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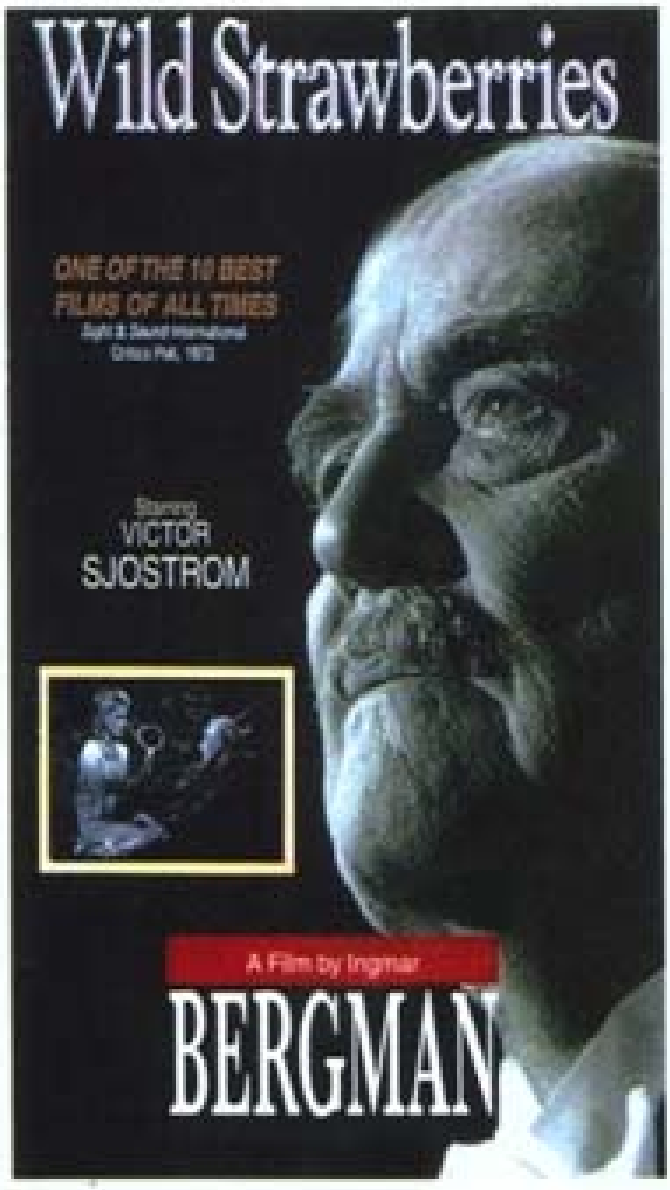

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Carboxytherapy: effects on microcirculation and its use in the treatment of severe lymphedema

A review

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Carboxytherapy refers to the administration of CO_2 for therapeutic purposes. It has been shown that, because of the interaction between CO_2 and regulating factors of tissue perfusion, Carboxytherapy acts on the microcirculation at the level of metarterioles, arterioles and precapillary shpincteres by increasing tissue flow velocity and consequently, by improving lymphatic drainage. Analysis of literature data shows a wide range of today applications for this treatment involving either phlebology or non-phlebology fields. Specifically, the positive effect on the increase of lymphatic drainage has more recently made Carboxytherapy useful for treatment of lymphatic stasis. Basic hemodynamic, histologyc and biochemical principles that explain the effects on microcirculation bed and lymphatic drainage are here analyzed to show how Carboxytherapy can be useful in the treatment of diseases such as severe lymphedema.

KEY WORDS: Carboxytherapy - Microcirculation - Lymphedema.

Carboxytherapy refers to the administration of CO_2 for therapeutic purposes.[1] Although this treatment originated in France in 1932 and was then introduced in Italy in 1990 by Belotti and De Bernardi,[2] it was only in 1995 that the term "Carboxytherapy" was coined by Luigi Parassoni during the XVI National Meeting of Esthetical Medicine, promoted at Rome by the Italian Society of Esthetical Medicine.[3-13] Indeed, this therapy was also known as "carbon dioxide therapy" because of the CO_2 molecular configuration including two oxygen and one hydrogen atoms.

The microcirculation

Hemodynamic essentials

The role of cardiovascular system is to provide oxygen, nutrients and hormones to, as well as remove carbon dioxide and wasted from the cells and to ensure "body homeostasis" by keeping the concentration of dissolved participles, the temperature, the volume and the pH level largely constant. The circulatory system can be subdivided into a pulmonary circulation and a systemic circulation, the last providing to the perfusion for all the tissues except for lungs and also called "big circulation" or "peripheral circulation".[5]

Due to dissimilarities concerning development and function, blood vessels are differentiated in arterials, arterioles, metarterioles, capillaries,venules and veins. In the "microcirculation" arterial tree divides into munerous arterioles which again divide into capillaries and sinusoids. Components of Microcirculation, that refers to the smallest blood vessels in the body, are shown in Figure 1.

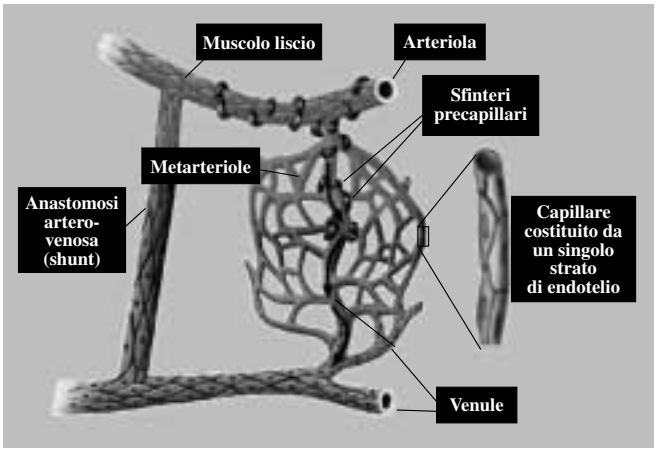

Figure 1.—Components of Microcirculation: arterioles, metarterioles, capillaries, precapillary sphincters, capillary shunt, venules.

