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Whole-Body Cryotherapy in Athletes

Giuseppe Banfi, Giovanni Lombardi, Alessandra Colombini and Gianluca Melegati

IRCCS Galeazzi, Milan, Italy

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Abstract

Cold therapy is commonly used as a procedure to relieve pain symptoms, particularly in inflammatory diseases, injuries and overuse symptoms. A peculiar form of cold therapy (or stimulation) was proposed 30 years ago for the treatment of rheumatic diseases. The therapy, called whole-body cryotherapy (WBC), consists of exposure to very cold air that is maintained at -110°C to -140°C in special temperature-controlled cryochambers, generally for 2 minutes. WBC is used to relieve pain and inflammatory symptoms caused by numerous disorders, particularly those associated with rheumatic conditions, and is recommended for the treatment of arthritis, fibromyalgia and ankylosing spondylitis. In sports medicine, WBC has gained wider acceptance as a method to improve recovery from muscle injury. Unfortunately, there are few papers concerning the application of the treatment on athletes. The study of possible enhancement of recovery from injuries and possible modification of physiological parameters, taking into consideration the limits imposed by antidoping rules, is crucial for athletes and sports physicians for judging the real benefits and/or limits of WBC.

According to the available literature, WBC is not harmful or detrimental in healthy subjects. The treatment does not enhance bone marrow production and could reduce the sport-induced haemolysis. WBC induces oxidative stress, but at a low level. Repeated treatments are apparently not able to induce cumulative effects; on the contrary, adaptive changes on antioxidant status are elicited – the adaptation is evident where WBC precedes or accompanies intense training. WBC is not characterized by modifications of immunological markers and leukocytes, and it seems to not be harmful to the immunological system. The WBC effect is probably linked to the modifications of immunological molecules having paracrine effects, and not to systemic immunological functions. In fact, there is an increase in anti-inflammatory cytokine interleukin (IL)-10, and a decrease in proinflammatory

cytokine IL-2 and chemokine IL-8. Moreover, the decrease in intercellular adhesion molecule-1 supported the anti-inflammatory response. Lysosomal membranes are stabilized by WBC, reducing potential negative effects on proteins of lysosomal enzymes. The cold stimulation shows positive effects on the muscular enzymes creatine kinase and lactate dehydrogenase, and it should be considered a procedure that facilitates athletes' recovery. Cardiac markers troponin I and high-sensitivity C-reactive protein, parameters linked to damage and necrosis of cardiac muscular tissue, but also to tissue repair, were unchanged, demonstrating that there was no damage, even minimal, in the heart during the treatment. N-Terminal pro B-type natriuretic peptide (NT-proBNP), a parameter linked to heart failure and ventricular power decrease, showed an increase, due to cold stress. However, the NT-proBNP concentrations observed after WBC were lower than those measured after a heavy training session, suggesting that the treatment limits the increase of the parameter that is typical of physical exercise. WBC did not stimulate the pituitary-adrenal cortex axis: the hormonal modifications are linked mainly to the body's adaptation to the stress, shown by an increase of noradrenaline (norepinephrine).

We conclude that WBC is not harmful and does not induce general or specific negative effects in athletes. The treatment does not induce modifications of biochemical and haematological parameters, which could be suspected in athletes who may be cheating. The published data are generally not controversial, but further studies are necessary to confirm the present observations.

Local cold therapy or cryotherapy is commonly used as a procedure to relieve pain symptoms, particularly in inflammatory diseases, injuries and overuse symptoms. A peculiar form of cold therapy or stimulation was proposed 30 years ago^[1] for the treatment of rheumatic diseases. The therapy consists of the brief exposure to very cold air in special temperature-controlled cryochambers, where the air is maintained at -110°C to -140°C . The treatment was named whole-body cryotherapy (WBC).

Exposure to WBC is usually for 2 minutes, but in some protocols it lasts 3 minutes. Exposure can be performed with a single subject, but entry of a small group of subjects, up to four, in the same chamber is permitted. Each participant's entry to the cryochamber is preceded by 30 seconds of temperature adaptation in a vestibule at a temperature of -60°C . During the exposure, the subjects have minimal clothing and to avoid frostbite they wear shorts (bathing suit), socks, clogs or shoes, surgical mask, gloves, and a hat (or headband) covering the auricles. Any sweat is removed from

the subjects before entering the cryochamber, where the air is clear and dry. While in the cryochamber, the subjects have to move their fingers and legs and avoid holding their breath. The system is automatically controlled, but safety personnel are always present (figure 1).

