Calf Raise Exercise Increases Walking Performance in Patients With Intermittent Claudication

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ABSTRACT

Background/Objective: Symptoms of intermittent claudication (IC) are improved by exercise. The improvement might be secondary to increased blood perfusion or increased muscular mitochondrial capacity. Ischemia followed by reperfusion, also named preconditioning, is known to stimulate the mitochondria. We focused on a calf raise exercise inducing preconditioning in the calf muscle of patients with IC. We hypothesized that eight weeks with this exercise would increase walking performance and mitochondrial capacity without a change in blood flow.

Methods: Patients with IC were randomized to either a calf raise exercise (n=14) or a traditional walking exercise group (n=15) The calf raise group was instructed to perform a specific type of

calf raise exercise three times a day. The walking group was instructed to walk near the pain threshold at least 30 minutes three times a week. Both interventions lasted eight weeks and were not supervised. Measurements of walking performance, mitochondrial capacity, peak oxygen uptake, peripheral hemodynamics and health-related quality of life were obtained on each patient before and after the intervention period. Adherence was measured by a training diary and an activity monitor was used.

Results: The calf raise group improved pain free walking distance by 44 meters (P=.04) and maximal walking distance by 99 meters (P=.047). Furthermore, claudication onset time increased by 123 seconds (P=.02) and peak walking time by 104 seconds (P=.01). The calf raise group increased the enzyme citrate synthase activity, which is a biomarker of mitochondrial volume-density in the muscle tissue (P=.02). The walking group did not increase any of these variables. Maximal blood flow, peak oxygen uptake and mitochondrial respiration did not change in either groups. The calf raise group experienced less disease anxiety (P<.01). Adherence to the instruction of exercise was 100% in the calf raise group and 80% in the walking group. The calf raise group maintained physical activity. A reduction in activity (P<.01) was found in the walking group.

Conclusion:Calf raise exercise improves walking performance and increases mitochondrial volume-density in the gastrocnemius muscle without increasing blood flow in patients with intermittent claudication.

Introduction:

Patients with intermittent claudication (IC) experience muscle ischemia during walking that is felt as cramping and aching in the affected muscle.[1] The prevalence of IC increases with age and

the number of patients with IC can be expected to increase further as the population of Europe and North America is aging. [2,3] Thereby, IC will lead to an increased burden for healthcare systems in the years to come. Patients with IC can benefit from exercise training, besides medical treatment, smoking cessation and interventions. [4] Supervised exercise programs are efficient, but the number of patients with IC makes it difficult to offer this type of training to everybody. [5] Structured home-based exercise programs also improve walking performance, but are less efficient. [6,7] To meet future demands, efficient and manageable home-based exercise programs are needed.

A hallmark of IC is ischemia due to reduced blood flow. Research also supports that patients with IC have reduced enzyme activity and respiratory defects in the muscle mitochondria. [8-10] Patients with IC seem to increase mitochondrial capacity after exercise training without increasing blood flow. [11,12] Exercise training till onset of ischemia is recommended for maximal effect in these patients. [1,13] Such training-induced ischemia followed by reperfusion, provokes preconditioning, which improves mitochondrial capacity both in skeletal muscle and heart muscle. [14,15]

The aim of the present study was therefore to test whether exposure to a single episode of instruction for daily training without supervision using preconditioning could improve walking performance and mitochondrial capacity in patients with IC, without increasing blood flow.

Methods

Patient screening. A total of twenty-nine PAD patients limited by intermittent claudication were enrolled into this study. The patients were recruited between February 2015 and January 2016 at the Department of Vascular Surgery, St Olavs University Hospital, Trondheim, Norway. All experimental protocols and procedures were approved by the regional committee of medical and health research ethics, central Norway (nr. 2011/2533) and conformed to the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was registered in ClinicalTrials.gov (ID: NCT 023110256).

All patients had a physical examination and medical history during baseline visit and were classified by the following criteria: (1) a history of intermittent claudication, (2) exercise tolerance limited by intermittent claudication during a screening treadmill test, (3) an ankle brachial index (ABI) at rest above 0.4 and below 0.9, (4) No age restrictions were applied and (5) vascular interventions over three months independent of indication (also critical limb ischemia) but resulting in stable intermittent claudication symptoms, which were of no hindrance. Patients were excluded from this study having (1) absence of PAD, (2) asymptomatic PAD, (3) critical limb ischemia; defined as those with ABI below 0.4 combined with rest pain or ischemic ulcer, (4) exercise tolerance limited by factors other than claudication (eg, coronary artery disease, dyspnea), (5) vascular interventions in the last three months, (6) usage of antiplatelet drug other than acetylsalicylic acid (eg, Plavix, Persantine), (7) usage of anticoagulants (eg, Warfarin), (8) diabetes mellitus, (9) active cancer and (10) renal- or liver disease which needed treatment or follow up.

