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Increased Fatigability of the Gastrocnemius Medialis Muscle in Individuals with Intermittent Claudication

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WHAT THIS PAPER ADDS

- The article reports the first tensiomyographic study on biomechanical properties of the gastrocnemius medialis muscle in patients with intermittent claudication. Compared to healthy individuals, the contraction properties of the gastrocnemius medialis muscle of claudicants have been found to be different regarding the time and velocity of electrically elicted contractions. The increased fatigability of the studied muscle found in claudicants could represent a novel target to study the effectiveness of different treatment modalities for patients with symptomatic peripheral arterial disease (PAD).

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ABSTRACT

Background: We attempted to identify possible differences in the contractility of the gastrocnemius medialis (GM) muscle between healthy controls and individuals with peripheral arterial disease (PAD) and intermittent lower-limb claudication.
Methods: The GM muscles of 17 PAD patients and 17 healthy controls were examined with tensiomyography. Single or multiple electrical impulses were used to trigger muscle contractions, and the time and amplitude of contractions were measured.
Results: After single-impulse stimulation, the GM muscles of PAD patients showed significantly shorter contraction times ($P < 0.001$) than the GM muscles of controls. During 1 min of repetitive electrical stimulation, the contraction velocity of the controls' GM muscles typically showed a sustained increase throughout the stimulation period, whereas in PAD patients, a significant decrease in contraction velocity was observed after 30 s. The onset of muscle fatigue was unrelated to the ankle brachial index (ABI) of the examined leg. When the legs of PAD patients with higher and lower ABIs were compared to each other, no significant differences were found regarding the time and amplitude of contraction after single-impulse stimulation.
Conclusions: The GM muscles of individuals with intermittent claudication contract more quickly and fatigue earlier than the GM muscles of healthy controls. Because the contraction time, measured with tensiomyography, reflects the individual's muscle fibre composition, our findings may reflect a shift from type I fibres to type II fibres in the GM muscles of PAD patients. Our data support the idea that calf myopathy is present in claudication-prone patients and, in part, determines the clinical manifestations of PAD.

Peripheral arterial disease (PAD) is a common disorder, affecting a fifth of the population older than 55 years.[1] The most common symptom of PAD is intermittent claudication, characterised by a cramp-like pain in the calves that occurs during walking and is relieved by rest.[2] Claudication symptoms are thought to be the

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result of reduced blood flow and, therefore, insufficient oxygen and substrate delivery to the working leg muscles.

Although lowered leg arterial pressure is emblematic of PAD, several studies have found little or no correlation between decreased leg perfusion and exercise performance in patients with PAD.[3–5] Improvements in claudication symptoms after exercise training also appear to be unrelated to changes in lower-limb arterial blood flow.[6,7] The current perspective on the pathogenesis of PAD, therefore, also includes abnormalities in the muscle's structure and metabolic function that may contribute to muscle weakness and walking limitations.[8] In fact, a new clinical entity called the 'myopathy of peripheral arterial disease' was recently introduced[9] to define this set of abnormalities. Understanding the specific role of the calf muscle in the pathophysiology of PAD could help to set new end points for evaluating the effectiveness of current therapies (e.g., exercise, percutaneous interventions, surgery and angiogenic gene therapy).

To elucidate the contraction characteristics of the gastrocnemius medialis (GM) muscle in patients with claudication, we compared the contractility and fatigability of the GM muscle between a group of PAD patients and a group of healthy individuals. To do this, we used a novel, non-invasive method called 'tensiomyography (TMG)' to measure the mechanical response of the GM muscle to single or multiple electrical stimuli. We hypothesised that the fatigability of the GM muscle is significantly more pronounced in PAD patients than in healthy individuals, and that the onset of muscle fatigue depends on the individual's ankle-brachial index (ABI). To our knowledge, this is the first TMG study of the biomechanical characteristics of calf muscles in symptomatic PAD patients.

Material and Methods

Participants

With approval from the National Medical Ethics Committee, 17 individuals with PAD and 17 healthy individuals (Table 1) were enrolled in the study over a period of 6 months. Patients with PAD were identified consecutively from a list of candidates waiting for a percutaneous intervention on the lower-limb arteries at the Clinical Department for Vascular Diseases at the University Clinical Center of Ljubljana, Slovenia. Controls were volunteers from the hospital staff. All participants were informed about the nature of the procedure, and written consent was obtained from each participant.

