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PERIPHERAL ARTERY DISEASE AND RESISTANCE TRAINING: IMPROVING PERFORMANCE AND HEALTH.

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Peripheral artery disease and resistance training: improving performance and health.

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SOMMARIO

L'arteriopatia periferica aterosclerotica degli arti inferiori (PAD) è una patologia che colpisce circa il 20% dei soggetti al di sopra dei 70 anni. La claudicatio intermittens (IC), che ne è il principale sintomo, è un dolore che si presenta tipicamente ai polpacci durante il cammino. Questo sintomo limita la capacità di camminare dei pazienti influendo negativamente sul loro ruolo sociale, sul livello di attività fisica, e sulla qualità di vita. La terapia principale per l'IC è l'esercizio fisico supervisionato. Tuttavia se è chiaro il ruolo che quest'ultimo svolge nell'incrementare la capacità di marcia dei pazienti, non è altrettanto chiara la tipologia ottimale di esercizio da proporre. Il trattamento abitualmente utilizzato include esclusivamente cammino ed esercizi aerobici, vi sono tuttavia le basi per suppore che i pazienti potrebbero beneficiare dell'aggiunta di esercizi anaerobici di forza al loro protocollo di allenamento.

Nel nostro studio si sono testati gli effetti di un protocollo di allenamento combinato, rispetto al protocollo standard attualmente in uso. 24 soggetti sono stati divisi in 2 gruppi ed hanno preso parte a 3 mesi di allenamento. All'inizio ed al termine dello stesso sono stati misurati: la capacità di marcia libera da dolore e totale, i principali parametri cardiovascolari durante esercizio, la forza, l'equilibrio, la variabilità del ciclo del passo e l'attività svolta quotidianamente.

I risultati ci hanno mostrato una simile capacità dei due protocolli d'incrementare la massima capacità di marcia. Abbiamo inoltre evidenziato il ruolo che le modifiche nella variabilità del ciclo del passo potrebbero avere in tale incremento. Il protocollo combinato si è dimostrato invece più efficace nell'incrementare la capacità di marcia libera da dolore. Inoltre l'allenamento combinato ha incrementato l'iperemia dell'arto in corso di attività fisica, così come ha migliorato importanti parametri di performance cardiorespiratoria (consumo di ossigeno, frequenza cardiaca, pressione arteriosa, estrazione di ossigeno). Abbiamo anche documentato la capacità dell'allenamento combinato di migliorare la forza muscolare e l'equilibrio riducendo il rischio di cadute. Abbiamo infine osservato il trasferimento degli effetti positivi nella vita quotidiana con uno stile di vita meno sedentario. L'allenamento combinato potrebbe quindi essere utilizzato nei pazienti con IC per migliorare non solo la performance ma anche la forza e l'equilibrio.

Possiamo quindi concludere che l'utilizzo del protocollo combinato, al posto del protocollo di allenamento standard, dovrebbe essere preso in considerazione nel trattamento dei pazienti con IC.

ABSTRACT

Peripheral artery disease (PAD) is a pathology of lower limbs affecting about 20% of people older than 70 years. Intermittent claudication (IC) is the most frequent symptom of PAD. IC is pain experienced during walking, typically in the calf. This limits the walking ability of patients and reduces social role, physical activity and quality of life. The main therapy for IC is supervised exercise. Whereas the primary role of supervised exercise in treatment of IC is well established there are some doubt about the best typology of exercise. Usually training includes only walking and aerobics exercises, but some elements suggested that for this patients adding strength exercises may be useful.

In our study we tested the effects of combined training against usually standard training. We divided 24 subjects in two groups and they were trained for 3 months. At the beginning and at the end of training we measured: total and pain free walking distance, main cardiovascular parameters during exercise, strength, balance, gait variability and daily physical activity.

According to our results the two different training protocols have similar power to improve total walking distance. We also underlined the role that changes in gait variability can play in this increment. Combined training is more effective in improving pain free walking distance. Moreover combined training increased limb's hyperemia during exercise, and improved important index of cardiorespiratory performance (oxygen consumption, heart rate, blood pressure, oxygen extraction). We also recorded the usefulness of combined training in improving strength and balance and lowering falls risk. We also observed the persistence of this effects in daily life, with a more active life style.

So combined protocol should be taken into account as alternative treatment for patients with IC.

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1. BACKGROUND

Peripheral arterial disease of lower extremity arteries (PAD) is a pathology included in the category of peripheral arterial disease, a range of noncoronary arterial syndromes that are caused by the altered structure and function of the arteries that supply the brain, visceral organs, and the limbs. It leads to progressive stenosis or occlusion of one or more arterial branches that supply lower extremities.
Arterial diseases include those disorders that cause either fixed obstruction or abnormal vascular reactivity of the arteries that supply a given tissue; the obstruction impairs blood delivery and can produce ischemia.
Numerous pathophysiological processes can contribute to the creation of stenosis/occlusions of the noncoronary arterial circulation, but atherosclerosis remains the most common disease process. Risk factors for atherosclerosis such as cigarette smoking, diabetes, dyslipidemia, hypertension and hyperhomocysteinemia increase the likelihood of developing lower extremity PAD[1,2].

1.1. Risk factors for peripheral arterial disease

1.1.1. Unchangeable risk factors

Race, gender, age, familiarity.

1.1.1.1. Race

The pathology has higher prevalence for Blacks (7,8%) than for Whites (4,4%), regardless of other risk factors[3].

1.1.1.2. Gender

The prevalence of PAD is slightly greater in men than women[2], the ratio of men to women is between 1:1 and 2:1.

1.1.1.3. Age

The prevalence of the pathology increases with increasing age[2]. The prevalence of asymptomatic PAD is in the range of 3% to 10% in the general population, increasing to 15% to 20% in persons over 70 years[4]. The symptomatic PAD with intermittent claudication (IC) has a prevalence of 3% at the age of 40, whereas it reaches 6% at the age of 60.

1.1.2. Changeable risk factors

Cigarette smoking, diabetes, dyslipidemia, hypertension, hyperhomocysteinemia, chronic inflammation, blood hyperviscosity and hypercoagulability.

1.1.2.1. Cigarette smoking

Cigarette smoking is an exceptionally powerful etiologic risk factor for lower extremity PAD, even more than for coronary artery disease. Large epidemiological studies have found that smoking increases the risk of lower extremity PAD by 2- to 6-fold and the risk of intermittent claudication by 3- to 10-fold[1,5,6]. More than 80% of patients with lower extremity PAD are current or former smokers[7]. The risk of lower extremity PAD increases in a powerful dose-dependent manner with the number of cigarettes smoked per day and the number of years smoked[8]. Smoking cessation is associated with a decline in the incidence of IC, the relative risk decreases from 3,7 in smokers to 3 in ex-smokers, who had discontinued smoking since less than 5 years[2].

1.1.2.2. Diabetes

Diabetes mellitus increases the risk of lower extremity PAD by 2- to 4-fold, and the risk of IC by 3,5-fold in men and 8,6-fold in women[1]. Between 12% and 20% of persons with lower extremity PAD are also diabetics[9]. The risk of developing lower extremity PAD is proportional to the severity and duration of diabetes[10], for every 1% increase in glycosylated hemoglobin there is a corresponding 26% increased risk of PAD[11]. PAD in patients with diabetes is more aggressive compared to non-diabetics, with early large vessel involvement coupled with distal symmetrical neuropathy[2]. The need for a major amputation is 7- to 15-times higher in diabetics than non-diabetics[12]. This is contributed to by sensory neuropathy and decreased resistance to infection. Very important is also the role played by insulin resistance[13], which is a risk factor for PAD even in subjects without diabetes, raising the risk approximately by 40% to 50%.

1.1.2.3. Dyslipidemia

Low-density lipoprotein (LDL) and total cholesterol levels are generally higher, and those of high-density lipoprotein (HDL) are lower, in patients with intermittent claudication than in age-matched controls. Elevated levels of triglycerides have been reported to be associated with lower extremity PAD in some studies but not in others. The risk of developing lower extremity PAD increases by approximately 5% to 10% for each 10 mg per dl rise in total cholesterol, and a fasting cholesterol level greater than 7 mmol/L (270 mg/dl) was associated with a doubling of the incidence of IC, but the ratio of total to HDL cholesterol was the best predictor of occurrence of PAD[1,2].

1.1.2.4. Hypertension

Hypertension is associated with lower extremity PAD, although the association is generally weaker than that with coronary artery disease. One study assessed that

hypertension increases the risk of IC 2,5- to 4-fold in men and women, respectively, and the risk is proportional to the severity of high blood pressure[6,1].

1.1.2.5. Hyperhomocysteinemia

Elevated levels of homocysteine (greater than 12,1 micromoles per liter) are associated with a 2- to 3-fold increased risk for developing atherosclerotic arterial disease, with an increased risk of approximately 1,5-fold for each 5 micromoles per liter increment in homocysteine level[14]. Hyperhomocysteinemia also appears to increase the risk of progression of lower extremity PAD[1].

1.1.2.6. Chronic inflammation

C-reactive protein is a serological marker of systemic inflammation. C-reactive protein concentrations in the highest quartile are associated with a 2,1-fold increased risk of developing lower extremity PAD. One study noted that C-reactive protein levels were higher in individuals who subsequently developed lower extremity PAD and highest in those who ultimately required vascular surgery[15].

1.1.2.7. Blood hyperviscosity and hypercoagulability

Raised hematocrit levels and hyperviscosity have been reported in patients with PAD, possibly as a consequence of smoking. Increased plasma levels of fibrinogen, which is also a risk factor for thrombosis, have been associated with PAD in several studies. Both hyperviscosity and hypercoagulability have also been shown to be markers or risk factors for a poor prognosis[2].

1.1.2.8. Low levels of physical activity

In healthy populations, higher physical activity levels are associated with lower all-cause and cardiovascular disease mortality[16]. Because ambulation is one of the primary physical activities performed by the elderly, it is not surprising that PAD subjects adopt a sedentary lifestyle. Indeed IC limits ambulatory ability of patients decreasing physical activity and affecting health related quality of life.

The poor prognosis of patients with PAD is further compounded by sedentary living. The mortality risk of sedentary PAD patients is higher than their more physically active counterparts[17]. Patients limited by intermittent claudication who engage in any amount of weekly physical activity beyond light intensity at baseline have a lower mortality rate than their sedentary counterparts who perform either no physical activity or only light-intensity activities. The protective effect of physical activity persists even after adjusting for other predictors of mortality.

1.2. Stages of pathology

The disease has different stages of severity and tends to progress, similarly to what happens to other diseases of atherosclerotic origin.

Few studies of the natural history of PAD have been performed to objectively quantify disease progression, claudication symptoms usually remain stable and do not worsen or improve at rapid rates. Thus, after 5 to 10 years, more than 70% of patients report either no change or improvement in their symptoms, while 20% to 30% have progressive symptoms and require intervention, and less than 10% need amputation[18].

Stages of pathology are described in the classification of Fontaine/ Rutherford (Figure1).

Pathology in the early stage is asymptomatic.

In the advanced stage, or with more important degrees of stenosis/occlusion, appears the main symptom of PAD, intermittent claudication (IC).

Claudication is defined as fatigue, discomfort, or pain that occurs in specific limb muscle groups during effort due to exercise-induced ischemia. Individuals with IC

have sufficient blood flow so that limb ischemic symptoms are absent at rest. With increased local muscular demand for metabolic support during exercise, the increase in blood flow limited by arterial occlusive lesions is inadequate to meet this demand, and limb muscular fatigue and/or pain results.

The anatomic site of the arterial stenosis is often associated with specific leg symptoms. Occlusive disease in the iliac arteries may produce hip, buttock, and thigh pain, as well as calf pain. Occlusive disease in the femoral and popliteal arteries is usually associated with calf pain. Occlusive disease in the tibial arteries may produce calf pain or, more rarely, foot pain and numbness.

Usually pain is relieved by rest within 10 minutes.

Some patients have atypical claudication symptoms, and typical claudication symptoms may not occur in patients who have co-morbidities that prevent sufficient activity to produce limb symptoms, or in patients who are so deconditioned that exercise is not performed.

When occlusion involves larger segments of the arterial tree, perfusion is progressively compromised, up to determine rest pain and subsequently ischemia with necrosis.

Fontaine

Stage	Clinical
I	Asymptomatic
IIa	Mild claudication
IIb	Moderate-severe claudication
III	Ischemic rest pain
IV	Ulceration or gangrene

Rutherford

Grade	Category	Clinical
0	0	Asymptomatic
I	1	Mild claudication
I	2	Moderate claudication
I	3	Severe claudication
II	4	Ischemic rest pain
III	5	Minor tissue loss
IV	6	Ulceration or gangrene

Figure 1: classification of disease severity according to Leriche-Fontaine's Stages and Rutherford's Categories.

1.3. Coexisting vascular disease

Because PAD is one of manifestations of atherosclerosis, it is not surprising that this disease commonly occurs together with others of same origin.

PAD is associated with coronary artery disease in 40% - 60% of patients.

The link with cerebral artery disease seems to be weaker, carotid artery disease occurs in 26% to 50% of patients with IC, but only about 5% of patients with PAD will have a history of any cerebrovascular event.

Between 23% and 43% of patients with PAD have renal artery stenosis of 50% or over[2].

As a consequence of coexisting coronary and cerebral artery disease, patients with PAD have an increased risk of myocardial infarction, ischemic stroke and vascular death.

There is a 20% to 60% increased risk for myocardial infarction and a 2- to 6-fold increased risk of death due to coronary heart disease events. The risk of stroke is increased by approximately 40%.

It is very likely that renal artery stenosis is also a partly independent risk factor for mortality in patients with PAD since renal artery stenosis of 50% or over is associated with a 3,3-fold higher mortality rate than in the general population[2].

The mortality rate of patients with PAD depends on the stage of the disease and is from 4% to 6% higher in those with more severe condition. The 1-year mortality rate in patients with critical limb ischemia is approximately 25% and may be as high as 45% in those who have undergone amputation[1].

1.4. Diagnostic methods

The vascular physical examination includes inspection of feet, evaluation of color, temperature, and integrity of the skin and intertriginous areas, and search for additional findings suggestive of severe PAD, including distal hair loss, trophic skin changes, hypertrophic nails, and the presence of ulcerations. Then blood pressure

is measured, the ankle-brachial index (ABI) is calculated and hemodynamic or imaging analysis are effectuated.

To make diagnosis of PAD numerous physiological noninvasive tests are available. These are relatively inexpensive, can be performed at no risk, and provide prognostic information. Some of these examinations permit to localize lesions to specific limb arterial segments. Moreover some second level invasive analysis such Computed Tomographic Angiography and Magnetic Resonance Angiography are available and should be effectuated in case of worsening of the disease.

1.4.1. Ankle-Brachial Index (ABI)

Measuring the pressure in the ankle arteries has become a standard part of the initial evaluation of patients with suspected PAD[19,20,21].

The ABI is a measurement that provides objective data that serve as the standard for the diagnosis of lower extremity PAD. It can be used either as a screening tool for lower extremity PAD or to monitor the efficacy of therapeutic interventions.

The ABI should become a routine measurement for asymptomatic subjects belonging to high risk groups, including all persons over the age of 70, patients aged 50–69 years who also had diabetes or a smoking history, and subjects younger than 49 years with an history of diabetes and another atherosclerotic risk factor[2].

The ABI is performed by measuring the systolic blood pressure from both brachial arteries and from both the dorsalis pedis and posterior tibial arteries after the patient has been at rest in the supine position for 10 minutes. The blood pressure ratio is calculated using the higher of the considered ankle systolic pressures (posterior tibial or dorsalis pedis) divided by the higher arm systolic pressure, left or right arm (in normal individuals, there should be a minimal interarm systolic pressure gradient, less than 12 mm Hg). Optimal recordings are obtained with blood pressure cuffs that are appropriately sized to the patient's lower calf, immediately above the ankle, and systolic pressures are recorded with a handheld 8- or 10-mHz doppler instrument[1].

Pulse wave reflection in healthy individuals causes the ankle pressure to be 10 to 15 mm Hg higher than the brachial arterial systolic pressure, and thus the normal ankle-arm brachial index systolic blood pressure ratio is **greater than 1,00** (calculated ABI values should be recorded to 2 decimal places).

The ABI provides considerable information. A reduced ABI in symptomatic patients confirms the existence of hemodynamically significant occlusive disease between the heart and the ankle, with a lower ABI indicating a greater hemodynamic severity of occlusive disease.

The threshold value of ABI to make diagnosis of PAD is ≤ 0,90 at rest.

Abnormal ABI values represent a continuous variable less than 0,90. ABI values are often considered to be mildly to moderately diminished when they are between 0,41 and 0,90 and severely decreased when less than or equal to 0,40. These relative categories have prognostic value and offers prognostic data that are useful to predict limb survival, wound healing, and patient survival.

From a systemic perspective, a reduced ABI is a potent predictor of the risk of future cardiovascular events. This risk is related to the degree of reduction of the ABI, lower ABI predicts higher risk, and is independent of other standard risk factors.

The presence of a severely decreased ABI thus identifies individuals who are at particularly high risk of subsequent development of rest pain, ischemic ulceration, or gangrene. For example, an ABI value greater than 0,50 suggests that progression to critical leg ischemia is unlikely during the subsequent 6,5 years of follow-up. In contrast, when the ABI is less than 0,40, patients are more likely to experience ischemic rest pain[22].

In patients with PAD who do not have classic claudication a reduced ABI is highly associated with reduced limb function. This is defined as reduced walking speed and a shortened walking distance during a timed 6-minute walk. The ABI can also serve as an aid in differential diagnosis, in those patients with exercise-related leg pain of non-vascular causes which have a normal ankle pressure at rest and after exercise.

In some patients with diabetes, renal insufficiency, or other diseases that cause vascular calcification, the tibial vessels at the ankle become non-compressible.

This leads to a false elevation of the ankle pressure. These patients typically have an ABI >1,40 and, in some of these patients, the doppler signal at the ankle cannot be obliterated even at cuff pressures of 300 mmHg. In these patients additional non-invasive diagnostic testing should be performed to evaluate the patient for PAD. Alternative tests include toe systolic pressures, pulse volume recordings, transcutaneous oxygen measurements or vascular imaging. When any of these tests is abnormal, a diagnosis of PAD can be reliably made.

1.4.2. Toe-Brachial Index

In individuals with noncompressible leg arterial segments, with an ABI greater than 1,3, the ABI is inadequate to make diagnosis of PAD. In such individuals, diagnostic information to establish the lower extremity PAD diagnosis can be obtained by the measurement of toe systolic pressure and calculation of the toe-brachial index, and toe-brachial index values less than 0,7 are usually considered diagnostic for lower extremity PAD. The toe pressure measurement remains a sensitive diagnostic test in such patients because digital arteries are usually spared the calcinosis that alters compressibility of more proximal arteries[23]. This test is performed by placement of a small occlusive cuff on the proximal portion of the great or second toe, with the return of toe pulsatility (which represents the systolic perfusion pressure) assessed by use of a plethysmographic detection device[1].

1.4.3. Continuous-Wave Doppler Ultrasound

Continuous-wave Doppler ultrasound is used to obtain velocity waveforms and to measure systolic blood pressure at sequential segments of the lower extremities. Use of this technique permits initial estimation of disease location and severity, follow-up of disease progression, and quantitation of the effects of revascularization therapies. One commonly used quantitative indirect measure for detection of proximal occlusive disease is the peak-to-peak pulsatility index, defined as the peak systolic velocity minus the minimum or most reversed diastolic velocity, divided by the mean blood flow velocity. Normally, the pulsatility index increases

from the more proximal to the more distal segments of the lower extremities. A decrease in the pulsatility index between adjacent proximal and distal anatomic segments implies the presence of occlusive disease between these 2 locations[24]. The degree of decline in the pulsatility index value is usually proportional to the severity of occlusive disease. However, downstream from moderate stenosis, the velocity pulse waveform may revert to a normal waveform within a short distance, depending on the severity of stenosis. This latter phenomenon of "pulse normalization" distal to some arterial stenosis is a diagnostic limitation. Thus, the presence of a high-resistance-type waveform does not provide irrefutable evidence of the absence of more proximal occlusive disease[1,25]. Doppler waveform analysis can also provide useful localizing information in patients with poorly compressible arteries and in patients with a normal resting ABI.

1.4.4. Exercise testing

Exercise testing may be extremely useful: in establishing the diagnosis of lower extremity PAD when resting measures of the ABI are normal, to objectively document the magnitude of symptom limitation in patients with lower extremity PAD and claudication, to objectively measure the functional improvement obtained in response to claudication interventions, and to individualize exercise prescriptions in patients with claudication before initiation of a formal program of exercise training.

Exercise testing for patients with lower extremity PAD and claudication should use motorized treadmills programmed to provide less intense progressive workloads than are commonly used for healthy individuals or patients with coronary heart disease. The most common testing protocol is walking on a treadmill at 3,2 km/h, 10% - 12% grade. The treadmill test should record the time of onset of leg symptoms, laterality and specific muscle groups involved, and the total walking time. During treadmill testing, the patient should be asked to indicate when any exercise-limiting symptoms occur; whether symptoms represent typical claudication, atypical limb discomfort, joint pain, or general fatigue; or if exercise is limited by chest pain or other cardiovascular symptoms.

Patients should be asked to walk to their maximally tolerated claudication symptom to most accurately define peak walking time during treadmill exercise.

Exercise should be stopped when mandated by symptoms or if objective signs of myocardial ischemia are observed. For patients who do not develop specific limb or cardiovascular symptoms, exercise may be terminated when patients achieve a high functional end point.

After completing the test, the ABI is recorded while the patient is resting in the supine position. Measurement of the ankle blood pressure and the ABI at rest and immediately after exercise yields objective data to grade the dynamic functional significance of an arterial stenosis. Walking induces profound peripheral vasodilatation and decreased leg peripheral resistance. In normal individuals, the brachial and ankle blood pressures rise together and maintain their normal relationship with exertion. In contrast, in the presence of arterial occlusive disease, an abnormal hemodynamic response results. In individuals with lower extremity PAD, despite the increased central blood pressure, maximal exercise-induced ischemic vasodilation in the claudicating limb is associated with development of a significant blood pressure gradient across the lower extremity arterial stenosis. Thus, in the individual with vasculogenic claudication, the post exercise ankle blood pressure, and usually the ABI, will fall from their baseline value. A decrease in ABI of 15% - 20% would be diagnostic of PAD[1,2].

Exercise ABI allows to individuate patients with pseudoclaudication due to other nonarterial functional limitation, that will demonstrate a normal post exercise ABI, and may be useful in establishing the diagnosis of lower extremity PAD when there is a high index of suspicion of lower extremity PAD, yet measures of the ABI at rest are normal. Both the absolute fall in post exercise ankle blood pressure and the percent fall in ABI value have been used as diagnostic criteria after exercise, with variable diagnostic thresholds[1].

A simplified form of exercise testing can utilize a pedal plantar flexion test when a treadmill is not available. In this test, patients are asked to stand flat-footed and perform 50 sequential, symptom limited ankle plantar flexions and thus raise the heels maximally off the floor. Post exercise ABI values measured with this “tip-toe” test are similar to those recorded after treadmill exercise[26].

An alternative test, which can be performed in patients with limitations to the use of treadmill testing, is corridor walking, such as that associated with the 6-minute walk test (6MWT). This test can potentially offer a more representative measure of walking ability during daily life and it is sensitive to change in walking endurance after exercise interventions[27]. Thus, a 6MWT can serve as an alternative objective method of assessing walking endurance, for example in older men and women, who can associate treadmill walking performance with significant anxiety[1].

In addition to clinical parameters, changes in the physical domains of the 36-item Short-Form Health Survey (SF-36) serve as patient-based measures of treatment effect[2].

1.5. Treatment

The treatment for PAD include interventions to reduce cardiovascular and atherosclerotic risk factors, and those to limits the specific symptom of PAD, i. e. IC. To reduce adverse cardiovascular events associated with lower extremity PAD, lifelong treatment should include modification or elimination of atherosclerotic risk factors, such as cigarette smoking, diabetes mellitus, dyslipidemia, and hypertension, and promotion of daily exercise and use of a nonatherogenic diet. Patients with claudication are physically impaired and, therefore, the specific treatment goals are to relieve symptoms, improve exercise performance and daily functional abilities.

1.5.1. Lipid lowering treatment

Treatment of dyslipidemia reduces the risk of adverse cardiovascular events in patients with atherosclerosis. In patients with coronary artery disease, cholesterol lowering therapy with statin reduces the risk of nonfatal myocardial infarction and cardiovascular death by 24% to 34%[1,28,29].

