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Chronic Transcutaneous Electrical Stimulation of Calf Muscles Improves Functional Capacity without Inducing Systemic Inflammation in Claudicants

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Objectives. *To assess whether electrical stimulation of ischaemic calf muscles in claudicants causes a systemic inflammatory response and to evaluate effects of its chronic application on muscle function and walking ability.*
Design. *Prospective randomised controlled trial of calf muscle stimulation.*
Materials and methods. *Stable claudicants were randomised to receive either active chronic low frequency (6 Hz) motor stimulation (n = 15) or, as a control treatment, submotor transcutaneous electrical nerve (TENS) stimulation (n = 15) of calf muscles in one leg, 3 × 20 min per day for four weeks. Leucocyte activation was quantified by changes in cell morphology, vascular permeability by urinary albumin:creatinine ratio (ACR), calf muscle function by isometric twitch contractions and walking ability by treadmill performance pre- and post-intervention.*
Results. *Acute active muscle stimulation activated leucocytes less (28% increase) than a standard treadmill test (81% increase) and did not increase ACR. Chronic calf muscle stimulation significantly increased pain-free walking distance by 35 m (95% CI 17, 52, $P < 0.001$) and maximum walking distance by 39 m (95% CI 7, 70, $P < 0.05$) while control treatment had no effect. Active stimulation prevented fatigue of calf muscles during isometric electrically evoked contractions by abolishing the slowing of relaxation that was responsible for loss of force.*
Conclusions. *Chronic electrical muscle stimulation is an effective treatment for alleviating intermittent claudication which, by targeted activation of a small muscle mass, does not engender a significant systemic inflammatory response.*

Key Words: Intermittent claudication; Muscle stimulation; Leucocyte activation.

Introduction

In patients with peripheral vascular disease (PVD) and intermittent claudication, leg muscles display changes characteristic not only of chronic ischaemia, but also of muscle disuse, which develops as pain on walking limits exercise capacity. These changes include impaired aerobic energy production,[1] decreased oxidative enzyme capacity and a shift towards less oxidative Type II muscle fibres[2–4] and fibre atrophy.[5] Exercise training of PVD patients is well known to alleviate some of these alterations by elevating activity of oxidative enzymes and improving aerobic metabolism and oxygen extraction, all of which are linked with significant increases in walking performance and hence functional status.[6–8]

Chronic low frequency electrical stimulation (CLFES) of fast muscles can mimic many of the effects of endurance exercise. In healthy animal muscles, it rapidly elevates oxidative enzyme capacity and capillary supply, thereby improving fatigue resistance.[9] Human muscle, although less amenable to the transforming effects of CLFES, can show similar responses. Stimulation of healthy human quadriceps for 6–8 weeks elevated levels of oxidative enzymes, increased capillarity around fast fibres, and improved resistance to fatigue.[10,11] On the basis of studies[12] showing that CLFES enhanced capillary supply and blood flow and reduced fatigue in chronically ischaemic rat muscles, Tsang *et al.*[13] stimulated the anterior and posterior tibial muscles of claudicants at a low frequency for short periods each day for four weeks. Muscle performance tested by measuring ankle dorsiflexor fatigue was improved after stimulation and maximum and pain-free walking distances were significantly greater.

For claudicants, one potential disadvantage of

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training using whole body exercise such as walking or cycling is that the ischaemic muscles experience repeated episodes of inadequate flow followed by reperfusion on cessation of exercise. These may engender a repetitive low-grade inflammatory response involving neutrophil activation[14–17] that can lead to a systemic increase in vascular permeability[18,19] and may be associated with the increased risk of cardiovascular events and mortality in this patient group.[8] CLFES offers an alternate means of exercising muscles in a targeted manner in comparison with whole body training, but is not known if it induces neutrophil activation in PVD patients which could increase cardiovascular risk.

