The effects of Crenotherapy and exercise in peripheral arterial occlusive disease.

A comparison with simple exercise training

Ann. Ital. Chir., 2017 88, 6: 469-477
pii: S0003469X17027853

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The effects of Crenotherapy and exercise in peripheral arterial occlusive disease. A comparison with simple exercise training.

BACKGROUND: *Conservative therapies for patients affected by Peripheral Arterial Occlusive Disease (PAOD) aim first to correct the risk factors and to slow down the disease progression. Among these, exercise has positive effects on blood flow, muscle metabolism and well demonstrated systemic effects. These include reduction of chronic inflammation markers, improvement of walking mechanics and heart function. Controlled physical training increases the ability to perform the daily activities improving life expectancy of these patients. The aim of this study is to evaluate the effects and the effectiveness of physical training performed in thermal water compared to traditional treadmill walking exercise.*
METHODS: *98 patients affected by IIb stage PAOD, according to Leriche-Fontaine classification, were enrolled. Patients were randomized into two groups: the first arm carried out an intensive training program under medical supervision (group A); the second one carried out a rehabilitative exercise associated with crenotherapy (group B). The following parameters were detected: Ankle-Brachial pressure index (ABI), actual claudication distance (ACD), maximum walking distance (MWD), flow mediated dilatation (FMD) and the intima-media thickness (IMT). All patients underwent Doppler echocardiography and complete biochemical assay.*
RESULTS: *In both groups, there was a statistically significant improvement of lipidaemia compared to baseline. When compared with each other, the two groups did not show statistically significant differences. There were no significant differences between the two groups regarding echocardiographic findings. Vascular reactivity study showed a statistically significant improvement of FMD after 3 months of exercise in both groups. In crenotherapy group (B) FMD values were significantly higher than the treadmill ones (A). In both groups a statistically significant improvement in ACD was observed*
CONCLUSIONS: *Our experience shows that crenotherapy has similar effects compared to traditional physical training in the treatment of PAOD, being equally well tolerated and safe; it gives an advantage over conventional physical training in terms of ACD and MWD improvement, although not statistically significant, and it is extremely welcome to patients compared to traditional physical training.*

KEY WORDS: Arterioscleroses, Intermittent Claudicatio, Peripheral Arterial Diseasen, Physical Exercise, Rehabilitation

Introduction

Peripheral arterial occlusive disease (PAOD) is a chronic condition caused by an obstruction to blood flow in the aortic-iliac-femoral-popliteal-tibial district.

Atherosclerosis is the main etiological factor [1]. According to the National Cholesterol Education Program - Adult Treatment Panel III (NCEP-ATP III), PAOD, as well as diabetes, abdominal aortic aneurysm and symptomatic carotid disease, determines a cardiovascular risk equivalent to that of coronary heart disease. The PAOD is a very common condition whose diagnosis is often underestimated; its prevalence, evaluated in different epidemiological studies [2-4] with specific tests such ABI (ankle-brachial-index), is of 3-10%, with a maximum of 15-20% in subjects with more than 70 years. The annual

Pervenuto in Redazione Ottobre 2017. Accettato per la pubblicazione Novembre 2017
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incidence increases with age: it is 0.6 in 45-54 years old patients, 2.5% in 55-64 ones and 8.8% in 65-74 ones. PAOD tends to be twice more frequent in males than women between 50-70 years, but this difference decreases in over 70s [5]. The ratio between symptomatic and asymptomatic form is of 1: 3 - 1: 4 and the majority of symptomatic patients is over 60.

Symptoms

Intermittent Claudication (IC) is the most common symptomatic form while critical limb ischemia (CLI) is less frequent, with a prevalence of 0.4% in a Swedish population over 60 [6-8]. IC is a muscle cramp pain downstream of the vascular lesion, where arterial pulses are constantly reduced or absent; nevertheless, diagnoses based only on this finding, tend to underestimate PAOD. Edinburgh Artery Study found that one-third of patients with asymptomatic PAOD has a complete occlusion of a main artery in a leg [9]. In autoptic studies, 15% of men and 5% of women, who were asymptomatic, had a > 50% stenosis [10]. The absence of CI in a patient with a severe PAOD can depend on the presence of other conditions, such as heart failure, pneumopathy, severe muscle-skeletal disease.

Risk Factors

The risk factors for the development of PAOD are in common to the other cardiovascular diseases. Among the modifiable risk factors, smoking diet is considered the most important [11,12], among the not modifiable ones, and diabetes [13,14] is proven to be associated with the development of PAOD [2,15]. Dyslipidaemia is present in about 50% of patients with PAOD. In the US Physicians Health Study [16], the total cholesterol / HDL ratio was found to be strongly related to the disease. In the Framingham study, a fasting total cholesterol greater than 270 mg/dl has been associated with a two-fold increasing of the incidence of Claudicatio Intermittens [17]. Hypertension is another common risk factor, although the association with PAOD is not strong as well as cerebrovascular disease. The hyperhomocysteinemia is a recent marker of atherosclerotic vasculopathy and is significantly increased in PAOD [18,19] as well as other hemostatic, rheological and inflammatory markers, as plasma fibrinogen and C-reactive protein, which showed in several studies an association with PAOD. Other not modifiable risk factors are advanced age, male sex and Black race, where the risk of developing PAOD is two-fold increased. Coronary artery disease is the cause of death in the 40-60% of patients with PAOD. Other vascular events, as aortic aneurysm rupture, cause approximately 10% of deaths. Therefore, only the 20-30% of patients with PAOD dies for non-cardiovascular causes [20].