On the basis of these findings, it is recommended that patients with PAD and an LDL cholesterol level of 100 mg per dl or greater be treated with a statin. The recommended LDL cholesterol goal is less than 100 mg per dl, but when the risk is very high, an LDL cholesterol goal of less than 70 mg per dl is preferable[1]. This therapeutic option may also extend to patients with lower extremity PAD who are at very high risk and who have a baseline LDL cholesterol less than 100 mg per dl.

1.5.2. Antihypertensive treatment

For the antihypertensive therapy, which should be administered in patients with PAD, the current recommendation is a goal of 140/90 mmHg if the patient is nondiabetic, and 130/80 mmHg if the patient also has diabetes or renal insufficiency. This reduces the risk of myocardial infarction, ischemic stroke and vascular death[30,31].

Antihypertensive therapy may decrease limb perfusion pressure and potentially exacerbate symptoms of claudication or critical limb ischemia. These possibilities should be taken into consideration when administering antihypertensive drugs to patients with PAD. However, most patients are able to tolerate therapy without worsening of symptoms and should be treated appropriately to reduce the risk of adverse cardiovascular events. Beta-blockers and Angiotensin-converting enzyme inhibitors can be used, in patients with PAD, to reduce blood pressure without worsening in walking ability[1].

1.5.3. Diabetes therapies

Aggressive treatment of diabetes does decrease the risk for microvascular events such as nephropathy and retinopathy[32,33]. Therefore, it is recommended[34] that diabetic patients with lower extremity PAD be treated aggressively to reduce their glycosylated hemoglobin to less than 7%. Meticulous attention to foot care is necessary to reduce the risk of skin ulceration, necrosis, and subsequent amputation.

This includes the use of appropriate footwear to avoid pressure injury, daily inspection and cleansing by the patient, the use of moisturizing cream to prevent dryness and fissuring[1,35].

1.5.4. Smoking cessation

Individuals with lower extremity PAD who smoke cigarettes or use other forms of tobacco should be advised by each of their clinicians to stop smoking and should be offered comprehensive smoking cessation interventions, including behavior modification therapy, nicotine replacement therapy, or bupropion[1].

Observational studies have found that the risk of death, myocardial infarction, and amputation is substantially greater in those individuals with PAD who continue to smoke than in those who stop smoking[36,37].

In some but not all studies, exercise time is greater in patients who discontinue smoking than in current smokers[38,39].

Higher 1-year smoking cessation rates are achieved by physician advice coupled with frequent follow-up, approximately 5% compared with only 0,1% in those attempting to quit smoking without a physician's intervention[40], and with pharmacological interventions[41] such as nicotine replacement therapy and bupropion, respectively 16% and 30%.

1.5.5. Antiplatelet drug therapy

Antiplatelet drug therapy in patients with PAD reduces the risk of myocardial infarction, ischemic stroke and vascular death. Aspirin in daily doses between 75 and 325 mg is suggested as a safe and effective therapy, which leads to a 23% risk reduction. Higher doses of aspirin result in increased risk of gastrointestinal side effects and bleeding rates[1].

Clopidogrel, in dose 75 mg per day, is recommended as an effective alternative antiplatelet therapy to aspirin, and one study showed the better efficacy of this one

to reduce the risk of myocardial infarction, stroke, or vascular death in individuals with atherosclerotic lower extremity PAD[42].

Several studies have suggested that antiplatelet therapy, in addition to reducing the overall cardiovascular risk, may act specifically on PAD, reducing the risk of progression to arterial occlusion by 30% over a 19 month period[43].

1.5.6. Exercise and Lower Extremity PAD Rehabilitation

Initial recommended treatment for patients with IC is a program of supervised exercise. Regular walking in a supervised claudication exercise program can be expected to result in an increase in the speed, distance, and duration walked, with decreased claudication symptoms at each workload or distance. These functional benefits accrue gradually and become evident over 4 to 8 weeks and increase progressively over 12 or more weeks.

Unsupervised exercise or simple walking advice are not effective[44].

The data supporting the efficacy of supervised exercise programs to alleviate claudication symptoms are robust. Results showed that pain free walking time improved by an average of 180% and maximal walking time increased by 120% (interval between 74% and 230%) in claudication patients who underwent exercise training[45,46]. Exercise induced improvements in walking ability translate to an increase of 38% in routine daily activity, as measured in one study by accelerometry[47]. Self-reported physical activity increased by 62%, which confirms that patients themselves appreciated this functional improvement[48]. In addition to the benefits of daily exercise on limb ischemic symptoms, regular exercise is associated with improved blood pressure, an improved serum lipid profile, including increased HDL values and decreased triglyceride values, and improved glycemic control[49].

Such sustained increases in physical activity, if associated with improvements in cardiovascular risk factors, have the potential to reduce the risk of cardiovascular ischemic events, thereby potentially improving the poor prognosis for survival in this population.

Supervised exercise can induce increases in maximal walking ability that exceed those attained with drug therapies, which have been estimated to result in improvements in maximal walking distance of 20% to 25% with pentoxifylline and 40% to 60% with cilostazol[50,51].

The potential beneficial synergy of exercise training and pharmacological therapies has been incompletely evaluated. Although there are biological reasons that could support potentially more rapid or sustained improvements in pain free or maximal walking distance when patients are treated concomitantly with exercise and claudication medications, there are currently inadequate data to support any efficacy conclusion[1].

Lack of responses to exercise and/or to drug therapy, or the presence of a stenosis in a proximal position, leads to the next level of intervention, which is limb revascularization.

Data confronting revascularization procedures, or arterial bypass, and exercise did not demonstrated superiority between each other. One recent multicenter study[52] demonstrated the superiority of exercise, compared with stent placement, in increasing total walking distance but not in increasing quality of life. It is likely that supervised exercise training can serve as a beneficial adjunct to further augment the improvements in walking that can be gained by both endovascular procedures and surgical bypass[53].

The data supporting the efficacy of supervised exercise programs to alleviate claudication symptoms are robust, therefore supervised exercise should be made available as part of the initial treatment for all patients with peripheral arterial disease. Many cardiac rehabilitation exercise programs can accommodate patients with claudication, providing an environment conducive for “lifestyle change” that underlies long-term compliance to exercise and risk factors modification.

The biological mechanisms underlying such reproducible benefit are complex. However, there is inadequate evidence to attribute this functional benefit, as is often believed, to the growth of new collaterals (angiogenesis); in contrast, clinical improvement is more likely to be due to alterations in skeletal muscle metabolism, muscle hypertrophy, improvements in endothelial function, or altered gait[1].

Because patients with claudication often have concomitant clinical or occult coronary artery disease, hypertension and diabetes, adverse cardiovascular and physiological responses during exercise training are possible, and this risk should be evaluated clinically before initiation of the therapeutic program. However, there is no evidence that patients with claudication need to undergo stress imaging or invasive angiographic studies before initiating an exercise program.

Such safety has been maintained, with serious adverse events rarely documented in clinical practice or in research investigations, by prudent application of an initial standard treadmill exercise test. This test should be performed with 12-lead electrocardiographic monitoring before a therapeutic exercise program is initiated, so that ischemic symptoms, ST–T-wave changes, or arrhythmias may be identified[1]. Although these patients will, by definition, have claudication-limited exercise, and therefore will not achieve a true maximal exercise performance, the findings from the exercise test can be used to determine that there are no untoward cardiovascular responses at the exercise level reached. The exercise test also provides information about claudication thresholds and heart rate and blood pressure responses for establishing an exercise prescription.

Patient enrollment in a medically supervised exercise program with electrocardiographic, heart rate, blood pressure, and blood glucose monitoring is encouraged. It is also prudent to use monitoring routinely during the initial exercise sessions; individual clinical responses then would determine the need for monitoring in subsequent sessions.

A typical supervised exercise program requires the performance of treadmill or track-based exercise for 45 to 60 minutes performed 3 or more times a week for a minimum of 12 weeks. Optimal workload is walking to near-maximal pain.

The initial workload of the treadmill is set to a speed and grade that elicit claudication symptoms within 3 to 5 minutes. Patients are asked to continue to walk at this workload until they achieve claudication of moderate severity. This is followed by a brief period of rest to permit symptoms to resolve.

The exercise-rest-exercise cycle is repeated several times during the hour of supervision.

This cycle of exercise and rest should last at least 35 minutes at the start of the program and increase to 50 minutes as the patient becomes comfortable with the exercise sessions. Workload should be modified in subsequent weeks, with an increase in grade speed or both, to allow patients to reach an elevated pain free and total walking distance[45].

Typical benefits of such a program include an improvement in peak exercise performance higher than 100%, significant improvements in walking speed and distance noted with the Walking Impairment Questionnaire and improvements in physical function and vitality on the SF-36 (36-item short-form health survey) questionnaire.

1.6. Rational of the study

Whereas numerous studies demonstrated the key role of supervised exercise compared to other exercise modalities, the best exercise typology is not equally well defined.

Treadmill walking seems to be more effective than other exercise modalities, maybe because it reproduces walking activity performed in daily life.

Former studies demonstrated that many patients with IC also have reduced muscle mass and a lack of muscle strength and endurance[54,55], and modification in muscles fibers composition[56], which exacerbates their physical impairment[57]. They found indeed a link between muscle mass and peak VO_2.

One study[58] also hypothesized a link between loss of muscle mass and the repeated exposition to ischemia-reperfusion during walking exercise. This may therefore also have negative effects on performance ability in subjects with PAD; similar negatives effects have been found on endothelial function[59].

Resistance training, is generally recommended for most individuals with other manifestations of cardiovascular disease because of its beneficial effects on strength and endurance, cardiovascular function, metabolism, coronary risk factors, and psychosocial wellbeing[60].

In healthy adults resistance training is used as a part of a program of general fitness, and specially in elderly to prevent strength loss[61,62,63]. Subjects with PAD

have reduced balance and increased fall risk[64], therefore they can benefit of resistance training also for falls prevention.

Nowadays few studies have been effectuated about resistance training as an alternative to walking on treadmill for subjects with PAD and results are contrasting[65,66]. There do not seem to be significant differences in the effect of the two different training modalities on walking ability, both pain free and total.

Also few studies on combined training are available[67,58], and none found positive results. This is surprising because combined training include walking on treadmill whose efficacy is proven. Lack of positives results may be due in the first case to different loads between control (only walking on treadmill) and combined group, indeed in the second case walking on treadmill was stopped at the onset of claudication pain, for author's choice, and this made the different groups not fully comparable.

For this reasons we decided to test the effects, on different aspects of physical function, of an experimental protocol of combined training, maintaining the same training loads on treadmill that are used nowadays in ours rehabilitative unit for standard training, and adding resistance training only for lower limbs.

2. MATHERIALS AND METHODS

2.1. Design of the study and inclusion criteria

The study protocol consists in a non-randomized clinical trial, with 2 parallel arms (ratio1:1), with a follow-up period of di 3 months.
The aim is to evaluate the superiority of a combined training protocol, walk plus strength exercise, with respect to that actually used, only walking and aerobic exercises, in improving walking endurance.
Secondary aims are: to collect additional information about mechanisms leading to improvements in walking ability, and about effects of training, especially resistance training, on some rarely studied aspects such as gait cycle variability and local oxygen extraction.
To evaluate the effects on cardiovascular function, static and dynamic balance, fall risk and to study effects on muscular strength.
Moreover effects on lipid profile, inflammation markers, daily physical activity and perceived quality of life will be evaluated.
Subjects were divided into 2 groups, the one performing the experimental combined protocol will be henceforth named strength group, the one performing the usual standard protocol will be henceforth named standard group.

2.1.1. Inclusion criteria

- clinical diagnosis of claudication intermittent (II stage of Leriche Fontaine's classification, code ICD9 440.21) by anamnesis and visit with measurement of the Ankle Brachial Index, and diagnostic confirmation of presence of peripheral arterial disease by Eco color Doppler of lower limbs;
- age between 40 and 80 years;
- ABI between 0,5 e 0,9 in the symptomatic limb;
- optimal control of hypertension, diabetes (glycated Hb<7%), dyslipidemia if presents.

2.1.2. Exclusion criteria

- advanced diabetic microangiopathy with peripheral neuropathy;
- acute myocardial infarction/stroke in the preceding 6 months;
- surgical revascularization of the lower limbs in the preceding 6 months;
- stable/unstable angina;
- cardiac dysfunction (ejection fraction <45%);
- renal impairment (creatinine >1.5 mg/dl);
- respiratory disease reducing physical performance and exercise tolerance;
- contraindications to exercise (for example orthopedic/neurologic pathology);
- cancer in active phase;
- severe depression.

2.1.3. Criteria for ongoing exclusion

- acute myocardial infarction/stroke;
- onset of orthopedic pathology or other contraindications to exercise;
- onset of cancer in active phase.

2.2. Methods

Each patient enrolled in the study was admitted to day hospital regime for three months, at the department of UOC Angiology/Vascular Rehabilitation of the general hospital of Verona. Blood analysis were effectuated as usual at the beginning and at the end of the recovery period.

Before being considered eligible for the enrolment in the study, patients underwent a routine medical examination with ABI measure, to confirm the diagnosis of PAD and to assess the eligibility to undergo the protocol. They also performed a familiarization pre-treadmill test. At entry and after 12 weeks of training the following was done:

- measure of weight, height, skinfolds, waist and hip circumference;

- ABI measure;
- **treadmill test** with measure of:
    - heart rate;
    - oxygen consumption;
    - blood pressure;
    - local oxygen extraction;
    - gait cycle;
- common femoral artery flow volume by Doppler, before and after treadmill test;
- **six minute walk test** (**6MWT**);
- **strength test**;
- **alternate step test**;
- **unipedal stance test**;
- **timed up and go**;
- **short physical performance battery** (**SPPB**);
- SF-36 questionnaire;
- chair sit and reach test;
- daily physical activity monitoring;
- blood analysis.

2.2.1. Day 1

Measure of weight, height, circumferences, skinfolds, compiling SF-36 questionnaire, treadmill test with laboratory examinations.

2.2.1.1. Treadmill test

Treadmill test was performed following standard protocol habitually used for incoming patients in the Unit of UOC Angiology/Vascular Rehabilitation, at constant speed and slope (speed of 3,2 km/h and slope of 10%), according to guidelines. All subjects already effectuated a pre-test in our department of UOC Angiology/Vascular Rehabilitation, to evaluate the possible occurrence of cardiovascu-

lar symptoms and the ability to walk at speed and slope required by the test. For those who were not able to follow the protocol, slope and/or speed were reduced to enable them to perform it. Indeed for subjects that didn't report any symptom during pre-test, speed was increased to 4,5 km/h and slope to 12%.

During the test, patients were encouraged to walk with symptoms of claudication up to the maximum tolerable pain, they were also required to report immediately to the operator the appearance of the first symptoms, or of other inconvenience limiting performance, creating discomfort or indicating cardio-vascular fatigue for example: dyspnea, fatigue, chest or joint pain.

By treadmill test we measured pain free walking distance (PFWD), which is the distance covered before the onset of claudication symptoms, and total walking distance (TWD), which is the total covered distance (for subjects that didn't report pain PFWD were equal to TWD). Both measures are reported in meters and calculated from treadmill speed and time reported by the operator.

During the test the evaluated parameters were constantly monitored.

Basal values were calculated as mean of values of at least 30 seconds before the start of the test.

Systolic and diastolic blood pressure were measured by Portapress®, and from the track provided by the instrument values at PFWD, which are the means of measures made in the 30 seconds before reaching the PFWD, and values at TWD, which are the means of measures made in the 30 seconds before the end of the test, were calculated.

VO_2 and respiratory quotient (R quotient VCO_2/VO_2, it indicates the energy source mainly used, with values around 1 indicating carbohydrates and values around 0,7 indicating fatty acids), the instrument also detects heart rate by the signal of the heart rate monitor. Values of these parameters at PFWD and TWD were calculated as mean of at least 15 breaths/beats preceding respective measures.

Levels of oxygenated, deoxygenated, total and percentage of oxygenated hemoglobin were recorded by NIRS in both calves. Values at TWD were calculated as mean of at least 30 seconds before the end of the test.

The length of different phases of gait cycle was calculated by Optogait® over all the treadmill test.

Stride time is the duration of the whole gait cycle, it starts with the first contact of the heel of one foot with the floor and it ends with the subsequent contact of the same foot at the end of the gait cycle. Stance is the first phase of gait cycle, it includes the whole contact phase from the heel to the tip, swing is the second phase, during this phase the foot is raised and it is moving in the air. Both measures can be expressed as absolute values or as percentage of stride time.

Usually the stance phase occupies about 60% and the swing phase 40% of stride time. As measure of gait, the variability coefficient of variation (CV) expressed as a percentage [(standard deviation/mean)*100] was calculated. Initial values were calculated as mean of at least 30 steps within those effectuated in the first minute of walk, final values were calculated as mean of at least 30 steps within those effectuated in the last minute of walk.

Before starting and immediately after the test, flow volume of common femoral artery was measured bilaterally by Doppler.

The measure was performed with the subject in the supine position, after a rest period of 5 minutes for the initial assessment, at the level of the common femoral artery of each leg. The instrument automatically calculates diameter, speed and flow volume. Post exercise measures were performed immediately after the test end. We calculated the differences between post treadmill and basal values.

2.2.1.2. Plicometry

Thickness of subscapular, tricep, chest, axilla, suprailiac, abdominal, and thigh skinfolds was measured by a plicometer. Body density and percentage of fat mass (%FM) were evaluated by the equation of Jakson and Pollock on the basis of 7 skinfolds, the equations are different for men and women.

Body density for men = 1.112 – (0.00043499 * sum of 7 skinfolds) + (0.00000055 * (square of the sum of 7 skinfolds)) – (0.00028826 * age).

Body density for women = 1.097 – (0.00046971 * sum of 7 skinfolds) + (0.00000056 * (square of the sum of 7 skinfolds)) – (0.00012828 * age).

%FM = (495/Body density) – 450.

2.2.1.3. Waist/hip ratio and body mass index

Body mass index (BMI) is calculated by dividing the individual's weight in kilograms by height in meters squared. It is widely accepted that being overweight, traditionally defined as having a BMI >25 kg/m^2, is a major risk factor for a wide range of chronic diseases and injuries including cardiovascular disease, type II diabetes, and certain site-specific cancers[68]. A reductions in life expectancy by 3 years in individuals with moderate obesity (BMI 30–35 kg/m^2), and up to 10 years in individuals with extreme obesity (BMI 40–50 kg/m^2) is estimated[69], the latter being equivalent to the years lost by lifetime smoking.

Waist/hip ratio (WHR) is calculated by dividing the waist circumference by that of the hip. Abdominal obesity assessed by this index is related to increased risk of all-cause mortality throughout the range of body mass index[70]. The waist/hip ratio seems to be better than BMI in predicting cardiovascular risk. Cut off value of WHR is 0,8 for women and 0,9 for men.

2.2.2. Day 2

Six minute walk test (6MWT), test included in short physical performance battery (SPPB), alternate step test, timed up and go, balance on force plates, joint mobility.

2.2.2.1. Six minute walk test (6MWT)

By six-minute walk test we measured pain free walking distance (PFWD) and total walking distance (TWD).

This test is easy to perform and does not require special equipment. It requires a stopwatch, a measurement tape, and cones to mark the path. The subjects has to cover back and forth, as many times as possible, the 30 meter corridor in the 6 minutes allowed. At the end of the six minutes, the distance covered in meters is measured. Moreover, patients with PAD era asked to report the onset of pain to measure the pain free walking distance. During the test the subjects walks at the

self-selected speed, nonetheless, before starting participants are advised that the goal of the 6MWT is to achieve the greatest distance possible, and therefore they should walk as fast as possible. Participants are allowed to rest during the test, but the clock continues to run while the participant rests. If the patient usually uses any gait aid during ambulation, such for example a cane, he can use it also during the test.

6MWT provides measures similar to those obtained during the treadmill tests, however, we decided to do this additional evaluation because walking on a treadmill requires dynamic balance and the ability to maintain a constant rhythmic gait in order to keep up with the treadmill's constant pace. Patients with PAD have specific impairments in balance and cognitive function that are likely to make the need for good balance and a rhythmic gait on the treadmill particularly difficult to achieve, therefore they frequently touch or hold on to the treadmill rail to maintain balance. Importantly, these skills are intrinsically linked to treadmill walking but do not apply to walking in daily life. Furthermore, among patients with PAD, handrail support is associated with a greater learning effect and longer maximal walking distances, compared to no handrail support[71].

In a corridor walk, such as the six-minute walk test, PAD patients who have difficulty walking can slow down, but keep going or even rest temporarily without stopping the test. In contrast, the treadmill test requires PAD participants to maintain or even increase their walking speed. PAD participants who cannot keep up with the treadmill must stop walking, thereby simultaneously ending the treadmill test. This is likely to result in longer walking distances in the six-minute walk than on the treadmill.

Another limitation of treadmill walking is that it is associated with a significant learning effect. Even without any therapeutic intervention, patients with PAD typically increase their maximum and pain-free walking distance between baseline and follow-up testing.

The extent of the improvement only due to learning effect has been estimated[71] between 17% and 26%. In contrast there aren't learning effects by repeating the 6MWT.

Among patients with PAD, the 6MWT detects and quantifies improvements due to a training cycle, or declines in walking endurance due to the worsening of the disease. Also a baseline 6MWT predicts rates of all-cause mortality, cardiovascular disease mortality, and mobility loss. Some studies, effectuated on both healthy subjects or patients, demonstrated the clinical meaningfulness of changes in 6MWT. A small meaningful change was defined as a change over 20 meters and a large meaningful change in 6MWT was defined as a change of 50 meters[71].

Furthermore, during treadmill test performed for our protocol, the subject would have been linked to numerous monitoring devices, which could accentuate the discomfort of the test.

For these reasons, we decided to assume results of 6MWT as reference to evaluate the effects of our training protocol, besides the traditional treadmill evaluation.

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2.2.2.2. Timed up and go

Timed up and go is a test of physical function which correlates well with activities in daily life[72] and is often used in elderly to evaluate fall risk. The test consists in rising from an arm chair, walk 3 meters, turn, walk back, and sit down again. Time employed from the "start" to when the subject is again properly seated, with his back against the chair, is timed. The patient may use any gait aid that he normally use during ambulation.

In addition to the time spent, we also record whether the subject has uncertainties in gait, loses his balance, makes short steps, holds on to the wall, doesn't move his arms, turns with a jerky motion, or doesn't use the aids correctly. The subject should be given a practice trial that is not timed before testing. The reference values for the time spent in test execution vary according to age, for the range 60-69 years they are comprised between 7,1 and 9 seconds with mean 8,1 seconds.

2.2.2.3. Alternate step test

Alternate step test is a test of dynamic balance which involves weight shifting and provides a measure of lateral stability. This test involves alternatively placing the entire left and right feet (shoes removed) as fast as possible onto a step that is 18 cm high and 40 cm deep. The time taken to complete eight steps, alternating between the left and right feet represents the test measure. Results obtained in this test were demonstrated to be significantly worse in patients who subsequent fell[73]. The cut off value between fall risk and normal subjects is 10 seconds.

2.2.2.4. Protocols of test on force plates

Static balance test was performed on force plates. It supplies additional information on displacements of the center of pressure (CoP).

2.2.2.4.1. Romberg test

This test includes 4 tasks with increasing level of difficulty.
The first level requires subject standing in an upright position with feet closed and eyes open for 10 seconds without swaying while holding both arms extended to the front with palms facing upward.
The second level requires subject standing in the same position, but with eyes closed.
The third level requires eyes open and feet in tandem stand, the tip of one foot in contact with the heel of the other.
The forth level is equal to the third but with eyes closed.
It is observed whether the subject is able to maintain the position for 10 seconds without moving his feet, lowering his arms or opening his eyes in the levels that provide eyes closed.

2.2.2.4.2. Unipedal stance test

Unipedal stance test is a test of unipedal balance. Subject were asked to maintain unipedal stance for as long as possible, they chose the leg they preferred for the test. The tip of the raised foot is about at the height of the contralateral ankle. The test is interrupted if the patient shifts the stance foot, places the lifted foot on the floor or moves the arms. The test was stopped and considered normal if it reached 45 seconds. Subjects were given three trials, and the longest one was considered, unless they achieved 45 seconds on the first or second trial. Results shorter than 30 seconds were demonstrated to be linked with an elevated fall risk, whereas those longer than 30 seconds were associated with low risk[74].

2.2.2.5. SPPB (Short Physical Performance Battery)

The SPPB scale is a short battery of tests established to evaluate the functionality of the lower limbs.

It consists in three sections: balance, walking speed and dynamic balance/strength of the lower limbs.