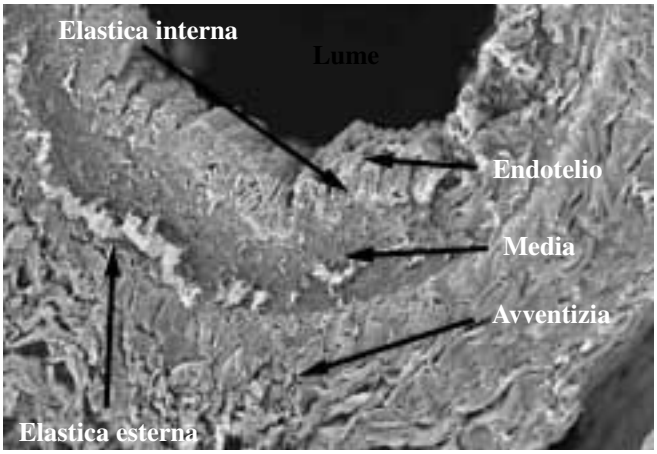

Figure 2.—Three-layer structure of arterial wall (microscopic view).

Arterial vessels supply high pressure blood flow from the heart to body tissues. The function of arterioles and metarterioles is to regulate blood flow through capillary bed and to ensure a "vis a tergo" that allows flow in the microcirculation. The principal function of capillaries is to permit the exchange of substances (water, electrolytes, nutrients, hormones) between tissues and blood. The venules collect blood from capillary bed and finally, the veins transport the blood back to heart and also serve as capacity vessels of large volume.

A common characteristic for all the types of circulatory vessels is the "distensible" property. In the arterial district, distensibility allows vessels to receive high pressure, pulsatile blood flow from the heart pump and to deliver while buffering these pulsatile excessive pressure and flow towards the small peripheral vessels. The highest distensibility is a property of the venous systems. Due to the large expansibility the veins represent a temporary reservoir of large blood volumes which are stored but can be utilized, when necessary, in whichever body district.[5]

The heart is filled with low pressure blood flow from the venous systems. By the contraction of the heart muscle, the blood is then driven rhythmically (at each systole) and at high pressure through arteries which, due to their elastic properties, represent a "pressure chamber". The most important function of large arteries is to reduce the impedance (dynamic resistance to the oscillatory components of pulsatile flow) to left ventricular outflow. This is accomplished directly by arterial expansion during systole and storage of blood for run-off in diastole. The elastic retractions of the arterial walls push and dampen the flow from the large arteries towards the microcirculation. Blood flow is subsequently collected into the low pressure system of the venules and veins.

Histology essentials

Elastic retraction is an essential property of arterial vessels in order to ensure a continuous blood flow in the microcirculation. Without this retraction, the blood could reach peripheral bed (microcirculation) exclusively during the systolic phase due to the propulsive heart force. Properties and functions of arterials are explained by the structure of the arterial wall.[5]

The wall of arterial vessels is quite thick and strong and is structured in a typical well distinct three-layer configuration that is common for all the arteries: an intimae layer (tunica intima) made up of endothelial cells, a medium layer (tunica media) mainly represented by transverse-oriented smooth muscle fibro cells and an adventitial external layer (tunica adventitia) formed by bundles of fibroblastic cells and collagen fibres mostly oriented with longitudinal direction. An internal elastic membrane is mostly present at the border between tunica intima and media. The external elastic membrane demarcates the border to the tunica adventitia (Figure 2). According to the wall composition two types of arteries, elastic (conductive arteries) and muscular (distributive arteries), can be identified.In elastic arteries, which include the largest arteries of the body, the borders between intima, media and adven-

titia are less distinct compared to muscular arteries, due to the presence of numerous fenestrated elastic lamellae in all three layers. In the medium layer up to 70 lamina of elastin with large fenestration can be found. Close to the heart, elasticity of the arteries is of great functional importance: during systole, the aorta and the elastic arteries dilate and then, during diastole, gradually return to their original size due to their elastic properties. By this means, the pulsatile blood flow is turned into a more steady flow, reducing blood pressure requirements.

The most common type of arterial vessels is represented by muscular arteries. In these, the medium layer is composed by variable strata of smooth muscle fibro cells ("myofibroblast"), ranging from 3-4 in the smallest arteries to 30-40 strata in the largest.

Arterioles and metarterioles are small muscular vessels (an endothelium surrounded by one or more layers of smooth muscle cells) and represent a major site for regulating systemic vascular resistance in the microcirculatory bed. These vessels are named as the "heart" of the microcirculatory system and provide the "vis a tergo" for microcirculation flow. Indeed, rhythmical contractions and relaxations in the arterioles walls are the source of the force ("vis a tergo") that drives and regulates the blood flow into the microcirculation bed. Fluctuations in systemic pressure are almost completely damped out by the arterioles at the level of microcirculatory flow (Figure 3).

Finally, in vein vessels the wall is quite thin but contains a muscular layer of smooth myocytes that allows the vessels to expand or to contract and therefore, to collect or release large blood volumes into the circulatory bed.

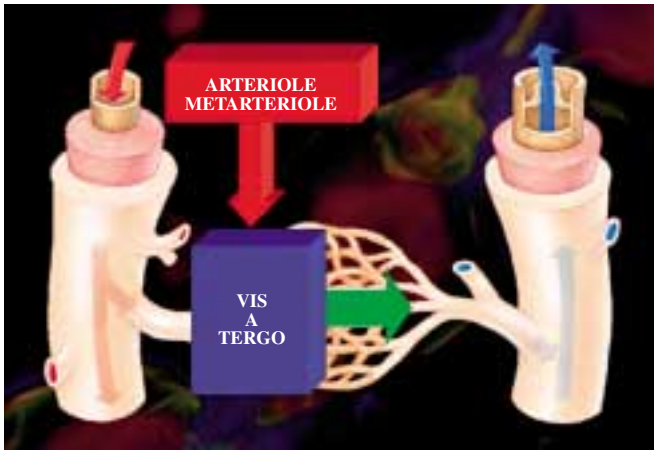

Figure 3.—Microcirculatory arterioles and metarterioles: vis a tergo. Arterioles and metarterioles represent a major site for regulating systemic vascular resistance in the microcirculatory bed. These vessels are the "heart" of the microcirculatory system and provide the "vis a tergo" for microcirculation flow. Indeed, rhythmical contractions and relaxations in the arterioles walls are the source of the force ("vis a tergo") that drives and regulates the blood flow into the microcirculation bed. Fluctuations in systemic pressure are completely damped out by the arterioles at the level of microcirculatory flow.

Microcirculation regulation

Under normal circumstances, continued adequate perfusion of individual vascular beds is assured by the capacity of blood vessels and heart contraction. Blood vessels are regulated by nervous, hormonal and local stimuli.[5]

Neurogenic regulation of blood circulation, provided by the autonomic nervous system, affects general functions such as the distribution of flow to different organs of the body, the strength the heart pump and the rapid regulation of blood pressure.