Procedures. Patients were evaluated during study visits at baseline and after eight weeks of the exercise intervention. During each visit, patients completed tests in the following order:

(1) physical examination including a review of current medication (2) questionnaires on quality of life and physical function; (3) collection of muscle biopsies (4) exercise and physical function test; (5) peripheral hemodynamics tests. During the baseline visit it was determined whether patients could comply with the exercise interventions and perform cardiopulmonary testing.

At the end of the baseline visit participants were randomized (Figure 1) between a calf raise exercise group (n=14) or a walking exercise group (n=15). An internet-based randomization database using a 1:1 allocation, offered by the Unit for Applied Clinical Research at our Medical Faculty was used. Assessors were blinded to the allocation of each participant.

There were no complications as a result of the exercise interventions. Two patients were excluded from analysis in the walking group; one patient suffered of a partial gastrocnemius muscle rupture on the day after the baseline testing and one patient developed critical ischemia and needed surgery.

Exercise interventions. The calf raise exercise group was instructed to perform calf raise exercise three times a day. Calf raise exercise consisted of the subject standing in front of a wall, which was used for support of the balance. The body was lifted using the calve muscles to the maximal height that the subject could achieve. This was repeated until pain was felt in the calf musculature. Following initiation of pain, the subject was instructed to perform five extra calf raises. The five extra calf raises secured ischemia followed by reperfusion of the muscle at rest. Thereby establishing a preconditioning situation. The exercise training was entitled “Five plus”.

The walking exercise group was instructed to walk near the pain threshold for 30 minutes, three times a week.

Both training regimens were home-based and without supervision for eight weeks.

No instructions were given on risk factor management or lifestyle modification to any of the two groups. It is normal practice at the vascular clinic that instruction in exercise is only provided once to patients, and it was the intent of the current study to reproduce this practice. We were mainly interest in the improvements after one single instruction of exercise and not the effect of different types of exercise. The latter would have needed supervision to guarantee adherence.

Adherence

An activity monitor (Garmin Vivofit) was placed on the arm, which was worn for the whole study period. This was done to monitor compliance. Furthermore, a training diary was given to the participants. The calf raise group registered the amount of calf raises performed during each session. The walking group registered the time and content of each activity. Participants in both groups registered each evening the total counts of activity registered by the monitor. Comparison of the average counts of activity per day in the first week was compared to the average count of the last week. Adherence was based on whether participants followed the instruction of exercise.

Measurements

Walking performance and oxygen uptake

6-minute walk test. A standardised protocol was followed. [16] The self-paced six-minute walk test assessed the pain free walking distance as the distance at which participants first reported pain and the distance covered during six minutes.

Maximal walking distance was the distance at which participants stopped because of claudication pain.

Cardio Pulmonary Exercise Testing (CPET). A treadmill (Woodway, USA) with a graded Gardner-Skinner protocol consisting of a constant speed of 3.2 $km \cdot h^{-1}$ was used for

cardio pulmonary testing together with the device METAMAX II and the METASOFT software (Cortex, Germany). [17] The inclination started at 0%, and increased by 2% every second minute until the end of the test. Peak oxygen uptake (VO_{2peak} = ml /kg/min) was determined as being the highest value obtained.

Claudication onset time was the onset of claudication pain and peak walking time was the time at which participants stopped the test, during the tests on the treadmill.

Peripheral hemodynamics

Ankle Brachial Index. Ankle brachial index, an evaluation of blood pressures in the arm and the ankle was performed at rest. [18]

Plethysmography: Plethysmograhy assessments were conducted as previously described. [19] The blood flow in the lower extremity was assessed with a strain-gauge plethysmography (Hokanson A16 Inc, Bellevue).