- The assessment of the balance is composed of three tests:
  - standing in an upright position with feet closed for 10 seconds;
  - standing in an upright position with feet in semi-tandem position, the big toe of one foot beside the heel of the other, for 10 seconds;
  - standing in an upright position with feet in tandem position, the tip of one foot in contact with the heel of the other, always for 10 seconds.

The score of this section goes from a minimum of 0, if the patient is not able to stand with feet closed for at least 10 seconds, to a maximum of 4, if he can successfully complete the three tests.

- The second test is designed to assess the walking speed on 4 linear meters.

The score of this section varies, on the basis of the time realized, from 0 if unable, to 1 if the performance lasts longer than 8,7 seconds, to a maximum of 4 if he can perform the task in less than 4,8 seconds.

- The third section evaluates the time taken to perform 5 times consecutively the sit to stand from a chair, without using the upper limbs, which must be crossed on the chest.

Also in this case the score goes from 0 if unable, or if the performance lasts longer than 60 seconds, to a maximum of 4 if it lasts less than 11,2 seconds.
The total score of the scale has therefore a range from 0 to 12.

2.2.2.6. Chair sit and reach

As a measure of joint mobility we utilized the chair sit and reach (CSR), a modified version of the sit and reach test. During the test the subject sits on the edge of a chair (placed against a wall for safety). One foot must remain flat on the floor. The other leg is extended forward with the knee straight, heel on the floor, and ankle bent at 90°. With one hand placed on top of the other with tips of the middle fingers even, the subject tries to reach forward toward the toes by bending at the hip, keeping the back and the knee straight and head up. The distance is measured between the tip of the fingertips and the toes. If the fingertips do not touch the score is negative, if they touch the toes the score is zero, if they overlap the score is positive. This test in older adults produces reasonably accurate and stable measures of hamstring and lower back flexibility. In addition, it appears to be safer and more comfortable to perform compared to the traditional floor sit-and-reach tests[75].

2.2.3. Day 3

2.2.3.1. Strength test

Strength was measured with the exercises that later would been part of the training protocol: **leg press**, **leg extension**, **leg curl**, **hip abduction**, **hip adduction**, and **calf press**.

Maximal dynamic strength (1-RM) was evaluated by indirect estimation and calculated by the formula of Brzycki on the basis of 10 repetitions[76].

During the test the subject performed for each exercise two series of warming and familiarization with a light weight. After a complete recovery we proceed with the test series, up to find out the load with which the subject was not able to make a number of repetitions exceeding 10. Between the series a complete recovery time of 3 minutes was observed. The same delay was provided between different exercises.

The most reliable method to evaluate the maximal dynamic strength of a muscle, or a group of muscles, is the test of 1 maximal repetition (1-RM). However untrained subjects may not be able to reach their 1-RM, therefore this test can be contraindicated for those who have no previous experience of weight lifting. Therefore in clinical practice, when performing tests of muscle strength in patients with low fitness condition, methods that indirectly estimate the maximal are preferable to 1-RM test.

Procedures for the estimation of 1-RM are reported in the literature, and their validity has been demonstrated for exercises such as squat, triceps press, bicep curl, leg press, hip flexion, hip extension, hip abduction, hip adduction, plantar flexion, and dorsiflexion[77]. There are various formulas for the estimation of 1-RM, among these the equation of Brzycki, which is commonly used for the estimation of 1-RM by a submaximal exercise of repetition (5-8 RM test), was demonstrated[78] to be the most reliable for the evaluation of 1-RM in the bench press exercise (r= 0,98), if the maximal number of repetitions during the test doesn't exceeds 10. This formula demonstrated to be reliable even if used in untrained or diseased subjects[77]. The equation used in the calculation is as follows: 1RM = W/[102.78–2.78(R)]/100 with W = weight lifted and R = number of repetitions effectuated.

2.2.4. Measure of daily activity

Patients wore an Armband® bracelet for a week. The device was placed according to the manufacturer instructions on the left triceps. This device measures biaxial acceleration, temperature and impedance, and through a series of algorithms it is

able to calculate the duration of daily physical activity, number of steps and to estimate the energy expenditure. This device was demonstrated to be reliable in assessing these data for low intensity activities of daily life[79].

2.2.5. Training protocols

2.2.5.1. Training protocol of the strength group

Training was performed 3 times a week, with sessions lasting 1 hour and 30 minutes, subdivided into about 30 minutes of walking on the treadmill and 1 hour of exercises with strength machines. We utilized 6 training machines: leg extension, leg curl, hip abduction, hip abduction, leg press, leg press for calves. For each exercise 3 sets of 10 repetitions were performed, with load of 70% of 1-RM. The load was monitored weekly and adjusted when the subject was able to perform more than 12 reps with the previous load. Between the series, and between the different exercises, a recovery period of 2 minutes was observed. The workout on the treadmill required walking until reaching a moderate pain, followed by rest until the pain resolution. The cycle was repeated 2-3 times. Workouts with machines and with treadmill were alternated, always respecting recovery periods.

2.2.5.2. Training protocol of the standard group

The workout on the treadmill required walking until reaching a moderate pain, followed by rest until the pain resolution. The cycle was repeated 2-3 times, as for the strength group. Also 40 minutes of light free body gymnastics and 20 minutes of winding-down cycling were performed.

2.3. Materials

The equipment available at the our Division and at the laboratories of Sports Science Faculty includes:

- Color Doppler Ultrasound GE Healthcare, model Vivid 7 Pro, with probes 10L, M4S, 4C, that we use to perform cardiac Doppler ultrasound examinations and those of the vessels of the lower limbs and carotids;
- Color Doppler Ultrasound Acuson, Sequoia 512 (Imagegate), with probes 3V2c, 8L5, 15L8w, that we use to perform ecocolordoppler of the vessels of the lower limbs and carotids;
- Doppler Basic S, Basicare 120032, that we use to measure ABI;
- Portapres Model-2 with data analysis software BeatScope 1.1 0344 CE Finapres Medical Systems Enschede, PH, Netherlands, to monitor beats to beats finger blood pressure;
- Quark PFT 0476 CE Cosmed Rome, RM, Italy, to monitor breath to breath respiratory exanges;
- Oxiplex ISS Near Infrared Tissue Oximeter Model 95306 with software OxiTS 3.1 for measuring the concentration of oxygenated/deoxygenated hemoglobin in the peripheral circulation;
- Plicometer GIMA, MI, Italy, for plicometry;
- Tredmill Runrace Technogym 770 CE to effettuate test and training;
- Macchines for strength training;
- Armband SenseWear Pro-3 version 6.1, BodyMedia, Pittsburgh, PA, USA.

2.3.1. Portapress

Portapress is a device which is able to monitor, beat to beat, the pressure wave form, and to derive systolic and diastolic pressure values, heart rate and to calculate other parameters, such as total peripheral resistances and cardiac output.
By an inflatable finger cuff, which comprises an infrared plethysmograph, appropriately sized to the patient's finger and placed around the medial phalanx of the index or the middle finger, the device measures blood pressure variations. The de-

vice automatically calibrates itself in about 1 minute at the beginnings of registration, and recalibrate, if necessary, during the test for few seconds, at approximately one-minute intervals. Blood pressure measured in fingers and in central arteries have different values, differences by -0,8 ± 11,7 mmHg for systolic pressure and by -1,6 ± 7,7 mmHg for diastolic pressure are estimated[80].

2.3.2. NIRS

NIRS is a device consisting of two pulsed light sources and two detectors avalanche photodiode. It uses intensity-modulated light at a frequency of 1 MHz and laser diodes at three wavelengths (905, 850 and 770 nm) corresponding to high absorption of oxyhemoglobin and deoxyhemoglobin. When near-infrared light propagates through biological tissues, it is partly absorbed or scattered by the tissues and partly recollected by the detector. The intensity of the recollected light provides information about oxyhemoglobin and deoxyhemoglobin concentrations. The device was switched on at least 30 minutes before the beginnings of the test and properly calibrated through a known absorption block. During the test one probe for each leg was secured to the medial side of the calf by fabric bands, and suitably covered with bandages to isolate it from the ambient light. In addition to the oxygenated and deoxygenated hemoglobin the instrument automatically calculates the total hemoglobin, which is the sum of the two, and the percentage of oxygenated hemoglobin.

2.3.3. Quark

Quark is a device that analyzes oxygen consumption and ventilation, through an appropriate size mask connected to a turbine and a sensor. The device was switched on at least 30 minutes before the beginnings of the test and properly calibrated through a gas sample of known concentration and the use of a three liter syringe.

2.3.4. Optogait

Optogait is an optical detection system consisting of two bars, a transmitter and a receiver one. The bars communicate between them via infrared light, through 96 LEDs. Interruption of the contact between the bars are detected, and allow to calculate the contact and the flight time during walking, with a time resolution of one thousandth of a second and a spatial resolution of one centimeter. The instrument is set with parameters of treadmill's speed and length of the subject's foot and doesn't require calibrations.

2.4. Statistical analysis

For statistical analysis we utilized SPSS Software version16.0 for Windows.
We performed analysis per protocol, so the 2 subjects who didn't completed the trial were excluded from the analysis. To exclude differences in baseline data we performed Student's t-tests for independent samples, or the Wilcoxon test, according to the type of data to analyze. We used Fischer's exact test to assess differences in the presence of diseases and in the number of smokers. Differences between groups pre – post training were analyzed by ANOVA test, Wilcoxon's test for nonparametric data or Student's t-test for paired data.
The differences are significant if $p \leq 0,05$.

3. RESULTS

3.1. Characterization of the patients

24 patient affected by peripheral arterial disease satisfying inclusion criteria were enrolled into the study: 12 subjects were assigned to the standard group, and 12 to the strength group. In the baseline condition, the patients of the two groups differed by age, however there were no differences in the presence of various diseases, the number of smokers, in walking ability, and in none of the studied parameters. 2 subjects of the strength group were excluded during the study, respectively for non-compliance and for an incoming disease that prevented the workout.

	Patients strength group n= 12	Patients standard group n= 12
Lost at follow-up	2	0
Men/Women	11/1	11/1
Age	49 – 74yo	59 – 77yo
Height	172 ± 6cm	168 ± 6cm
Smokers	3	2
Ex-smokers	5	9
Diabetes	4	8
Hypertension	6	10
Dyslipidemia	2	4

Table 1: baseline conditions of patients.

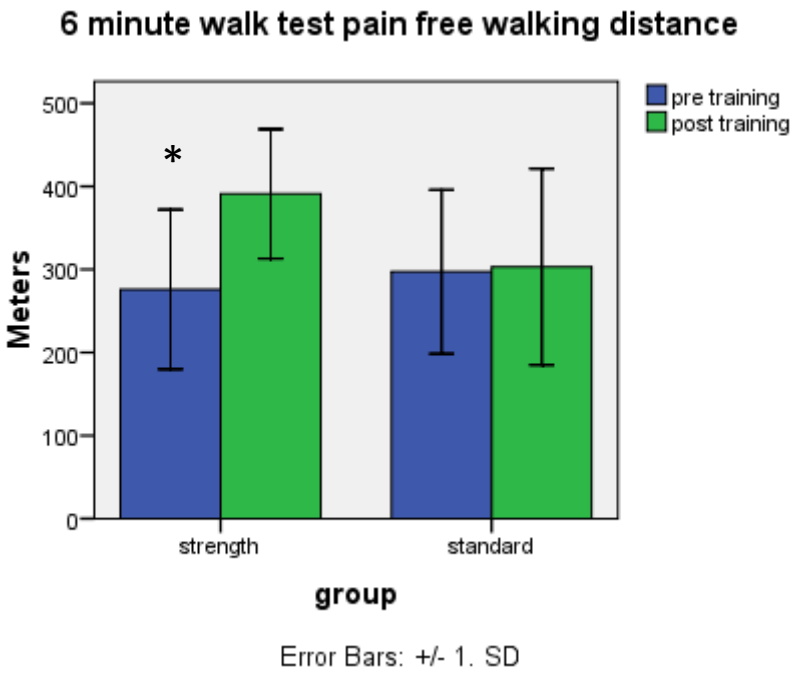
Variations after 36 training sessions:

	Forza		Standard	
	pre training	post training	pre training	post training
Weight	81 ± 9kg	80 ± 8kg*	85 ± 20kg	85 ± 20kg
BMI	28 ± 4kg/cm²	27 ± 3kg/cm²	30 ± 6kg/cm²	30 ± 6kg/cm²
%FM	35 ± 8%	33 ± 9*%	33 ± 12%	35 ± 10%
WHR	1,0 ± 0,04	0,9 ± 0,06	1,0 ± 0,06	1,0 ± 0,04
CSR	-9 ± 8cm	-5 ± 10cm*	-16 ± 9cm	-14 ± 11cm

Table 2: body composition and joint mobility * difference pre-post training p<0,05.

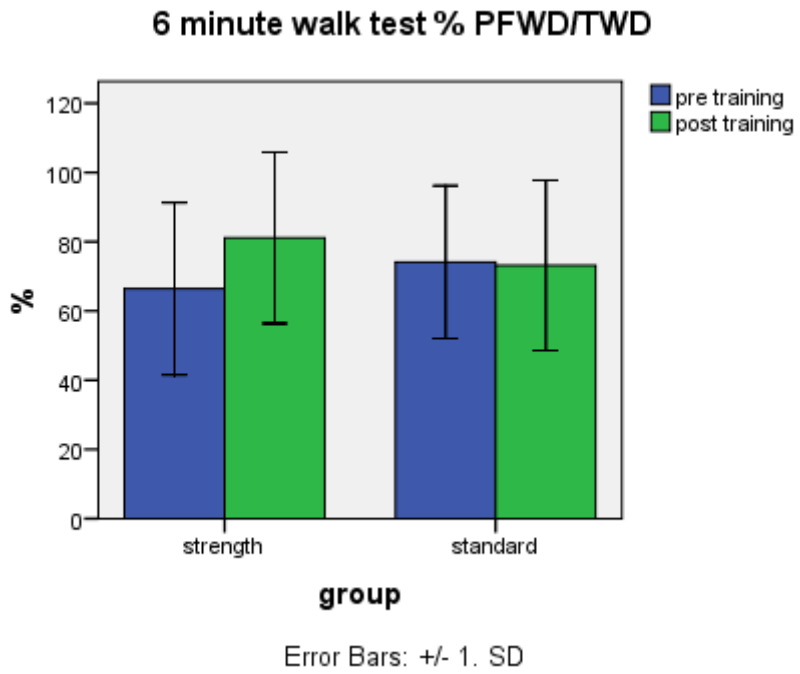
3.2. Data about walking

3.2.1. Data of 6 minute walk test



Graph 1: mean of pain free walking distance as measured by 6MWT, * between groups interaction p<0,05.

Graph 2: mean of total walking distance as measured by 6MWT, # p<0,05 for time factor.



Graph 3: mean of percentage of pain free on total walking distance during 6MWT.

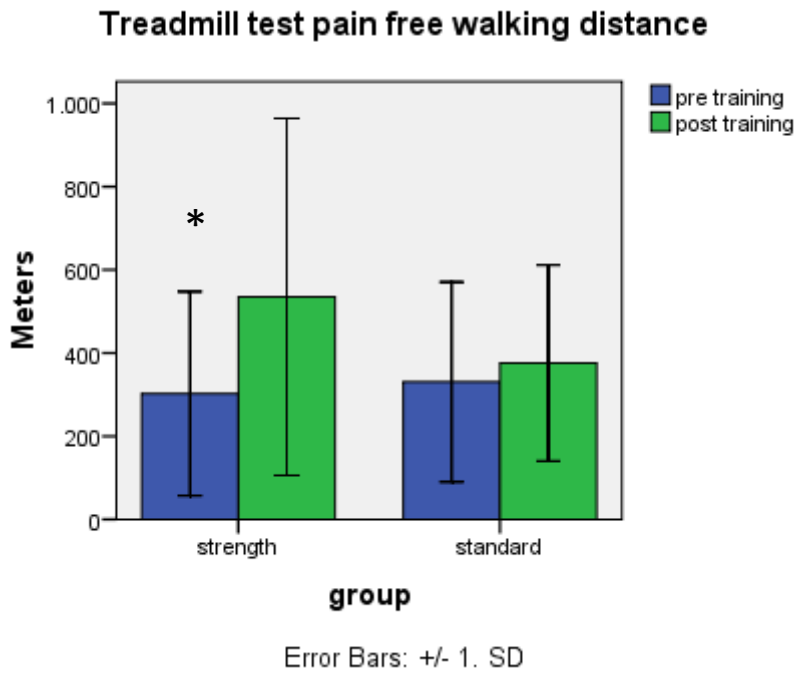
The mean of the values of pain free walking distance, measured by 6MWT, significantly increased in the strength group from 276 ± 96 meters to 390 ± 77 me-

ters, whereas in the standard group it increased from 297 ± 99 meters to 303 ± 118 meters.

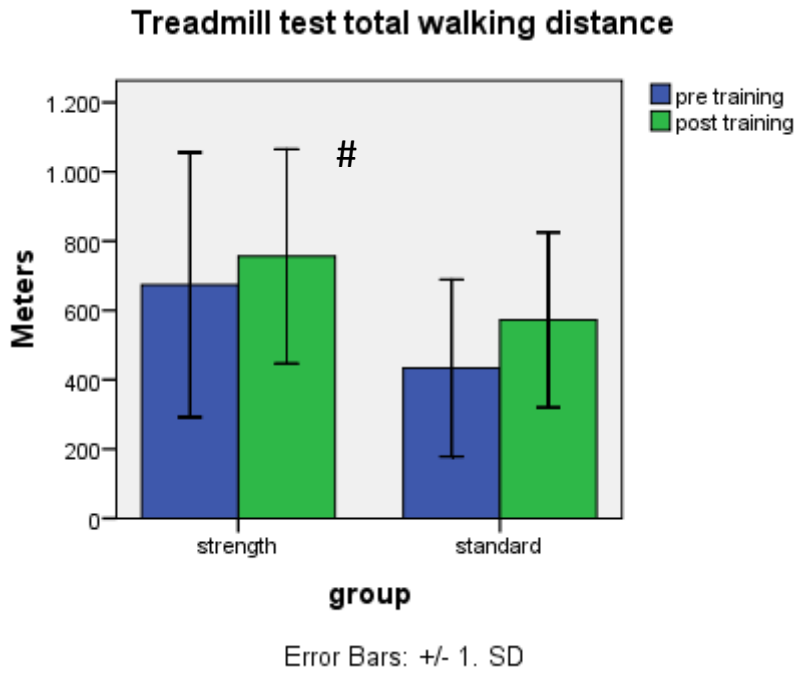
The mean of the values of total walking distance significantly increased in time, from 436 ± 68 meters to 465 ± 62 meters in the strength group, and from 399 ± 60 meters to 420 ± 71 meters in the standard group.

The mean of percentage of pain free on total walking distance increased in the strength group from 66 ± 25 % to 81 ± 25 %, whereas in the standard group it decreased from 74 ± 22 % to 73 ± 25 %.

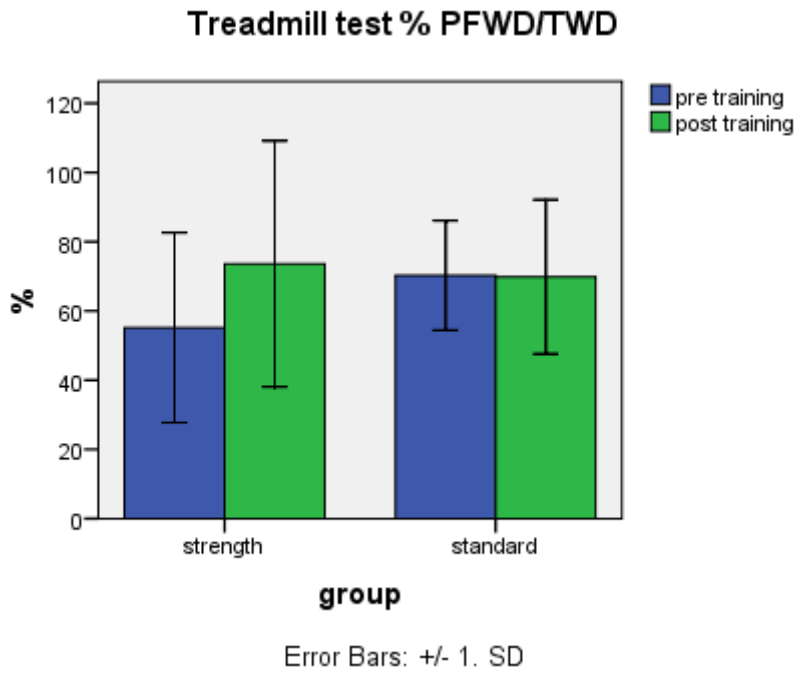
3.2.2. Data of treadmill test



Graph 4: mean of pain free walking distance as measured by treadmill test, * between groups interaction p<0,05.



Graph 5: mean of total walking distance as measured by treadmill test, # p<0,05 for time factor.



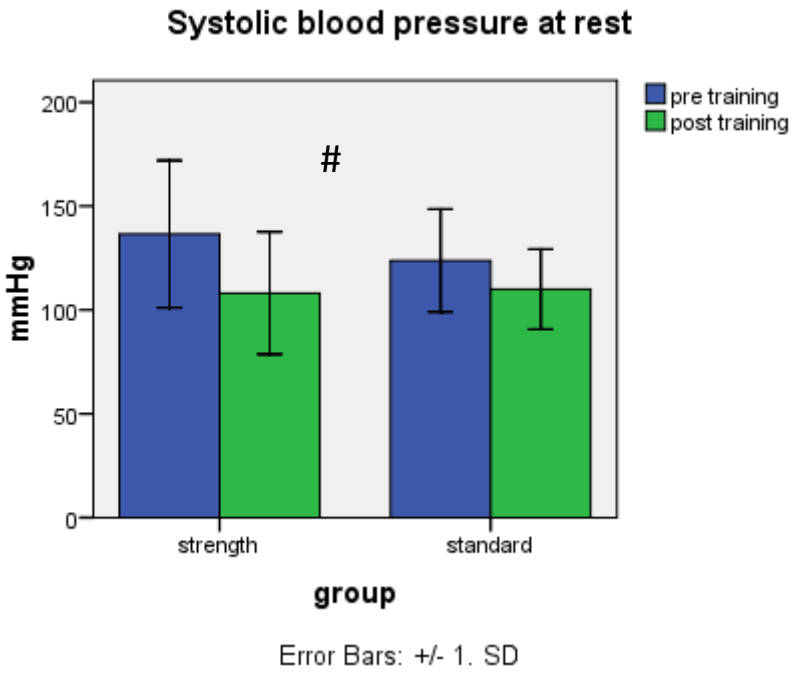
Graph 6: mean of percentage of pain free on total walking distance during treadmill test.

The mean of the values of pain free walking distance, measured by treadmill test, significantly increased in the strength group from 302 ± 245 meters to 535 ± 429 meters, whereas in the standard group it increased from 330 ± 240 meters to 376 ± 236 meters.

The mean of the values of total walking distance significantly increased in time, from 674 ± 382 meters to 756 ± 309 meters in the strength group, and from 434 ± 255 meters to 573 ± 252 meters in the standard group.

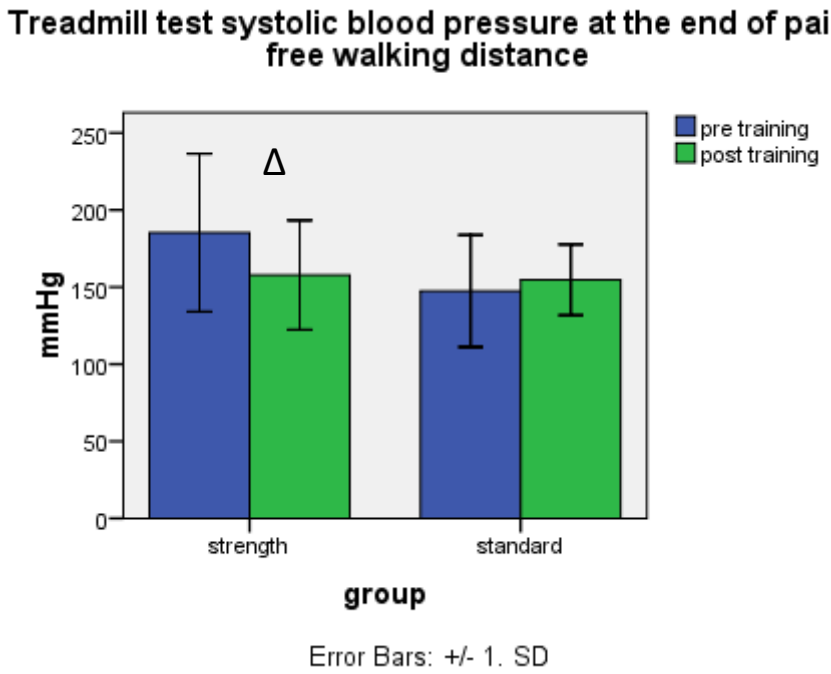
The mean of percentage of pain free on total walking distance increased in the strength group from 55 ± 27 % to 74 ± 36 %, whereas in the standard group it remained unchanged from 70 ± 16 % to 70 ± 22 %.