The present study was therefore carried out to investigate leucocyte activation in response to calf muscle stimulation in patients with intermittent claudication, and to see whether application of muscle stimulation on a chronic basis could improve walking ability and calf muscle function as endpoints without adverse effects on leucocytes. The large muscle group of the calf was chosen for treatment because it comprises the major synergists for plantar flexion, plays a prominent role in walking, and is the common site of ischaemic pain limiting exercise performance.

Method

Patient population

With approval from South Birmingham Local Research Ethics Committee, 30 patient volunteers were recruited over a period of 18 months from those attending the Vascular Assessment Clinic at University Hospital Trust, Selly Oak, Birmingham. Patients were included in the single blind study if they had stable claudication for 3 months or longer, had a post-exercise ankle-brachial pressure index (ABPI) of <0.8 and were able to walk between 50 and 350 m on a standard treadmill test.

Study protocol

Patients attended the laboratory at the hospital for five visits at fortnightly intervals, the first of these being an habituation session the data from which was excluded from final analysis. At the second visit, patients were assessed by treadmill to determine claudication and maximal walking distances, and supine resting and post-exercise ABPIs. Calf muscle function of the worse affected leg was objectively evaluated by measuring fatigue during a test of electrically evoked isometric contractions. Blood samples from an antecubital vein and mid-stream urine specimens were collected before and 1 h after completion of the muscle function test. Patients were then randomly assigned, 15 in each group, to receive four weeks of either active CLFES treatment or control treatment, the latter provided by transcutaneous electrical nerve stimulation (TENS) machines (see below). They returned after two weeks for a muscle function test only, and after four weeks, all tests (treadmill, ABPIs, muscle function, blood and urine sampling) were performed as final outcome measures. Two weeks after cessation of treatment, a test of muscle function was performed to evaluate the persistence of any CLFES effects.

Testing procedures

Treadmill performance and ABPI

After 1 h of rest, ABPIs were measured in the supine position immediately before and after a standardised maximum walking test performed on a Powerjog E10 treadmill (Sport Engineering Ltd., UK), 10% incline at a speed of 2.5 km h^{-1}. Claudication distance was defined by the initial onset of claudicating pain and maximum walking distance by that achieved before pain became too severe to continue.

Calf muscle function

Calf muscle function was tested during 5 min of electrically-evoked twitch contractions. Patients were seated in a custom-made chair with the lower leg under investigation fixed by a knee clamp and the foot resting on a plate to which strain gauges were attached for measurement of isometric plantar flexion torque.[20] Calf muscles were contracted at frequencies of 3.5–5 Hz by transcutaneous electrical motor stimulation (pulse duration 50 μs, 100 V) using a Devices DS7 constant voltage stimulator (Devices Instruments, Welwyn Garden City, UK) controlled through a MacLab 4e system (ADInstruments Ltd., Hastings, UK). To avoid high contraction forces that would not be tolerable for ischaemic muscles in the patients, initial torque was set by adjusting stimulus intensity to produce about half the value used in healthy subjects,[20] i.e. as close to 7.5 Nm as possible. Muscle torque was recorded throughout 5 min stimulation at a frequency which gave repeated unfused twitch contractions.

Torque was averaged from 5 twitches every 10 s throughout the test and muscle function was assessed as torque measured 10 s prior to the end of the test as a percentage of that measured 10 s after the start, to avoid any movement artefacts. The time course of

individual twitch contractions was evaluated by expanding recordings taken throughout the test and measuring time to peak contraction (TTP) and time to half relaxation (1/2RT).

Blood and urine testing

To investigate whether muscle electrical stimulation had any effect on systemic leucocyte activation, mixed venous blood samples and urine specimens were taken before and 1 h after the calf muscle function testing and the treadmill test. Blood was collected into EDTA tubes and processed immediately after collection to extract leucocytes according to the method described by McCarthy *et al*.[21] Briefly, 1 ml samples were fixed with paraformaldehyde, lysed and centrifuged to remove red cells and platelets to yield a suspension of leucocytes which were then stained with Toluidine blue. The external morphology of cells was examined by light microscopy ($\times 400$) and the proportion of activated cells (ruffled or irregular surface appearance) as opposed to quiescent cells (smooth round surface) was determined in the whole sample.[21,22]