The treatment is applied to relieve pain and inflammatory symptoms caused by numerous disorders, particularly those associated with rheumatic conditions, and it is recommended for the treatment of arthritis, fibromyalgia and ankylosing spondylitis. WBC has been shown to be not deleterious to lung function;^[2] a sudden exposure to cold water or air may elicit several effects on the respiratory system, such as a gasp response, increase in ventilation and bronchoconstriction, but repeated treatments are not harmful to local reactions and do not impair the local immunological barrier.

Despite the wealth of literature on rehabilitation techniques, published data on WBC in physiology or rehabilitation programmes are scarce. The scientific information is based on pilot studies,

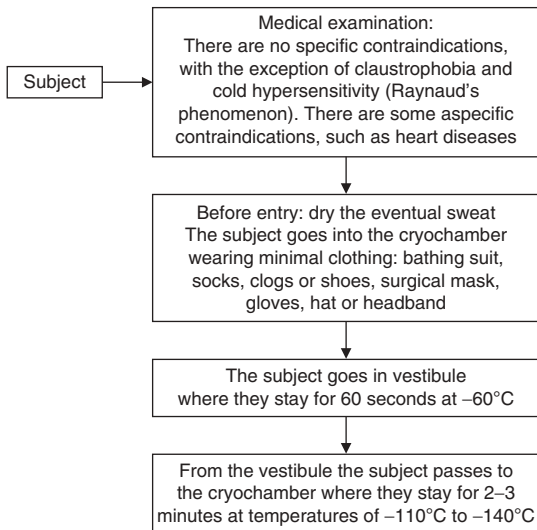


Fig. 1. Preparation of patients and treatment with whole-body cryotherapy.

abstracts of congresses and reports in journals, but are not generally in the English language.

In sports medicine, WBC has gained wider acceptance as a method to improve recovery from muscle injury. The enhancing effects of WBC are anecdotally widely used for recovering from traumas and for preventing overtraining symptoms. The enhancement of the cardiovascular system, amelioration of muscular activation, limitation of sport-induced haemolysis, potent anti-inflammatory effect and additional potentially beneficial effects of WBC could be useful for athletes.

The study of WBC effects can have a practical value not only for many physiological and clinical purposes, but also for determining clinical significance in the context of antidoping testing, since techniques that accelerate recovery may be classified as prohibited. Furthermore, post-WBC treatment changes in biochemical and haematological parameters could lie outside the threshold range imposed by sports federations and official control agencies for athletes classified as being doped, or they could be interpreted as an attempt to mask changes caused by illicit treatment.

There are few studies on the effects of WBC on athletes, but it is interesting to review the data

observed to date in physically-active subjects to describe WBC effects and possible modifications of physiological parameters in order to stimulate further studies in the field.

We evaluated the medical literature available on PubMed using the keywords “whole body cryotherapy” and “whole body cryostimulation”. Papers concerning only patients were not evaluated. Those involving healthy subjects and sportsmen were evaluated. Additional references were identified from the reference lists of published articles selected in the PubMed search. Only papers concerning a treatment involving a cryochamber, and which described the protocol treatment and ethical assessment, were evaluated.

1. Haematology

The haematological parameters before and after 1 week of WBC treatment have been evaluated in top-level rugby players.^[3] Originally, the aim of the study was to determine the potential risk of bone marrow-boosting, which could be induced by WBC treatment, as suggested in the media.

From 30 rugby players who underwent WBC treatment, 10 athletes from the Italian national team were randomly selected to be studied before and after a WBC treatment cycle performed at Spała, Poland. The treatment was applied once a day for 5 days; during this period, the athletes trained regularly and following the same protocol used during the previous weeks.

The analyses were performed with a Coulter® LH 750 haematology analyser and showed no modifications of leukocytes, platelets and reticulocytes. As far as erythrocytes are concerned, their cell count did not show modifications, but a significant decrease of haemoglobinization appeared. Haemoglobin decreased from a mean of 15.8 g/dL to 15.5 g/dL, and mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration parameters were also reduced. The effect, probably due to the relatively short period of treatment, did not influence the mean corpuscular volume, and the significant decrease in mean reticulocyte volume did not affect erythrocyte volume or reticulocyte counts.