3.3. Data about blood pressure

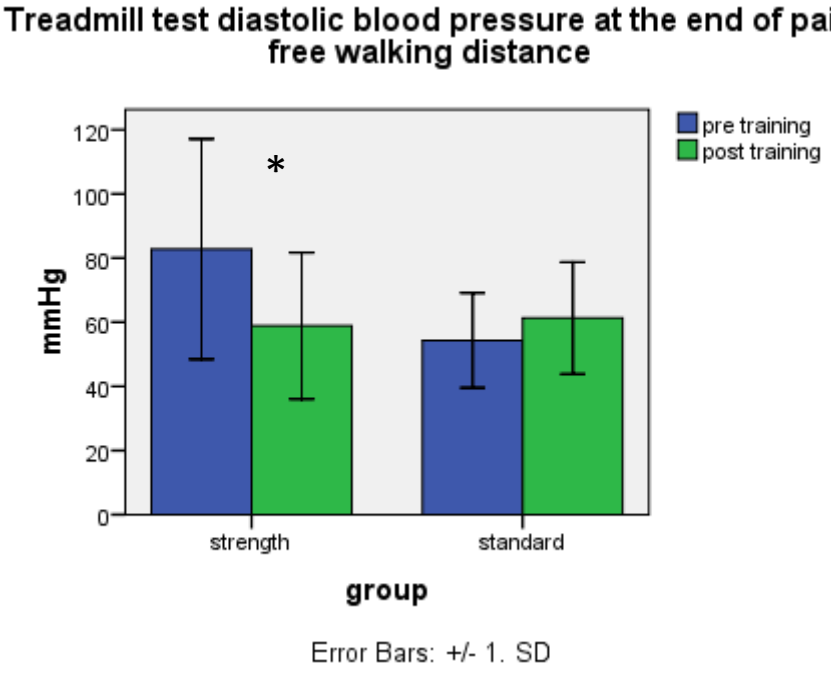


Graph 7: mean of systolic blood pressure at rest, # p<0,05 for time factor.

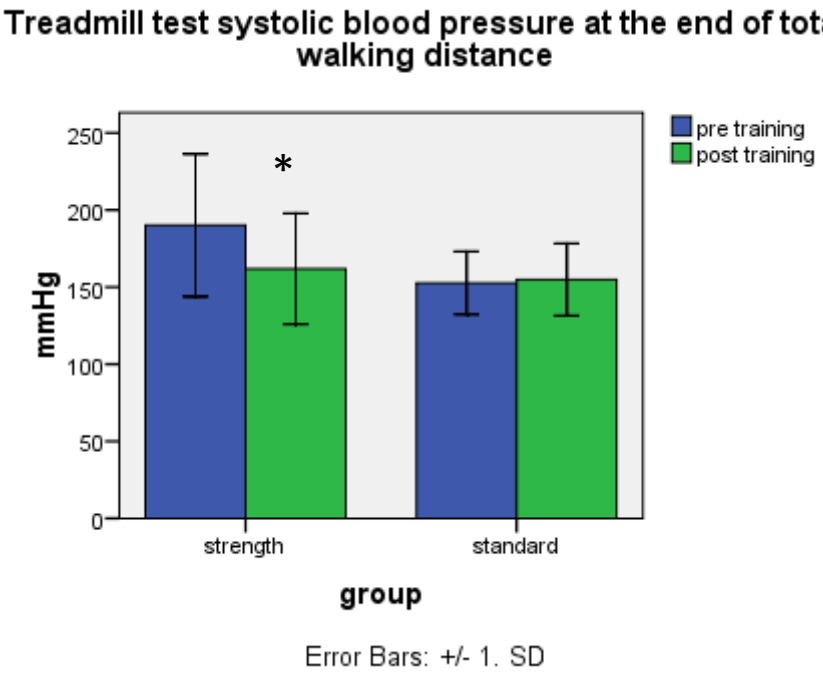
The mean of the values of systolic blood pressure at rest significantly decreased in time, from 137 ± 33 to 114 ± 32 mmHg in the strength group, and from 128 ± 26 to 112 ± 21 mmHg in the standard group.



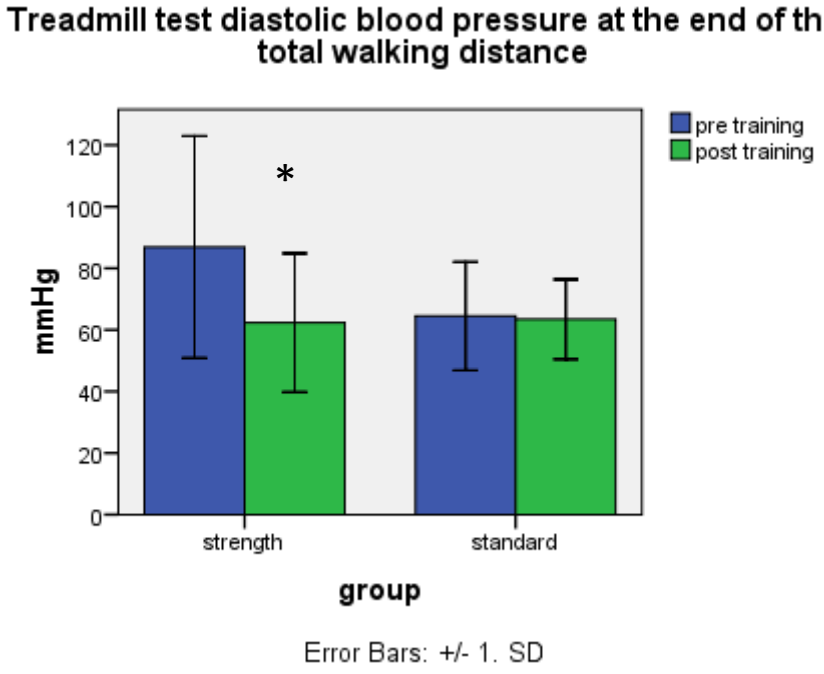
Graph 8: mean of systolic blood pressure measured at the end of pain free walking distance by treadmill test, Δ significance trend for between groups interaction p= 0,057.



Graph 9: mean of diastolic blood pressure measured at the end of pain free walking distance by treadmill test, * between groups interaction p<0,05.



Graph 10: mean of systolic blood pressure measured at the end of treadmill test, * between groups interaction p<0,05.



Graph 11: mean of diastolic blood pressure measured at the end of treadmill test, * between groups interaction p<0,05.

The mean of the values of systolic blood pressure, measured in the last minute of pain free walking distance by the treadmill test, decreased in the strength group

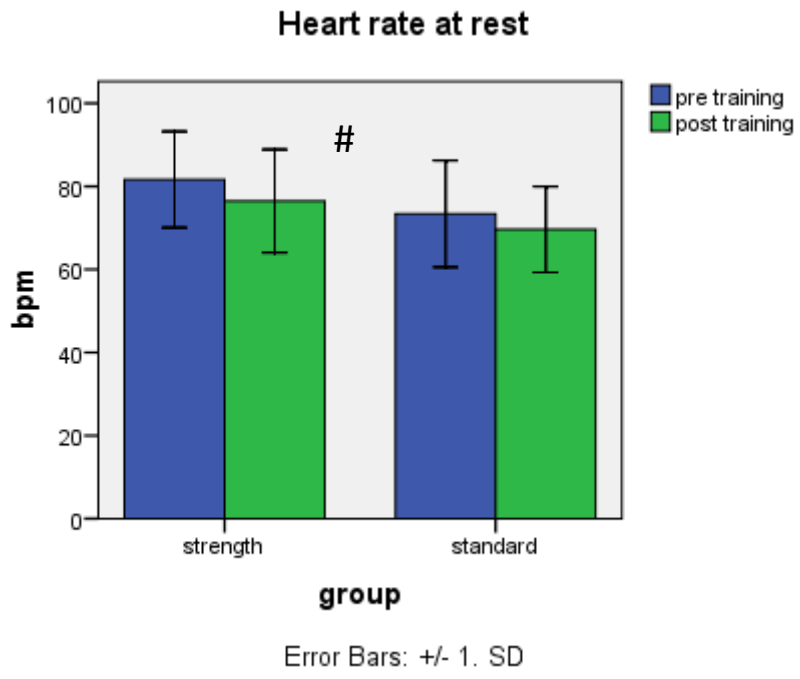
with a significant trend from 193 ± 52 mmHg to ± 164 ± 34 mmHg, whereas in the standard group it increased from 146 ± 35 mmHg to 151 ± 25 mmHg.

The mean of the values of diastolic blood pressure, measured in the last minute of pain free walking distance by the treadmill test, decreased significantly in the strength group from 83 ± 34 mmHg to 59 ± 23 mmHg, whereas in the standard group it increased from 54 ± 15 mmHg to 61 ± 17 mmHg.

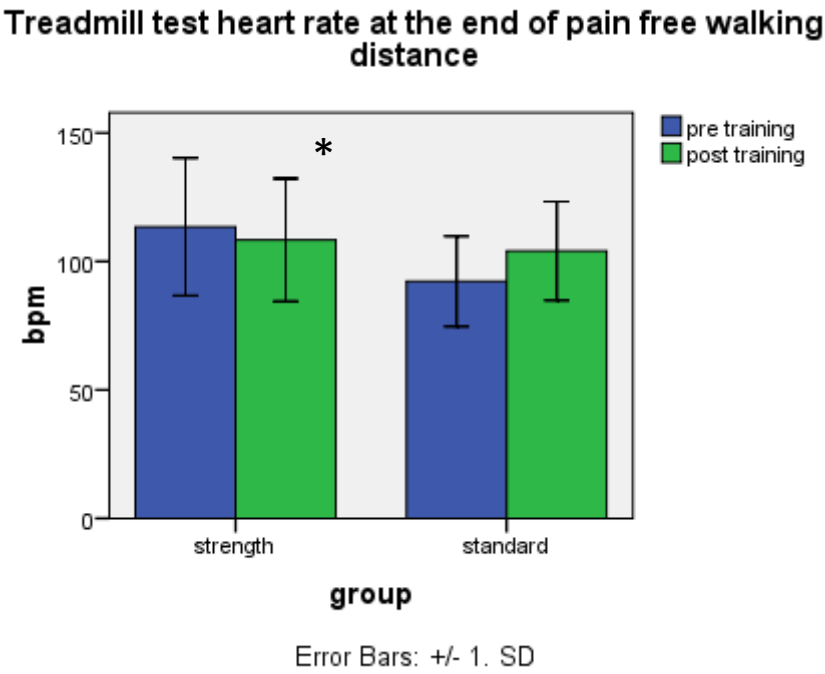
The mean of the values of systolic blood pressure, measured in the last minute of total walking distance by the treadmill test, decreased significantly in the strength group from 190 ± 46 mmHg to 162 ± 34 mmHg, whereas in the standard group it increased from 153 ± 20 to 155 ± 23 mmHg.

The mean of the values of diastolic blood pressure, measured in the last minute of total walking distance by the treadmill test, decreased significantly in the strength group from 87 ± 36 mmHg to 62 ± 23 mmHg whereas in the standard group it decreased from 65 ± 18 mmHg to 63 ± 13 mmHg.

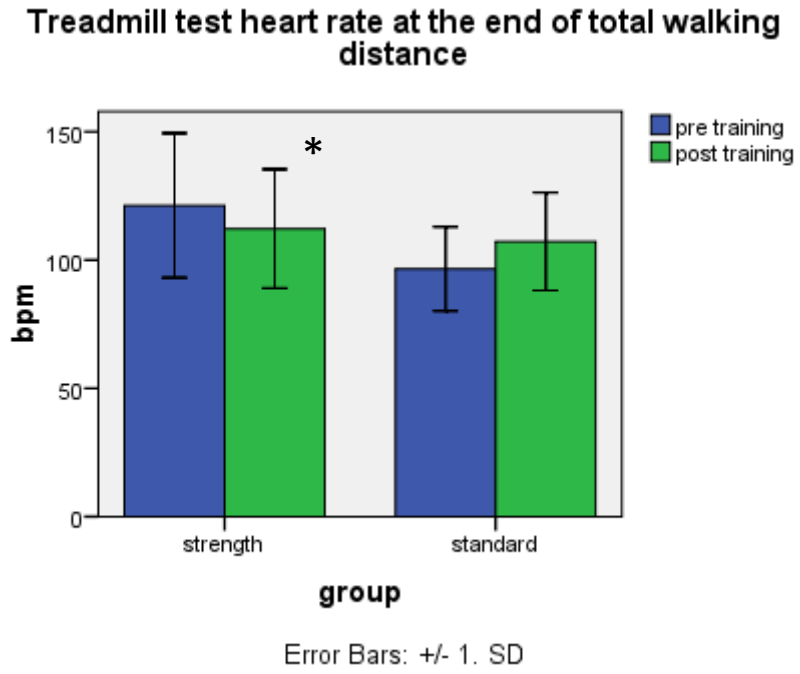
3.4. Data about heart rate



Graph 12: mean of heart rate at rest, # p<0,05 for time factor.



Graph 13: mean of heart rate measured at the end of pain free walking distance by treadmill test, * between groups interaction p<0,05.



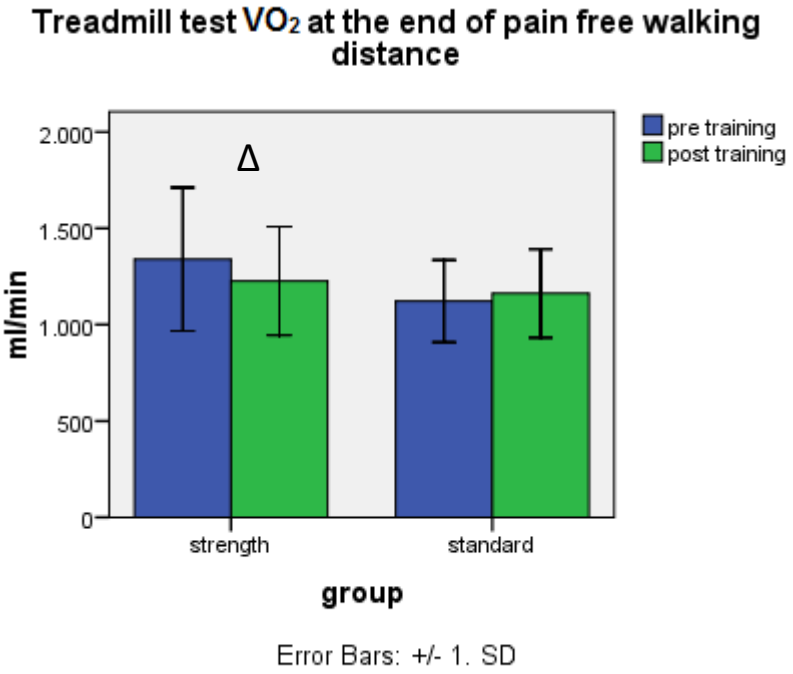
Graph 14: mean of heart rate measured at the end of treadmill test, * between groups interaction p<0,05.

The mean of the values of heart rate at rest significantly decreased in time, from 82 ± 12 bpm to 76 ± 12 bpm in the strength group, and from 73 ± 13 bpm to 70 ± 10 bpm in the standard group.

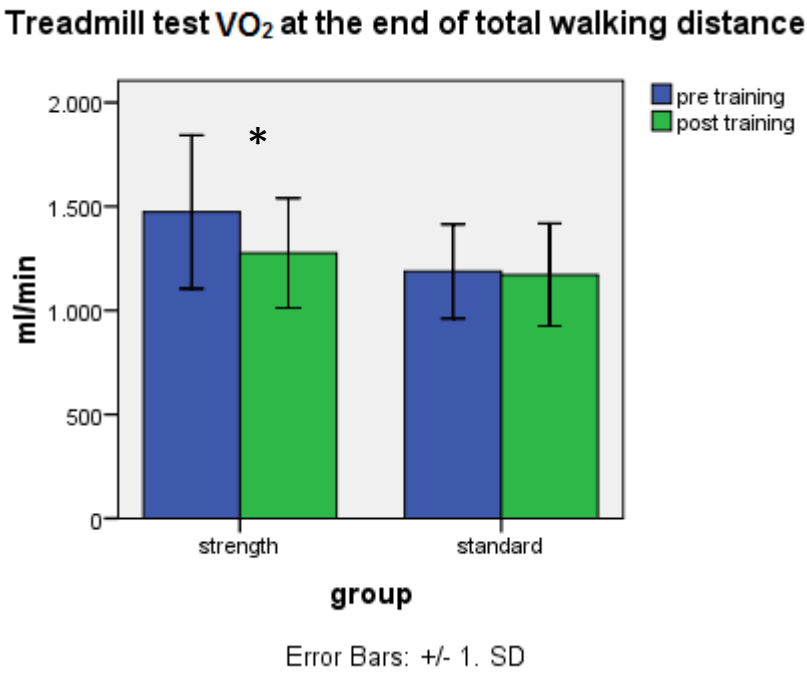
The mean of the values of heart rate, measured in the last minute of pain free walking distance by the treadmill test, significantly decreased in the strength group from 113 ± 27 bpm to 108 ± 24 bpm, whereas in the standard group it increased from 92 ± 18 bpm to 104 ± 19 bpm.

The mean of the values of heart rate, measured in the last minute of total walking distance by the treadmill test, significantly decreased in the strength group from 121 ± 28 bpm to 112 ± 23 bpm, whereas in the standard group it increased from 97 ± 16 bpm to 107 ± 19 bpm.

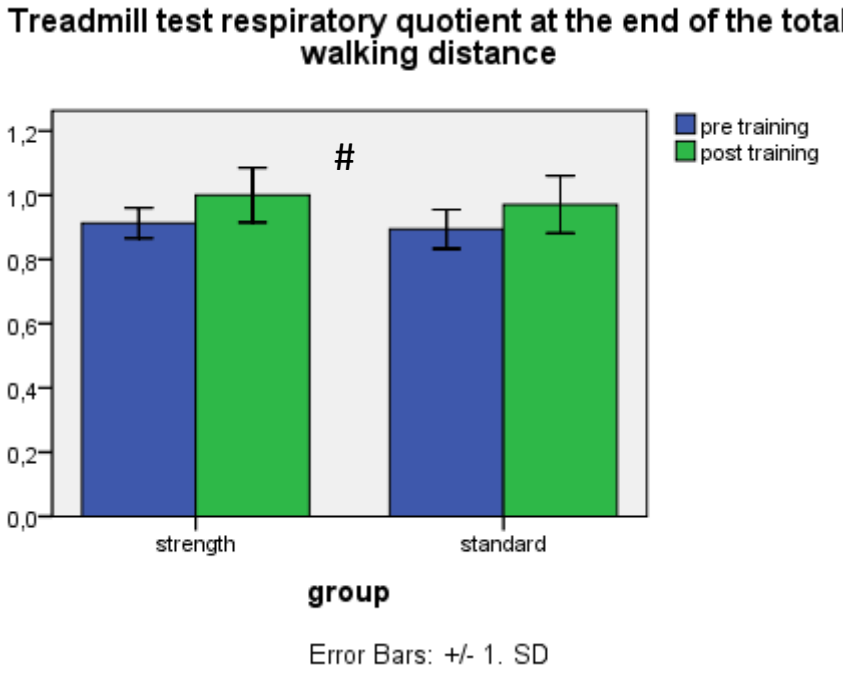
3.5. Data about oxygen consumption



Graph 15: mean of VO_2 measured at the end of pain free walking distance by treadmill test, Δ significance trend for between groups interaction p= 0,059.



Graph 16: mean of VO_2 measured at the end of treadmill test, * between groups interaction p<0,05.



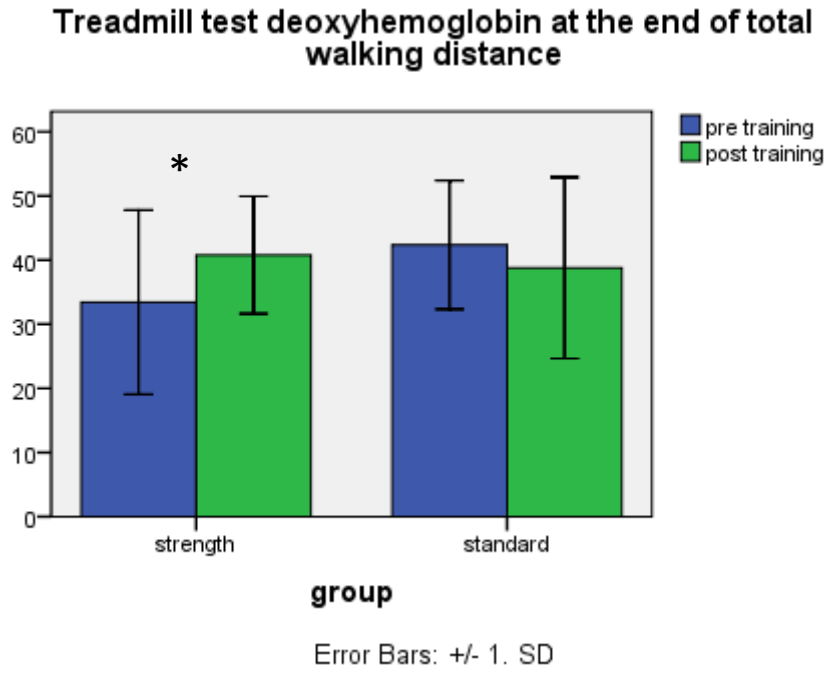
Graph 17: mean of respiratory quotient at the end of treadmill test, # p<0,05 for time factor.

The mean of the values of VO_2, measured in the last minute of pain free walking distance by the treadmill test, decreased in the strength group with a significant trend from 1339 ± 372 ml/min to 1227 ± 281 ml/min, whereas in the standard group it increased from 1122 ± 213 ml/min to 1162 ± 229 ml/min.

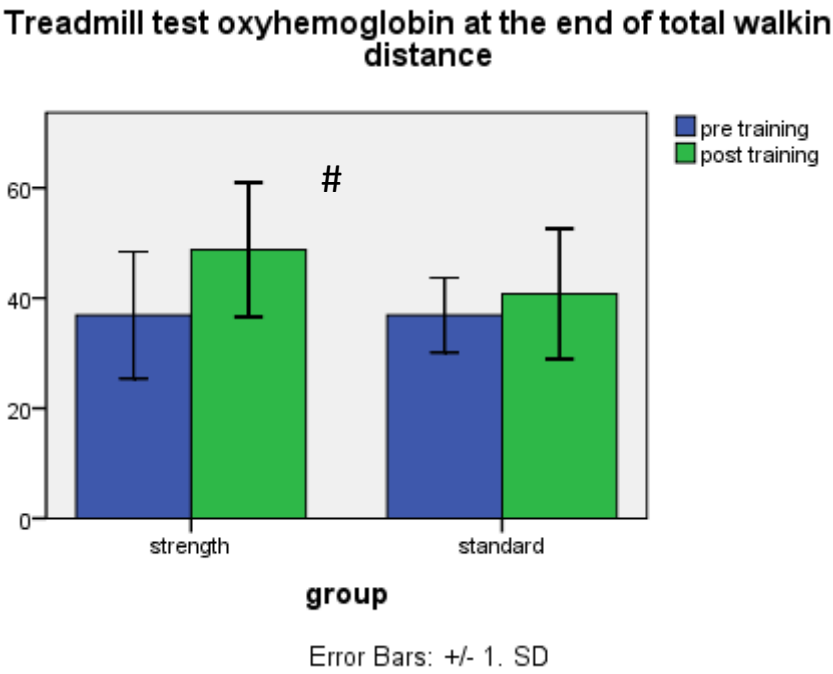
The mean of the values of VO_2, measured in the last minute of total walking distance by the treadmill test, significantly decreased in the strength group from 1473 ± 369 ml/min to 1276 ± 264 ml/min, whereas in the standard group it decreased from 1188 ± 226 ml/min to 1171 ± 246 ml/min.

The mean of the values of the respiratory quotient, measured in the last minute of total walking distance by the treadmill test, significantly increased in time, from 0,91 ± 0,04 to 1 ± 0,08 in the strength group, and from 0,89 ± 0,06 a 0,97 ± 0,09 in the standard group.