The autonomic regulation of circulatory vessels is mainly due to sympathetic adrenergic fibres, while the parasympathetic nerves mainly act on the heart functions and exert only a minor effect on the vessels. Sympathetic vasomotor fibres originated in the spinal cord travel with the thoracic nerves and the two upper lumbar nerves to reach the ganglia of sympathetic chain from which they depart using two different ways to innervate the circulatory system: 1. "specific sympathetic nerves" to supply the heart and the major vessels 2. "spinal nerves" to regulate peripheral territories.[5]

The Sympathetic system provides innervations for all the vascular systems (including arterial and venous systems) with the only exception of capillaries, precapillary sphincters and the majority of metarterioles which are mostly regulated by humoral local factors (Figure 4).

The vasomotor centre (located in the reticular area of the medulla and in the third inferior of the pons) repeatedly transmits signals to the sympathetic vasoconstrictive fibres at a rate of 0.5-2 per second. This continuous discharge is called as sympathetic vasomotor tone and allows a persistent partial contraction of the vessels wall.

Any increase in sympathetic autonomic system output enhances wall vessel contractility (vasomotor effect), peripheral resistance and cardiac filling pressure and decreases vessel capacitance and circulating blood mass. The nervous autonomic system plays a

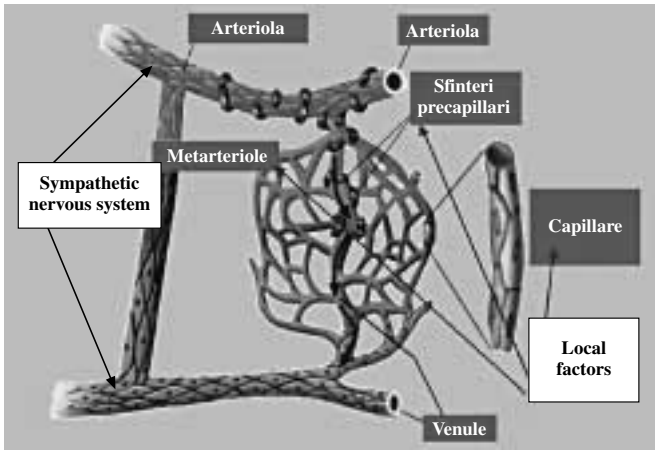

Figure 4.—Interaction between Sympathetic innervations and local factors in vessels regulation.

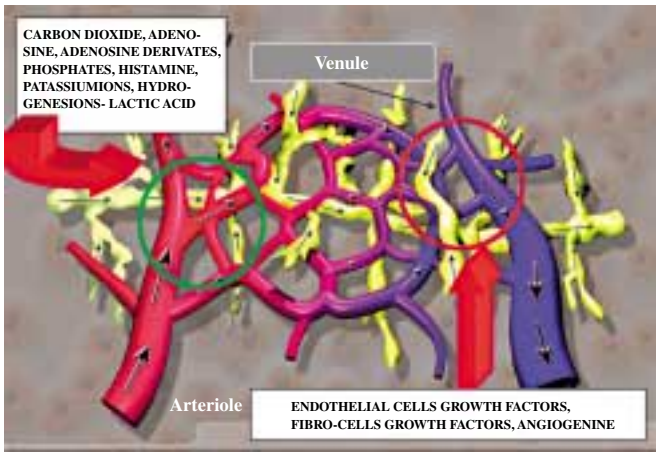

Figure 5.—Microcirculatory network and regulating factors. Top, left:short term regulating factors. Bottom on the right: long term regulating factors.

main regulatory function by inducing significant changes in filling and capacity.

However, autonomic system is only just involved in the changes of tissue blood flow, the main regulation of tissue perfusion being driven by local factors (Figure 5).

An essential principle of the circulatory function is the ability for each circulatory district to regulate local perfusion according to the specific metabolic necessity. Since in each tissue metabolic requirements vary over the time, local flow accordingly changes. Indeed, local flow needs depend from variable tissue metabolic requirements such as O_2 provision, nutrients supply (glucose, lipid, amino acid….), CO_2 removal, preservation of an appropriate ionic tissue concentration and hormones transportation.

Furthermore, there are some tissues with specific functions and requirements. Blood vessels of the skin need to fulfil at least two functions: nourishment and thermoregulation. By regulating skin capillary perfusion, heat loss may significantly vary. At the renal level, local circulation allows the removal of metabolic wasted contributing to the emunctories (depurative) function.

As a general rule, the higher the metabolic tissue activity, the higher the local blood flow requirement. At rest, in the muscle tissue there is little metabolic activity and consequently, the basic flow is very low (4 ml/minute/100 g). However, with strong physical activity, muscle metabolic requirements can increase for more than 60 folds and blood flow can increase for more than 20 folds (up to 80 ml/minute per 100 g).[5]

Tissue flow is regulated by the release of several local factors (Figure 5).

All these local factors contribute to regulate local perfusion in short and long term.

Factors regulating short term local perfusion include: CO_2 (carbon dioxide), lactic acid, adenosine derivates, phosphates, histamine, potassium, hydrogen ions.

Local factors regulating tissue flow in the long term include endothelial growth factors, fibro cells growth factors and angiogenine.

Short term regulation of tissue perfusion (by CO_2 and adenosine) is determined by the enhancement in rhythmic dilations and constrictions of vessel wall at the level of arterioles, metarterioles and precapillary sphincters ("increased vasomotion") with correspondent flow modifications. These changes of local flow occur within few seconds or minutes with the purpose to maintain perfusion appropriate to local metabolic requirements.

Long term regulation of local flow (mediated by growth factors) is managed by the expansion of the microcirculatory bed ("true" and "false" angiogenesis). This vascular bed increase may require few days, weeks or some months.

Vasomotion

A single metarteriola, a single capillary and the surrounding tissue form a tissue unit. Precapillary sphincters are located at the proximal level of the capillary vessels while several smooth muscle fibrocells surround the metarteriola.

Experimental models on wing bat under microscopy magnification, have shown that precapillary sphincters may be opened or closed and the metarteriole tone (persistent basal contraction of muscle cells in vessel wall) may change over the time. At a definite time, the number of opened precapillary sphincters corresponds to the tissue requirements for oxygen and nutrients in that time. Furthermore, sphincters and metarterioles can open and close cyclically, several times per minutes. This rhythmic (at regular intervals) opening and closing of metarterioles and precapillary sphincters is named vasomotion.

Since smooth muscle cells require oxygen to begin and maintain contraction, the contractile strength of precapillary sphincters increases when oxygen availability rises.