Urine samples were stored with sodium azide at 4 °C until microalbuminurea could be determined by standard radioimmunoassay (Double Antibody Albumin, Euro/D.P.C. Ltd., UK). Creatinine content was also estimated (kit from Synermed Europe Ltd., UK) so that urinary albumin:creatinine ratio (ACR) could be calculated.[18]

Chronic calf muscle stimulation

Treatment, carried out by patients at home, was applied unilaterally to the calf muscle of the leg which had the lowest ABPI values on baseline testing. Active CLFES treatment involved using Compex stimulators (MediCompex SA, Ecublens, Switzerland) programmed to deliver square wave pulses (250 μs duration, 100 V) at a frequency of 6 Hz for 20 min periods. Patients were instructed in placement of electrodes over the motor point and belly of triceps surae and in adjusting stimulation intensity to produce visible pain-free calf muscle contractions. They used the stimulators three times per day with an interval of at least 2 h between sessions, usage being registered by the stimulator programme card. Control treatment involved using TENS machines (SKF Services Ltd., Kent, UK) which were set to deliver an output at 50 μs, 100 V and 90 Hz. Patients were again instructed in positioning of electrodes and were asked to increase stimulus intensity until a perceived sensation of tingling without muscle contraction was obtained, and perform three sessions each of 20 min duration per day. TENS machines were used as a placebo treatment that did not induce active muscle contraction.

Statistical analysis

Comparisons of blood and urine data were made within active or control treatment groups by Wilcoxon signed rank test and between patient treatment groups by Mann–Whitney U tests for non-parametrically distributed data, which is, therefore, presented in the results as median (interquartile range (IQR)). Analysis of variance (ANOVA) with Scheffe's post hoc tests was performed on parametric treadmill, muscle function and ABPI data where two or more samples were compared. These data are presented as means (standard deviations (SD)). Analyses were performed using Statview software (Abacus Concepts, USA) with significance in all cases set at the 5% level.

Results

There were no significant differences in baseline descriptors of the patients assigned to active or control treatment groups (Table 1). The most common site of the limiting occlusion was the popliteal artery in 10 of the active group and nine of the control group, the remaining occlusions located in the femoral (active 1, control 2) or tibial (active 4, control 3) arteries. Mean (SD) ankle-brachial pressure indices in the worst affected leg, 0.64(0.14) for all patients, classed them as having mild claudication and following treadmill exercise, ABPIs decreased on average by 30(51)% ($P < 0.005$). Baseline claudication and maximum walking distances were similar for the groups (Table 1), with the total duration of treadmill exercise not exceeding 3 min.

Significant improvements in treadmill performance were observed after chronic muscle stimulation (Table 2). After active treatment, claudicating and maximum walking distances were 93(37) and 150(63) metres, respectively, increases of 82(108)% ($P < 0.01$) and 44(50)% ($P < 0.05$) from baseline. By contrast, patients receiving the control treatment showed no significant change from baseline in either claudicating (64(38) m, NS) or maximum walking (114(100) m, NS) distances. After CLFES, the improvements in pain-free walking were more marked in seven patients with unilateral disease in whom treatment was targeted at the affected leg (distance increased 102(155)%) than in the remaining eight with bilateral disease where treatment was only applied to the worse affected leg (increase of 64(42)%). Better walking ability in the active group was not related to changes in ABPIs, since values in the treated leg were

Table 1. Patient descriptors for active and control treatment groups at the start of the study. Data are given as means (standard deviations). Ranges are given in parentheses below where appropriate.

	Active treatment	Control treatment
Number	15	15
Age in years	66(6) (53–80)	71(5) (61–80)
Male:female ratio	2:1	4:1
Current smokers	2	2
Duration of symptoms in years	4.9(3.9) (1–15)	5.1(4.8) (0.5–20)
Ankle-brachial pressure index at rest	0.64(0.10)	0.63(0.18)
Claudicating distances in metres	58(28)	64(37)
Maximum walking distance in metres	111(42)	115(82)

not different at rest nor was the fall post-treadmill exercise altered (0.60(0.13) and 36(29)%, respectively, NS vs. baseline testing).