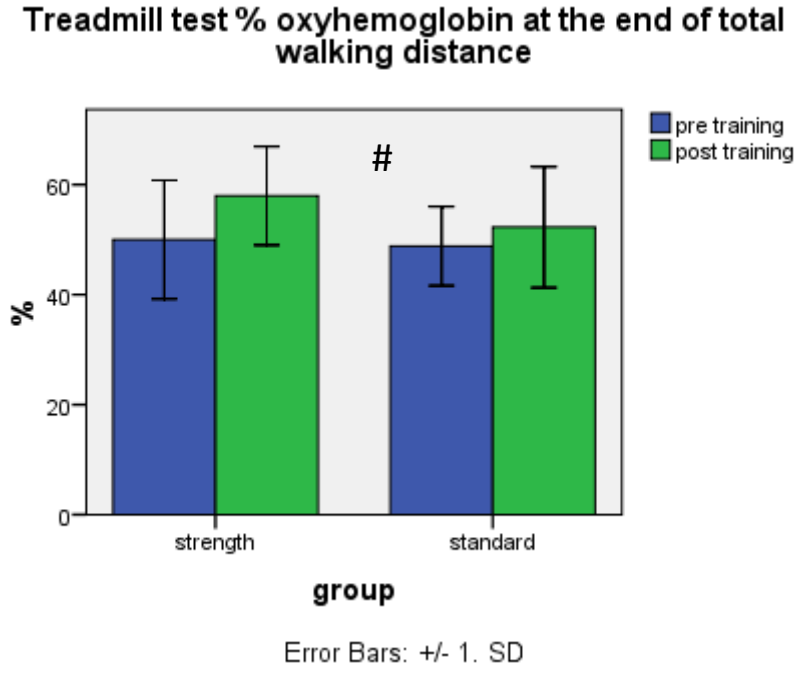
3.6. Data about local oxygenation



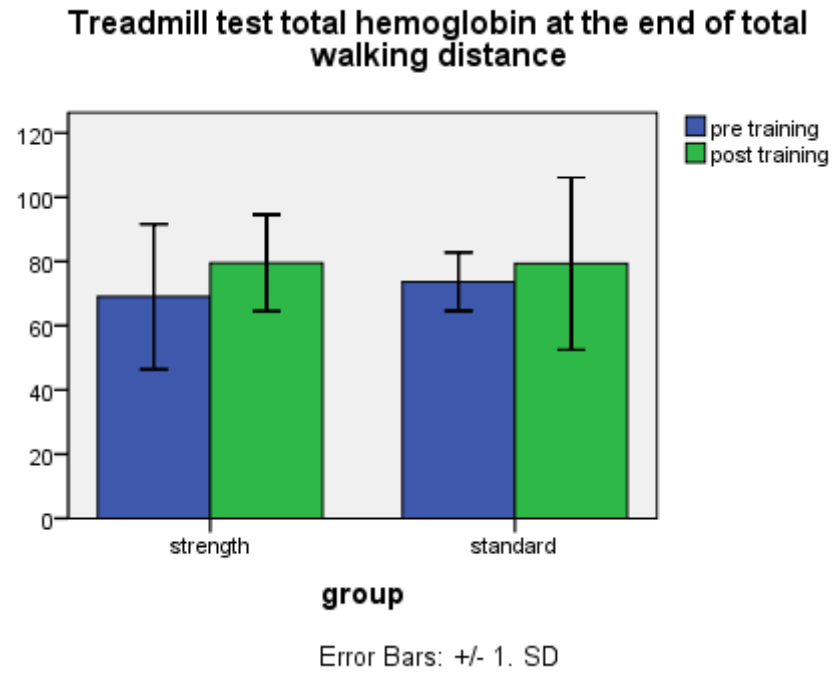
Graph 18: mean of deoxyhemoglobin at the end of treadmill test, * between groups interaction p<0,05.



Graph 19: mean of oxyhemoglobin at the end treadmill test, # p<0,05 for time factor.



Graph 20: mean of percentage of oxyhemoglobin at the end of treadmill test, # p<0,05 for time factor.



Graph 21: mean of total hemoglobin at the end of treadmill test.

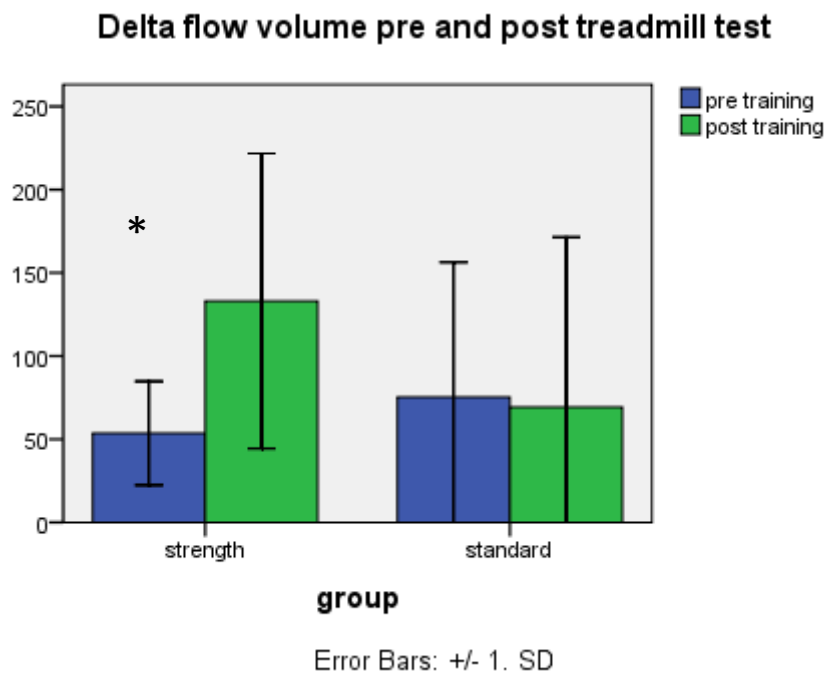
The mean of the values of deoxyhemoglobin, measured in the last minute of total walking distance by the treadmill test, significantly increased in the strength group from 33 ± 14 to 41 ± 9, whereas in the standard group it decreased from 42 ± 10 to 39 ± 14.

The mean of the values of oxyhemoglobin, measured in the last minute of total walking distance by the treadmill test, significantly increased in time, from 37 ± 12 to 49 ± 12 in the strength group, and from 37 ± 7 to 41 ± 12 in the standard group.

The mean of the percentage of oxyhemoglobin, measured in the last minute of total walking distance by the treadmill test, significantly increased in time, from 50 ± 11 % to 58 ± 9 % in the strength group, and from 49 ± 7 % to 52 ± 11 % in the standard group.

The mean of the values of total hemoglobin, measured in the last minute of total walking distance by the treadmill test, increased in the strength from 69 ± 23 to 80 ± 15, whereas in the standard group it increased from 74 ± 9 to 79 ± 27.

3.7. Data about flow volume



Graph 22: mean of the difference between flow volume before and after treadmill test, * between groups interaction p<0,05.

The mean of the differences between values of flow volume measured before and after the treadmill test significantly increased in the strength group from 54 ± 31 to 133 ± 88, whereas in the standard group it decreased from 75 ± 81 to 69 ± 102.

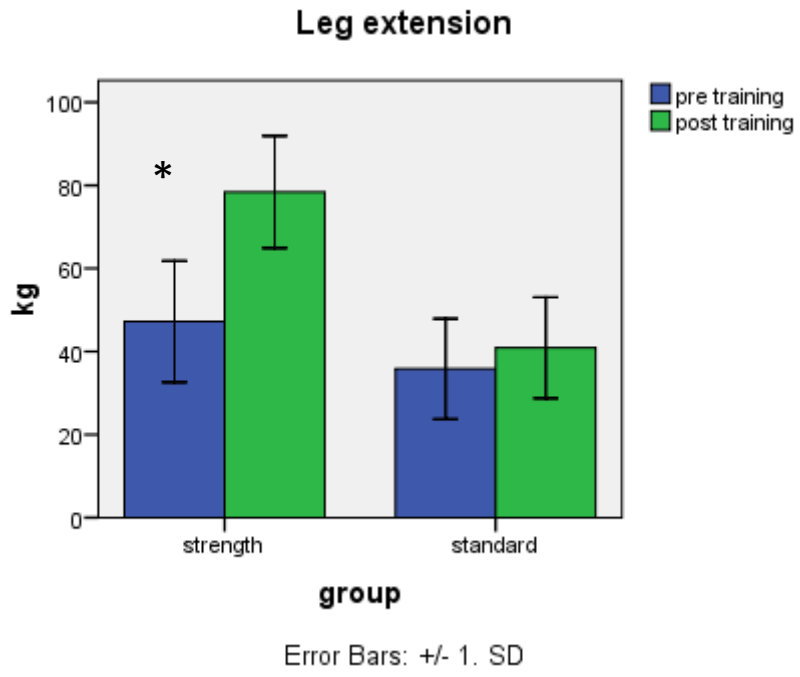
3.8. Data about strength

The mean of the values of maximal strength estimated by the leg press significantly increased in the strength group from 187 ± 54 kg to 292 ± 59 kg, whereas in the standard group it increased from 151 ± 65 kg to 153± 47 kg.

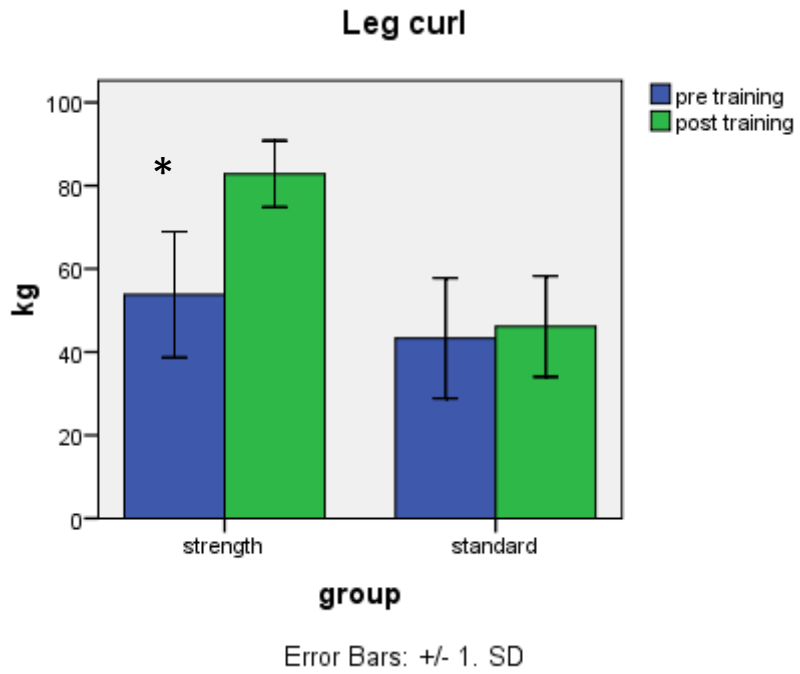
The mean of the values of maximal strength estimated by the leg extension significantly increased in the strength group from 47 ± 14 kg to 78 ± 13 kg, whereas in the standard group it increased from 35 ± 12 kg to 40 ± 12 kg.

The mean of the values of maximal strength estimated by the leg curl significantly increased in the strength group from 54 ± 15 kg to 83 ± 8 kg, whereas in the standard group it increased from 43 ± 14 kg to 46 ± 12 kg.

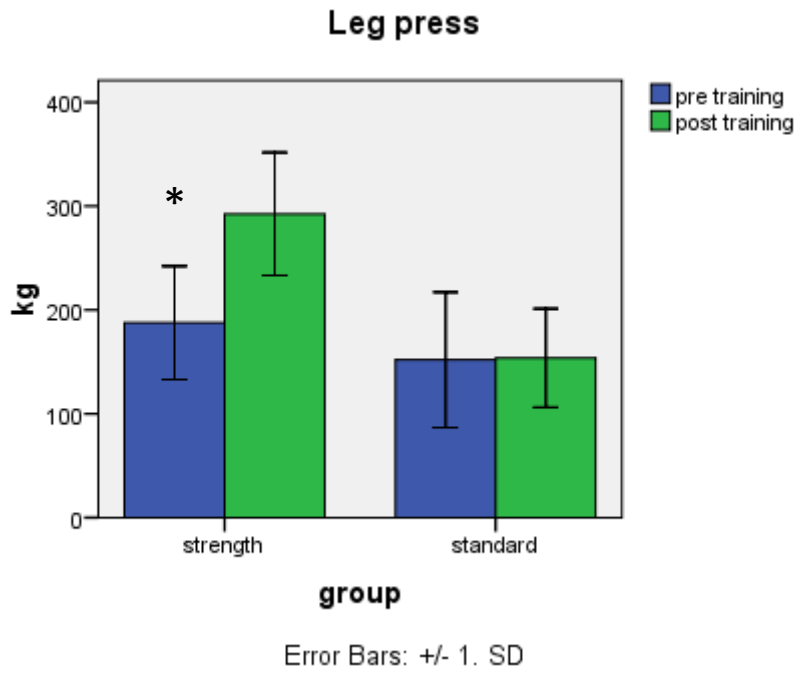
The mean of the values of maximal strength estimated by the hip abduction significantly increased in the strength group from 72 ± 18 kg to 100 ± 15 kg, whereas in the standard group it remained unchanged from 61 ± 19 kg to 61 ± 17 kg.
The mean of the values of maximal strength estimated by the hip adduction significantly increased in the strength group from 58 ±18 kg to 95 ± 18, whereas in the standard group it increased from 49 ±13 kg to 56 ± 17 kg.
The mean of the values of maximal strength estimated by the leg press for calves significantly increased in the strength group from 175 ± 43 kg to 258 ± 62 kg, whereas in the standard group it increased from 121 ± 32 kg to 133 ± 40 kg.



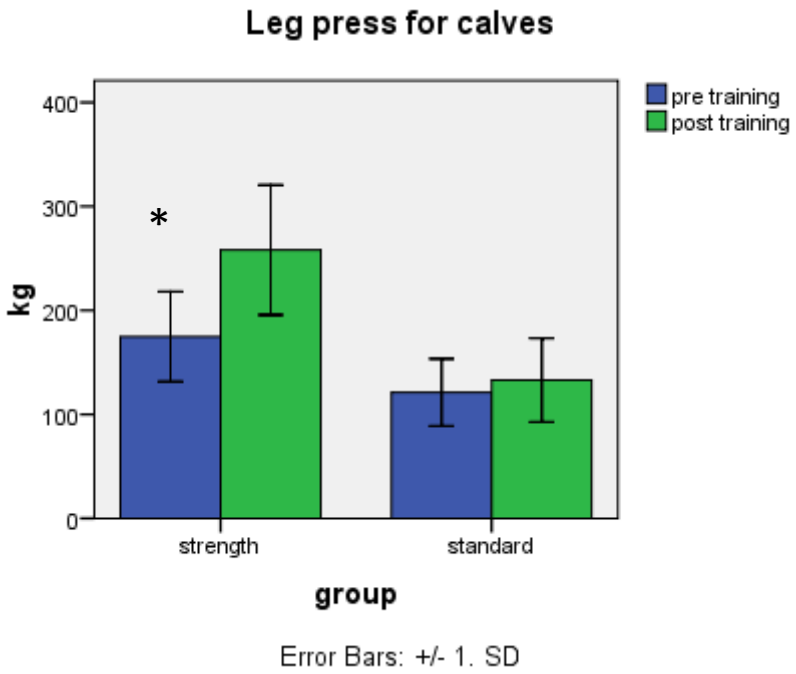
Graph 23: mean of load estimated for 1RM on the leg extension, * between groups interaction p<0,05.



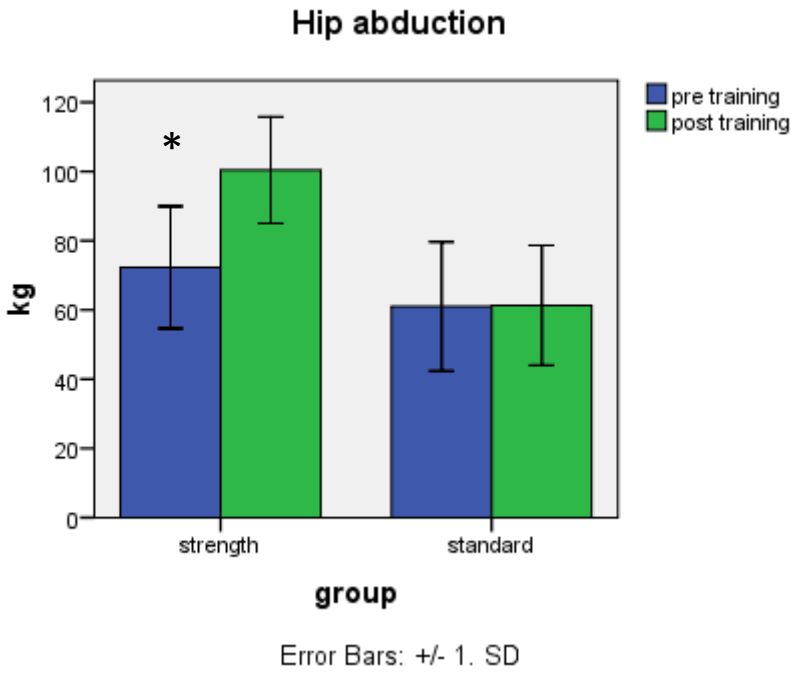
Graph 24: mean of load estimated for 1RM on the leg curl, * between groups interaction p<0,05.



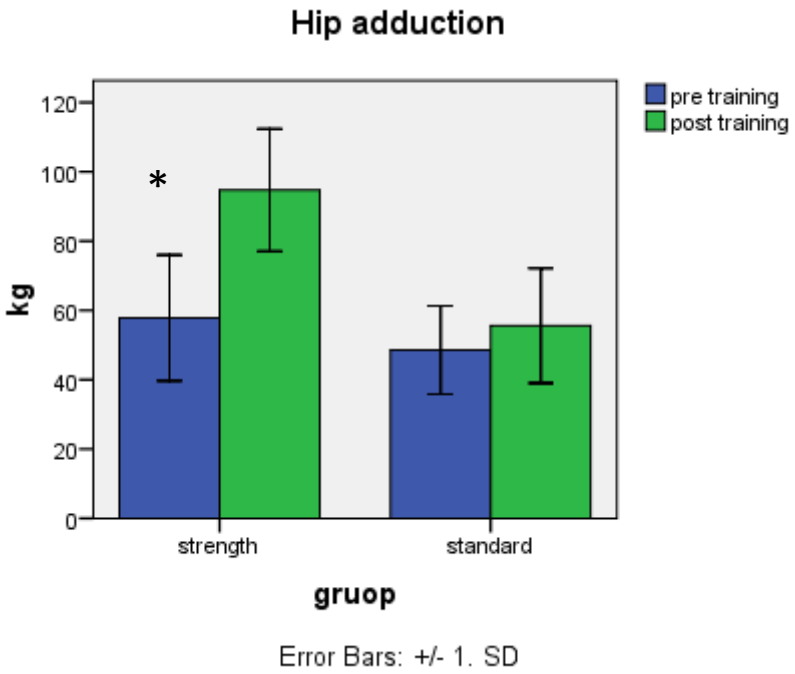
Graph 25: mean of load estimated for 1RM on the leg press, * between groups interaction p<0,05.



Graph 26: mean of load estimated for 1RM on the leg press for calves, * between groups interaction p<0,05.

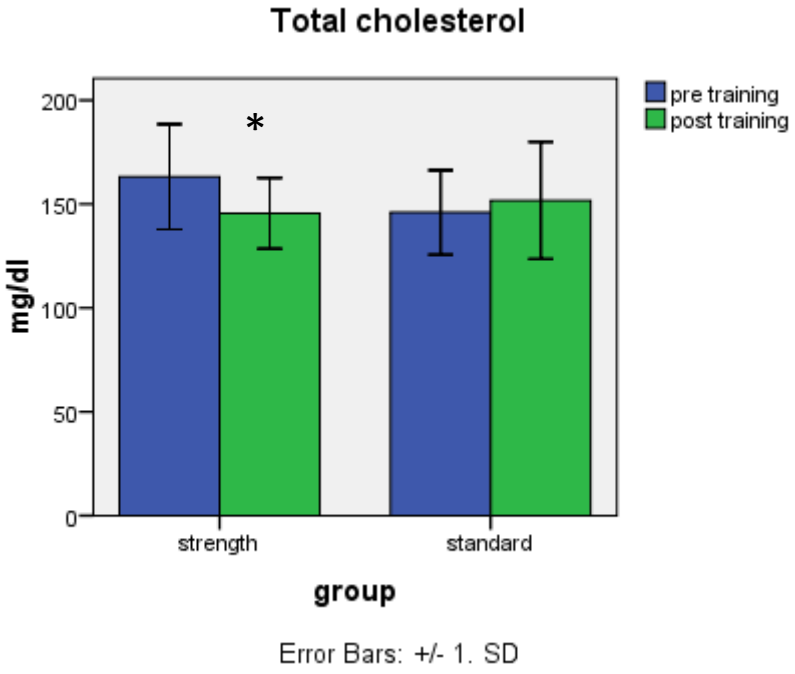


Graph 27: mean of load estimated for 1RM on the hip abduction, * between groups interaction p<0,05.

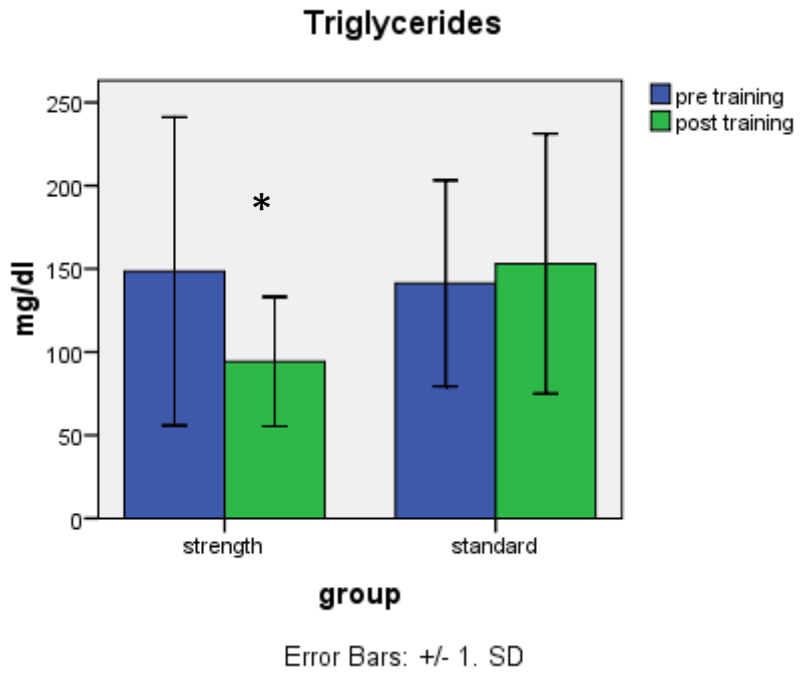


Graph 28: mean of load estimated for 1RM on the hip adduction, * between groups interaction p<0,05.