Therapy

Therapeutic strategies for patients with PAOD are classified in conservative and invasive. Among invasive therapies, endovascular surgery techniques, such as percutaneous transluminal angioplasty (PTA), gave the best results [21,22]. Nevertheless, a correct approach should aim to prevent the onset of the pathology and treat PAOD stopping its natural course in the stages which does not require any invasive treatment. That is why conservative medical therapeutic strategies are of primarily importance.

Conservative therapies aim first to correct the risk factors and to slow down the progression. The most recent guidelines [23] prescribe the suspension of smoking diet, weight loss in obese or overweight patients, treatment of hypercholesterolemia and hypertension.

Physical exercise is a part of the treatments which correct the modifiable risk factors.

The American College of Cardiology/American Heart Association recommended a LDL cholesterol level of <2.59 mmol/l (100mg /dl) in all patients with PAOD. In patients with multidistrict vascular disease the goal should be <1.81 mmol/l (<70 mg/dl) of LDL cholesterol. The target pressure should be <140/90 mmHg or <130 / 80mmhg if the patient is also diabetic or has renal failure.

In PAOD patients the anti platelet therapy is also indicated: a meta-analysis on clinical trials in which patients with PAOD were treated with ASA, or clopidogrel, ticlopidine, dipiridamole and picotamide, showed a reduction of 23% of ischemic events. There is not a clearly established efficacy for the drugs which relieve the symptom of IC. The only drugs with a demonstrated clinical evidence are cilostazol and naftidrofuryl [10,24].

Aims

The aim of the study was to investigate, among conservative therapy options, the effects of crenotherapy applied to physical exercise in subjects with Stage IIb PAOD and to compare it with a traditional treadmill training cycle. Actual Claudication Distance (ACD) and Maximum Walking Distance (MWD) were considered Primary Endpoint.

As secondary endpoint, we evaluated the impact of crenotherapy on major markers and risk factors for atherosclerotic disease as lipid, cholesterol, triglyceride, C-Reactive Protein, and total antioxidant status (TAS).

Patients and Methods

98 patients affected by IIb stage PAOD, according to Leriche-Fontaine classification, were enrolled. The study was conducted in our tertiary referral centre at the University of Naples Federico II in collaboration with

"Terme di Agnano S.p.A.". Patients were randomized into two groups: the first arm carried out an intensive vascular training program under medical supervision (group A); the second one carried out a rehabilitative exercise associated to chrenotherapy, (group B). All patients were treated with an oral dose of pentoxifilline (600mg BID) + an oral dose of ASA (100mg / day). If antiplatelet therapy was different from ASA, it was not replaced with ASA. Suspension of smoking diet was considered an integral part of treatment. Smoking diet was reported in the records of the patient. Other any medical therapies were maintained. Cilostazol was considered among the exclusion criteria, since cilostazol could not be administrated in all patients of the two groups, representing a potential bias. PAOD diagnosis was made on the basis of medical history and physical examination, ABI (ankle-brachial pressure index) and lower limb US scanning.

Were considered appropriate for the enrollment:

– all patients with ABI <0.8 at rest and a decrease of the ankle systolic pressure > 30 mmHg after 40 seconds from an exercise on the treadmill with a slope of 10% and at a speed 3.2 km / hours;

– all patients with CI stable for at least three months and where the actual Claudicatio Distance ACD, was between 100 and 200 m.

All patients were submitted to a cardiac assessment, with EKG and complete doppler echocardiography and complete biochemical assay at baseline, 3, 6 and 12 months, in order to avoid the onset of cardiovascular adverse events.

Exclusion criteria were:

– severe claudication, (less than 50 meters without pain symptoms);

– recent (<6 months) acute myocardial infarction;

– unstable angina;

– history of cerebrovascular accident;

– major surgery in the past three months;

– severe comorbidity (including neurological disorders associated with difficulty in walking, heart failure in class NYHA II-IV, important pneumopathy with dyspnea or severe arthropathy);

– inability to perform an exercise program;

– age > 80 years;

– treatment with cilostazol or with oral anticoagulants.

During the first and third week of rehabilitation program, the following parameters were detected:

– ankle brachial pressure index (ABI)

– actual claudication distance (ACD)

– maximum walking distance (MWD),

– flow mediated dilatation (FMD)

– carotid artery Intima-media Thickness (IMT)

The exercise rehabilitation sessions had the duration of an hour, at least three times a week for 12 weeks. The exercise was supervised by medical personnel. Each session was preceded by 5 minutes training on exercise bike and muscular stretching exercises, which included lifting on heels, step-up and sit to stand, each repeated 10 times. Patients in the group A walked on a treadmill at a 3,2 km/h speed for an hour without any incline. Patients in the group B walked into two 50-60 cm depth pools arranged in sequence. Both the pools had 4 steps at their extremities. The two pools were filled with different temperature salso-Bromo-iodine water, according to the Kneipp protocol. The first pool water had a temperature of 28-30°C, the second one of 24-26°C. The crenotherapy exercise has been performed in 3 sessions per week, for 3 months; every session had a duration of an hour. Patients were therefore evaluated once again by measurement of ABI, ACD, MWD and FMD after 3, 6 and 12 months [25].