Therefore, when oxygen concentration increases above a threshold level, precapillary sphincters and metarterioles close down and tissue flow slows until the excess of oxygen is completely utilized by the tissue cells. On the contrary, any decrease in oxygen concentration causes the release and complete opening of metarterioles and precapillary sphincters with a subsequent enhancement in flow velocity and tissue perfusion.

Short term local flow regulation

Short term regulation of tissue flow is mainly determined by local factors: CO_2, adenosine, lactic acid, phosphates, histamine, potassium, hydrogen ions.

This short term regulation is mediated by vasodilatation at metarterioles and arterioles level due to the relaxation of smooth muscle cells in the vessels wall and the enhanced vasomotion. These adjustments occur within few seconds or minutes to rapidly adapt local flow to tissue requirements. Short term regulation causes abrupt modification of local perfusion due to a very consistent change in the contractile tone of arterioles, metarterioles and precapillary sphincters.

Several factors interact for short term regulation, including tissue metabolism, oxygen and nutrients availability.

Tissue metabolism: It has been shown that 8 folds increase in tissue metabolism is followed by 4 times increase in the local flow.

Consequences of Oxygen and metabolic availability on local flow: at any time local oxygen availability decreases, such as in the altitudes, during pneumonia and in poisoning from carbon monoxide (that reduces haemoglobin ability of carrying oxygen) or cyanides (that reduce oxygen utilization from tissues), as a consequence local blood flow significantly increases.

Two different theories are suggested to explain flow changes due to metabolic or oxygen variations:

— Flow dependent regulation (Vasodilatation hypothesis);

— Oxygen requirement hypothesis.

THE FLOW DEPENDENT REGULATION OF VASOMOTOR TONE (VASODILATATION HYPOTHESIS)

According with the vasodilatation theory, the greater the metabolism or the lower the oxygen availability, the higher the release of strong vasodilator factors that act by increasing arterioles, metarterioles and precapillary sphincters relaxation. This allows substantial vasodilatation and enhanced flow. Several factors have been described as putative mediators of the coupling between oxygen consumption, metabolism increase and vasomotor tone (vasodilatation stimulating factors, released in condition of increased metabolism or decreased oxygen availability) such as adenosine, CO_2, lactic acid, adenosine derivates, phosphates, histamine, potassium and hydrogen ions.[5]

Recently, particular attention has been paid to the role of adenosine as one of the most powerful vasodilators acting directly (via the A2 receptors) upon the smooth muscle cells of coronary arteries. The hypothesis that adenosine could represent a major signal coupling the myocardial metabolism and the coronary flow is supported by the observation that its actual concentration in myocardial interstitium is indeed within the range of its vasoactive effect. When coronary flow is reduced, interstitial adenosine levels increase: this is followed by active coronary artery vasodilatation and re-established coronary normal flow. Similarly, in cases of heart hyperactivity and increased metabolites and oxygen consumption, an increase in tissue ATP degradation with increased adenosine production has been observed. It has been hypothesized that the released adenosine, in part, passes outside the muscle cell and stimulates coronary arteries vasodilatation in order to adapt coronary flow to the increased oxygen requirements due to the increased heart activity.[5]

Many Authors suppose that the CO_2 promotes vasodilatation at arterioles and metarterioles levels

using a mechanism similar to that of adenosine. Increase in interstitial CO_2 tension has indeed been considered a potential signal responsible for the metabolic control of the coronary blood flow. The CO_2 produced during increased myocardial activity directly stimulates coronary vasodilatation in order to increase the coronary flow and to adapt this to the increased cardiac oxygen requirements consequence of the increased activity.

Both, adenosine and CO_2, are physiologic factors used for the "autoregulation" of local flow, that is the adjustment of local perfusion to metabolic and oxygen tissue requirements.

THE OXYGEN REQUIREMENT HYPOTHESIS

The flow dependent regulation of vasomotor tone is accepted by many Authors. However, Others support a different mechanism to explain changes in tissue flow: the hypothesis of "the oxygen requirement", or better, "the nutrients requirement" since besides oxygen, many other local nutrients may be probably involved in local flow regulation.[5]

Oxygen (such as other nutrients) is needed to maintain the contraction of smooth muscle cells of the arterial wall (vasomotor tone). In situations of scarce availability of oxygen (and other nutrients), arterial vessels tend to relax and dilate. Similarly, during increased metabolic activity, the augmented oxygen consumption could cause a decrease in oxygen availability for vessels smooth muscle fibro cells with consequent local arterial vasodilatation.

Long term tissue flow regulation

The regulation of tissue flow in the long term is based on the enlargement of the microcirculatory vascular bed. A "true Angiogenesis" and a "false Angiogenesis" are involved in obtaining the expansion of microcirculatory bed.[9]

True angiogenesis

The term "angiogenesis" refers to the growth, expansion and remodelling of vessels. This process occurs as a consequence of various angiogenic factors that may be released from ischemic tissues, rapid growing tissues or tissues with increased metabolic activity.

Today more than 12 angiogenesis factors have been individuated. All of these are small peptides. The three most known factors being:

— endothelial cell growth factors;
— fibroblast growth factors;
— angiogenine.

These factors have been isolated from tumoral cells or ischemic tissues. It has been hypothesized that the lack of tissue oxygen is the main cause for the production of angiogenic factors. Each of these factors promotes development of new vessels by the same mechanism of sprouting, growing and remodelling from venules and, more rarely, from capillaries. New arterioles and metarterioles are indeed generated.[5]

Hypoxia is a strong stimulus for angiogenesis and therefore, for the regulation of flow in the long term. Indeed, an increase in microcirculatory bed has been found in several animals living in altitudes where atmosphere oxygen concentration is low. Other experimental studies have been performed in chicken embryos incubated in low oxygen atmospheres: development of microcirculatory bed has been doubled with respect to normal animals.

Furthermore, in immature neonates who undergo oxygentherapy it has been shown that the increase in oxygen availability causes a rapid interruption in the physiologic process of retinal vessels neoformation, and even the degeneration of some formed capillaries. Subsequently, when the cycle of oxygen-therapy is completed and the newborn returns to a normal oxygen atmosphere concentration, the new condition of relative hypoxia provokes an impulsive fast growth of new vessels. In some circumstances this growth may be excessively chaotic to cause invasion of the vitreous from the neogenerated vessels and consequently blindness (retrolenticular fibroplasias).

The Angiogenesis process can be stimulated by decreased availability of oxygen and, consequently by an excess of CO_2. CO_2 may promote the release of local growth factors able to stimulate angiogenesis.