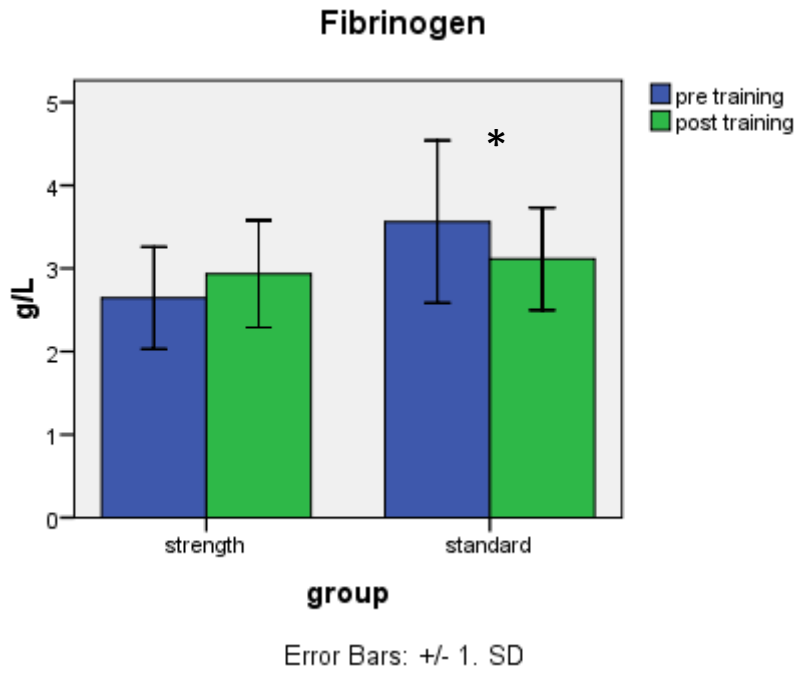
3.9. Data about blood parameters



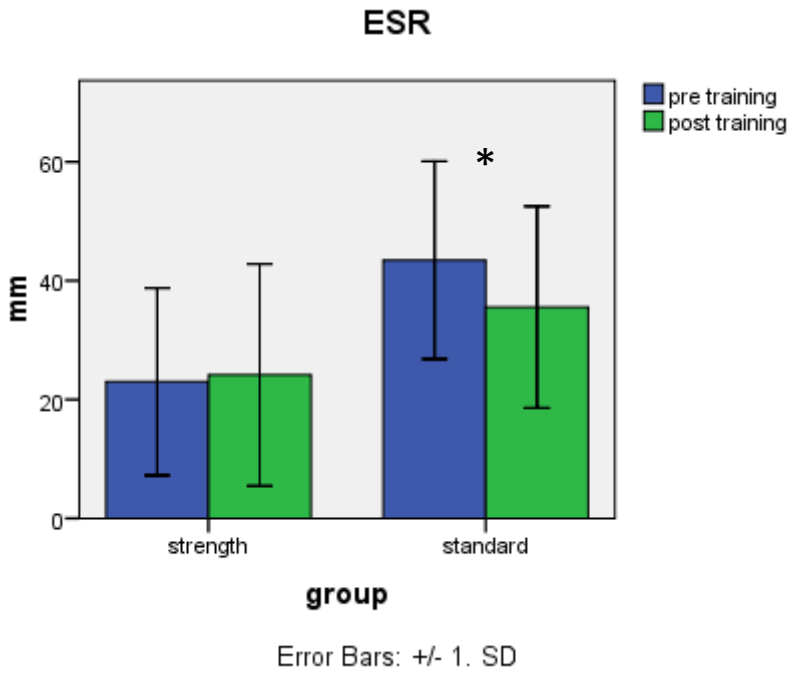
Graph 29: mean of total cholesterol, * between groups interaction p<0,05.



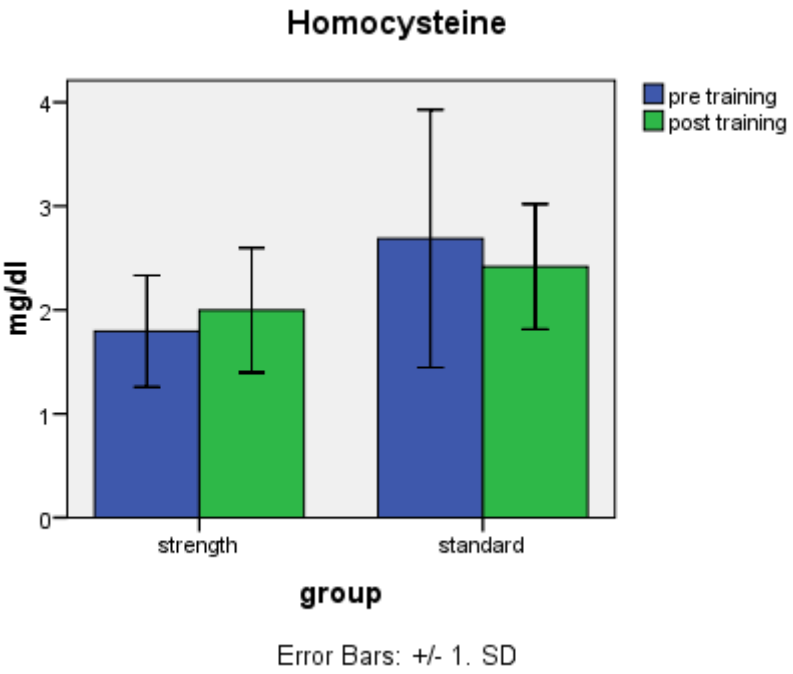
Graph 30: mean of triglycerides, * between groups interaction p<0,05.



Graph 31: mean of fibrinogen, * between groups interaction p<0,05.



Graph 32: mean of erythrocytes segmentation rate, * between groups interaction p<0,05.



Graph 33: mean of homocysteine.

The mean of the values of total cholesterol significantly decreased in the strength group from 163 ± 25 mg/dl to 146 ± 17 mg/dl, whereas in the standard group it increased from 146 ± 20 mg/dl to 152 ± 28 mg/dl.

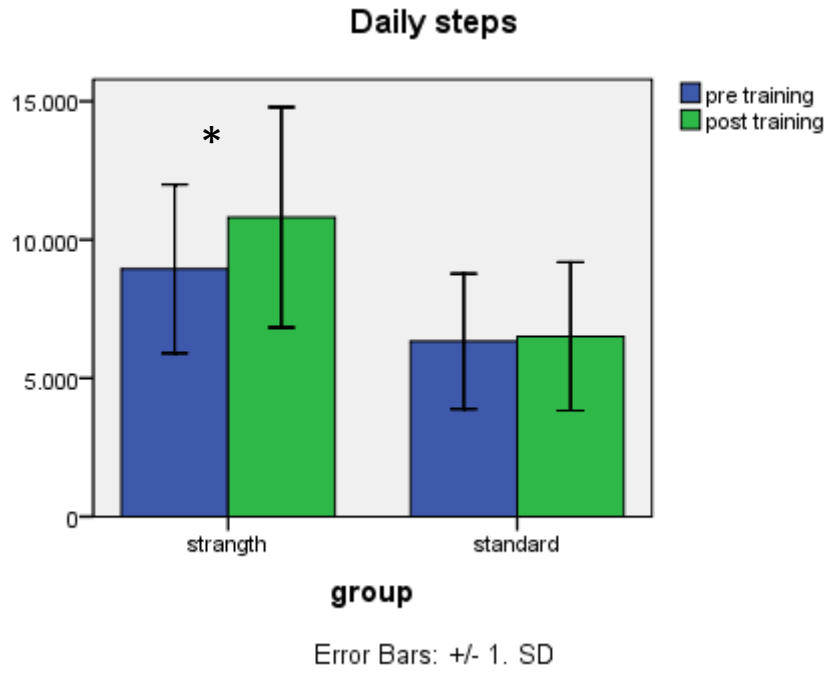
The mean of the values of triglycerides significantly decreased in the strength group from 149 ± 93 mg/dl to 94 ± 39 mg/dl, whereas in the standard group it increased from 141 ± 62 to 153 ± 75 mg/dl.

The mean of the values of erythrocytes segmentation rate (ESR) increased in the strength group from 23 ± 16 mm to 24 ± 19 mm, whereas it significantly decreased in the standard group from 43 ± 17 mm to 35 ± 17 mm.

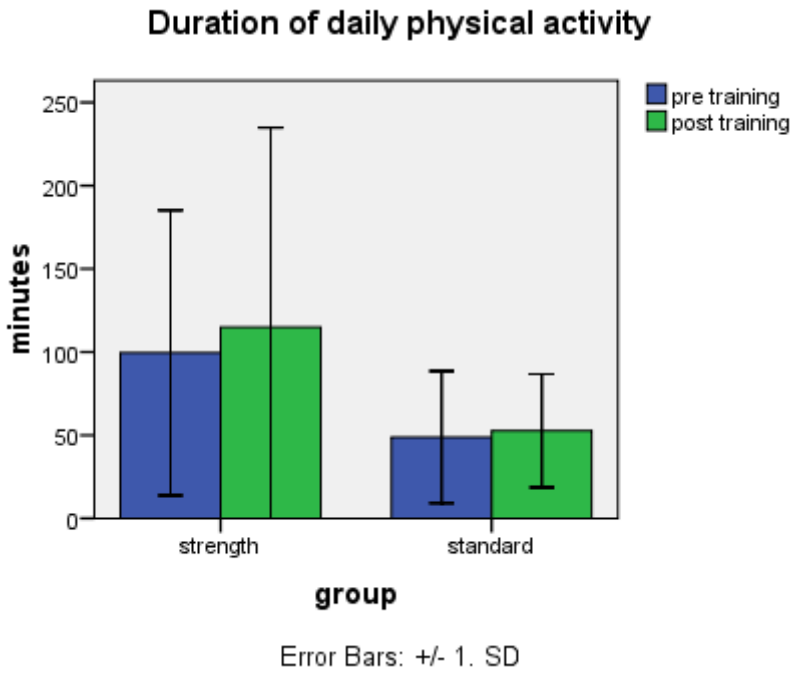
The mean of the values of fibrinogen increased in the strength group from 2,6 ± 0,6 g/L to 2,9 ± 0,6 g/L, whereas it significantly decreased in the standard group from 3,5 ± 0,9 g/L to 3,1 ± 0,6 g/L.

The mean of the values of homocysteine increased in the strength group from 1,84 ± 0,5 mg/dl to 1,95 ± 0,6 mg/dl, whereas it decreased in the standard group from 2,68 ± 1,2 mg/dl to 2,4 ± 0,6 mg/dl.

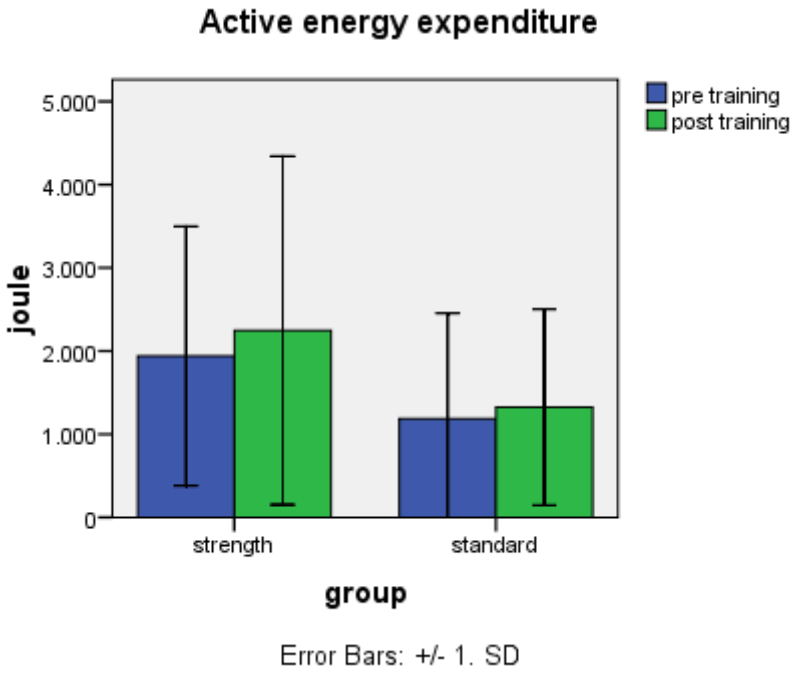
3.10. Data about physical activity in daily life



Graph 34: mean of daily taken steps, * between groups interaction p<0,05.



Graph 35: mean of the duration of daily physical activity.



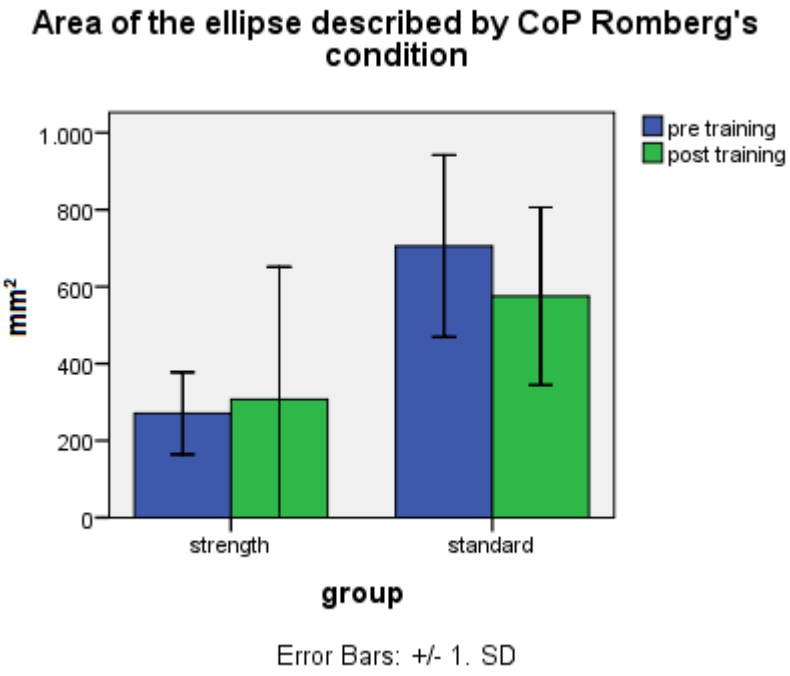
Graph 36: mean of daily active energy expenditure for activities over moderate intensity.

The mean of number of daily steps significantly increased in the strength group from 8946 ± 3046 to 10809 ± 3981, whereas in the standard group it increased from 6331 ± 2445 to 6507 ± 2680.

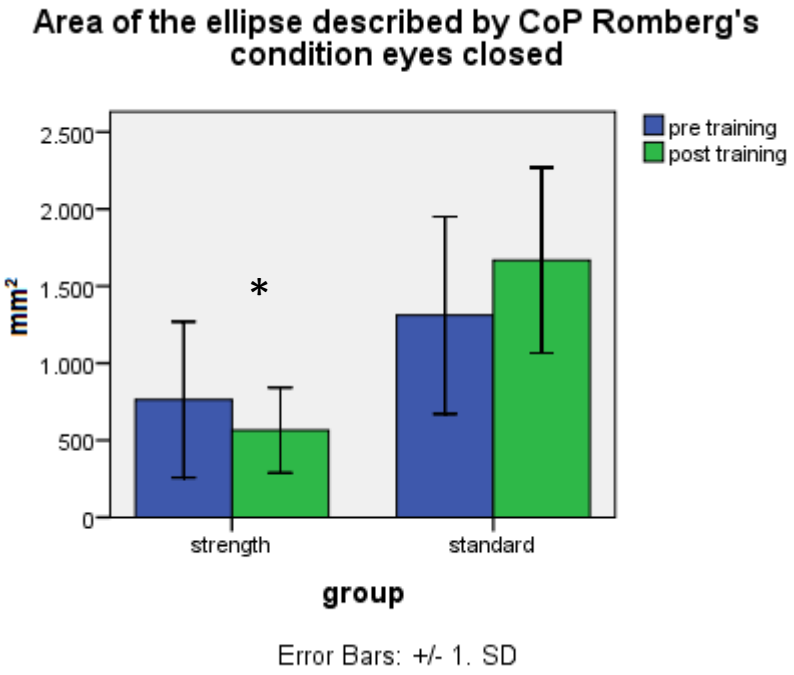
The mean of the duration of daily physical activity increased in the strength group from 100 ± 86 minutes to 115 ± 120 minutes, whereas in the standard group it increased from 49 ± 40 minutes to 53 ± 30 minutes.

The mean of the values of daily active energy expenditure increased in the strength group from 1939 ± 1558 joule to 2247 ± 2095 joule, whereas in the standard group it increased from 1187 ± 1269 joule to 1325 ± 1179 joule.

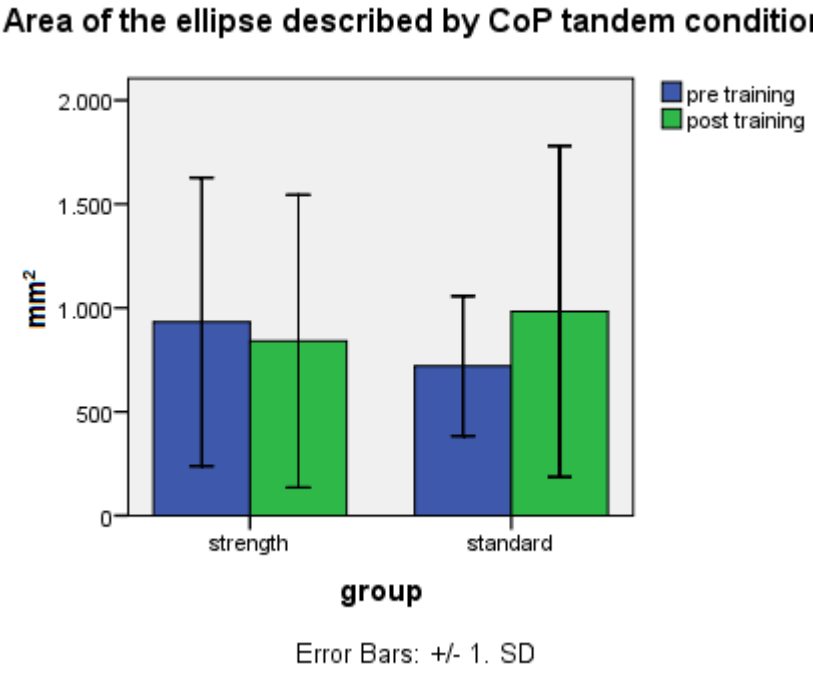
3.11. Data about force plates



Graph 37: mean of the area of the ellipse described by CoP in Romberg's condition.



Graph 38: mean of the area of the ellipse described by CoP in Romberg's condition eyes closed, * between groups interaction p<0,05.



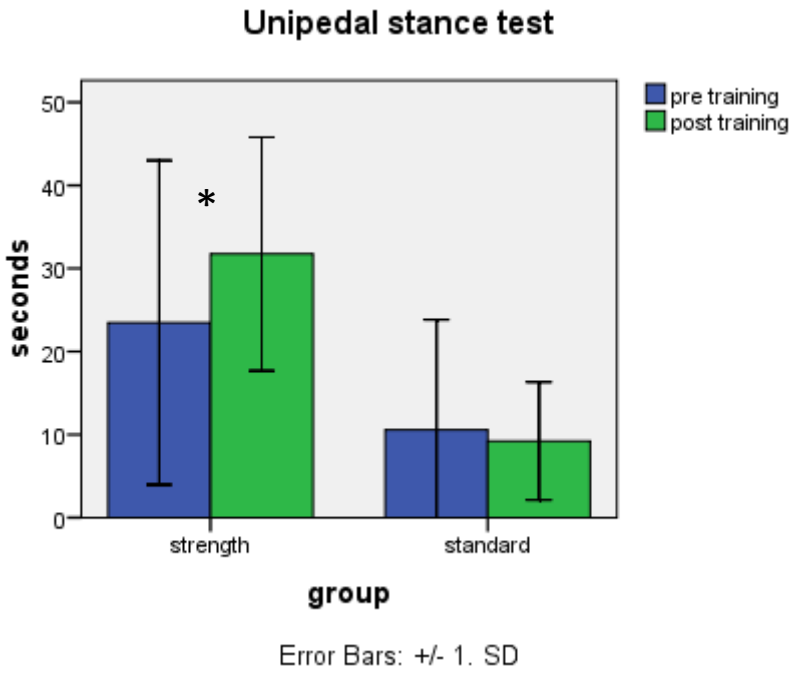
Graph 39: mean of the area of the ellipse described by CoP in tandem condition.

The mean of the values of the area of the ellipse described by CoP in Romberg's condition increased in the strength group from 271 ± 107 mm² to 307 ± 344 mm², whereas in the standard group it decreased from 706 ± 237 mm² to 576 ± 231 mm².

The mean of the values of the area of the ellipse described by CoP in Romberg's condition with eyes closed significantly decreased in the strength group from 763 ± 505 mm² to 565 ± 271 mm², whereas in the standard group it increased from 1311 ± 640 mm² to 1667 ± 601mm².

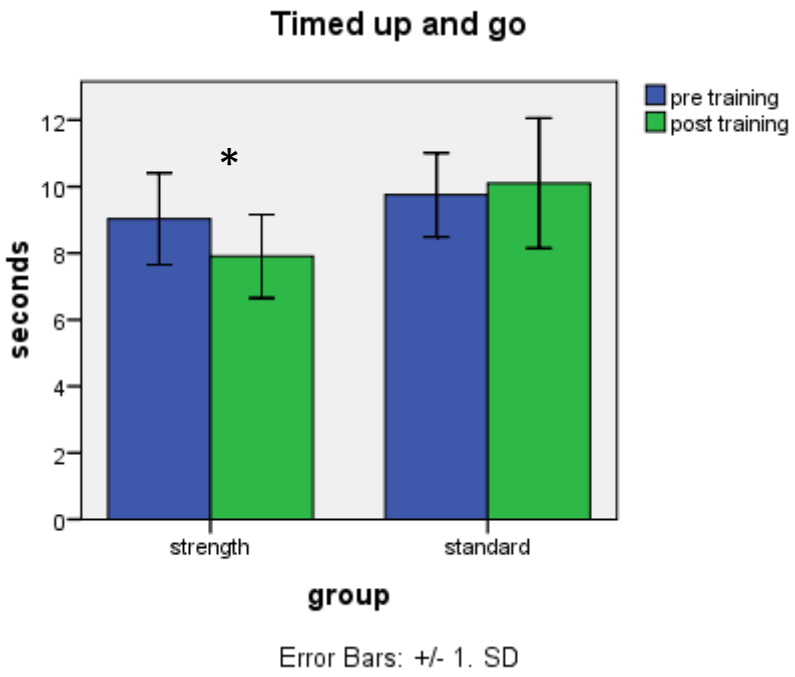
The mean of the values of the area of the ellipse described by CoP in tandem condition decreased in the strength group from 932 ± 694 mm² to 841 ± 704 mm², whereas in the standard group it increased from 720 ± 337 mm² to 983 ± 797mm².

3.12. Data about physical function

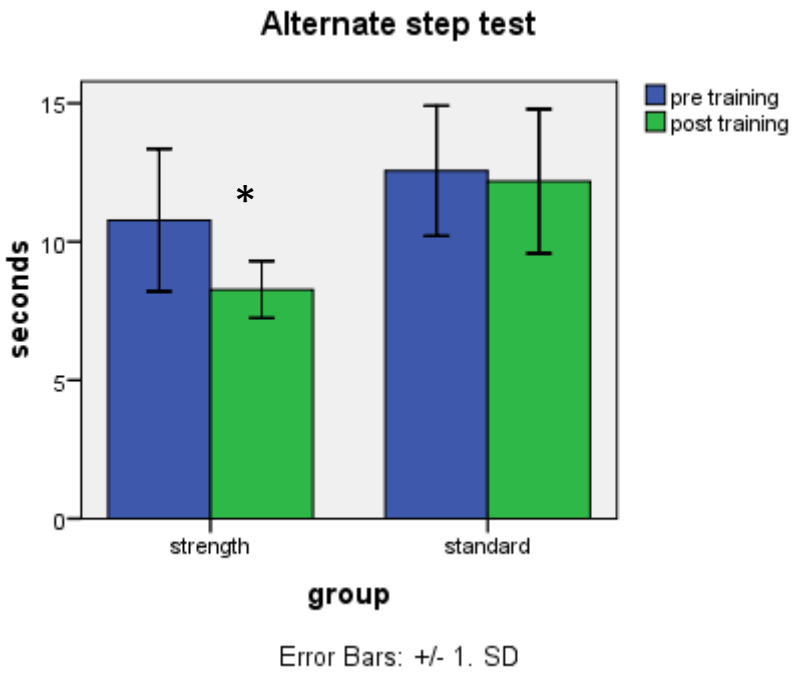


Graph 40: mean of unipedal stance test, * between groups interaction p<0,05.

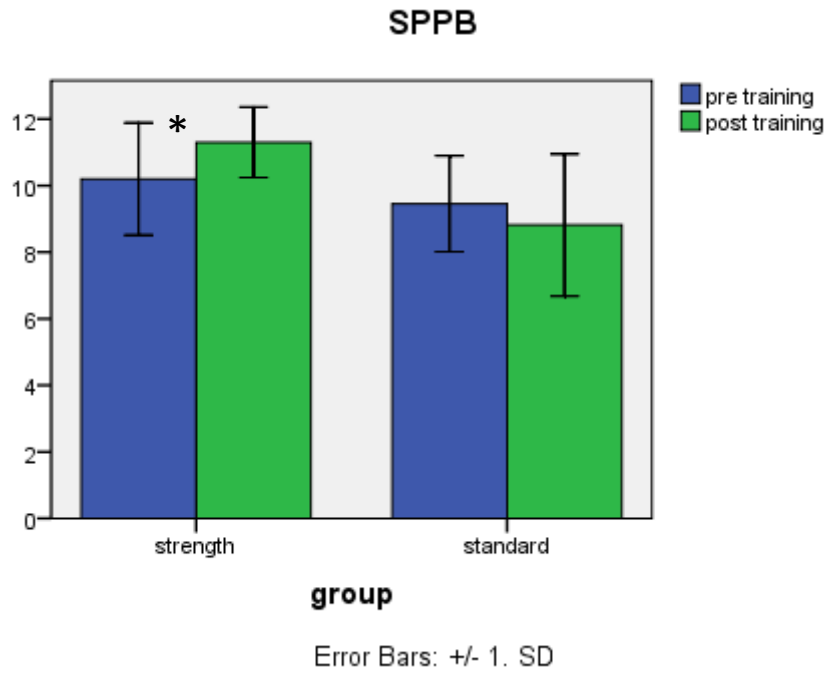
The mean of the duration of the unipedal stance test significantly increased in the strength group from 23 ± 20 seconds to 32 ± 14 seconds, whereas in the standard group it decreased from 11 ± 13 seconds to 9 ± 7 seconds.