Fig. 1(A) shows a typical example of torque recording during the calf muscle function test from one patient at baseline. At the start of the baseline test torque was 7.2(0.9) Nm in the active group and 7.4(1.4) Nm in the control group. Six patients were unable to complete the full 5 min test, one ceasing after 1 minute, one after 2 minutes and four between 4 and 5 min. For all other patients who completed the baseline test, twitch torque had declined to 73(30)% of initial values by 5 min because the muscles progressively fatigued. They were unable to relax quickly enough to generate single twitches, and contractions became partially fused such that basal torque had risen above zero by 80–90 s into the test (Figs. 1(A) and 2(A)). The changes in contraction times underlying this effect were a prolongation of half relaxation time from 78(16) ms at the start of the test to 159(133) ms at the end ($P < 0.01$), whereas times to peak contraction were unaltered (86(14) ms at the start, 84(22) ms at the end, NS).

All patients who received active CLFES treatment for 4 weeks were able to complete the full 5 min muscle function test. After two weeks, the marked slowing of relaxation time and rise in basal torque during the electrically evoked contractions were less (Fig. 2(B)) and after four weeks, they no longer occurred (Figs. 1(B) and 2(C)). There was no twitch fusion and in consequence, force was maintained throughout the test. By contrast, muscle function in patients receiving the control TENS stimulation showed the same slowing and fatigue as they had at baseline testing (Fig. 2(D)). When patients who had received active treatment were tested two weeks after its cessation, muscle force traces showed a reversion to the baseline pattern (data not shown).

At baseline testing, median (IQR) values for morphological signs of cell activation under resting conditions were 10.5(9.7–12.8)% of leucocytes sampled from all patients and this increased to 13.9(12.5–15.6)% in blood taken 1 h after electrical stimulation for the calf function test ($P < 0.001$, Fig. 3). Cell activation was, however, even greater in samples taken 1 h after a treadmill test where the proportion of activated leucocytes was 18.5(16.3–19.6)% ($P < 0.001$) even though the duration of treadmill exercise (<3 min) was shorter than that of the chair test (4–5 min) (Fig. 3). Urinary ACRs were 1.27(0.63–3.77) mg $mmol^{-1}$ before and 1.38(0.55–2.78) mg $mmol^{-1}$ after calf muscle testing (NS) showing that acute stimulation did not cause any change in systemic microvascular permeability. Ratios were higher after the treadmill than the chair test (3.85(1.09–8.68) mg $mmol^{-1}$) but the increase was not significant due to large inter-subject variation. After chronic active stimulation for 4 weeks, the proportion of activated leucocytes at rest (10.1(8.5–11.2)%) was not different from baseline and the

Table 2. The effects of 4 weeks of chronic calf muscle motor stimulation (active treatment) versus sub-motor TENS stimulation (control treatment) on treadmill performance.

	Active treatment	Control treatment
Change in claudicating distances in metres	+35(31)* (CI +17, +52)	−0.1(5) (CI −3, +3)
Change in maximum walking distance in metres	+39(57)** (CI +7, +70)	−1(27) (CI −17, +15)

Data are given as means (standard deviations) with 95% confidence intervals. *$P < 0.001$, **$P < 0.05$ active vs. control treatments.