At the time of the recruitment, after 3 (12 weeks of exercise), 6 and 12 months, the patients were subjected to echocardiography and routine blood sampling, (full blood count, lipid panel, glucose, liver and renal functionality test) and a not-routinary dosage of inflammatory cytokines, protein C-reactive, igf1, TNF Alpha and VGFA. Specific markers for inflammatory lipids were also evaluated: resolvine d1, leukotriene b4 and prostaglandin 2. Inflammatory biomarkers were analyzed by ELISA. "Federico II" University hospital ethical committee approved the study protocol and all patients signed a written informed consent [26].

Continuous variables are expressed as means ± standard error of the mean (SEM). Categorical variables were expressed as percentage. Normally distributed variables were compared between two groups using the two-ties, unpaired Student's t-test with the assumption of unequal variance.

Rates and proportions were compared between groups of interest using the chi-square test. Statistical analysis was performed using the SPSS version 16.0 package (SPSS Inc., Chicago).

Results

Baseline characteristics of the two groups are shown in Table I.

As regards primary endpoints ACD and MVD has been performed a comparison between the mean values obtained at baseline and after 3 months of training and 3 and 6 months after the end of that. There is a statistically significant difference between the mean values of ACD and MWD at baseline and after 3 months of training both in the crenotherapy and exercise groups ($p<0.0001$). The mean values after 3 and 9 months of follow up after the end of the training still remain statistically different with the respective baseline values; nevertheless does not exist any statistically relevant difference between the values found at the end of training

TABLE I - *Baseline characteristics of the study population*

	Crenotherapy (n=47)	Treadmill (n=45)
Age (years)	69±11	68±7
Weight (kg)	81,7 ±12	82,2 ±9,7
Winsor index	0,73±0.15	0.71 ±0,11
FMD		
ACD (m)	160±34	159±32
MWD (m)	245±69	242±44
Sex (Male %)	78	72
Triglicerids (mg/dL)	212±77	215±48
Total Cholesterol (mg/dL)	205±24	200±26
C-HDL (mg/dL)	44±844	49±5
C-LDL (mg/dL)	118±12	108±10
LDL/HDL	4,65±1,1	4,08,±0,46
Glucose (mg/dL)	97±25	96 ±18

and 3 and 9 months after, between the crenotherapy and treadmill group.

The results about the impact of crenotherapy+physical exercise and simple physical exercise on the secondary endpoints such as cholesterol, triglyceride, fasting glucose, are the following (for complete results see tables III and IV):

– During the 3 months training there is a statistically significant decreasing, referring to the baseline, in the values of Total Cholesterol both in the crenotherapy and the physical exercise group (respectively p<0.0001 and =0.024);

– Fasting glucose does not show any statistical significant changing neither in the same group nor between the two groups;

– Triglyceride have the same variations as cholesterol with values which still keep a statistical significant reduction in respect of the baseline ones after 12 months from the beginning of the study (respectively p=0,0002 <0.0001 after the training, p<0.00001 for both the groups after 12 months).

To recap, in both groups, there was a statistically significant improvement of lipidaemia compared to baseline. When compared with each other, the two groups did not show statistically significant differences, although there was a more favourable trend in the group that also performed crenotherapy.

There were no statistically significant differences neither within the groups nor between the two groups regarding the ejection fraction (EF) or the left ventricular mass and thicknesses. Even diastolic and filling pressure were not statistically significant although a positive trend for IRVT (isovolumetric relaxation time) and ventricular filling pressure index (E/E') could be observed. The results, evident after 3 months of training, lasted even after 6 months and 12 months.

Vascular reactivity study showed an evident improvement of FMD after 3 months of exercise in both groups. This improvement is also evident at 6 and 12 months. The effect was more evident in the crenotherapy group (B) where FMD values were statistically significant (p<00001, Table II) higher than the treadmill one (A). No differences were observed for carotid intima/media thickness (c-IMT).

With regard to biochemical data, patients showed an alteration of TNF-alpha, hsCRP, VEGF-A values characteristic of the inflammatory pattern described in patients with PAOD [27]. The lipid mediators that affect the 5-lipoxygenase system were unchanged neither in the two groups nor in relation to exercise. IGF-1 increases slightly in both groups, but its changes are not statistically significant. Even with regard to the VEGF-A angio-