Thus, this treatment does not accelerate erythrocyte maturation or haemoglobin production. Conversely, a slight decrease of haemoglobinization was found.^[3]

These results are similar to those described in 11 professional field hockey players from the Polish national team. WBC was administered twice a day for a total of 18 procedures. The athletes regularly trained after treatment. Blood was drawn before the treatment cycle and after the series of treatments. A decrease of erythrocytes, haemoglobin and haematocrit was shown; however, the decrease was transient. Subjects recovered their basal levels after 1 week for erythrocytes and haematocrit, and overwhelmed the baseline concentration of haemoglobin.^[4]

An interesting result of WBC is the reduction of haemolysis, which usually accompanies intense physical exercise and possibly induces a decrease in haemoglobin, a condition universally known as sports anaemia. A specific effect of cold temperatures in reducing haemolysis after intense exercise has already been described where cold water immersion of the legs was used in 30 top-level rugby players.^[5]

The difference between mean corpuscular volume, which remains stable, and mean sphered corpuscular volume (MSCV), which decreases when haemolysis occurs, was statistically significant in the whole group of athletes and in the subgroup who performed passive recovery after an intense bout of training. Conversely, in the other two subgroups, who performed active recovery (i.e. cycling at maximal power followed by leg immersion in iced water or *vice versa*), the difference was not significant.^[5]

After the 1 week of WBC treatment, the rugby players had a significant increase in haptoglobin mean values, from 56.6 mg/L to 75.2 mg/L. Haptoglobin is a protein that blocks the free haemoglobin released by broken erythrocytes; its increase is linked to a decrease in haemolysis. The MSCV values also increased from a mean of 84.6 fL to 87.6 fL, whilst the difference between mean corpuscular volume and MSCV also decreased, confirming the reduction of sport-induced haemolysis, usually linked to an increase of membrane peroxidation from reactive oxygen

species, which are produced in high amounts during exercise.^[6]

In conclusion:

1. WBC does not enhance bone marrow production.
2. WBC could reduce the sport-induced haemolysis.

2. Antioxidant Capacity

Physical exercise leads to increased production and release of reactive oxygen species, which induce oxidative stress mainly as lipid peroxidation and, consequently, membrane damage. The body responds to this via the antioxidant system, including non-enzymatic scavengers and some enzymes, which reduce the potentially dangerous effect of oxidant molecules. The WBC effect on pro-oxidant-antioxidant balance was studied in 20 top-level kayakers from the Polish Olympic team and in 10 untrained men.^[7]

The kayakers completed a 10-day programme with training sessions twice a day. The first training session each day was preceded by one WBC treatment, and the second by two WBC treatments, whereas controls received only one WBC treatment. Blood samples were taken before training, and after 6 and 10 days of training with WBC. Blood samples for the controls were taken 3 days before and immediately after the treatment. The athletes also performed an identical training cycle that was not accompanied by WBC treatment.

In non-athletes, some oxidant reagents, such as conjugated dienes in plasma and erythrocytes, increased, whereas other oxidant species, such as thiobarbituric acid-reactive substances (TBARs), did not. Conversely, the activity of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) increased, whereas that of catalase did not. A similar scenario appeared in athletes on days 6 and 10 of training without WBC treatment (i.e. there was an increase in plasma and erythrocyte conjugate dienes, and an increase in SOD and GPx). Moreover, plasma and erythrocyte TBARs were also increased in athletes as a result of intensified lipid peroxidation, but probably also because of a

different rate of metabolization of these products during intensive physical exercise. However, the differences between the results observed during training cycles, with and without WBC, were the relative decrease in conjugated dienes in plasma and erythrocytes, and the relative increase of TBARs. After training without WBC, the activity of SOD and GPx significantly increased. Conversely, after training that is preceded by WBC, only GPx activity significantly increased. When comparing the two training models, the SOD activity was 47% lower after the 6th day with WBC and the GPx activity more than halved after the 10th day. The WBC treatment induced reactive oxygen species generation, probably by muscle shivering or metabolism of intensified oxidation of catecholamines released by cold stress. A single application of WBC induced an increase in conjugated diene concentration, and SOD and GPx activity in sedentary subjects. However, the lower activity of antioxidant enzymes during training accompanied by WBC, at the same time as a lower concentration of lipid peroxidation molecules, indicates a decrease in the generation of reactive oxygen species. The low temperatures induced adaptation of the body to ensure a correct balance between pro-oxidant-antioxidant reactants during intense training. WBC preceding kayakers' training induced positive adaptive changes in cells protecting organisms from pro-oxidative-antioxidative equilibrium disturbances.^[7-9]

WBC influences the equilibrium between oxidant and antioxidant species. Some experiments have been conducted in healthy, but non-physically active, individuals. Lubkowska et al.^[10] showed that one WBC session causes disturbances of the oxidant-antioxidant balance. The concentration of peroxides, a sign of total oxidant status, was decreased 30 minutes after leaving the cryochamber in 15 young men (mean age 21 years), and it remained low after 24 hours, whereas the level of total antioxidant status decreased immediately after cold exposure, but had increased after 24 hours.