Inclusion and exclusion criteria

Patients with PAD were included in the study if they had persistent claudication for 3 months or longer, had an ABI of less than 0.8 and were able to walk between 50 and 350 m. Exclusion criteria were serious co-morbidities that interfered with walking, insulin-dependent diabetes mellitus, advanced neuromuscular disease, significant leg oedema, critical limb ischaemia and prior lower-limb surgical revascularisation. Healthy individuals (controls) were included if their resting ABI was normal and if they had no history of diabetes, leg swelling or cardiovascular or neuromuscular disease.

Table 1
Clinical Patient Characteristics.

Patient Characteristics	PAD Patients	Controls
No. patients	17	17
Male/Female	14/3	12/5
Age, yr	62.7 ± 10.3	58.3 ± 8.2
Height, cm	172.0 ± 9.9	172.2 ± 5.3
Weight, kg	76.2 ± 13.6	80.5 ± 16.4
BMI, kg/m^2	25.6 ± 3.1	27.2 ± 4.0
Hypertension,N (%)	14 (82)	0
Diabetes, N (%)	5 (29)	0
Hyperlipidaemia, N (%)	12 (71)	0
Smokers, N (%)	12 (71)	0
Lower ABI leg	0.50 ± 0.16	/
Higher ABI leg	0.81 ± 0.16	1.07 ± 0.10

PAD = peripheral arterial disease; BMI = body mass index; ABI = ankle brachial index; Data are presented as number, as mean ± SD, or as percentage.

ABI measurement

Before starting the ABI measurement, participants rested supine for 5 min. By an established method,[10] a hand-held Doppler probe (Super-Dopplex II, Huntleigh Healthcare, Cardiff, UK) was used to obtain systolic pressure values on the dorsalis pedis and tibialis posterior arteries of both legs and on both brachial arteries. Appropriately sized cuffs were applied and deflated at a rate of 3 mmHg s^{-1}. The ABI was calculated for each leg by dividing the average pressure in each leg by the average brachial pressure. If the two average brachial pressures differed by 10 mmHg or more, the higher value was used.[11] In PAD patients, the leg with the lower ABI was labelled the lower-ABI leg and the other the higher-ABI leg.

Tensiomyography

TMG is a non-invasive method for investigating the contraction properties of skeletal muscles.[12] It measures changes in the diameter of the muscle belly during an electrically evoked isometric contraction. The thickening of the muscle belly is recorded with a mechanical displacement sensor (Fig. 1) connected to a computer (Fig. 2).[13] Every contraction is displayed as a displacement-over-time curve, which is then used to calculate different parameters (Fig. 3). In previous studies,[14–16] 2 parameters were found to be particularly useful: the time of contraction (Tc) and the maximal radial displacement (Dm) of the muscle belly. The Tc has a strong correlation ($r = 0.93$) with the percentage of type I (fatigue-resistant) fibres in the muscles studied.[17] This relationship allows indirect estimation of the fibre-type composition of the studied muscles. The clinical significance of the Dm of the muscle belly is less clear, but it has been correlated with the muscle's architecture[15] and longitudinal twitch torque.[12] We calculated the velocity of contraction (Vc) from the Dm and Tc parameters (Vc = Dm/Tc). A decrease of more than 20% of maximum Vc during repetitive

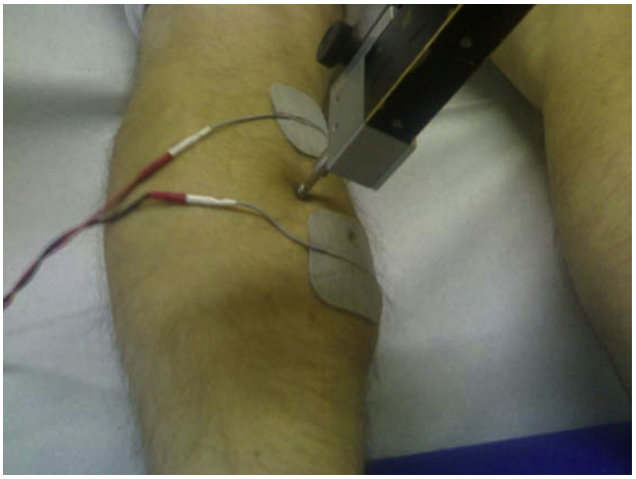

Figure 1. Electrodes and displacement sensor positioned for TMG measurements on the gastrocnemius medialis muscle.