TABLE II - *Morphological Parameters*

	Crenotherapy				Treadmill			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
FMD (%)	16.4±1.2	20.7±1*#	22.9±4*#	22.1±3*#	16.7±1.3	19.1.±1.1*	20.5±.9*	20.6±.5*
IMT (mm)	0.98±0.4	0.99±0.3	0.98±0.1	0.99±0.2	0.97±0.3	0.97±0.2	0.98±0.2	0.97±0.2
ABI	1.1±0.6	1.2±0.7	1.3±0.7	1.3±0.3	1.1±0.6	1.1±0.6	1.1±0.7	1.2±0.9
LV End-diastolic volume index (mL/m^2)	68±6	69±8	70±1	70±2	69±2	69±2	70±1	70±2
LV End-systolic volume index (mL/m^2)	25±2	25±2	24±1	23±3	24±6	24±8	24±5	25±4
LV mass index (g/m^2)	72±4	72±4	72±1	72±3	71±8	72±7	71±3	71±2
Relative wall thickness	0.38±.07	0.37±.09	0.37±.08	0.37±.07	0.37±.09	0.36±.08	0.36±.09	0.36±.08
Ejection fraction (%)	57±1	57±4	56±3	57±1	60±2	59±1	59±1	59±1
Deceleration time (ms)	190±25	151±24*	150±18*	151±14*	181±33	168±32*	164±18*	160±18*
Isovolumic relaxation time (ms)	111±8	86±3*	83±6*	84±6*	112 ±5	98 ±3	95 ±2*	90 ±3*
E/A1.8±0.3	2.2±0.5	1.9±0.5	1.9±0.6	1.9±0.3	2.1±0.3	2±0.6	1.99±0.6	
E/E'	6.9±0.5	5.1*±0.6	6*±0.4	6*±0.3	6.9±0.7	6.3±0.8	6.3±0.1	6.2±0.4
PVa (m/s) ns	0.30±.04	0.31±.03	0.31±.02	0.31±.02	0.30±.02	0.30±.05	0.30±.04	0.30±.03

*Paired two-tie Student's T Test: *P<0,001 **P<0,05 comparison with baseline within a single group*
 # comparison with the same time point of the other group

TABLE III - *Distance parameters and Lipid levels*

	Crenotherapy				Treadmill			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
ACD (m)	160±34	221±45*	228±30*	230±15*	159±32	209±33*	215±22*	227±18*
MWD (m)	245±69	359±56*	360±50*	355±35*	242±44	300±43*	320±41*	330±23*
Triglicerids (mg/dL)	212±77	160±49**	160±38**	160±23**	215±48	168±43**	165±23**	168±12**
Total Cholesterol (mg/dL)	205±24	190±22*	180±21*	179±32*	200±26	188±25*	185±15*	180±12*
C-HDL (mg/dL)	44±8	42,4±6,5**	47,4±7,7**	46,5±3,6**	49±5	45±5	47±8	47±7
C-LDL (mg/dL)	118±12	116±14**	110±13**	105±21**	118±10	124±17	120±21	115±5
Glucose (mg/dL)	97±25	97±51	98±22	96±10	96 ±18	95 ±18	93 ±12	94±6

*Paired two-tie Student's T Test: *P<0,001 **P<0,05 comparison with baseline within a single group*
 # comparison with the same time point of the other group

TABLE IV - *Inflammation markers*

	Crenotherapy				Treadmill			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
Resolvin D1 (pg/ml)	51±18	55±10	55±17	54±15	51±14	50±16	51±16	52±18
Leukotriene B4 (pg/ml)	34±16	35±17	36±15	35±16	35±18	36±16	32±19	33±18
Prostaglandin E (pg/ml)	16±5	15±4	16±3	16±2	16±5	17±5	16±3	16±3
IGF-1 (ng/ml)	91±15	100±23	101±18	97±14	96±11	105±15	100±15	100±12
VEGF-A (pg/ml)	31±7	30±7,5	31,4±7,7	31±5,6	30±6	29±5	31±7	31±7
TNF-alpha n(pg/ml)	21±11	13±8**	11±7**	10.5±8**	22±10	18±10#	14±7*#	11.5±5*
hsCRP (mg/ml)	8.7±6	6.5±4.3*	6±4.1*	5.8±3.6*	8.7 ±3.7	6.5 ±4.9#	6.8 ±5.6*	6.3±4*

*Paired two-tie Student's T Test: *P<0,001 **P<0,05 comparison with baseline within a single group*
 # comparison with the same time point of the other group

genetic factor there are no significant changes following the exercise protocol.
Regarding TNF-alpha and C reactive pro-vitamin, a 3-months and persistent 6 and 12months statistically significant reduction was observed in both groups compared to baseline. When compared with each other, the two groups showed a statistically significant 3-month difference favourable to crenotherapy group.
This difference decreases after 6 and 12 months. At these time-points, the differences between the two groups are no longer statistically significant although there is a positive trend in favour of the crenotherapy group.

Discussion

The positive effects of exercise in patients affected by PAOD are described in numerous studies [28-31].

Role of Physical Exercise

Exercise has positive effects on blood flow, muscle metabolism and also systemic effects. Among positive effects on the blood flow, there is a better redistribution of blood to the various active muscle groups, higher levels of nitric oxide, neoangiogenesis, improvement of rheology and the induction of endothelium-mediated vasodilation. The effects on muscle metabolism include, on the other hand, the increase in extractive and oxidative ability and the improvement of carnitine metabolism. Systemic effects include the reduction of chronic inflammation markers, the improvement of the walking mechanics and heart function. Controlled physical training increases the ability to perform the daily activities, a factor that could improve the life expectancy of these patients [32]. The best benefits of physical rehabilitation have been reported in programs lasting 3-6 months with at least 3 sessions weekly. The first protocols required a walking with an intensity lower than the threshold of the appearance of pain. This mode has the advantage of being well accepted, promote possible aerobic adaptations and has a low cardiovascular commitment. On the other hand, this mode does not promote tolerance to ischemic pain and should promote a reduced angiogenetic stimulus. The most recent indications suggest performing exercises at intensities which could evoke pain and, then, resting the minimum time required to allow the regression of symptom and resume the exercise. The aforementioned

mode, carried out under medical supervision and at least three times a week for a minimum 12 weeks, on treadmill or track, is adopted by the current protocols and currently registered.
International literature data refer mainly to symptomatic disease, while little is known about the possible benefits that exercise could have in asymptomatic patients. In our population, we have observed that the training program improves the lipid profile, according to previous data [33,35].
Interestingly, the beneficial effects of physical exercise have been highlighted also in the crenotherapy group, showing that this therapeutic mode, which combines the principles of physical training with the properties of thermal medicine, is not less than the already proven effective training.
Cardiovascular effects of immersion in water can be distinguished in:
– effects related to the immersion itself;
– temperature related;
– water composition related.