To assess maximal hyperemic response to ischemia (ml/100ml/min) to the calf the thigh cuff was inflated to 220 mmHg for five minutes, resulting in total arterial and venous occlusion of the leg. During the last minute of arterial occlusion, the ankle cuff was, as above, inflated to 250 mmHg. The thigh cuff was deflated to initiate reactive hyperaemia. A series of ten to 15 measurements was performed during which each measurement the thigh cuff was again inflated for ten seconds at 40 mmHg to block the venous reflux. Thereby the arterial inflow to the calves was measured at rest and as a maximal response in the hyperaemic phase.

Mitochondrial function

Muscle biopsy. Biopsies were collected from the lateral part of the gastrocnemius muscle. A micro biopsy technique was conducted to obtain muscle tissue as previously described. [20 21]

Permeabilized skeletal muscle fiber preparation. The muscle tissue was treated, in order1 to permeabilize the extracellular membranes of the muscle fibers leaving intracellular membranes of the mitochondria intact. [21,22] The wet weight of muscle fibers (1-3mg) was measured on a microbalance (Sartorius ME235P-SD; Sartorius AG, Goettingen, Germany) immediately before assessment of mitochondrial respiration.

Mitochondrial respirometry. The muscle fibers were analyzed by high-resolution respirometry measurements (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria). The oxygen concentration and oxygen consumption were continuously recorded in the chamber. Oxygen consumption per second, per milligram of wet weight of muscle fibers was addressed as mitochondrial respiration (pmol O_2 / s / mg wet weight of muscle fibers). Measurements were performed at 37°C. All experiments were carried out in hyper-oxygenated chambers (250-500 uM oxygen).. The Substrate, Uncoupler and Inhibitor Titration (SUIT) protocol was used to examine different branches of the electron transfer system as previously described. [21-23] This was achieved by adding inductive or blocking substrates to the chamber. All respirometric analyses were made in duplicates. The LEAK state (presented with subscript $_L$) represents the resting mitochondrial respiration of an unaltered and intact electron transport system in the absence of ADP. The LEAK state is measured from the electron flow through complex I (CI) and electron transferring flavoprotein (ETF). Convergent electron flow into the Q-junction from both complex I (CI) and ETF(ETF+CI)$_L$.was induced with the addition of octanoylcarnitine (0.2 mM) and malate (2 mM)

OXPHOS state (presented with subscript $_P$) represents maximal electron flow through the electron transfer system in the presence of ADP. Electron transferring-flavoprotein capacity $(ETF+CI)_P$ was determined following the addition of ADP (5 mM). Mitochondrial respiration specific to complex I $(CI+ETF)_P$ was induced by the addition of glutamate (10 mM). Respiration supported by complex I and complex II $(CI+CII+ETF)_P$, was then induced with the addition of succinate (10 mM). $(CI+CII+ETF)_P$.[22]

Electron transfer system (presented with subscript $_E$) state represents the electron transport through the electron transfer system, when it is uncoupled from ATPase (complex V). Electron transfer system capacity $(CI+CII+ETF)_E$ was assessed through titration of the proton ionophore, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP: 0.5 M stepwise titration to optimum concentrations ranging from 1.5 to 3 M). Rotenone (0.5 M) was added to inhibit complex I, thereby electron flow specific to complex II $(CII)_E$ can be measured. Finally, malonic acid (5mM) and antimycin A (2.5 M) were added.

Citrate synthase activity. (mitochondrial content) Citrate synthase activity is a biomarker that is representative for the total concentration / volume-density of the mitochondria present in the muscle tissue. [24] Citrate synthase activity was assayed as previously described in homogenates of the permeabilized fibers used in the respiration measurements. [21,23]

Health related Quality of life.

Short Health Form 36 Survey (SF36): Self-reported physical function was assessed with SF 36: consisting of the eight subscales: physical function, physical role, bodily pain, general health, vitality, social function, emotional role and mental health.

Claudication Scale (CLAU-S): Self-reported walking ability, specific for patients with intermittent claudication was assessed with CLAU-S: consisting of the five subscales: daily life, pain, social life, disease-specific anxiety and psychological wellbeing.

The scores of the SF-36 and CLAU-S were notified separately. Both SF-36 and CLAU-S are validated instruments. [25,26]

Statistical analyses. The primary analysis was based on an intention to treat basis. Primary endpoints were maximal walking distance/peak walking time and mitochondrial respiration supported by electron transfer flavoprotein and complex I. Secondary endpoints were citrate synthase activity, maximal hyperemic response to ischemia, peak oxygen uptake and quality of life (CLAU-S and SF36).