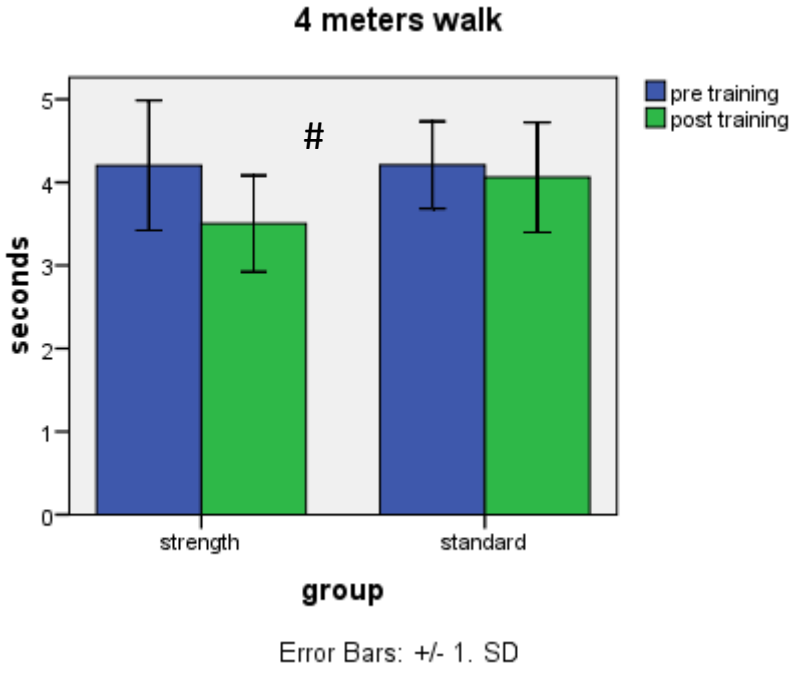
Graph 41: mean of timed up and go test, * between groups interaction p<0,05.



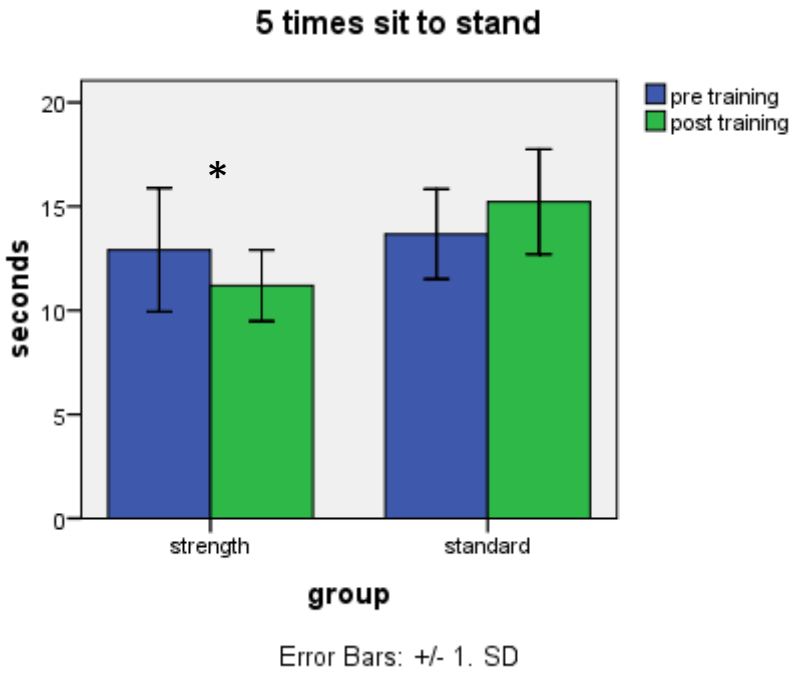
Graph 42: mean of alternate step test, * between groups interaction p<0,05.



Graph 43: mean of scores of SPPB, * between groups interaction p<0,05.



Graph 44: mean of 4 meters walk, # p<0,05 for time factor.



Graph 45: mean of 5 times sit to stand test, * between groups interaction p<0,05.

The mean of the duration of the timed up and go test significantly decreased in the strength group from 8,9 ± 1,4 seconds to 8 ± 1,2 seconds, whereas in the standard group it increased from 9,7 ± 1,3 seconds to 10,1 ± 2 seconds.

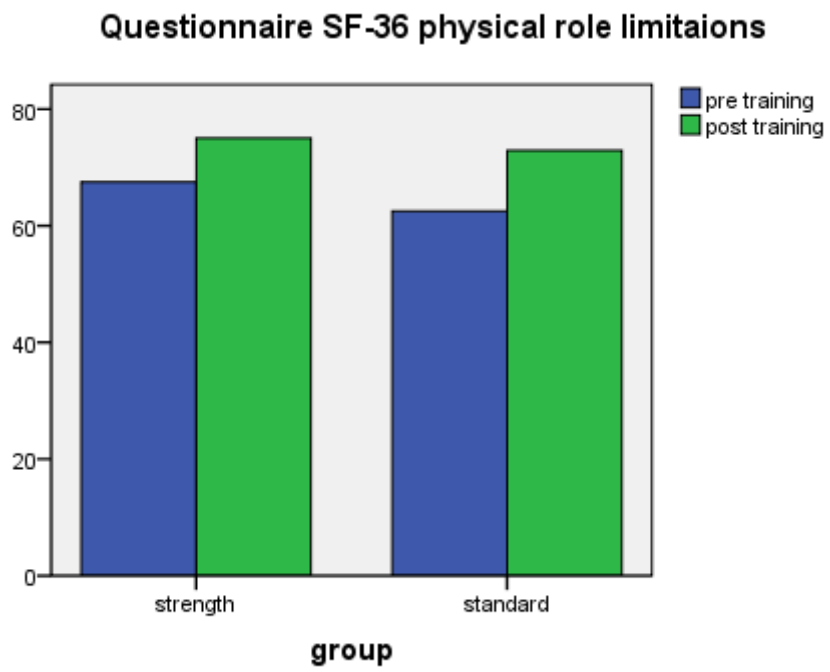
The mean of the duration of the alternate step test significantly decreased in the strength group from 10,5 ± 2,6 seconds to 8,4 ± 1,1 seconds, whereas in the standard group it decreased from 12,6 ± 2,4 seconds to 12,2 ± 2,6 seconds.

The mean of total scores of the SPPB scale significantly increased in the strength group from 10,2 ± 1,7 to 11,3 ± 1, whereas in the standard group it decreased from 9,5 ± 1,4 to 8,8 ± 2,1.

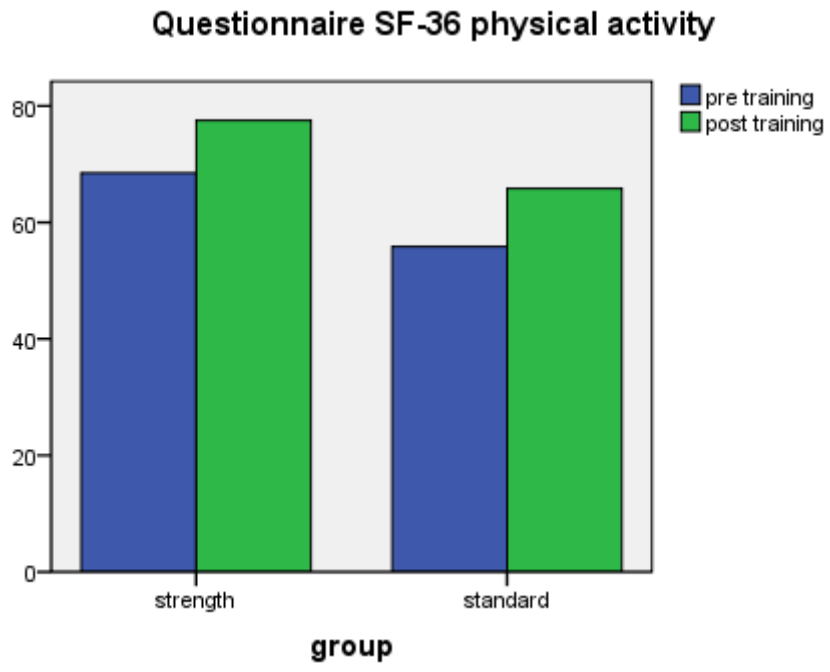
The mean of the duration of 4 meters walk significantly increased in time, from 4,2 ± 0,7 seconds to 3,5 ± 0,6 seconds in the strength group, and from 4,2 ± 0,5 seconds to 4 ± 0,7 seconds in the standard group.

The mean of the duration of the sit to stand test significantly decreased in the strength group from 13 ± 3 seconds to 11 ± 2 seconds, whereas in the standard group it increased from 14 ± 2 seconds to 15 ± 3 seconds.

3.13. Data about SF-36 questionnaire



Graph 46: mean of the score of physical role limitations of SF-36 questionnaire.

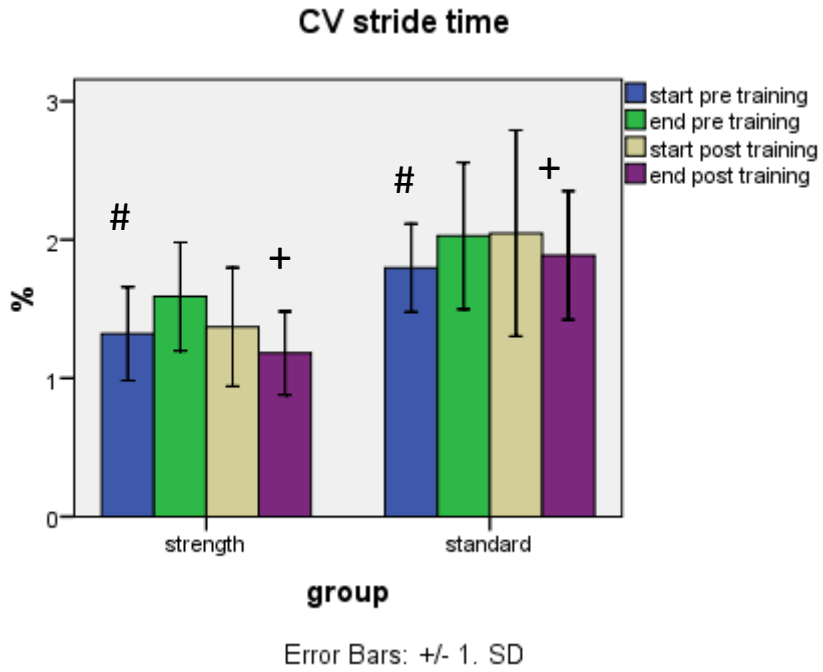


Graph 47: mean of score of physical activity of SF-36 questionnaire.

The mean of the score of physical role limitation of the SF-36 questionnaire increased in the strength group from 68 to 75, whereas in the standard group it increased from 63 to 73.

The mean of the score of physical activity of the SF-36 questionnaire increased in the strength group from 69 to 78, whereas in the standard group it increased from 56 to 66.

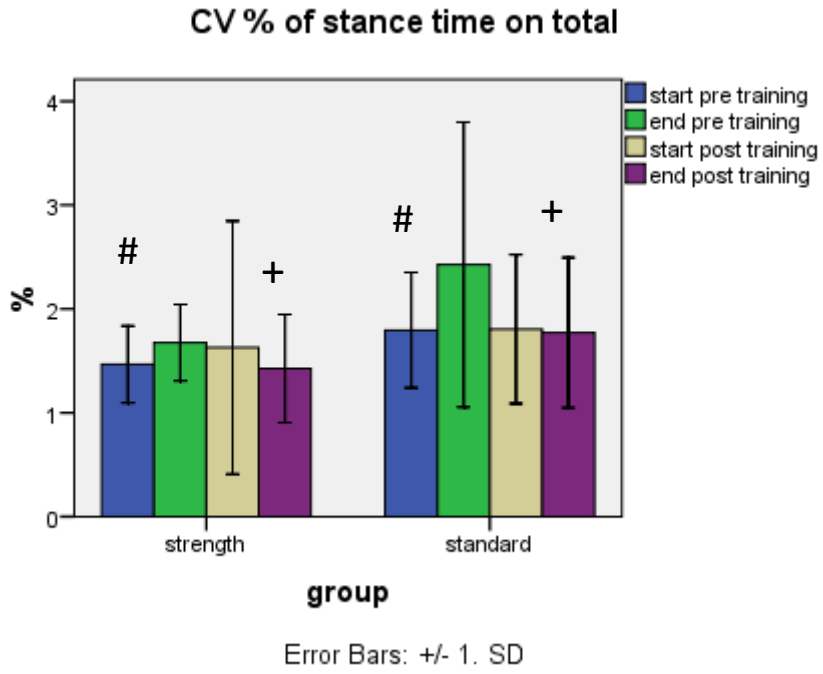
3.14. Data about gait cycle



Graph 48: mean of the coefficient of variation of stride time, # comparison between the initial and the final part of the entry test p<0,05 for time factor, + comparison between the final part of the entry del test and the final part of the exit test p<0,05 for time factor.

The mean of the coefficient of variation of the stride time by the entry test significantly increased in time between the initial and the final part of the test, from 1,6 ± 0,7 % to 1,9 ± 0,8 % in the strength group, and from 1,9 ± 0,3 % to 2 ± 0,5 % in the standard group, whereas by the exit test it decreased from 1,4 ± 0,4 % to 1,2 ± 0,3 % in the strength group and from 1,9 ± 0,7 % to 1,8 ± 0,5 % in the standard group.

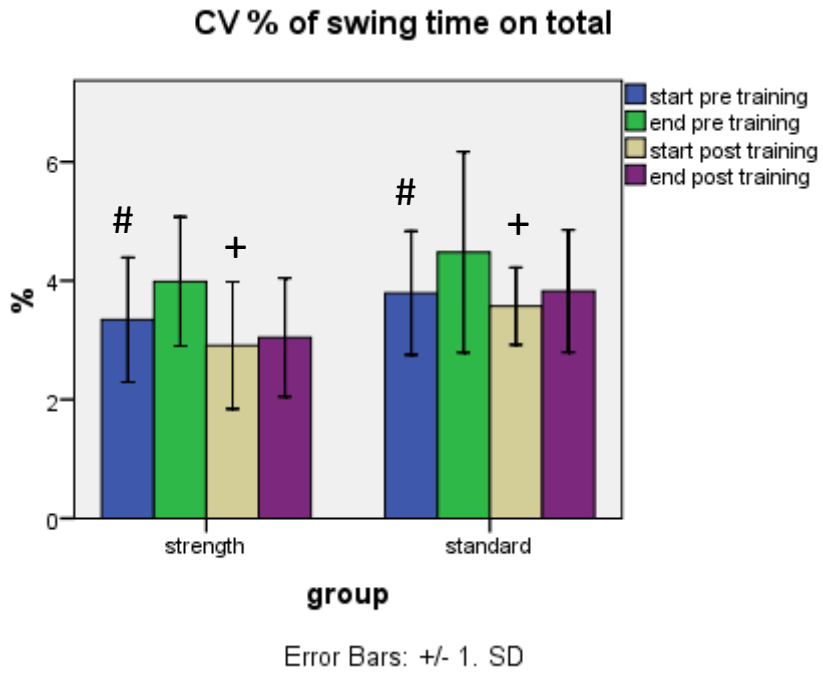
There were no significant differences between the initial and the final part of the exit test, the final part of the test significantly decreased in time between pre and post training.



Graph 49: mean of the coefficient of variation of the percentage of stance time on total, # comparison between the initial and the final part of the entry test p<0,05 for time factor, + comparison between the final part of the entry del test and the final part of the exit test p<0,05 for time factor.

The mean of the coefficient of variation of the percentage of stance time on total by the entry test significantly increased in time between the initial and the final part of the test, from 1,6 ± 0,4 % to 1,8 ± 0,5 % in the strength group, and from 1,8 ± 0,5 % to 2,4 ± 1,3 % in the standard group, whereas by the exit test it decreased from 1,6 ± 1,2 % to 1,4 ± 0,5 % in the strength group and remained unchanged from 1,8 ± 0,7 % to 1,8 ± 0,7 % in the standard group.

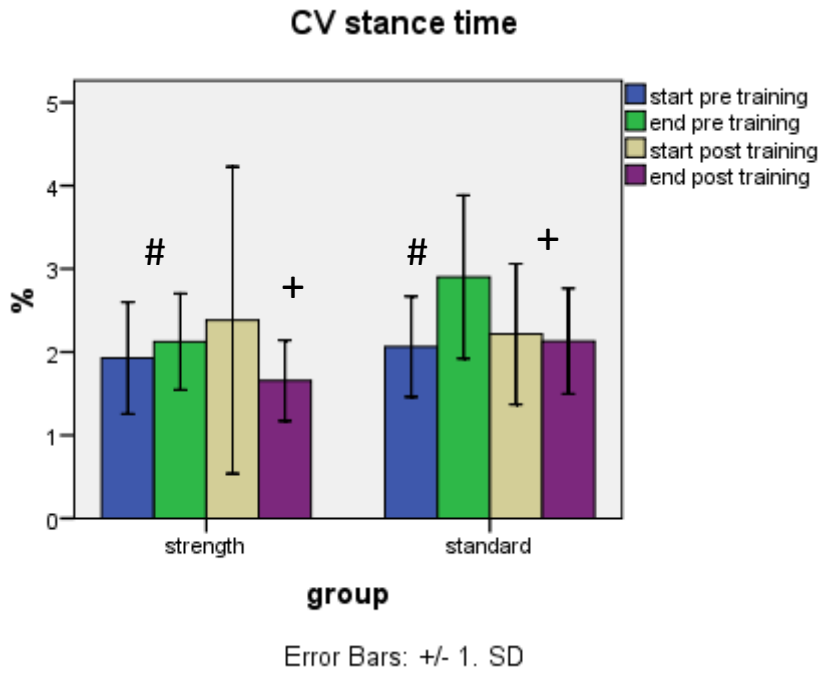
There were no significant differences between the initial and the final part of the exit test, the final part of the test significantly decreased in time between pre and post training.



Graph 50: mean of the coefficient of variation of the percentage of swing time on total, # comparison between the initial and the final part of the entry test p<0,05 for time factor, + comparison between the final part of the entry del test and the final part of the exit test p<0,05 for time factor.

The mean of the coefficient of variation of the percentage of swing time on total by the entry test significantly increased in time between the initial and the final part of the test, from 3,6 ± 1% to 4,4 ± 1,3% in the strength group and from 3,8 ± 0,9 % to 4,5 ± 1,6 % in the standard group, whereas by the exit test it increased from 2,9 ± 1 % to 3,1 ± 0,9 % in the strength group, and from 3,5 ± 0,6 % to 3,8 ± 1% in the standard group.

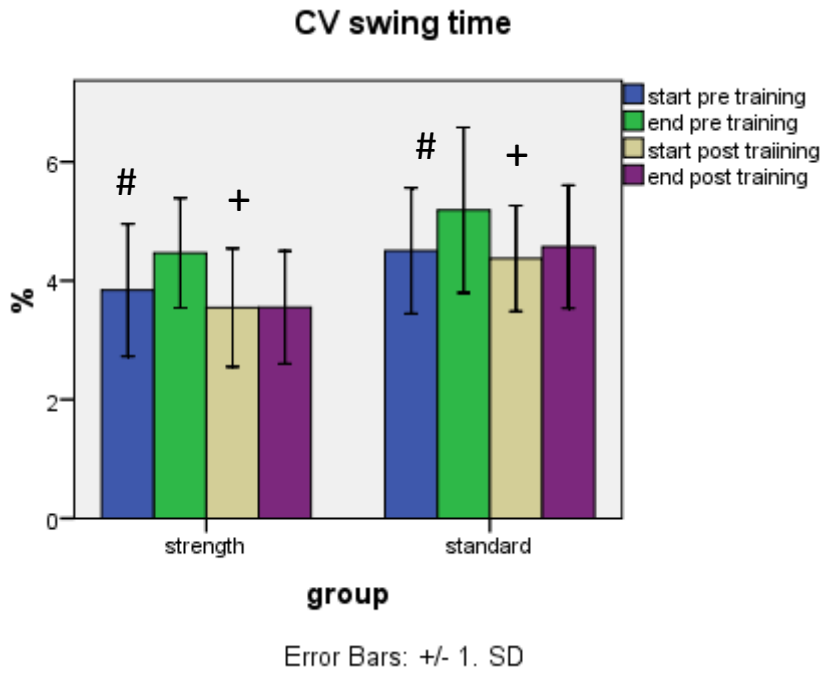
There were no significant differences between the initial and the final part of the exit test, the final part of the test significantly decreased in time between pre and post training.



Graph 51: mean of the coefficient of variation of stance time, # comparison between the initial and the final part of the entry test p<0,05 for time factor, + comparison between the final part of the entry del test and the final part of the exit test p<0,05 for time factor.

The mean of the coefficient of variation of the stance time by the entry test significantly increased in time between the initial and the final part of the test, from 2,1 ± 0,8 % to 2,4± 0,8 % in the strength group, and from 2,1 ± 0,6 % to 2,8 ± 0,9 % in the standard group, whereas by the exit test it decreased from 2,3 ± 1,8 % to 1,7 ± 0,5 % in the strength group and from 2,2 ± 0,8 to 2,1 ± 0,6 % in the standard group.

There were no significant differences between the initial and the final part of the exit test, the final part of the test significantly decreased in time between pre and post training.



Graph 52: mean of the coefficient of variation of swing time, # comparison between the initial and the final part of the entry test p<0,05 for time factor, + comparison between the final part of the entry test and the final part of the exit test p<0,05 for time factor.

The mean of the coefficient of variation of the stance time by the entry test significantly increased in time between the initial and the final part of the test, from 4,2 ± 1,2 % to 5 ± 1,4 % in the strength group, and from 4,6 ± 1 % to 5,3 ± 1,4 % in the standard group, whereas by the exit test it increased from 3,5 ± 1 % to 3,7 ± 1% in the strength group and from 4,3 ± 0,8 % to 4,5 ± 1% in the standard group. There were no significant differences between the initial and the final part of the exit test, the final part of the test significantly decreased in time between pre and post training.

4. DISCUSSION

Total walking distance, which is the primary end point of our study, significantly increased in both groups, as it could be expected on the basis of the literature[65].
Pain free walking distance that increased only in the strength group, seems to indicate greater effectiveness of combined training respect to walking only. Indeed pain free walking distance highlights the walking ability in 'valid' perfusion conditions, not in ischemia. This finding has therefore a special significance respect to total walking distance, being directly linked to the effective walking autonomy in everyday life, and thus highlighting the actual functional limitations of the patient.

Data present in literature[81] about the effects, on cardiovascular parameters of arteriopathic subjects, of a standard walking training respect of an experimental protocol of only strength exercises, show a decrease in systolic blood pressure at rest similar in both groups. Our data behave in a similar manner.
The same study indeed didn't find any difference for both groups in maximal pressure values. Our results also detected no difference in the standard group, while differences were found in the strength group. This leads us to think of an enhanced effect of combined training on cardiovascular parameters respect to walking only as well as resistance training alone. Indeed a lower systolic and diastolic pressure in the strength group, both at pain free walking distance (diastolic) and at the end of the test, indicates a positive adjustment to the effort as a result of the training period, and shows greater levels of fitness of the subjects and a potential reduction of cardiovascular risk factors.

The reduction in heart rate during exercise indicates a positive adaptation to the training. The previous study[81] found differences in the heart rate at rest similar to those we detected. Instead there were no differences in maximal values, whereas our data show significant between groups differences, with a reduction of values in the strength group. This data further confirm the enhanced effect of combined training respect to each method alone.
Variations in heart rate also resulted to by different between PAD patients and healthy adults in the early stages of an incremental walk test, and links were found

between these variations and those of values of oxygenated and deoxygenated hemoglobin[82], with a reduction in submaximal heart rate linked to an increase in values of oxyhemoglobin or oxygen extraction ability. Such mechanisms might underlie the different between groups heart rate responses, measured in our study at the end of pain free walking distance.