Immersion in thermal water at a temperature of 35 ° C induces an increase of the cardiac index with no change in heart rate and differential blood pressure. Immersion also results in a decrease in vascular resistance, a reduction in plasma viscosity and albuminemia. The mechanism of action has been attributed to a change in the breakdown of intracorporeal fluids since immersion involves mobilization of peripheral venous blood, an increase in circulating blood volume, diuresis and cardiac debt. With regard to the pathophysiology underpinning these effects, some authors [36,37] emphasized the importance of Atrial Natriuretic Factor, which was significantly increased after only 15 minutes of diving. On the other hand, Epstein [38,39] proposed other mechanisms involving the Prostaglandine and the Kinin-kallikrein system. Moreover, thermal stress results in an increase in plasma β-endorphins and ACTH levels, with increased collagen-rich tissues extensibility and a reduced viscosity of synovial fluid. In this sense, physical exercise in thermal waters is easier for elderly patients with concomitant articular pathology.
The composition of thermal waters [40] is extremely variable but there is no evidence in the literature of a close link between the chemical-physical composition of thermal waters and therapeutic effects. The above-mentioned mechanisms of action provide a theoretical presupposition for hypothesising the beneficial effects of immersion in thermal waters in venous and arterial diseases. Studies assessing the effects of immersion in thermal waters in patients with venous-lymphatic and arterial disorders, are not conclusive and require further, more extensive evaluations. However, none of the studies reported in the literature evaluates the combined effect of physical exercise and immersion in thermal waters, nor comparative studies have been conducted comparing physical exercise and physical exercise by immersion in thermal water under medical surveillance.
Crenotherapy has shown to increase the distance of ACD and MWD as well as traditional training. Interestingly, crenotherapy has shown to have an immediate (although not statistically significant compared to treadmill group) effect, which improves to 3 months and then stabilizes after 6 months while traditional training improves these two parameters progressively,
Data on inflammatory markers may help explain this effect. In our patients, there was a decrease in TNF alpha and reactive protein C. It is well known that TNF-alpha and hsCRP are both elevated in PAOD [41,42] and both physical exercise and spa therapy reduce plasma levels. The more rapid decrease of these markers observed in patients underwent spa therapy may be due to the particular chemical composition of the waters used for balneotherapy. In a number of studies, it has been shown that the use of thermal mud and bromo-iodine salt water results in a drastic decrease in hsCRP and TNF-alpha [43,27]. Of note, considering that neither the 5-lipoxygenase system, of which the resolvine D1 is a known marker, nor the leukotriene B4 underwent to substantial variations during the study, it is possible to speculate that the observed improvement was not due to classical mechanisms that attenuate inflammatory processes involved in atherosclerotic processes.
Several studies are focusing on other new potential arterial inflammation markers. The most promising of them are Glycated Albumin (GA) [44] and, in general, circulating soluble receptor for advanced glycation end products (sRAGE) [45], and Malondialdehyde-modified low-density lipoprotein (MDA-LDL) [46]. Nevertheless, despite their potential role, we choose not to analyse them for the not yet completely clarified role in pathogenesis of arterial wall inflammation and arteriosclerosis, focusing on a commonly ascertained validity inflammation cytochines panel.
IGF-1, which mediates most of GH actions [43,47] increases significantly in both groups and this effect could be explained with a moderate stimulation of the GH / IGF-1 axis by exercise. Considering the importance of this pathway in cardiovascular disease [48,49], this is an important finding that needs further investigation. Our patients showed low VEGF-A value at baseline that lasts unchanged during the study in both groups. VEGF-A is an angiogenic factor that is altered in patients with PAOD and in several studies it has been observed a significant increase in physical exercise [50-52]. Our finding could be related to the intensity of the exercise or its duration, as well as the frequency of blood sampling. With regard to ultrasound findings, although no improvement in morphology and systolic and diastolic function of the left ventricle has been observed, a positive trend was showed with respect to IVRT and left ventricular filling pressures (E/E ratio). This allows to extend positive observations even at the level of the cen-

tral cardiovascular system and, therefore, not only peripherally. FMD showed a positive trend in both groups. Interestingly, crenotherapy group showed a more effective improvement.

Conclusions

The hypothesis that exercise-associated crenotherapy may contribute to enhancing its effect, allowing a faster improvement in cardiovascular risk and symptomatology, is very promising. Further explanations can be provided by the results of levels of Cytokines and growth factors, as they could provide pathophysiological explanations about the pleiotropic effects of training and crenotherapy in association.
Our experience shows that crenotherapy have similar effects of traditional physical training in the treatment of PAOD, being equally tolerated and safe; it gives an advantage over conventional physical training in terms of ACD and MWD improvement, although not statistically significant, and is extremely welcome to patients compared to traditional physical training with the only negative consideration of the difficulty in reaching the Thermal Centres.