The sample size was based on maximal walking distance/peak walking time data from previous studies [6,13] and unpublished data, from our group, on improvements in mitochondrial respiration after exercise in older adults. A two-sided significance level of .05 and a power of the test of 80% indicated a need of 14 participants to be included in each group.

The primary objective of this study was to compare changes within each group (pre versus post). Comparisons within the groups before and after exercise were done using paired t-test. The randomization procedure was used to secure similar baseline characteristics. Continuous data were reported as the mean with standard deviation or as the mean with the standard error of the mean. Significance was considered at P value < .05. All analyses were performed using STATA 13th edition (StataCorp LP, Texas, USA).

Results

Demographics

The randomization resulted in similar baseline clinical characteristics between the calf raise group and the walking group (Table I) except for that the calf raise group was heavier than the walking group.

Walking performance

The groups were similar at baseline on each measure of walking performance except for that the calf raise group had a shorter pain free walking distance. The calf raise group increased (Table II) pain free walking distance (P=.04), maximal walking distance (P=.047), claudication onset time (P=.02) and peak walking time (P =.01). No changes in walking performance were found in the walking group.

Adherence

Adherence to the instruction of exercise was 100% in the calf raise group and 80% in the walking group. The calf raise group maintained physical activity. A reduction in activity (P<.01) was found in the walking group. (Table II)

Oxygen uptake and peripheral hemodynamics

The groups were similar (Table III) at baseline on oxygen uptake, ankle brachial index and maximal hyperemic response to ischemia. Neither group had any significant change in these measures.

Mitochondrial function

The groups were similar at baseline on each measure of mitochondrial function. Mitochondrial respiration did not change within any group (Table IV). Citrate synthase activity increased (P=.02) significantly within the calf raise group.

Health related Quality of life.

The groups were similar at baseline on each measure of quality of life (Table V and VI). The calf raise group increased the subscale `disease anxiety` (P<.01), meaning that they experienced less anxiety.

Discussion

Calf raise exercise with five extra repetitions, entitled “Five plus”, over a period of eight weeks improved walking performance, measured as maximal walking distance and peak walking time on a treadmill. Calf raise exercise increased citrate synthase activity, a biomarker of muscle mitochondrial volume-density, without changing mitochondrial respiration and maximal hyperemic response to ischemia in patients with intermittent claudication. No significant changes in walking performance, mitochondrial respiration, volume-density and blood flow were observed in the walking group.

Up to 20% of patients with peripheral arterial disease experience symptoms of intermittent claudication (IC). [2] The reduced walking performance in these patients results in reduced quality of life. [26] Patients with IC have a 2.5-fold increase in cardiovascular morbidity and mortality compared with an age-matched population. [1] Exercise training has shown to improve walking performance in patients with IC and to reduce cardiac risk and mortality. [13,27]

Introduction of supervised training programs are lacking in Europe and North America, even though supervised exercise is cost-effective. [27-29] The programs seem not to be feasible due to the number of individuals having IC. Some home-based exercise programs have shown to increase walking performance while others do not. [6,7,25] Walking advice alone has been compared with home-based structured exercise, and the latter has shown better results. [6,7] We propose a new home-based calf raise exercise training focusing on the metabolism in the leg muscles of interest, thereby excluding classical training goals as cardio-pulmonary function, but trying to improve the mitochondrial metabolism, through preconditioning. The training concept is easy to perform and self-regulatory since the instruction to perform five extra calf raises after debut of pain will remain unchanged with any improvement. It is of value that an exercise program without supervision is followed. Other home-based exercise programs showed an adherence of approximately 80%. [6,7] In contrary to this all the participants in the calf raise group adhered to the given instruction. Furthermore, the average age was higher and the ankle brachial index was lower in our calf raise group compared to the home-based studies. This supports that our calf raise exercise has an impact also in older individuals and in those that have a more severe PAD. But this study is not able to clarify whether the response differs in different age groups and different degrees of claudication intermittens.