The extend of tissue vascularization (angiogenesis) can increase or decrease according with the metabolic activity of the same tissue. If metabolism increases (and therefore the release of CO_2 is high), tissue vascularization expands; vice versa, when metabolic activity decreases (and the CO_2 concentration is low), tissue vascularization concurrently shrinks. In conclusion, in true angiogenesis there is a constant remodeling of microcirculatory vessels according with tissue metabolic requirements.

False angiogenesis

The theory of "false angiogenesis" was ideated by Curri.[11] The Author suggested that CO_2 promotes the expansion of microcirculatory bed by the recanalization of virtual capillaries. This "false angiogenesis" is not directly stimulated by CO_2 but is the consequence of the increased tissue flow. The term "false angiogenesis" was used because the increase in vascular bed was not due to neoformation of new vessels promoted by growth factors, but for the recanalization of pre-existent virtual capillaries.

True and False angiogenesis might cooperate in determining the increase of microcirculatory vascular bed. Indeed this expansion in vascularization may be consequence from the combination of:

— a true angiogenesis (vessels neoformation) stimulated by endothelial and fibroblast growth factors and angiogenine;[5]

— a false angiogenesis, that is the recanalization of virtual capillaries stimulated by the notably increased local flow velocity and volume.[9]

Carbon DIOXIDE (CO_2) metabolism and excretion: biochemical essentials

Carbon dioxide (CO_2) formed during cellular metabolism moves across the cell wall (from inside to outside) in a gaseous status to be removed. This is because only a very small amount of CO_2 can diffuse as bicarbonate ions that are almost completely impermeable to the cell wall.[7] After entering into the capillary bed, CO_2 undergoes several physical and biochemical reactions that allow its transformation and delivery to tissues.

— A small fraction (~7%) of the overall plasmatic CO_2 is carried to the lungs as a dissolved gas (in solution).

— The majority (~70%) of CO_2 reacts with plasmatic water to form carbonic acid : $CO_2+H_2O \rightarrow H_2CO_3$. This reaction, naturally occurs so slowly (1-3 minutes) in the plasma to loose any relevance. At the opposite, the Erythrocytes contain a specific catalytic enzyme, the "carbonic anhydrase", that is able to increase for about 5000 times the reaction speedy. Due to carbonic anhydrase the reaction becomes so fast to reach the steady state in less than 1 second. [7] Therefore, a large fraction of CO_2 is already transformed in carbonic acid into the red cells before to leave the capillary bed. Within another fraction of a second, carbonic acid dissociates by loosing a proton to become bicarbonate: $H_2CO_3 \rightarrow H^+ + HCO_3^-$. Most of the protons (Hydrogen ions) then combine with haemoglobin in one of the main buffer systems of the red cells. Bicarbonate is rapidly carried outside the red cells with the help of a carrier protein located on the cell wall that allows rapid exchange, in opposite directions, between bicarbonate ions that are expelled and Chloride ions that are carried in ("Chloride ions exchange"). Therefore, chloride ions concentration in the red cells of venous blood is lower that in the red cells of the arterial blood. This reversible binding between CO_2 and water within the erythrocytes is the main carrier used in human body to carry 70% of CO_2 from tissues to lungs. It has been shown in experimental models that by using an inhibitor (e.g., acetazolamide) to block carbonic anhydrase activity in the red cells, the CO_2 removal is significantly lowered and levels of tissue PCO_2 can increase from 45mmHg to 80 mmHg!.

— Another fraction of CO_2 binds to the ammine radicals (heme sites) of haemoglobin to form carbamylhemoglobin (CO_2Hb). This is a weak and reversible binding that allows the rapid release of CO_2 in the pulmonary alveoli where PCO_2 is lower than in the capillary bed.

— Finally, a small fraction of CO_2 combines with plasmatic proteins by the same bindings used with haemoglobin. However, these reactions between CO_2 and plasmatic proteins are significantly slower than that catalyzed in erythrocytes by carbonic anhydrase and can contribute for only 20% of CO_2 transportation.

At the capillary level, carbonic acid produced from CO_2 dissociation decreases the pH of the blood. However, the interaction between this acid and plasmatic buffers prevents the excessive increase in blood protons level and therefore, avoids acidosis.

The effects of carboxytherapy on the microcirculation

The purpose of Carboxytherapy is to improve or restore microcirculation function when damaged. The final effect is that of a rehabilitation therapy for microcirculation.

The results of CO_2 administration are not only due

to the improvement of local parameters of circulation and tissue perfusion but also to the instigation of a partial increase in oxygen tension as a consequence of : a hypercapnia-induced rise in capillary blood flow; a drop in cutaneous oxygen consumption; or a right shift of the O_2 dissociation curve (Bohr effect).

The effects of Carboxytherapy in instigating vasodilation are due to interactions between the released CO_2 and factors regulating tissue flow either in short or long term. It has been shown that CO_2 administration can determine:

Improvement in local tissue blood flow velocity;

Increase in microcirculatory vascular bed (angiogenesis).

1) *The increase in tissue blood flow velocity* are due to the effects of CO_2 at different levels:

— CO_2 interferes with the "vis a tergo" of the microcirculatory system by increasing the elastic retraction in arterioles/metartaerioles and indeed by inducing vasodilatation;[14]

— CO_2 relaxes smooth muscle fibro cells of precapillary sphincters allowing opening of sphincters and therefore an increase in tissue flow velocity;

— CO_2 enhances erythrocytes deformability.[10, 11]

The contraction of smooth muscle cells within the muscular layer of vessel wall requires oxygen. Therefore, oxygen provokes contraction of the metarterioles and precapillary sphincters with a consequent vessel narrowing and a decrease in tissue blood velocity and flow.

On the contrary, CO_2 administration determines relaxation of the smooth muscle cells in the metarterioles and precapillary sphincters with a consequent increase in the flow velocity and an overall improvement in the tissue perfusion. This enhanced vasomotion is the critical factor in determining the increase in local tissue perfusion. Indeed, this vasomotion represents the "vis a tergo" of the microcirculation that drives blood flow towards the capillary bed.

2) *The effect of CO_2 as a main regulating factor* inducing true and false angiogenesis has been detailed above.