Acknowlegements

The authors would thank dr. Mariagrazia Arrichiello for supervising patients and clinical data collection and Dr. Antonio Della Gatta for clinical data collection and analysing.
This no-profit study has been co-financed by Italian Foundation for Scientific Research on Thermalism – FoRST.

Riassunto

Le terapie conservative per i pazienti affetti da Arteriopatia Obliterante Periferica mirano, a correggere I fattori di rischio e a rallentare la progressione della storia naturale della malattia. Tra queste, l'esercizio fisico ha effetti positive sul flusso sanguigno, il metabolismo muscolare ed altri ben dimostrati meccanismi sistemici che includono la riduzione dei markers di infiammazione cronica, il miglioramento della meccanica del cammino e la funzione cardiaca. L'esercizio fisico controllato incrementa l'abilità di svolgere le comuni attività quotidiane, migliorando l'aspettativa di vita di questi pazienti. Lo scopo di questo studio è valutare gli effetti e l'efficacia dell'esercizio fisico eseguito in acqua termale confrontato con l'esercizio tradizionale eseguito su tapis roulant.
Sono stati arruolati 98 pazienti affetti da Arteriopatia Obliterante Periferica allo stadio IIb della classificazione di Leriche-Fontaine. Dopo la randomizzazione, i pazienti sono stati divisi in due gruppi: il primo braccio ha eseguito un programma di allenamento intensive sotto supervisione medica (gruppo A); il secondo ha eseguito un allenamento riabilitativo associate a crenoterapia (gruppo B). Sono stati rilevati i seguenti parametri: Indice Caviglia/Braccio (ABI), Actual Claudication Distance (ACD), Maximum Walking Distance (MWD), Flow-Mediated Dilation (FMD) e lo Spessore Medio-Intimale (IMT). Tutti i pazienti sono stati sottoposti ad un Ecografia Color Doppler Cardiaca e un completo assessment biochimico ematico.
In entrambi i gruppi, esiste un miglioramento statisticamente significativo della lipidemia in confronto al tempo 0. Quando confrontati tra di loro, i due gruppi non dimostrano differenze statisticamente significative. Non esistono differenze statisticamente significative tra i due gruppi per quanto riguarda i reperti ecocardiografici. Lo studio di reattività vascolare ha dimostrato un miglioramento statisticamente significativo del FMD dopo 3 mesi di allenamento. Nel gruppo B i valori sono significativamente più alti rispetto a quelli ottenuti nel gruppo A. In entrambi i gruppi sono stati osservati incrementi statisticamente significativi dell'ACD.
La nostra esperienza ha dimostrato che la crenoterapia associate all'esercizio fisico ha effetti simili comparati al tradizionale allenamento riabilitativo nei pazienti affetti da Arteriopatia Obliterante Periferica, essendo egualmente ben tollerato e sicuro; la crenoterapia conferisce all'esercizio fisico un vantaggio rispetto all'esercizio tradizionale in termini di miglioramento dell'ACD e dell'MWD, sebbene non statisticamente significativo; inoltre è meglio accettato e tollerato dai pazienti rispetto all'esercizio tradizionale.

References

1. Hirsch AT, Haskal ZJ, Hertzer NR, et al.: *ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilita*tion; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; *TransAtlantic InterSociety Consensus; and Vascular Disease Foundation.* Circulation, 2006; 113:e463- 654. Review.

2. Criqui MH, Fronek A, Barrett-Connor E, Klauber MR, Gabriel S, Goodman D: *The prevalence of peripheral arterial disease in a defined population*. Circulation, 1985; 71:510-51.

3. Hiatt WR, Hoag S, Hamman RF: *Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis Valley Diabetes Study*. Circulation, 1995; 91:1472-479.

4. Selvin E, Erlinger TP: P*revalence of and risk factors for peripheral arterial disease in the United States: Results fromthe National Health and Nutrition Examination Survey,1999-2000.* Circulation, 2004; 110:738-43.

5. Meijer WT, Hoes AW, Rutgers D, et al.: *Peripheral arterial disease in the elderly*: *The Rotterdam Study. Arteriosclerosis.* Thromb Vasc Biol, 1998; 18:185-92.

6. Sigvant B, Wiberg-Hedman K, Bergqvist D, Rolandsson O, Andersson B, Persson E, Wahlberg E: *A population-based study of peripheral arterial disease prevalence with special focus on critical limb ischemia and sex differences.* J Vasc Surg, 2007; 45:1185-191.

7. Kannel WB, McGee DL: *Update on some epidemiologic features of intermittent claudication: The Framingham Study*. J Am Geriatr Soc, 1985; 33:13-8.

8. Smith GD, Shipley MJ, Rose G: *Intermittent claudication, heart disease risk factors, and mortality. The Whitehall Study*. Circulation, 1990; 82:1925-931.

9. Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV, *Prescott RJ*: *Edinburgh Artery Study: Prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population*. Int J Epidemiol, 1991; 20:384-92.

10. Norgren LL, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG; TASC II Working Group: *Inter-Society consensus for the management of peripheral arterial disease (TASC II)*. J Vasc Surg, 2007; 45 Suppl S:S5-67.

11. Price JF, Mowbray PI, Lee AJ, et al.: *Relationship between smoking and cardiovascular risk factors in the development of peripheral arterial disease and coronary artery disease: Edinburgh Artery Study*. Eur Heart J, 1999; 20:344-53.