The calf raise group had similar improvements in claudication onset time and peak walking time after eight weeks as the other home-based programs. [6,7] Claudication onset time and peak walking time are assessed on a treadmill with increased inclination during testing. Strength training programs [30-32] have shown similar improvements in walking performance as measured by treadmill. One can speculate whether calf raises exercise strengthens the leg muscle and thereby increases walking performance at steeper grounds. The walking group did not increase

claudication onset time and peak walking time after eight weeks. The structured home based exercise programs consisted of walking exercise and showed increases in these variables. The difference to our walking group was that participants were contacted several times by the investigators in order to control whether they adhered to the walking exercise. The calf raise group did not increase activity which is in line with the other home-based studies.[6] The walking group decreased their activity, which shows that instruction of walking exercise alone is not sufficient to maintain activity and external control is needed.

The pathophysiology of intermittent claudication is mainly explained as a result of the reduced blood flow. A meta-analysis could however not find any increase in blood flow after exercise training. [33] This is in accordance to our observations with no significant changes in blood flow in either groups. In patients with IC, besides reduced blood flow, metabolic changes in the muscle mitochondria also occur expressed as lower mitochondrial enzyme activity. [8,9,34] A more effective usage by the mitochondria of the oxygen that is delivered to the muscle might therefore be an alternative explanation to the improvement after exercise. [35] Exercise training of healthy elderly with normal blood flow increases muscle mitochondrial capacity. [36] Less is known about mitochondrial adaptations after exercise in patients with reduced blood flow.

We assessed mitochondrial respiration, being a functional assay of mitochondrial oxygen consumption of the complexes collectively during oxidative phosphorylation. This includes a direct analysis of complex I to IV. We did not find any increase in mitochondrial respiration. After dynamic leg exercise of patient with IC mitochondrial enzymes, that indirect reflect complex II (succinic oxidase activity) and complex IV function (cytochrome C oxidase) [11,12] have shown to increase. The only mitochondrial enzyme assessed in this study was citrate synthase activity, which was increased significantly in the calf raise group, but not in the walking

group. Increased citrate synthase activity after exercise of strength has previously been shown after exercise of strength in patients relatively and directly. [11,37,]Our calf raise could therefore be characterized as an exercise of strength, possibly resulting in increased mitochondrial content. Others did not find changes in citrate synthase after walking exercise. [38] Walking exercise focuses on endurance, thereby possibly leading to increased mitochondrial efficiency of the mitochondria present and not leading to increased mitochondrial content.

The six-minute walking distance did not change indicating that walking speed remained the same. Walking speed has been shown to correlate with mitochondrial ATP production. [36] The lack of change in mitochondrial respiration in our groups might mirror similar mitochondrial energy production per time unit resulting in unchanged walking speed. The increased mitochondrial content in the calf raise group might improve energy production over time resulting in an increased maximal walking distance and peak walking time at a similar walking speed. Further research is necessary to clarify the importance of endurance and strength exercise in patients with intermittent claudication and the resulting mitochondrial responses.

Regarding quality of life a significant change in disease specific anxiety was found in the calf raise group. This supports the psychological value of home-based, self-regulated training.

Limitations

This is a small study that requires replication in external sites with larger N and longer follow up to determine the external validity of the findings. The calf raise group was heavier at baseline and they had a lower pain free walking distance and claudication onset time. Thereby these patients might have been de-conditioned and have more to gain from exercise. Mitochondrial respiration was assessed under hyper-oxygenated conditions. Mitochondrial respiration assessed under hypoxic conditions might better represent the physiological adaptations that occur after an

exercise intervention. Diabetics were excluded because they differ in mitochondrial bioenergetics compared to non-diabetics and in relation to the degree of the disease which would have been an confounding factor. [39] The mitochondrial response to exercise might therefore differ in diabetics.

Conclusion

Calf raise exercise improves walking performance. The training increases a biomarker of mitochondrial volume-density but not in mitochondrial respiration or maximal hyperemic response to ischemia. “Five plus” may be an easy, home-based and efficient exercise training for patients with IC as an alternative to the usual advice to “stop smoking and keep walking”.