Carboxytherapy: contraindications and side effects

Side effects from Carboxytherapy include minor pain, aches, ecchymoses, and local crackling or burning sensation. Major adverse events are uncommon. Indeed, it has been demonstrated in studies on gynaecologic laparoscopic surgery that even large amounts of CO_2 can be used to expand abdominal cavity (even 2-4 liters of CO_2) without any toxic effect. At rest, with normal ventilation (ventilation 6 l/min) the human body consumes 250 mL/min of Oxygen (transported from lungs to tissues) and exhales 200 mL/min of CO_2 (removed from tissues and excreted through pulmonary alveoli). In condition of hyperventilation, Oxygen consumption can increase up to 4000-5000 ml /minute and CO_2 can be formed up to 4000-4500 ml/minute. (5) During carboxytherapy an average of 30-50 ml/minute CO_2 are administrated per session: the mild increase in CO_2 levels are easily and therefore promptly resolvable by a mild hyperventilation at the end of treatment without risk of hypercapnia and respiratory acidosis.[10]

Nevertheless, in patients with severe respiratory insufficiency, severe renal failure or chronic congestive heart failure Carboxytherapy should be contraindicated. The main excretion of CO_2 and protons (H+) is performed by the kidneys and the lungs. Severe damages of these organs (renal or respiratory failure) may cause excessive accumulation of CO_2. Furthermore, in congestive heart failure, vessel circulations markedly slow down and consequently, the amount of CO_2 removal from tissues is significantly lowered. Chronic congestive heart failure is another pathophysiologic condition causing CO_2 accumulation in which carboxytherapy is questionable.[14]

Carboxytherapy should be also avoided in patients under treatment with carbonic anhydrase inhibitors (acetazolamide, diclofenamide, etc..). Indeed, the use of the inhibitor obstacles the main process by which CO_2 can be carried to be excreted trough lungs and kidneys. Therefore, an excessive increase in CO_2 levels during carboxytherapy might occur.

Severe anaemia might be another contraindication. This is because the significant reduction in erythrocytes and haemoglobin levels of anaemic conditions translate into a significant deficit of two of the main physiologic systems used for CO_2 elimination: the binding with water at the level of the erythrocytes and the direct interaction with plasmatic haemoglobin. 1. Severe anaemia implies a significant decrease in red cells number, therefore a significant decline in the availability of the main carrier by which CO_2 can be usually transferred to the lungs and kidneys to be eliminated 2. Severe anaemia implies also a decrease in

the haemoglobin levels and this reduces also the possibility of CO_2 binding to form carbamylhemoglobin plasmatic, the second most relevant CO_2 carrier from tissues to lungs and kidneys. Therefore, in condition of severe anaemia, CO_2 administration to perform carboxytherapy can further and dangerously increase CO_2 levels and cause severe hypercapnia and acidosis.[14]

A decrease in plasmatic protein levels (and consequently in carbamylhemoglobin and plasmatic proteins) may be caused by chronic liver insufficiency that, indeed, constitutes another contraindication for carboxytherapy.

Other contraindication is the presence of gaseous gangrene. This severe infectious disease is caused by anaerobic bacteria entered in the body through skin wounds and is characterized by extensive tissue damages, necrosis, oedema, and severe generalised deteriorated conditions. The infection from anaerobic bacteria (Welchia perfringens, Clostridium septicum, Clostridium novyi, Clostridium sporogenes, Clostridium hystoliticum) may be further enhanced by the increasing in CO_2 level that favours anaerobic environment (because of the concomitant decrease in O_2 concentration).

In conclusion, in physiological status any mild hypercapnia is rapidly compensated by an increase in the pulmonary ventilation that increases CO_2 excretion. Therefore, severe hypercapnia and acidosis never occur also after an excessive CO_2 administration. In normal condition (health patients) there is no possibility to cause severe hypercapnia by carboxytherapy.

Hypercapnia occurs when the lungs are unable to excrete CO_2 and therefore pCO2 significantly increases at the alveoli level. When alveolar PCO_2 reaches 60-75 mmHg the patient experiences severe dyspnoea. With further increase up to 80-100mmHg he becomes drowsy and lethargic (hypercapnic coma). With PCO_2 alveolar levels of 120-150mmHg the excessive CO_2 dampens respiratory bulbar center (normally small constant CO_2 levels are necessary to stimulate the centre) and can cause death.[5]

Carboxytherapy: clinical indications in phlebology

For all the effects on the microcirculation Carboxytherapy can be used to treat:

— chronic venous insufficiency (CVI);
— chronic veno-lymphatic insufficiency (CVLI);
— venous ulcers.

Other indications

— Localized adiposities (cellulitis);
— psoriasis;
— arterial vascular pathologies (Buerger disease, diabetic ulcers, acrocyanosis, atherosclerotic ulcers);
— aesthetic body disease (skin laxity, skin aging);
— rheumatism (acute or chronic).

Carboxytherapy: administration techniques

Two methods for CO_2 administration are employed:
— percutaneous;
— subcutaneous injection.

Percuteneous administration

Includes: gaseous baths and gaseous shower.

For gaseous baths CO_2 may be by administered as:
— true gas spring (carbon dioxide fumarole);
— carbonated water (carbon dioxide water).

Gaseous baths may be generalized or partial. For generalized administration, the patient lies supine or sit down. The entire lower body of the patient is drawn in a hermetically closed plastic bag where CO_2 is insufflated in order to create a saturated CO_2 atmosphere around the patient' legs. These generalized true gas sessions are usually applied for 20-30 minutes per day and repeated per 20-30 days.

Partial administration technique is applied in cases of localized functional or organic, arterial or lymphatic, disease: a single limb (or part of this) is drawn in the plastic bag that is then hermetically ensured around the limb and insufflated with CO_2.

An example of ambulatory gas bath application is the Hydrocarboxytherapy (Figure 6).

Carbonated water baths are applied with the full immersion of the patient in carbonated water at a temperature of 34°. It can be used natural carbonated water or water artificially enriched with CO_2 jets. Treatment is applied for mean of about 20-30 minutes per day and repeated for about 20-30 consecutive days.[11]

Gaseous showers are usually applied for the treatment of lower limbs dystrophic ulcers and include:

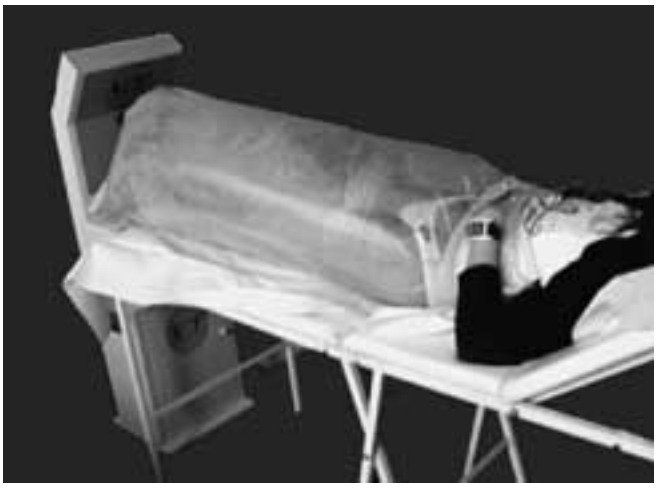

Figure 6.—Hydrocarboxytherapy (ME.DI.TER).