12. Fowkes FG, Housley E, Riemersma RA, et al.: *Smoking, lipids, glucose intolerance, and blood pressure as risk factors for peripheral atherosclerosis compared with ischemic heart disease in the Edinburgh Artery Study*. Am J Epidemiol, 1992; 135:331-40.

13. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al.: *Meta-analysis: Glycosylated hemoglobin and cardiovascular disease in diabetes mellitus.* Ann Intern Med, 2004; 141: 421-31.

14. ADA: *Peripheral arterial disease in people with diabetes.* Diabetes Care, 2003; 26: 3333 341.

15. Beks PJ, Mackaay AJ, de Neeling JN, et al.: *Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn study*. Diabetologia, 1995; 38:86-96.

16. Ridker PM, Stampfer MJ, Rifai N.: *Novel risk factors for systemic atherosclerosis: A comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease.* JAMA, 2001; 285:2481-485.

17. Kannel WB, Skinner JJ Jr, Schwartz MJ, Shurtleff D, Kannel WB, Skinner JJ Jr, Schwartz MJ, Shurtleff D: *Intermittent claudication. Incidence in the Framingham Study*. Circulation, 1970; 41:875-83.

18. Graham IM, Daly LE, Refsum HM, et al.: *Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project.* JAMA, 1997; 277:1775-781.

19. Quarto G, Genovese G, Apperti M, Amato B, Benassai G, Furino E: *Is the fibrotic parietal thickening a reliable parameter for diagnosing previous asymptomatic deep vein thrombosis?* Ann Ital Chir, 2015; 86:427-31.

20. Quarto G, Benassai G, Gallinoro E, Amato B, Furino E: Is *surgery always the best treatment for severe carotid stenosis in the frail elderly?* Ann Ital Chir, 2015; 86(1):9-12; discussion 12-3. PubMed

21. Feinglass J, McCarthy WJ, Slavensky R, Manheim LM, Martin GJ: *Functional status and walking ability after lower extremity bypass grafting or angioplasty for intermittent claudication: results from a prospective outcomes study.* J Vasc Surg, 2000; 31:93-103.

22. Creasy TS. McMilan PJ, Fletcher EWL, et al.: *Is percutaneous transluminal angioplasty better than exercise for claudication? Preliminary results from a prospective randomised* trial. Eur J Vasc Surg, 1990; 4:135-140.

23. L. Anderson J, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Guyton RA, Hochman JS,.Kovacs RJ, Magnus Ohman E, Pressler, SJ Sellke FW, Win-Kuang Shen: *Management of Patients With Peripheral Artery Disease (Compilation of 2005 and 2011 ACCF/AHA Guideline Recommendations): A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines.*

24. De Backer TL, Vander Stichele RH, Van Bortel LM: *Bias in benefit–risk appraisal in older products: The case of buflomedil for intermittent claudication.* Drug Saf, 2009; 32:283-29.

25. Harris RA, Nishiyama SK, Wray DW, Richardson, RS: *Ultrasound assessment of flow-mediated dilation.* Hypertension, 2010; 55:1075-85.

26. Ferrarese A, Pozzi G, Borghi F, Pellegrino L, Di Lorenzo P, Amato B, Santangelo M, Niola M, Martino V, Capasso E: *Informed consent in robotic surgery: Quality of information and patient perception.* Open Med (Wars), 2016; 11(1):279-85. doi: 10.1515/med-2016-0054.

27. Oláh M, Koncz A, Fehér J, Kálmánczhey J, Oláh C, Balogh S, Nagy G, Bender T: *The effect of balneotherapy on C-reactive protein, serum cholesterol, triglyceride, total antioxidant status and HSP-60 levels.* Int J Biometeorol, 2010; 54:249-54.

28. Watson L, Ellis B, Leng GC: *Exercise for intermittent claudication.* Cochrane Database Syst Rev, 2008; 8; (4):CD000990.

29. Regensteiner JG: *Exercise in the treatment of claudication: Assessment and treatment of functional impairment*. Vasc Med, 1997; 2:238-42.

30. Gardner AW, Poehlman ET: *Exercise rehabilitation programs for the treatment of claudication pain: A meta-analysis.* JAMA, 1995; 274:975-80.

31. Stewart KJ, Hiatt WR, Regensteiner JG, Hirsch AT: *Exercise training for claudication.* N Engl J Med, 2002; 347:1941-951.

32. Regensteiner JG, Steiner JF, Hiatt WR: *Exercise training improves functional status in patient with peripheral arterial disease.* J Vasc Surg, 1996; 23:104-15.

33. Mika P, Wilk B, Mika A, Marchewka A, Nizankowski R: *The effect of pain-free treadmill training on fibrinogen, haematocrit, and lipid profile in patients with claudication*. Eur J Cardiovasc Prev Rehabil, 2011; 18:754-60.

34. Izquierdo-Porrera AM, Gardner AW, Powell CC, Katzel LI.: *Effects of exercise rehabilitation on cardiovascular risk factors in older patients with peripheral arterial occlusive disease*. J Vasc Surg, 2000; 31:670-77.

35. Mohammadi HR, Khoshnam E, Jahromi MK, Khoshnam MS,

Karampour: *The Effect of 12-Week of Aerobic Training on Homocysteine, Lipoprotein A and Lipid Profile Levels in Sedentary Middle-aged Men*. E Int J Prev Med, 2014; 5:1060-66.