Siems and Brenke^[11] observed that acute cold stimulation (winter swimming) induced a decrease in plasma antioxidants (ascorbic acid, uric

acid) and an increase in the concentrations of a molecule that is a marker of lipid peroxidation, hydroxynonenal.

Dugué et al.^[12] observed a significant increase in total peroxyl radical trapping antioxidant capacity of plasma 2 minutes after cold stress in healthy women in the first 4 weeks over a 12-week treatment period. Thirty-five minutes after the application of cold stress, the effect was not apparent. The 20 women underwent either winter swimming or WBC, which included three exposures per week for 3 months. The authors remarked that the increase of trapping antioxidant capacity of plasma was unexpected and, moreover, that interindividual biological variation of this marker was high, as also outlined for total antioxidant status in another study.^[13]

The WBC effect resulted in immediate and significant increase in peroxidase and glutathione reductase activities, and a decrease in catalase and glutathione transferase in erythrocytes in healthy subjects. A single treatment induced oxidation stress, but the level of the oxidative stress was not high.^[13]

In conclusion:

1. WBC induces oxidative stress.
2. A single treatment can induce oxidative stress, but at low level.
3. Repeated treatments are not apparently able to induce cumulative effects; on the contrary, adaptive changes of antioxidant status are elicited.
4. WBC is not harmful for oxidative stress in healthy subjects.
5. The adaptation is evident where WBC precedes or accompanies intense training.

3. Immunology and Inflammation

Classical immunological markers such as immunoglobulins and C-reactive protein (CRP) were measured in athletes before and after a treatment cycle. These markers are both regularly and easily evaluated in the general population and also in athletes as markers of acute or chronic infection and/or inflammation. Moreover, these parameters are universally available in clinical laboratories. In effect, lymphocyte and monocyte counts and plasma IL-6 concentration were

higher in habitual winter swimmers than in inexperienced winter swimmers; the difference was probably due to the long-lasting exercise performed in the cold environment by experienced subjects.^[14]

Immunoglobulins were slightly, but not significantly, increased, and CRP showed a slight, but also not significant, decrease in rugby players who underwent WBC treatment. The lymphocyte and monocyte counts did not change: 44.7% (SD 8.2) for lymphocytes before WBC and 37.8% (SD 10.6) after (p-value not significant), and 9.6% for monocytes in both the blood drawings (SD 1.7 before, 3.5 after; p-value not significant).^[15]

Thus, WBC is not characterized by modifications of immunological markers and it does not seem to be harmful to the immunological system.

Data suggest that WBC does not have a detrimental effect on immunological parameters, although the period of observation, in this study, was too short to evaluate modifications of lymphocyte involvement and function. In fact, long-term cold water immersions of healthy males resulted in slight elevations in plasma tumour necrosis factor- α , and lymphocyte and monocyte counts.^[16]

Cold exposure has an immunostimulating effect possibly related to the enhanced noradrenaline (norepinephrine) response to cold. In effect, there is, in general, limited evidence of immunosuppression from short- or long-term cold exposure. On the contrary, a stimulating effect of cold exposure could be argued, which is dependent on the relationship between core temperature decrease and duration of exposure.^[17]

The WBC effect is probably linked to the modifications of immunological molecules having paracrine effects, rather than effects to systemic immunological functions.

In fact, there is an increase in the anti-inflammatory cytokine interleukin (IL)-10, and a decrease in the pro-inflammatory cytokine IL-2 and chemokine IL-8. Moreover, the decrease in intercellular adhesion molecule 1 (ICAM-1) supported the anti-inflammatory response. The contemporary decrease in prostaglandin E₂, which is widely synthesized at sites of inflammation where it induces vasodilation and the

increase of vascular permeability, confirmed that the treatment induces an anti-inflammatory protection.^[15]

The cytokine IL-1 β did not show modification after 18 WBC exposures during a 9-day cycle in professional field hockey players.^[4] The observed values of cytokines confirm the positive effect of WBC on immunological stimulation and/or protection. Some studies were performed to investigate the possible influence of WBC on inflammatory mechanisms.