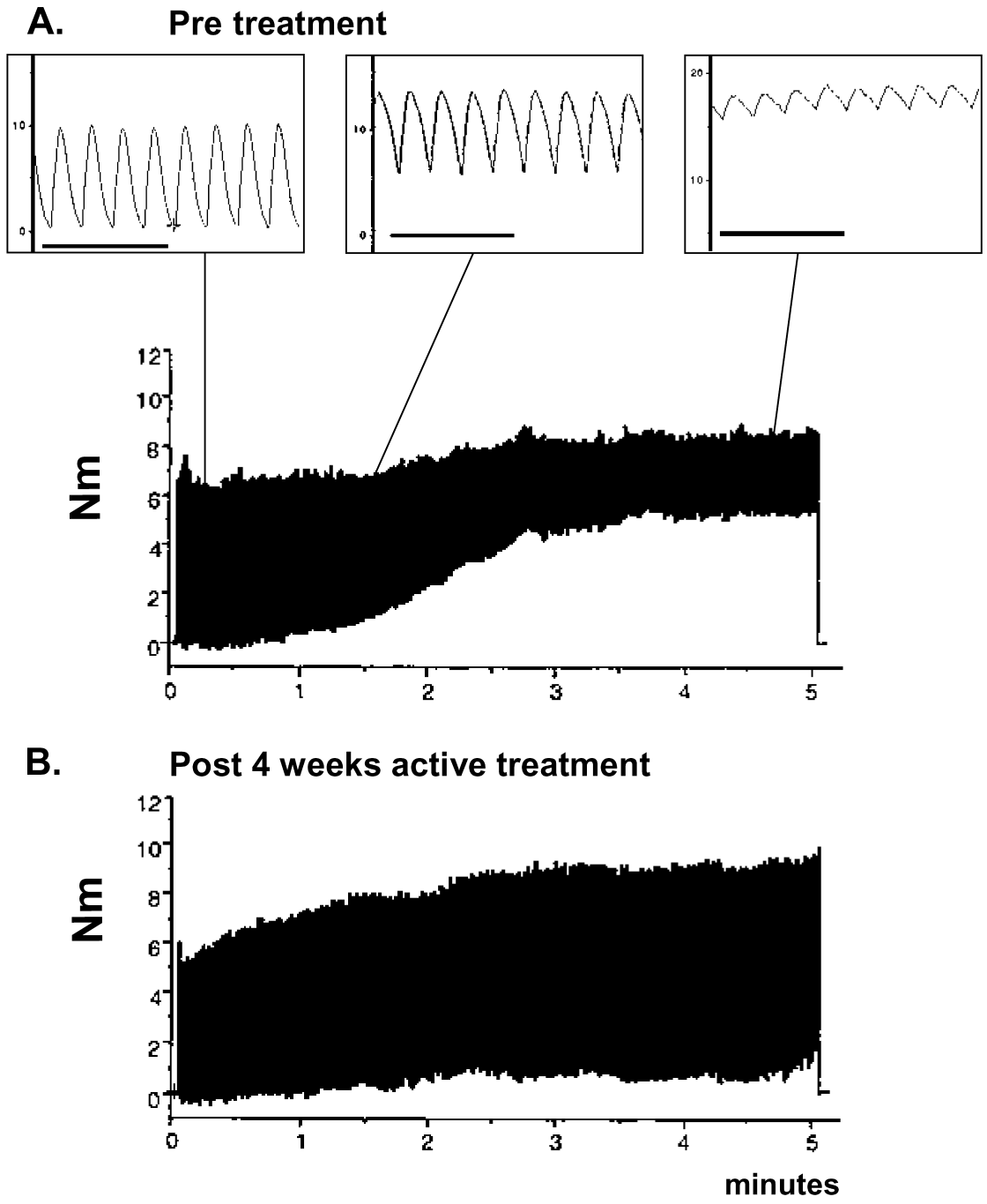

Fig. 1. Records of electrically-evoked isometric torque (Nm) developed during the test of calf muscle function from one patient pre (A) and post (B) 4 weeks CLFES. At baseline testing (A), the force of individual twitch contractions declined after 2 min due to slowing of the twitch relaxation and failure of the muscle to relax to zero torque (see inset figures, bar = 1 s). This no longer happened after CLFES. Any torque shown below zero represents dorsi-flexion rather than plantar flexion, usually due to small adjustments in body position by the patient.

increase following a treadmill test (17.3(16.4–19.0)%) was also unchanged. Urinary ACRs were not significantly altered at rest (0.60(0.42–6.21) mg $mmol^{-1}$) or in response to treadmill exercise (2.61(1.20–10.47) mg $mmol^{-1}$).