36. Kurabayashi H, Tamura K, Tmaura J et al.: *The effects of hydraulic pressure on atrial natriuretic peptide during rehabilitative head-out water immersion*. Life Sci, 2001; 69:1017-21.

37. Suffran J, Galen FX, Habrioux G, et al.: *Le facteur natriurétique atrial: un des mécanismes d'action du bain de Phlébologie à Barbotan*. J Mal Vasc, 1991; 16:99-104.

38. Epstein M, Lifschitz M, Hoffman D, et al.: *The relationship between renal prostaglandin E and renal sodium handling during water immersion in normal man.* Circ Res, 1979; 45:71-80.

39. Fioravanti A, Cantarini L, Guidelli GM, Galeazzi M: *Mechanisms of action of spa therapies in rheumatic diseases: What scientific evidence is there?* Rheumatol Int, 2011; 31:1-8.

40. Petraccia L, Liberati G, Masciullo SG, et al.: *Water, mineral water and health*. Clin Nutr, 2006; 25:377-85.

41. Signorelli SS, Mazzarino MC, Di Pino L, Malaponte G, Porto C, Pennisi G, Marchese G, Costa MP, Digrandi D, Celotta G, Virgilio V: *High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test.* Vasc Med, 2003; 8:15-19.

42. Gherman CD, Mironiuc AI: *Evaluation of serum adipokines in peripheral arterial occlusive disease.* Mediators Inflamm, 2012; 2012:1-8.

43. Bellometti S, Tassoni T, Maidecchi A, et al.: *Chronic venous insufficiency: Influence of thermal treatment and phytotherapic substances on clinical signs and on biochemical markers of endothelial function*. Clin Term, 2006; 53(3-4):113-26.

44. Roohk HV, Zaidi AR, Patel D: *Glycated albumin (GA) and inflammation: role of GA as a potential marker of inflammation.* Inflamm Res, 2017 oct 11 [Epub ahead of print].

45. Wannamethee SG, Welsh P, Papacosta O, Ellins EA, Halcox JPJ, Whincup PH, Sattar N: *Circulating soluble receptor for advanced glycation end product: Cross-sectional associations with cardiac markers and subclinical vascular disease in older men with and without diabetes.* Atherosclerosis, 2017; 264:36-43.

46. Takamura TA, Tsuchiya T, Oda M, Watanabe M, Saito R, Sato-Ishida R, Akao H, Kaway Y, Kitayama M, Kajinami K: *Circulating malondialdehyde-modified low-density lipoprotein (MDA-LDL) as a novel predictor of clinical outcome after endovascular therapy in patients with peripheral artery disease (PAD).* Aterosclerosis, 2017; 263:192-97.

47. Arcopinto M, Salzano A, Giallauria F, Bossone E, Isgaard J, Marra AM, Bobbio E, Vriz O, Åberg DN, Masarone D, De Paulis A, Saldamarco L, Vigorito C, Formisano P, Niola M, Perticone F, Bonaduce D, Saccà L, Colao A, Cittadini A; T.O.S.CA: (*Trattamento Ormonale Scompenso Cardiaco) Investigators. Growth hormone deficiency is associated with worse cardiac function, physical performance, and outcome in chronic heart failure: Insights from the T.O.S.CA. GHD Study*. PLoS One, 2017; 12(1):e0170058. doi: 10.1371/journal.pone.0170058.

48. Marra AM, Arcopinto M, Bobbio E, Salzano A, Saccà L, Cittadini A: *An unusual case of dilated cardiomyopathy associated with partial hypopituitarism.* Intern Emerg Med, 2012; 7 (Suppl 2):85-7. Epub 2011 Jun 29.

49. Arcopinto M, Salzano A, Isgaard J, Cittadini A: *Hormone replacement therapy in heart failure*. Curr Opin Cardiol, 2015; 30(3):277-84.

50. Arcopinto M, Salzano A, Bossone E, Ferrara F, Bobbio E, Sirico D, Vriz O, De Vincentiis C, Matarazzo M, Saldamarco L, Saccà F, Napoli R, Iacoviello M, Triggiani V, Isidori AM, Vigorito C, Isgaard J, Cittadini A: *Multiple hormone deficiencies in chronic heart failure.* Int J Cardiol, 2015:184:421-23.

51. Salzano A, Marra AM, Ferrara F, Arcopinto M, Bobbio E, Valente P, Polizzi R, De Vincentiis C, Matarazzo M, Saldamarco L, Saccà F, Napoli R, Monti MG, D'Assante R, Isidori AM, Isgaard J, Ferrara N, Perrone Filardi P, Perticone F, Vigorito F, Vigorito C, Bossone E, Citadini A, on behalf of T.O.S.CA.: *Investigators: Multiple hormone deficiency syndrome in heart failure with preserved ejection fraction.* Int J Cardiol. 2016; 225:1-3. doi: 10.1016/j.ijcard.2016.09.085.

52. Vital TM, Stein AM, de Melo Coelho FG, Arantes FJ, Teodorov E, Santos-Galduróz RF: *Physical exercise and vascular endothelial growth factor (VEGF) in elderly: A systematic review.* Arch Gerontol Geriatr. 2014; 59(2):234-39.