WBC effects on lysosomal enzymes were studied in 21 kayakers from the Polish Olympic team compared with 10 untrained men. The athletes were submitted to a 10-day training cycle where training sessions were preceded by WBC treatment three times a day. The athletes were also examined during a training cycle without WBC. Blood was taken before training and at days 6 and 10 of the cycle. The authors studied lysosomal enzymes because these molecules are involved in the hydrolysis of proteins from the injured muscle fibres. The increased muscle activity induced increases in levels of enzyme activity in blood and also enzyme release from monocytes and macrophages, which are involved in tissue repair. WBC does not induce increases in lysosomal enzymes: a single treatment with non-athletes did not change the levels of enzyme activities. Thus, WBC is not harmful to the lysosomal membrane and it does not facilitate the release of lysosomal enzymes. On the contrary, WBC seems to stabilize lysosomal membranes. In fact, the activity of acid phosphatase, arylsulphatase and cathepsin D was lower after the 6th day of training preceded by WBC, than after the 6th day of training without treatment.^[18]

An anti-inflammatory effect of WBC was found in rugby players who were treated for 1 week, where an increase in the anti-inflammatory cytokine IL-10, and a decrease in the pro-inflammatory cytokine IL-2 and chemokine IL-8 was seen. Moreover, the decrease in ICAM-1 supported the anti-inflammatory response.^[15]

Plasma IL-1 β , IL-6 and tumour necrosis factor- α (signals of inflammation) did not show changes after cold exposure during 12 weeks of exposure to winter swimming or WBC in

20 healthy females.^[19] The reduction of inflammation could be proposed as the principal effect of WBC, enhancing and influencing the positive effects on various pathways of metabolism.

In conclusion:

1. WBC has no detrimental effect on immunological parameters.
2. WBC has an immunostimulating effect.
3. WBC induces an increase in anti-inflammatory cytokine IL-10 and a decrease in proinflammatory cytokine IL-2 and chemokine IL-8, and also in prostaglandin E₂.
4. WBC does not induce the release of lysosomal enzymes and stabilizes the lysosomal membranes.

4. Muscular Enzymes

An increase in serum creatine kinase (CK) is the most typical sign of exertional rhabdomyolysis, and it could be used as a measure to predict physical workload, recovery and possible overtraining. The beneficial effects of active recovery have been described in top-level rugby players after training, where the immersion of legs in cold water resulted in a decrease in serum total CK concentration compared with passive recovery.^[20] This confirmed the results of Gill et al.,^[21] who also observed CK in interstitial muscular fluid of rugby players. WBC induced a clear and significant decrease in the mean values of CK and lactate dehydrogenase (LDH) after 1 week of treatment in professional rugby players.^[15]

It seems that short-time cold air exposure induces an enhancement of muscle fibre repair, reducing the breakdown of the cell membrane or reducing its increased permeability, which is generally caused by oxidant agents produced by physical exercise. Since the athletes did not change their training scheme or load during the period of WBC treatment, the significant decrease of serum total CK and LDH concentrations resulted in proper and rapid recovery of muscular damage.^[16]

The mechanism inducing the decrease of muscular enzymes could also be related to a thyroid response via the decreased sensitivity of mitochondria to adenosine diphosphate, creatine and mitochondrial CK, which influences the entire CK metabolism. In addition, membrane sta-

bilization could explain the limited increase of muscular enzyme in the plasma.

The reduction of microinjuries to muscle fibres caused by exercise (shown by a decrease in CK serum concentration) was confirmed in 21 kayakers performing two 10-day training cycles, one cycle without treatment and the other with WBC preceding each training session. The CK values in both groups were higher than those before training, as expected. The values during training with WBC were significantly lower than those observed during training without WBC. For example, the activity of CK after the 6th day of training with WBC was 34% lower than after training without treatment.^[18]

In conclusion:

1. WBC shows positive effects on muscular enzymes CK and LDH (i.e. a limitation of their increase), which are typical indicators of muscular involvement during physical exercise.
2. WBC is a procedure that facilitates athletic recovery.