Discussion

This study shows that low frequency electrical stimulation of calf muscles in PVD patients has minimal effects on leucocyte activation, assessed by

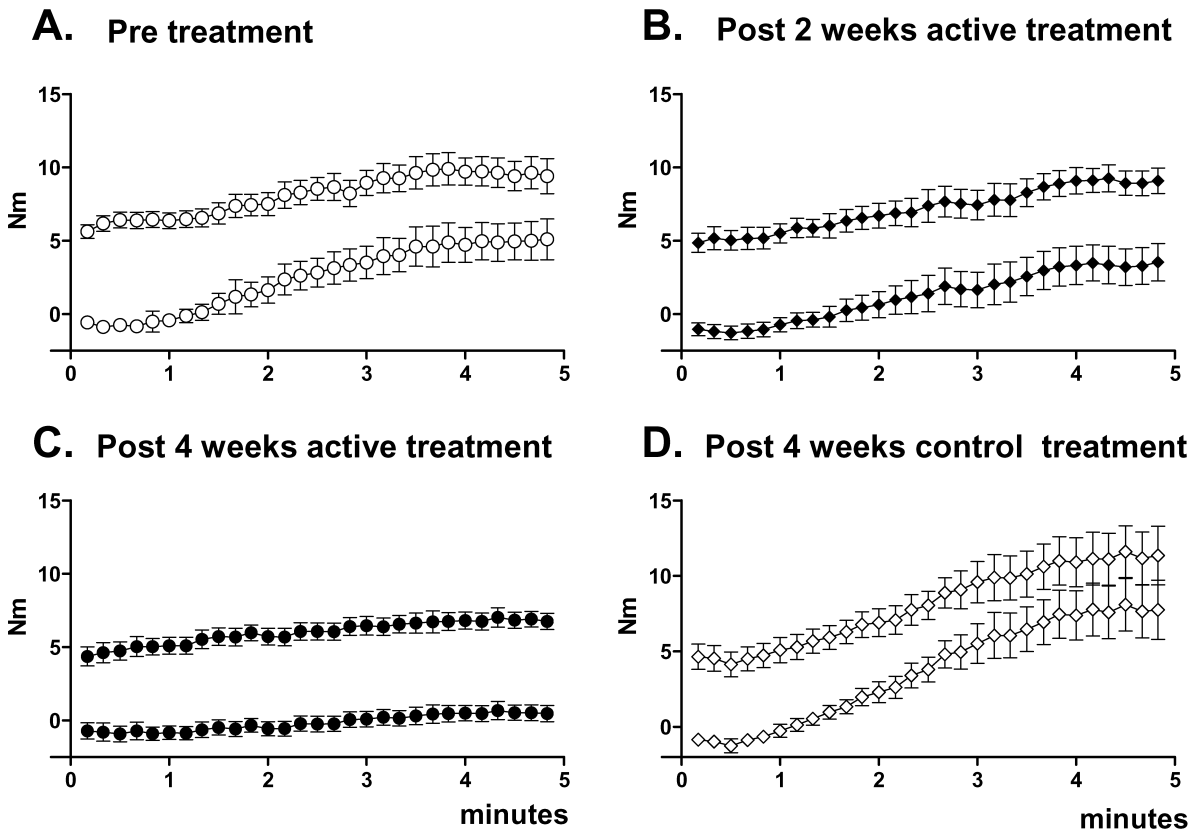

Fig. 2. Panels show grouped data for average peak and base torque values for 5 twitch contractions measured every 10 s during the chair test. (A) For all patients pre treatment, (B) for patients receiving active stimulation after 2 weeks, (C) for patients receiving active treatment after 4 weeks, (D) for patients receiving 4 weeks of control treatment (D).

their morphology, and does not influence systemic vascular permeability when applied acutely, nor did it have any deleterious consequences for leucocyte activation or systemic microvascular permeability when used as a chronic treatment. CLFES was, however, highly effective at inducing improvements in functional walking capacity of patients.