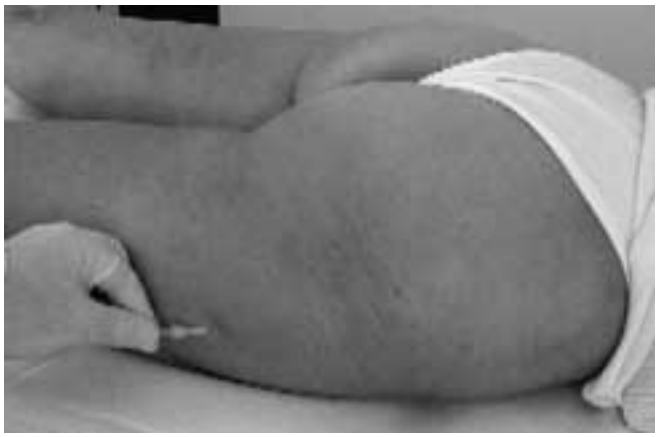

Figure 7.—Subcutaneous injection: Subtrochanteric site.

— point gas showers: CO_2 is released throw a small hole at the tip of a pipe. This technique is used for treatment of isolated small ulcers;

— loco-regional gas showers: CO_2 jets are provided from a multi –holes spring ramp where the limb is located after being draped and closed in a plastic bag.

Gas showers are applied for a mean of 20-30 minutes per day in cycles of 20-30 days.

Subcutaneous injections

The most common employed method to administrate Carboxytherapy is by subcutaneous injection (hypodermic injection). While CO_2 baths and showers (either gaseous or carbonated water) are applied as thermal therapeutic practice (balneotherapy), CO_2 injection can be administered exclusively in medical settings such as ambulatory practice. Since its first introduction, technology has evolved: in the first times gas bottles and syringes were used. Today, electronic programmable devices are available that allow accurate release of purified and precise CO_2 volumes. A number of filters, located inside the device, separate CO_2 from contaminates such as Clostridium sporogenes spores. Perfectly measured volumes of CO_2 gas are indeed released in a well depurated form to reach the delivery system provided with 30 G (13 mm) terminal needle.Once the CO_2 supply starts, few seconds are needed to completely fill the device and to ensure a saturated CO2 atmosphere within the delivery system. Therefore, the needle is introduced into the hypodermic layer and the CO_2 injections causes subcutaneous emphysema. The needle is ensured to the skin with a bandage, while CO_2 continues to spread within the subcutaneous tissue following virtual routes until the achievement of the predefinite volume. CO_2 diffusion and velocity of hypodermic dispersion strictly depends on the laxity of subcutaneous tissues and may vary from patient to patient.

Dosage

From few mL up to 2000 mL of CO_2 are administrated in each carboxytherapy session. Usually the medium CO_2 flow is of 30-50 mL/minute. But, in the first 2-3 minutes lower flows can be used (10-20 ml/min) in order to reduce the itchiness or pain sensation that could be evocated when CO_2 starts to push the way through the subcutaneous layer. After this first phase (about 2-5 minutes) CO_2 flow can be safely increased to 30-50 ml/minutes.[9]

Sites of injection

In condition of lower limbs severe lymphatic stasis, subcutaneous injections are applied at subtrochanteres levels: anteriorly and posteriorly, at the medium, medial and lateral thirds of the thigh (Figure 7). Needles are ensured to the skin and the CO_2 is realeased to reach the established dosage.

Literature data and personal experience

Recent studies have confirmed the vasomotor effects of CO_2, in addition to the absence of relevant side-

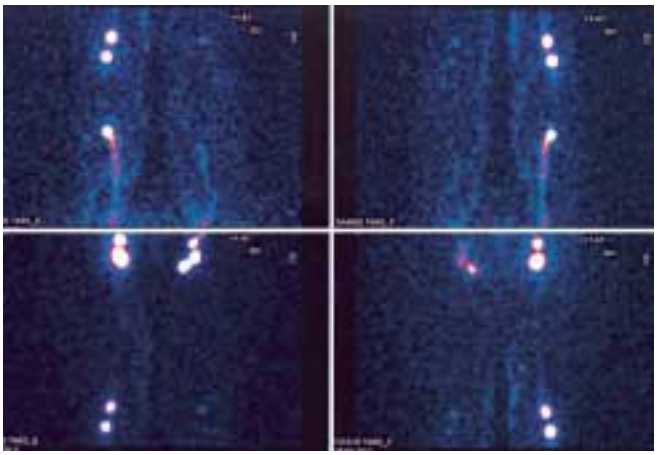

Figure 8.—Lymphoscintigraphy: basal radionuclide scan. Top, left: legs, anterior view. Top, right: legs by posterior view. Bottom, left: thighs anterior view. Bottom, right: thighs, posterior view.

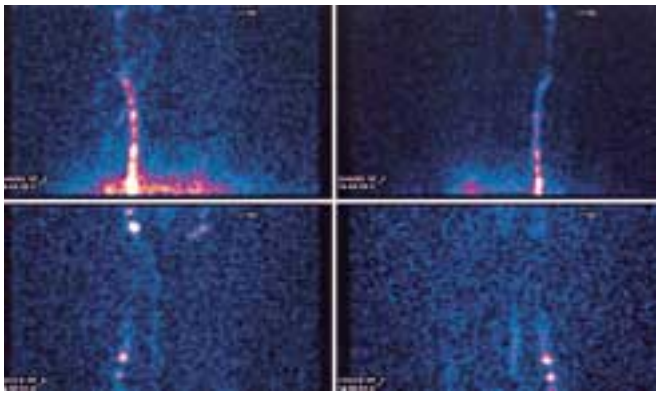

Figure 9.—Lymphoscintigraphy: radionuclide scan after walking (30 minutes). Top, left : legs, anterior view. Top, right: legs by posterior view. Bottom, left: thighs, anterior view. Bottom, right: thighs, posterior view.