5. Cardiac Markers

WBC and acute cooling induce an increase in high frequency power and an increase in cardiac parasympathetic modulation. However, after 3 months of repeated WBC, the increase in parasympathetic tone in healthy females was limited as a result of adaptation.^[22]

Thus, the treatment should not be harmful to cardiac function in healthy people. A study was conducted in top-level athletes who were treated once a day for 1 week with WBC. Measurement was taken of the cardiac markers troponin I and high sensitivity C-reactive protein (hsCRP) [parameters linked to damage and necrosis of cardiac muscular tissue, but also to tissue repair] and N-terminal pro B-type natriuretic peptide (NT-proBNP) [a parameter linked to heart failure and ventricular power decrease]. Troponin I and hsCRP were unchanged, demonstrating that there was no damage, even minimal, in the heart during the treatment. NT-proBNP increased from a mean of 19.7 pg/mL before WBC treatment to a mean of 31.3 pg/mL after treatment. The training workload was the same as that administered in

the weeks preceding the treatment; therefore, the increase in NT-proBNP was due to cold stress. However, the NT-proBNP concentrations observed after WBC were lower than those measured after a heavy training session in the same group of athletes. WBC possibly limits the NT-proBNP increase, which is a typical indicator of physical exercise.^[23,24]

In conclusion:

1. WBC does not appear to be deleterious for athletes' cardiac function.

6. Hormones

Hormonal homeostasis was studied in 22 elite soccer players who completed ten WBC sessions accompanied by kinesitherapy following each WBC session. Blood was collected before and 2 days after the treatment. After the treatment, a significant decrease in the concentration of testosterone and estradiol was found, whereas dehydroepiandrosterone sulphate and luteinizing hormone were unchanged. A possible influence of WBC on aromatization could be suggested.^[25]

No changes in serum concentration of growth hormone, thyroid stimulating hormone, prolactin or free thyroid hormones were found during 12 weeks of WBC treatment (three treatments per week) in six healthy females.^[26]

In another study, cortisol levels did not change after a single WBC treatment in untrained men. The concentration of cortisol increased by 23% after the first 6 days of training without treatment and remained at that level after the 10th day, whereas changes in the concentration after training with WBC were not significant.^[18]

WBC did not stimulate the pituitary-adrenal cortex axis during a 12-week treatment period in 20 healthy females who underwent winter swimming or WBC three times a week. Plasma adrenocorticotrophic hormone and cortisol concentrations in weeks 4–12 were significantly lower than in week 1 as a result of to habituation.^[19]

It is not possible to argue potential hormonal modifications by WBC from the limited published data. However, it seems that it does not directly influence pituitary function. Possible hormone modifications are linked to habituation

and adaptation of the body to cold stress; plasma adrenaline was unchanged, but noradrenaline increased 2- or 3-fold at each exposure to cold temperatures in healthy females undergoing 12 weeks of treatment.^[19]

In conclusion:

1. WBC does not induce modifications of pituitary and thyroid hormones.
2. WBC induces a decrease in testosterone and estradiol.
3. WBC does not stimulate the pituitary-adrenal cortex axis or cortisol release.
4. WBC stimulates the release of noradrenaline.

7. Conclusions

WBC is not harmful and does not induce negative general and specific effects in athletes. The published studies concentrate on physiological, biochemical and haematological parameters, which are not negatively modified; however, specific studies on physical parameters and the effects on recovery from injuries should be performed. WBC reduces proinflammatory responses, decreases pro-oxidant molecular species and stabilizes membranes, resulting in high potential beneficial effects on sports-induced haemolysis, and cell and tissue damage, which is characteristic of heavy physical exercise. Conversely, it does not influence immunological or hormonal responses, with the exception of testosterone and estradiol, or myocardial cell metabolism. Interleukin concentrations are modified by WBC, which induces an anti-inflammatory response.

The treatment does not induce modifications of biochemical or haematological parameters that would be suspected in cheating athletes.

The published data are generally not controversial, but further studies are necessary to confirm the present observations. Standardization of exposure times and the number of treatments during each cycle could improve data comparison. Intensities and frequencies of treatments are quite similar in the different studies, but a specific effort to standardize protocols in patients with various pathologies, and especially in athletes, should be encouraged. Standardization

could further reduce the possible discrepancies among results from different studies.

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Correspondence: Professor *Giuseppe Banfi*, IRCCS Galeazzi, School of Medicine, University of Milan, Via R Galeazzi, 4 - 20161 Milan, Italy.
E-mail: giuseppebanfi@supereva.it