involved only female participants. In literature (Cuttel et al., 2017) has been observed sex differences in mean skin and mean body temperature following very-low temperature exposures, that is why gender differences should be taken into account in order to optimize treatment. Further researches are required to investigate more deeply the influence of sex and body composition on the effects of cryotherapy, in order to take into account these differences in an attempt to optimize treatment, when cryotherapy is prescribed.

An interesting way to assess the efficiency of cryotherapy and to compare the effects described in different studies is to evaluate the variations in skin temperature induced by exposure to very low temperature. In study II skin thermal responses were measured, in order to assess the cooling of the low-back area where the pain-pressure test was applied. Indeed, the absence of this measure in experimental procedures based on cryotherapy technologies represents a possible limitation, because reporting as many data as possible about the cooling of skin and muscles after a determined protocol of PBC it could be the better way to contribute to the standardization of cryotherapy/ cryostimulation exposure.

In all studies we did not carried out a follow-up investigation. As a consequence, we can only talk about acute effects of a single or multiple exposures to very-low temperatures. Promote the knowledge about the lasting of the benefits of cryostimulation procedures should be one of the objectives of our further studies.

8. PERSPECTIVES

Even if the molecular mechanisms activated, or inhibited, by whole- and partial-body cryostimulation are still unknow, these cold-treatments are experiencing considerable development in several domains such as medicine, health and sports. However, as shown in previous chapters of this work, there is a lack of information concerning the optimal protocols in terms of temperature inside the devices, time of exposure and number of sessions. Even if in the last decade there has been an increasing number of scientific articles on this topic, it is still necessary establish a method to ensure first of all the safety and consequently the therapeutic efficacy for the final users.

This work contributes to the optimisation of partial-body cryotherapy treatments, especially in the field of isometric strength recovery, low-back pain management and quality of life assessment in patients suffering of fibromyalgia.

For our experiments we choose protocols of exposure reported in previous published articles. It would be interesting to study the effects of different protocols in different populations, considering also the anthropometric characteristics and gender of the people recruited.

From a clinical point of view, comparing our findings with those published by other authors, whole- and partial-body cryotherapy are clearly effective in improving the pain sensation, the clinical condition and the quality of life both in healthy people and specially in several inflammatory and autoimmune diseases. That is why we agree that the introduction of very-low temperatures exposures into the standard physiotherapy protocols for patients with inflammatory diseases in fully justified.

In the last decade the number of facilities offering cryostimulation devices has been grown exponentially, so it make sense to think that once the exposure protocols will be validated, an increasing number of people will have a marked improvement in the quality of life thanks to this kind of treatments.

Also in the field of sport recovery there is a growing interesting about these kind of techniques. At the beginning rugby players was the first to enter in cryochambers to optimize recovery (Banfi et al., 2010): nowadays cryochambers and cryocabins can be found in a multitude of locations closed to training centers and sport facilities around the world. Elite athletes are increasingly undergoing to cryostimulation sessions, so consequently a large number of sportsmen who usually take their favorite champions as examples, have started trying cryostimulation. The periodization of the training load and of the recovery sessions is crucial for every kind of athlete, so, to ascertain the appropriation of its use, future investigations should seek to assess the use of cryostimulation at various phases of competitive sports season.

The role of the scientific community it will be to develop research to demonstrate the benefits of whole- and partial-body cryotherapy in many fields of interest and try also to establish a certification not only for the devices, but also for the operators, which they must master the fundamentals concerning human thermogenesis, physiology and safety rules of cryostimulation.

9. CONCLUSIONS

The main findings and conclusions of this thesis are as follows:

a. A single session of partial-body cryotherapy leads to increase muscle isometric strength in healthy people. This finding provides new information on the important effects that both whole-body and partial-body cryotherapy can induce in the context of physical exercise as well as in exercise recovery. This is of practical value for coaches and practitioners, aiming to include this treatment in order to improve physiological performances, such as isometric strength. Particularly, PBC could be performed before a training session or a competition, such as, for example, climbing and gymnastics ring performances, where isometric strength is required.

b. A protocol of repeated exposures to partial-body cryotherapy in healthy women was shown to be efficient in decreasing the skin temperature in the low-back region, and to induce a significant increase in pain-pressure threshold. It is to be stressed the importance, for future studies, of describing the exposure protocols, to evaluate the relationship between temperature, duration, number of repetitions and the treatments' desired effects. These studies will allow to optimize the use of cryotherapy/cryostimulation under different physiological and pathophysiological conditions.

c. The same protocol of repeated exposures to PBC induced significant improvements for all indexes and sub-indexes of the quality of life questionnaires assessed in fibromyalgic patients, compared with the control group. Our results demonstrate the positive effects of PBC on the perceived health and quality of life of fibromyalgic patients and they are consistent with those obtained with whole-body cryotherapy. In view of the systematic multimodal approach to the treatment of fibromyalgia, widely accepted in the last few years, our findings suggest that partial body cryotherapy can be effectively recommended as a supportive approach in the improvement of health-related quality of life in fibromyalgic patients.

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