effects and toxicity. Curri et al.,[1, 4] by using Doppler and Laser Doppler and Albergati and Parassoni et al.[1] by using optic video capillaroscopy (VCSO) verified the increased vasodilatation in the arterioles and metarterioles and the increased vasomotion. An increase in femoral blood flow of te lower limbs, as well as an improvement in treadmill test perimeters were observed by Hartmann and Savin who proposed this treatment for obliterating arteriopathies.[6, 12] Brandi et al.[3] found that the effect of Carboxytherapy on microcirculation was a positive tool in the physiological oxidative lipolytic process and therefore suggested this gas for the treatment of localized adiposities. Indeed local adiposities are commonly associated with alterations in blood and lymphatic drainage. They reported significant variations in the maximum circumference of lower limbs, in the microcirculation studied by oxygen tension ($tcPO_2$) and Laser Doppler, and in the histological analysis of adipose and connective tissues, after this treatment.[3]

More recently, studies have focused on the effect of Carboxytherapy on lymphatic drainage. Indeed, the positive effect of Carboxytherapy in improving microcirculatory flow was found to translate into a similar benefit in increasing the lymphatic drainage. In 2006, Manzo, Villeggia and Verlaro showed by lymphoscintigraphy the improvement in local parameters of circulation and tissue perfusion caused by Carboxytherapy in patients with severe lymphatic stasis.[8] A significant decrease of lymphatic stasis and a documented re-establishment of the physiological lymphatic drainage was found in the treated cases. Furthermore, there was a clinical improvement due to a significant limb volume and circumference reduction. The Authors experience is detailed here following.

Figure 10.—Carbomed CDT Evolution (Carboxytherapy Italian device).

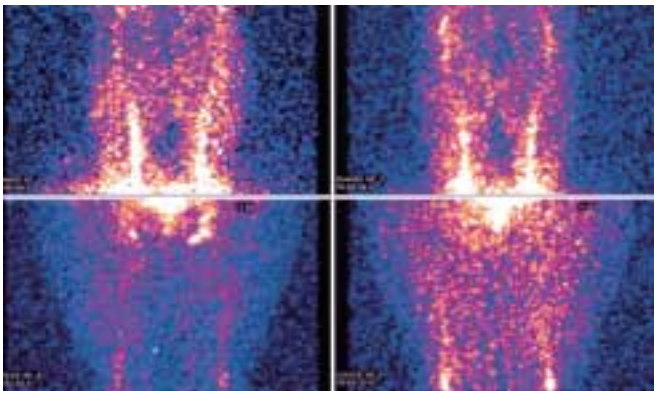

Figure 11.—Lymphoscintigraphy: radionuclide scan after 10 sessions of subcutaneous Carboxytherapy (1000 mL CO_2 by side). Top, left : legs, anterior view. Top, right: legs by posterior view. Bottom, left: thighs anterior view. Bottom, right: thighs, posterior view.

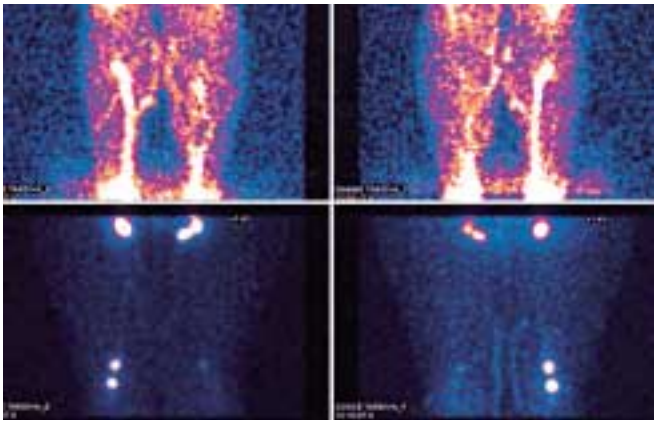

Figure 12.—Lymphoscintigraphy: final radionuclide scan after walking (30 minutes) + 10 sessions of subcutaneous Carboxytherapy (1000 mL CO_2 by side). Top, left : legs, anterior view. Top, right: legs by posterior view. Bottom, left: thighs, anterior view. Bottom, right: thighs, posterior view.

In a two years period (2004-2006) a total of 15 female patients affected by severe limphedema were treated by Carboxytherapy: the effects on the lymphatic flow were analysed. Before treatment, all the patients were evaluated by clinical examination, echocolor Doppler and lymphoscintigraphy. Lymphoscintigraphy was performed using computerised gamma camera with double rectangular head (GE Xeleris) by administrating 0.1 ml ^{99m}Tc-Albumin monocolloid through the 1st interdigital dorsal space of the foot (Figures 8, 9). For each patient 10 weekly sessions of subcutaneous Carboxytherapy using a programmable CO_2 apparatus (Carbomed CDT Evolution by Laboratory Electronics Designer, CE0051-ISO 9001-csq-csq med-Iqnet; Figure 10) were applied. The total quantity of CO_2 administrated by week was of 1000ml per side. In all the patients elastic compression was also applied before treatment and maintained for all the period of the study and thereafter. After a week from the last Carboxy-therapy session patients underwent a new lymphoscintigraphy examination. According with the final radionuclide scan imaging in all the patients the physiologic lymphatic flow was restored in lower limbs. This result was associated with a decrease in limb volume and lymphedema reduction. Post-treatment radionuclide results are shown in Figures 11, 12.

Conclusions

Due to the vasomotor effects of CO_2 and the absence of relevant major side-effects and toxicity, Carboxhytherapy has become increasingly diffused for the treatment of a variety of microcirculatory diseases. Specifically, the positive effect on lymphatic drainage in microcirculation let to use Carboxytherapy as an effective therapy for patients with lymphatic stasis.

Riassunto

Carbossiterapia: effetti sulla microcircolazione e suo uso nel trattamento dell'edema linfatico grave. Una review

Per carbossiterapia si intende l'utilizzo dell'anidride carbonica allo stato gassoso a scopo terapeutico. La carbossiterapia esplica i suoi effetti interferendo con i fattori che regolano a breve e a lungo termine il flusso ematico tessutale locale. La carbossiterapia esplica i suoi effetti a livello di microcircolazione: a livello delle metarteriole, delle arteriole, degli sfinteri precapillari determinando un aumento della velocità del flusso ematico tessutale locale e quindi un aumento del flusso linfatico. Una revisione della letteratura mostra come oggi la Carbossiterapia rappresenti un campo in continua espansione per il trattamento di patologie sia flebologiche che non flebologiche. Vengono qui riportati i principi fondamentali di emodinamica, istologia e biochimica che spiegano l'effetto della Carbossiterapia sul microcircolo e sul drenaggio linfatico. In particolare l'effetto positivo della Carbossiterapia sull'incremento del drenaggio linfatico tissutale (a livello del microcircolo) ha portato ad una sua rivalutazione nel trattamento dell'edema linfatico degli arti inferiori.

Parole chiave: Carbossiterapia - Microcircolo - Linfedema.

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