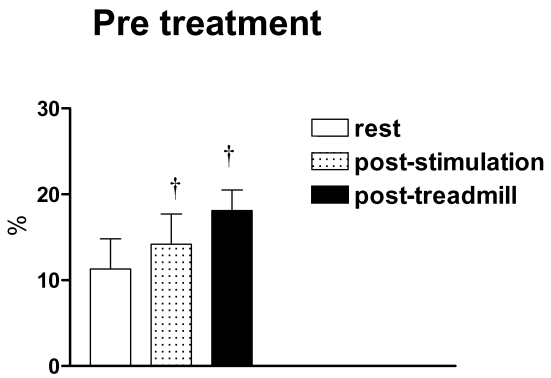

Fig. 3. Percentage of activated leucocytes, assessed by morphological cell shape change, in venous blood taken 1 h after either acute calf muscle stimulation (max 5 min) or treadmill exercise (max 3 min) from all claudicants pre-treatment. †$p < 0.001$ versus rest, paired *t*-test.

Enhancement of walking was particularly evident in cases of unilateral disease where the affected leg had been specifically targeted by stimulation and its hindrance to walking performance thereby ameliorated.

These positive effects of calf muscle stimulation confirm the findings of Tsang *et al.*[13] who stimulated anterior tibial muscles for four weeks, but the improvement in pain-free walking was even greater, 82% compared with 26%. This is likely because treatment was applied to the larger triceps surae muscle group which makes a more prominent contribution during the gait cycle.[23] The local nature of the treatment effect is shown by the fact that maximum walking distances, which would be curtailed by the untreated leg as well as being influenced by cardiorespiratory and motivational limitations, increased similarity in the two studies (44 and 34% respectively). Only one other study has reported positive effects of gastrocnemius muscle electrical stimulation in patients with PVD, using it in conjunction with an exercise training programme applied to the leg remaining after amputation.[24] It reported that claudication was alleviated by stimulation combined

with exercise but not by exercise alone but the stimulation protocol was more intensive, 2 h per day over 8 weeks, than presently used and the effect of stimulation per se was not evaluated.

Neutrophil activation in claudicants following exercise[17,25] is a consequence of ischaemia-reperfusion and has been considered to present a significant risk by contributing to the likelihood of their adherence to vascular endothelium and to a generalised increase in systemic vascular permeability[15,16] which has been shown in claudicants by urinary microalbuminuria.[18,19] In the present patient population, a single bout of calf electrical stimulation lasting 4–5 min for the muscle function test had significantly less effect on leucocyte activation (28% increase) than did treadmill walking for the shorter period of <3 min (81%). Neumann *et al*.[25] observed that total neutrophil numbers as well as the proportion of activated neutrophils were significantly elevated in venous blood leaving the legs compared to arterial blood after toe raise exercise to claudication, indicating a release of cells 'washed out' from the ischaemic limb itself by post-exercise reperfusion. Calf stimulation at submaximal intensity may involve a relatively small proportion of the muscle group compared with leg exercise so that any such reperfusion effects are minor. Furthermore, the small increase in leucocyte activation during stimulation did not induce systemic vascular permeability, based on the lack of change in ACRs, in contrast to the known effects of treadmill exercise. The increase in ACRs after treadmill was not significant in the present study most likely because of small patient numbers.[15] The local nature of electrically-evoked muscle contractions in comparison with whole body exercise therefore, means that CLFES as a treatment does not pose any risk to patients of aggravating inflammatory status, neither does it modulate the leucocyte activation response to treadmill exercise. A further possible benefit of CLFES is that a smaller degree of leucocyte activation than during exercise training would have less adverse effects on adhesion molecule expression which is associated with endothelial dysfunction in claudicants.[26]

The total treatment time with chronic stimulation (20 min three times daily, 7 days per week, 4 weeks) amounted to 28 h. Compliance was greater than 95% in both active and control treatment groups, as registered by stimulator cards or by self-report. Although exercise training programmes may result in greater improvements in pain-free and maximum walking distances than the present study,[7] the minimum duration required, using sessions of 30 min up to 1 h which may be repeated daily or several times per week, is of the order of 3–6 months.[7,14] Set against this, the magnitude of functional improvements in the present study show self-administered chronic stimulation to be a very effective treatment for claudicants. CLFES can be carried out by patients themselves after minimal tutoring in equipment use, and does not require attendance at a supervised exercise class, the usual recommendation for ensuring patient adherence to a physical activity programme.