Previous studies[82,83] used NIRS to evaluate values of oxygenated, deoxygenated and total hemoglobin in subjects with PAD. The values of the patients resulted significantly different respect to those of healthy controls, except total hemoglobin.

Also differences were found, after a period of unsupervised walking training, in values of oxyhemoglobin only in patients. The same study also found an increase in oxygen extraction ability at rest, especially in those subjects who didn't increased values of oxyhemoglobin.

Our results show similar increase in the values of oxyhemoglobin by treadmill test, that probably indicates an increase of muscle vascularization. Our data also don't show any significant increase in total hemoglobin, whose values don't seem to be modified by the pathology. Only in the strength group there was an increase of the values of deoxyhemoglobin, which correspond to an increase in muscle oxygen extraction ability, this should be linked to the increase in pain free walking distance.

This effect, given the simultaneous increase in oxygen availability, seems to be a direct consequence of resistance training, and we assume a better muscle metabolic and microcirculatory adaptation.

One study[84] reported a reduction of values of VO_2 after an unsupervised walking training period, but at submaximal levels, indeed there doesn't seem to be any difference at maximal levels.

Also our results at total walking distance don't show differences in the standard group, we found indeed a reduction of values of VO_2 in the strength group. The reduction of oxygen consumption at the end of the test indicates an improvement in walking economy. This could be the result of the delayed onset of pain in this

group, which may in turn imply a gait closer to normality, with less energy expenditure.
The respiratory quotient at the end of training period increased in both groups, in concordance with literature. This indicates the ability of subjects to achieve a higher stress level.

Data present in litterature[84] don't report differences between the values of flow volume at rest and post exercise. Our results are in agreement in the absence of differences in the standard group, whereas we found differences in the response following exercise in the strength group, possible consequence of increased vascular reactivity, probably due to the improvement of endothelial function of vasodilatation and recruitment of microcirculatory units. The data is particularly significant with respect to the ability to generate hyperemia in the course of exercise, in order to meet the increased metabolic demands with strictly controlled vascular adaptations.

Data present in litterature[84] about lipid profile don't report differences subsequent to standard training for total cholesterol and triglycerides, instead they found an increase in the values of HDL. Similarly, our data don't report changes in the standard group in the values of total cholesterol and triglycerides, whereas both significantly decreased in the strength group. This indicates an improvement in lipid profile and cardiovascular risk reduction. Instead our results didn't find any difference in either group for HDL cholesterol.
One study[85] found an increase in the levels of homocysteine, which indicates an inflammatory condition, following walking training and not following combined training. In our study, however, we found no significant differences in homocysteine levels for either group.

As a direct result of strength training we found an increase in maximal strength values in all muscle groups subjected to training. This is useful to contrast the loss of strength and muscle mass, due to aging and physical inactivity related to the disease, with positive effects on reducing the fall risk.

An high variability in the gait cycle parameter was linked to neuromotor control disorders, and is associated with an elevated fall risk[86,87,88]. Artheriopathic subjects showed greater variability than healthy subjects, even walking without pain[89]. Few studies handled the effects of training on gait parameters[59], and no differences were found.

Our data show an increase in variability in the end of the entry test, with the onset of symptoms, this lets us assume a deterioration in the gait mechanics in the presence of pain. The same differences are not observed after training. We hypothesize that patients adapted to the exercise, by adopting a correct gait even in the presence of symptoms. This may lead to a better walking economy and could have a role in increasing total walking distance. The same differences are not found in the initial part of the test, which lets us exclude a simple learning effect. The difference between our results and the data of the literature, may be due to different methods used, or to the fact that our analysis was performed specifically on a range affected by claudication, and not on the total walk.

Worst performances in the timed up and go test were associated with more severe stages of PAD[90], and in the elderly higher executions times are associated with a higher fall risk[72]. In our study, the significant reduction of the execution time in the strength group indicates an improvement in dynamic balance and a decrease in the fall risk.

The alternate step test is utilized in the general elderly population to identify subjects whit high fall risk[73], with more elevated execution times corresponding to worse performances. The data of the strength group show a significant reduction, going from higher to lower values than the threshold that discriminates subjects at high fall risk from general population.

In subjects with PAD with an ABI severely decreased[91] it was demonstrated a low speed in the 4 meters walking test. The increased performance observed in both groups subsequent to training confirms the improvements noted in walking autonomy. In contrast, the overall scores of SPPB scale, of which this test is part, increased more in the strength group, showing an improvement in the static and dy-

namic balance, as suggested by the reduction of execution times of 5 time sit to stand test.

The area of the ellipse, described by CoP during the different positions of the balance test, shows a significant decrease in the strength group in the closed eye condition and similar, but not significant, results in the tandem condition. There is no difference in any of the two groups in the Romberg's open eye condition. The decrease in the area of the ellipse in the closed eye condition suggests an improvement in static balance, which is usually decreased in PAD subjects[91]. The lack of differences in the initial conditions can be explained by the low difficulty of the test.

Similar conclusions can be drawn from the results of the unipedal stance test, in which the data of the strength group passes from lower to higher value than the threshold that discriminates individuals at fall risk against the general population.

From the results of tests that measure static and dynamic balance (timed up and go, alternate step, scale SPPB, unipedal stance, Romberg), improvements in the strength group emerge, which support the hypothesis of reducing fall risk.

A study[92] demonstrated that artheriopathic subjects every day make fewer steps and spend less time walking respect to healthy controls. Our data about daily activity show an increase in the number of daily steps made in the strength group while there are no significant differences in the duration of physical activity, including all periods of moderate or superior intensity activity, in any group. Previous studies[48], similarly, found no difference in the duration of physical activity after a training period of 12 weeks. Instead increases were found after a 6 months period[47]. This results indicate the poor ability of artheriopathic subjects to tolerate long periods of activity also after training. The increase in the number of steps demonstrate how, reducing claudication, patients were stimulated to have a more active life style, which is known to affects life expectations of this subjects.

The scores of SF-36 questionnaire about physical activity and physical role limitations increased in both groups but differences aren't significant. This is surprising because of the positive results obtained in the most part of the parameters.

We hypothesize that the lack of results is not due to poor perception of improvements by patients, but to the fact that the questionnaire is not specific for the pa-

thology and scores of the majority of the subjects were already in line with the average of the population by age group.

4.1. Limits of the study

This study presents some limits. The main is the reduced number of patients involved. Then there is the absence of deep analysis on bio humoral parameters and, lastly, the fact that it is a non-randomized study.

5. CONCLUSIONS

In patients affected by PAD with intermittent claudication both combined and standard training demonstrated to be effective in increasing the principal autonomy parameter : total distance walking. Combined training demonstrated to be more effective than the standard training to increase pain free walking distance. Moreover, combined training increased hyperemia of the limb during physical activity, as well as improved important parameters of cardiorespiratory performance (oxygen consumption, heart rate, blood pressure, oxygen extraction). We also have documented the ability of combined training to increase muscle strength and balance, reducing the fall risk. We finally observed the transfer in the everyday life of positive effects, with a less sedentary life style. Therefore combined training could be utilized in patients with IC to improve not only performances but also strength and equilibrium.

6. PARALLEL STUDIES

Before the start of the main study we performed a preliminary study on the effects of resistance training on the skin microcirculation.

Parallel to the main study, we performed a detailed study of platelet activation.

We also performed a study on the effects of exercise in patients with systemic sclerosis.

6.1. Effects of acute resistance exercise on microcirculation flux at lower limbs

Microcirculation is essential for adequate blood supply and oxygenation to tissues, in particular when exposed to ischaemia. Optimal microvascular reactivity in response to ischaemia is crucial to limit the extent of tissue injury. Strong associations between endothelial dysfunction in the peripheral circulation and both atherosclerotic risk and future cardiovascular events have been described[124].

The important role of microcirculation in the pathophysiology and symptoms of PAD has been progressively emphasized during the past twenty years, thanks to the use of different non-invasive methods, such as capillaroscopy, laser Doppler (LD) flowmetry and transcutaneous measurement of oxygen tension (tcPO2)[117].

Even though Laser Doppler studies showed a normal baseline leg skin perfusion in stage II PAD patients, impaired post ischemic hyperemic reaction, an abnormal flow motion pattern, and impeded vascular reactivity have been shown[118].

Cutaneous circulation seems to be an interesting witness of hemodynamic modification acting in lower limb also during muscles activation[112,113,114,115] so we proposed a test consisting in a series of elevations on the forefoot repeated in a rapid sequence, standing with similar work amount compared to a sub-maximal exercise on treadmill as used in training protocol.

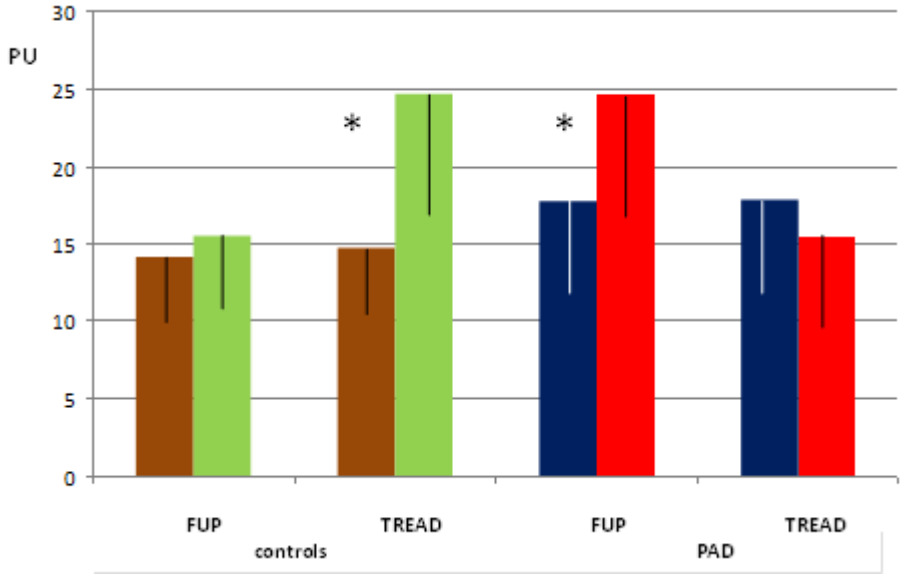
The aim is to evaluate the effects on cutaneous microcirculation, of a single bout of moderate strength exercise in PAD patients compared with aerobic treadmill exercise and controlled with healthy subjects.

We enrolled 14 patients, with PAD at Fontaine stage II (pain free walking distance 130 ± 45 m - ABI 0,75 ± 11), aged 73 ± 4 y, no diabetic/neuropathic pa-

tients were allowed; all subjects had a superficial artery occlusion in the symptomatic leg with concomitant 1 or 2 tibial vessels occlusion. They underwent a series of up-standing on forefoot (FUP), successively they performed treadmill exercise (3,2 Km/h, 0% slope) to 70% of maximal walking distance; the two exercise were comparable for energy consumption calculated (on treadmill= 0,50 x km x kg of body weight, on heel elevation = kg of body weight x m heel elevation x n° of elevation - respectively 61 ± 22 kcal and 55 ± 11 kcal, p=0,73); 10 healthy matched subjects performed FUP treadmill based on the same energy expenditure. We analyzed microcirculatory flux by means of Laser Doppler flowmetry with probe placed on forefoot of symptomatic leg in patients and on left leg in healthy volunteer; measurements were done at rest and after 1 minute after tests end and with a 2 minutes monitoring. Data were analyzed with Student's t test for paired data.

FUP caused a significant increase in microcirculatory flux in the recovery period in PAD patients (*p<0,05 vs rest – graph 53) while it caused no significant changes in healthy; treadmill caused a slight decrease in flux at PAD patient's forefoot, while it caused an increase in healthy (*p<0,05 vs rest- graph 53).

Our study has demonstrated that strength exercise in PAD patients with claudication causes an increase of cutaneous flux, while no significant changes in healthy. Treadmill causes a decrease (not significant) in the period of monitoring as reported in literature in PAD subjects. In healthy volunteers an increased flux after treadmill may be caused by hyperemia in the leg, elicited by exercise with a preserved inflow and a correct distribution in tissues[115,116]; a component of thermoregulatory vasodilation may be evoked. FUP in controls did not produce a relevant phenomenon perhaps for sympathetic tone prevalence and a reduced overall hyperemia. In PAD patients the increase after FUP may be due to a more pronounced ischemia and a more enhanced and concentrated vasodilation in a distal segment with sympathetic blunting and a more pronounced post exercise hyperemia due to a compensatory vasodilation, involving also cutaneous district. This data may enforce the proposal for dedicated moderate-strength exercises for distal muscles groups in PAD patients.



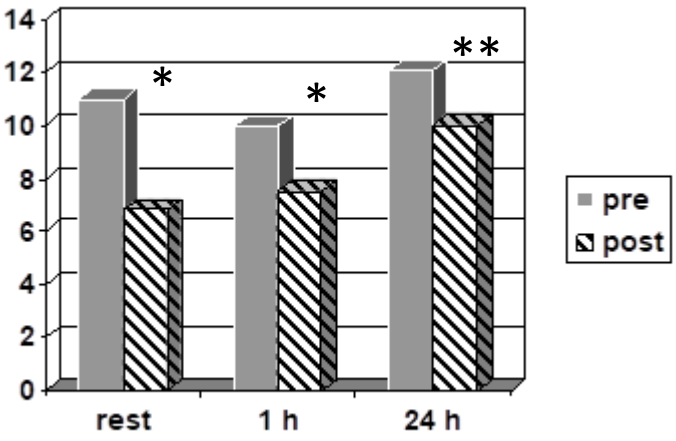
Graph 53: microcirculatory flux, * p<0,05 vs rest.

6.2. Effects of training on reticulated platelets and red blood cells phragments

Platelet activation plays a pivotal role in atherosclerosis progression and cardiovascular events. Reticulated platelets (IPF) are newly-formed platelets with high granule content and a residual amount of megakaryocyte-derived mRNA. Detection of these platelets in the circulating blood reflects the increased platelet production from megakaryocytes in the bone marrow and hence the rate of platelet turnover[125]. IPF recently have been associated to cardiovascular complications and atherosclerosis with unstable conditions (e.g. acute coronary syndrome).
Red blood cell distribution width (RDW) is a measure of red blood cell volume variations (anisocytosis) and is reported as part of a standard complete blood count. RDW is defined as the quotient of standard deviation of red blood cell volume and its mean volume and is expressed as a percentage according to the following formula: RDW = (standard deviation of red blood cell volume/mean cell volume) × 100. Higher RDW values reflect greater variations in red blood cell volume[127]. Presence of a RDW is considered recently as a prognostic factor for coronary artery disease.
A high RDW value depends greatly on presence of red blood cells fragmentation (FRC); this parameter may depend on different conditions such as inflammation, and oxidation and is connected with different risk factors such as hypertension and diabetes. Few data can be found for patients with peripheral arterial disease on training. We aimed to evaluate the effects of aerobic training on IPF and FRC at rest and after maximal walking exercise before and after training.

We enrolled 12 patients with intermittent claudication. They were submitted to a 15 days aerobic training period (cycling and treadmill exercise under maximal walking capacity). IPF, MPV, PLT count and FRC were analyzed at rest, 1 hour after maximal treadmill test and after 24 hours, these evaluations were performed at the beginning and at the end of the training period. The Lab parameters were analyzed with impedentiometry, fluorimetry (oxazyne) and optical methods (Sysmex Xn-1000, Sysmex Corporation, Kobe, Japan). Walking distance was measured with treadmill (3,2 km/h, 2-10% slope), maximal test was prolonged to the maximal tolerated claudication pain.

Platelets count was within normal range (216,9 ± 40 109/l) and did not change throughout the study; also MPV was unchanged (11,6 ± 1,9 vs 11.45 ± 0,8 fl) before and after the training; plateletcrit was slightly reduced (0,246 ± 0,061 vs 0,282 ± 0,018 %). IPF count (graph 54) slightly changed during maximal stress at the beginning of training with increase after 24 hours; after training the count decreased significantly (*p<0,05) at rest and 1 hour after, while it increased significantly after 24 hours (**p<0,05 vs rest ad vs 24 h-pre) but less than before training.



Graph 54: IPF count *pre-post p<0,05, ** p<0,05 vs rest ad vs 24 h-pre.

FRC decreased after training (0,381 ± 0,121 vs 0,542 ± 0,220 %; p<0,05), maximal test slightly increased FRC after 1 hour, no significant change after 24 hours.

At the end of training, absolute walking distance increased (450 ± 180 vs 250 ± 108 m; p<0,05).

Training reduces IPF in patients with peripheral arterial disease, IPF increase after acute maximal test and this phenomenon can be attenuated by training. We also observed a reduction in FRC. Presence of FRC in these patients may be caused by mechanical forces throughout a large surface of atherosclerotic plaques fragmenting red cells, ischemia reperfusion in claudication is another mechanism that can

elicit formation of FRC and in addition high oxidative stress may contribute. IPF are associated with an increase platelets activity and a higher turnover; in this pathology both these condition can be found associated with oxidative stress, inflammation and endothelial dysfunction. Training improves oxidation, inflammation and endothelium function with favorable effects on platelets activation and turnover, furthermore these parameters may influence also FRC count.

Training in PAD patients reduces IPF and FRC with potential improvement in risk profile for atherosclerosis progression and reduction of cardiovascular events.

6.3. Exercise and hypertone blunting in Systemic sclerosis

Systemic sclerosis (SSc) is a multi-organ system disease characterized by activation of immune cells, production of auto-antibodies, vasculopathy, and fibrosis. Although it is heterogeneous in extent of organ involvement and prognosis, it is accepted that SSc has a progressive and most often a devastating course.

One of the first symptoms in the course of systemic sclerosis includes lesions in blood vessels of skin and internal organs. Lesions occurring in skin mcrocirculation manifest clinically as Raynaud's phenomenon (RP) that consists of reversible contraction of microcirculatory vessels in response to cold or emotional stress. It represents a perturbation of digital resistance artery vascular reactivity likely due to alterations in the balance between vasoconstrictor and vasodilator signaling. Frequent relapses of Raynaud's phenomenon result in interrupted blood inflow to tissues and, consequently, their ischaemia involving formation of necrosis and painful trophic ulcers on finger and toe pulps. In more advanced cases it may result in the lysis of distal phalanxes.

Systemic sclerosis can be divided into two basic categories: limited systemic sclerosis or acrosclerosis (lSSc), where skin hardening lesions do not exceed 1/3 of the forearm length and occur also on the face and diffuse systemic sclerosis (dSSc) with generalised hardening[135].

Vascular alterations are important features in pathogenesis of SSc being consequence of vascular damage induced by inflammation and promoting tissue damage through an ischemic/hypoxic recurrence that enhances fibrotic modifications.

In SSc, RP-related impairment of tissue perfusion leads to tissue hypoxia, endothelial cell (EC) damage and dysfunction, and the promotion of vascular leak, immune activation, and fibrosis that are important for dictating the pace of vasculopathy. Importantly, EC damage with apoptosis coupled to insufficient compensatory repair results in the pathognomonic end-stage vascular abnormalities. Over time, this abnormal perpetual perfusion results in both functional and structural disease[135].

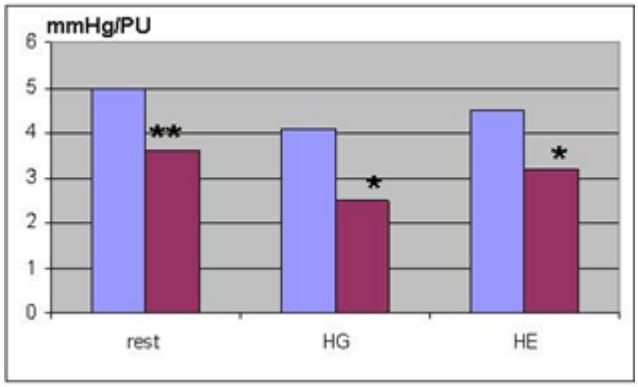
The decrease of tissue perfusion due to a progressive capillary reduction must be accompanied and anticipated by significant and progressive alterations of perfusion “quality”. Several authors have demonstrated a progressive decrease in endo-

thelial dependent dilation and reduction in hyperemic function[134,137,138]. Reactive hyperemia, an increase in peripheral blood flow due to a brief ischemic bout, has classically been utilized as a marker of peripheral resistance artery vasoreactivity and is predictive of future CVD events. It is dependent on a multitude of vasoactive factors which include, but are not limited to, endothelial-derived nitric oxide and prostaglandins, tissue-released adenosine, and myogenic tone.

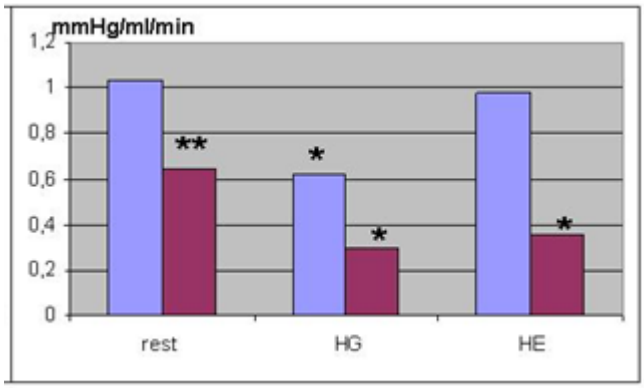
We hypothesize that also vasodilation due to exercise and sympathetic stimulation may be blunted in these patients with a progressive failure in adaptation to metabolic requests from tissues.
The aim of this study is to evaluate the effects on hand cutaneous microcirculation of ischemia, handgrip and exercise in Ssc patients.

We enrolled 12 adult, aged 61 ± 6 y, non-obese, non-smoker, non-diabetic, and non-hypertensive women, who fulfilled the American College of Rheumatology criteria for the diagnosis of Ssc. Mean time from diagnosis was 11,5 ± 4,2 y. They were all without ulcers; 2/12 had previous digital ulcers healed. Latest iloprost infusion was at least 28 days before the exams (28-32 days); no pulmonary hypertension signs were detected by means of echocardiography; no vasoactive drugs were assumed the day of exams. No obstructive pathology at upper limb was detected using color-Doppler ultrasound, while all had thickening of radial and ulnar vessels with rare dotted calcifications. 6 healthy, age matched subjects were enrolled. We analyzed microcirculatory flux by means of Laser Doppler flowmetry (LD) with probe placed on volar face of the right hand; flow at humeral artery was measured with ultrasound.
Blood pressure was measured with oscillometric device on the same arm at the end of each stimulation. Resistances were calculated as mean pressure/flow. Post ischemic hyperemia was evaluated after 3 minutes ischemia obtained with cuff placed on brachial artery and inflated 20 mmHg over systolic value; hand grip (HG) was determined with fist clutching on cuff with exerted pressure 70% of maximum for 3 minutes on the other hand; hand exertion (HE) was done with repeated near-maximal fist clutching for 3 minutes (at least 1 /sec). Data were analyzed with Student's t test for paired data.

LD flow at rest was slightly lower in Ssc (19,7 ± 9,2 vs 25,4 ± 11,7 PU ns); post ischemic hyperemia showed a lower microcirculatory peak flow with LD in Ssc patients (41,5 ± 8,6 vs 65 ± 11,3% increase p<0,05); HG determined an increase in LD flow only in healthy (p<0,05), while a slight decrease in Ssc; HE showed an increase in LD flow only in healthy (p<0,05). Microcirculatory resistances were increased in Ssc patients and slightly reduced only by HG; in healthy they were reduced both by HG and HE. Forearm resistances were significantly lower at rest in healthy (0,64 ± 0,02 vs 1,03 ± 0,06 mmHg/ml/min p<0,05 – graph 4), they were reduced by HG in both groups (p<0,05), while HE reduced them only in healthy (p<0,05);



Graph 55: microcirculatory resistances *p<0,05 vs correspondent rest; **p<0,05 vs SSc patients.



Graph 56: forearm resistances *p<0,05 vs correspondent rest; **p<0,05 vs SSc patients.

Our study demonstrates that SSc patient have impairment in microcirculatory flux and increased micro/macro-circulatory resistances at rest. Post ischemic hyperemia and the answer to exertion is impaired in SSc as well. HG can reduce resistances at the forearm also in SSc patients with a slight increase in microcirculatory flux. These data show a reduced adaptation SSc flow regulation when increase in requests occur. HG evokes a noradrenergic stimulation with a better response in Ssc that probably is due to abrupt blunting after sustained stimulation over a chronic hyperactivity, we can also hypothesize positive effect of noradrenaline in reactive vasodilation post-exertion, still working in SSc. Furthermore HG hyperemia is sustained also by NO production by flow stimulation of endothelial cells. As a conclusion we can hypothesize that HG exertion may be useful in SSc patients for improving forearm perfusion.

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