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Table I: Baseline clinical characteristics of patients with intermittent claudication who participated in a calf raise group and a walking group

	Calf raise group (n=14)		**Walking group (n=13)**	
	mean (SD)	range	mean (SD)	range
Female sex, n	7		6	
Age	66 (9.3)	46-86	70 (8.2)	52-77
Height	171 (6)	160-180	170 (9)	159-187
Weight	80 (11)	64-100	72 (11)	57-97
Ankle brachial index	0.58 (0.11)	0.41-0.77	0.57 (0.21)	0.32-0.93
Systolic blood pressure	143 (22)	115-180	145 (21)	120-190
Diastolic blood pressure	82 (13)	60-110	82 (6)	70-95
Drug therapy				
• Statin, n	8		8	
• Acetylsalicylic acid, n	9		10	
Comorbidity				
• Previous peripheral vascular intervention, n	4		6	

• Previous myocardial infarction/ intervention, n	2	2
• Current smoker, n	3	5
• Previous smoker, n	6	5

SD = standard deviation, n=number

Table II: Walking performance in patients with intermittent claudication who performed calf raise exercise (n=14) or walking exercise (n=13)

Variables	*Pre-test*	*Post-test*	*Change*	*P value*
	mean (SEM)	mean (SEM)	mean (SEM)	
Pain free walking distance (m)				
Calf raise group	177 (18)	221 (22)	44 (22)	.04
Walking group	305 (108)	352 (62)	46 (115)	.34
Maximal walking distance (m)				
Calf raise group	535 (39)	634 (85)	98 (54)	.047
Walking group	619 (68)	700 (145)	74 (108)	.25
6 minute walking distance (m)				
Calf raise group	466 (14)	480 (17)	15 (11)	.11
Walking group	452 (14)	462 (14)	10 (15)	.27
Claudication onset time (sec)				
Calf raise group	255 (45)	378 (61)	123 (51)	.02
Walking group	309 (31)	354 (59)	45 (48)	.19
Peak walking time (sec)				

Calf raise group	709 (98)	813 (113)	104 (39)	.01
Walking group	595 (64)	610 (66)	15 (43)	.37
Activity monitor (counts per day) [b]				
Calf raise group	9170 (974)	9567 (1029)	396 (458)	.21
Walking group	7238 (1278)	6271 (1174)	-967(299)	<.01

SEM = standard error of the mean

Table III: Peak oxygen uptake and peripheral hemodynamics in patients with intermittent claudication who performed calf raise exercise (n=14) or walking exercise (n=13)

Variables	***Pre-test***	***Post-test***	***Change***	***P value***
	mean (SEM)	mean (SEM)	mean (SEM)	
Peak Oxygen uptake (ml/kg/min)				
Calf raise group	18.7 (1.5)	19.0 (1.6)	0.4 (0.5)	.24
Walking group	18.1 (0.8)	17.9 (0.8)	- 0.2 (0.9)	.57
Ankle Brachial Index				
Calf raise group	0.58 (0.03)	0.58 (0.03)	0.00 (0.03)	.91
Walking group	0.57 (0.06)	0.57 (0.05)	0.00 (0.04)	.95
Maximal hyperemic response to ischemia (ml/100ml/min)				
Calf raise group	11.8 (1.2)	11.6 (1.4)	0.23 (0.4)	.29
Walking group	10.0 (1.3)	10.0 (1.4)	-0.01 (0.4)	.51

SEM = standard error of the mean

Table IV: Mitochondrial function: mitochondrial respiration (pmol/mg/s) and citrate synthase activity (μmol/min/mg of protein) in patients with intermittent claudication who performed calf raise exercise (n=14) or walking exercise (n=13)

Variables	*Pre-test*	*Post-test*	*Change*	*P value*
	mean (SEM)	mean (SEM)	mean (SEM)	
Respiration supported by $(ETF+CI)_L$				
Calf raise group	10.1 (1.2)	9.6 (0.8)	-0.5 (1.5)	
Walking group	10.7 (0.6)	10.2 (1.3)	-0.5 (1.2)	
Respiration supported by $(ETF+CI)_P$				
Calf raise group	18.9 (1.5)	17.2 (2.7)	-1.7 (2.2)	
Walking group	20.3 (0.6)	19.6 (1.8)	-0.7 (1.5)	
Respiration supported by $(CI+ETF)_P$				
Calf raise group	36.1 (1.8)	33.9 (2.5)	-2.2 (2.2)	
Walking group	39.7 (3.2)	38.1 (3.2)	-1.6 (1.9)	
Respiration supported by $(CI+CII+ETF)_P$,				
Calf raise group	70.8 (4.2)	66.4 (4.7)	-4.4 (3.4)	
Walking group	74.8 (5.7)	70.1 (5.5)	-4.7 (3.5)	
ETS capacity supported by $(CI+CII+ETF)_E$,				
Calf raise group	87.6 (5.4)	85.2 (7.3)	-2.4 (6.6)	
Walking group	95.7 (7.8)	90.3 (8.4)	-5.4 (4.7)	
Respiration supported by $(CII)_E$				
Calf raise group	51.2 (2.9)	50.3 (3.7)	-0.9 (3.9)	