Stimulation could exert its beneficial effects on walking ability by altering metabolic efficiency of the targeted muscle. Fatigue during electrically evoked twitch contractions in patients prior to treatment was evident as an inability to regain baseline between contractions due to slowing of muscle relaxation. This differed from the response during the chair test of healthy calf muscle with restricted blood flow in which force production declined without any slowing of contraction times.[20] The contractile properties of calf muscles in the patient group at baseline were significantly faster than times to peak contraction ($\sim$170 ms) and half relaxation (130 ms) reported for plantar flexor muscles in healthy elderly individuals,[27] consistent with the observed increase in fast myosin heavy chains[4] and change in fibre population towards the fast type in ischaemic muscles of PVD patients.[2,3] In fatigued muscle, slower relaxation has been attributed to prolongation of the Ca^{2+} transient due in part to the effect of elevated H^+ concentration on the Ca^{2+} pump rate of the sarcoplasmic reticulum[28] and during calf exercise, muscle pH falls to a greater extent in claudicants than healthy age-matched controls.[1] Whether the elimination of slowing of relaxation in treated muscles implies that CLFES had negated metabolic inefficiency independently of any vascular effects remains to be investigated.

With regard to the possibility that CLFES has vascular effects, the fact that ankle-brachial pressure indices for the stimulated leg were not changed by CLFES either at rest or post-exercise would suggest that overall perfusion had not been influenced, and indeed, the majority of exercise training studies in PVD patients also show that limb blood flow is not enhanced.[6] However, while ABPI measurements are good at predicting the severity of arterial disease they cannot discriminate redistribution of perfusion within the ischaemic limb. Low frequency electrical stimulation of anterior tibial muscles for several weeks in PVD patients improved skin oxygen saturation and led to healing of ulcers,[29] which implies a cutaneous vascular effect. In animals with stenosed arteries supplying the lower limbs, exercise training led to better walking performance which was not associated with higher limb blood flow but was thought to be linked with more homogeneous distribution of flow

and better oxygen delivery among muscles.[30] This would necessitate alterations in the fine control of arterial vessels that regulate perfusion through individual muscle capillary beds. Disturbances of resistance vessel control have been demonstrated in PVD patients as reduced calf reactive hyperaemic responses to thigh occlusion[31] and depressed endothelial and smooth muscle function in resistance arteries from patients with critical limb ischaemia.[32,33] Our own work in a rat model of intermittent claudication (iliac artery ligation) showed that impaired vasodilator responses of the smallest arterioles in ischaemic limb skeletal muscle is a very early feature,[34] whereas changes in fibre type or metabolism are not evident even after several weeks.[35,36] Furthermore, we have evidence that CLES applied intermittently to ischaemic rat hindlimb muscles can restore dilator capacity to these vessels,[37] and can also enhance hyperaemia in response to muscle contractions.[12] A vascular effect of stimulation that would optimise calf muscle perfusion is, therefore, a possible contributory factor in the improved walking ability of patients receiving the active treatment in this study.

In conclusion, CLFES is a simple and effective treatment for claudication that can be targeted to affected muscles, and improves patient walking ability without inducing a systemic inflammatory response. As a therapeutic modality, it is uses simple resources, involves little daily time and requires minimal supervision. The mechanism of action of stimulation on ischaemic muscles is likely to be a combination of enhanced oxygen delivery by restoration of vasodilator capacity and improved metabolic efficiency.

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