Walking group	52.6 (4.6)	53.7 (3.8)	1.1 (3.7)
Citrate synthase activity			
Calf raise group	4.74 (0.1)	4.82 (0.12)	0.08 (0.03)
Walking group	4.80 (0.07)	4.88 (0.2)	0.08 (0.06)

ETF = Electron Transferring Flavoprotein; CI = Complex I, CII = Complex II, ETS = Electron transfer system; The subscripts L,P,E indicate the LEAK state, (OX) PHOS state and ETS capacity. SEM = standard error of the mean.

Table V: Health related quality of Life measured by the questionnaires SF-36 in patients with intermittent claudication who performed calf raise exercise (n=14) or walking exercise (n=13).

Variables	*Pre-test*	*Post-test*	*Change*	*P value*
	mean (SEM)	mean (SEM)	mean (SEM)	
SF 36				
Pain				
Calf raise group	59.0 (6.4)	62.3 (5.7)	3.3 (6.1)	.31
Walking group	57.7 (6.6)	60.0 (6.3)	2.3 (8.6)	.40
General health				
Calf raise group	77.9 (4.5)	72.6 (4.6)	-5.3 (4.5)	.13
Walking group	58.5 (3.8)	64.2 (5.7)	5.7 (4.3)	.11
Vitality				
Calf raise group	59.4 (4.6)	57.8 (3.1)	-1.6 (3.2)	.32
Walking group	59.1 (3.9)	62.0 (6.4)	2.9 (6.7)	.33
Mental health				
Calf raise group	82.2 (4.3)	84.2 (3.2)	1.9 (2.4)	.22
Walking group	75.0 (5.0)	80.9 (5.0)	5.9 (7.4)	.22
Social function				
Calf raise group	87.5 (4)	87.5 (4.5)	0 (3.5)	1.00
Walking group	85.0 (5.2)	91.3 (4.2)	6.3 (5.7)	.11
Emotional role				
Calf raise group	80.1 (6.9)	85.3 (6.9)	5.1 (8.5)	.28
Walking group	77.1(8.7)	81.3 (8.7)	4.2 (6.5)	.27

Physical function				
Calf raise group	64.2 (4.9)	64.2 (2.8)	0 (3.2)	1.00
Walking group	64.2 (4.1)	6.7 (4.9)	2.9 (4.2)	.25
Physical role				
Calf raise group	68.3 (6.8)	74.5(5.0)	6.3 (5.2)	.13
Walking group	61.4 (6.9)	67.2 (6.9)	5.7 (7.3)	.22

1 SEM = standard error of the mean

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Table VI: Disease specific quality of Life measured by the questionnaires CLAU-S in patients with intermittent claudication who performed calf raise exercise (n=14) or walking exercise (n=13).

Variables	*Pre-test*	*Post-test*	*Change*	*P value*
	mean (SEM)	mean (SEM)	mean (SEM)	
CLAU-S				
Pain				
Calf raise group	59.6 (2.9)	57.1 (4.0)	-2.5 (3.3)	.23
Walking group	61.1 (5.3)	63.5 (5.5)	2.3 (6.8)	.37
Daily life				
Calf raise group	73.7 (5.6)	73.4 (5.4)	- 0.3 (3.1)	.10
Walking group	76.4 (4.3)	69.0 (6.5)	- 7.4 (5.5)	.47
Social life				
Calf raise group	93.8 (1.4)	95.2 (2.5)	1.4 (2.0)	.25
Walking group	90.1 (4.3)	90.1 (3.6)	0.0 (2.4)	1.00
Disease Anxiety				
Calf raise group	75.6 (5.9)	85.7 (5.7)	9.1 (2.3)	<.01
Walking group	74.8 (5.0)	80.9 (7.8)	6.2 (8.8)	.25
Psychological welbeing				
Calf raise group	83.7 (2.7)	83.7 (2.8)	0.4 (3.5)	.46
Walking group	87.6 (3.1)	84.7 (5.5)	-2.9 (4.6)	.27

SEM = standard error of the mean

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