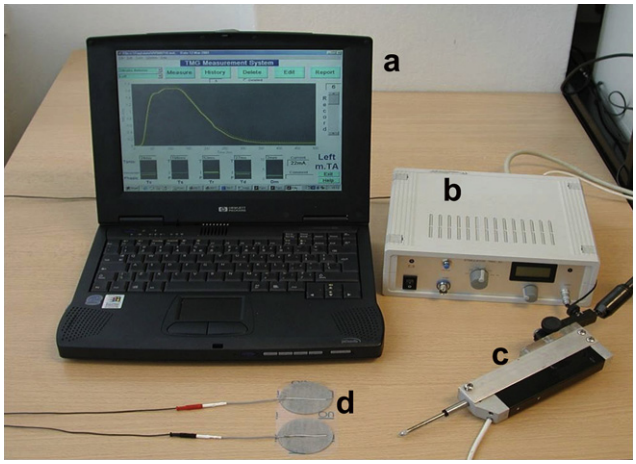

Figure 2. Equipment used for TMG measurements – a. computer for data analysis, b. generator of electrical impulses, c. muscle belly displacement sensor, d. stimulating electrodes.

muscle contractions was used to define and mark the beginning of muscle fatigue.[18]

After a 5-min rest, the participant was turned face down on a standard examination table with his or her ankles supported by a 10-cm-high pad. A digital displacement sensor (G40, RLS Inc., Ljubljana, Slovenia) was applied perpendicular to the outermost part of the GM muscle. The tip of the sensor was pressed against the skin with an initial contact pressure of 77 N mm^{-2}, as was done in a previous study.[13] From this measuring point, two rounded, 5-cm, self-adhesive electrodes (PALS Axelgaard Manufacturing Co. Ltd., Fallbrook, California, USA) were placed 5 cm distally (anode) and 5 cm proximally (cathode) over the muscle.

Single-impulse stimulation

A single contraction of the passive GM muscle was elicited with a square-wave electric pulse of 1 ms duration, delivered transcutaneously by a direct current stimulator (TMGZD1, EMF-Furlan & Co., Ljubljana, Slovenia) controlled by a computer. Stepwise increments in current were delivered, separated by rest periods of 30 s, until no further increase in twitch amplitude was seen. This method was previously described by Russ et al.[18] Three maximal twitches were then averaged and used for further analysis.

Multiple-impulse stimulation

After a 5-min rest, we continued with a multiple electrical stimulations protocol, which consisted of 1 min of intermittent (1-Hz) electrical stimulation of the GM muscle. The intensity used was the same as described for single-impulse stimulations. For each single muscle twitch, the Tc and the Dm were measured. The computer calculated the Vc for each muscle twitch, and the data were plotted on a velocity/time graph (Figs. 5 and 6). Fatigue time (FT) was defined as the time elapsed from the start of tetanic stimulation until Vc had decreased to more than 20% of its maximum value.

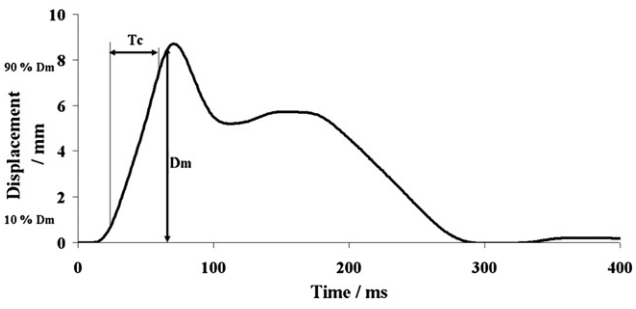

Figure 3. Typical curve obtained with tensiomyography after single-twitch stimulation of a normal skeletal muscle. Dm = Maximal radial displacement; Tc = Contraction time between the 10% and 90% Dm.

Statistics

All data were tested for homogeneity by using the Kolmogorov–Smirnov test before further statistical analysis was conducted. Means and standard deviations were used to describe patient characteristics and control parameters, whereas means and standard error were used to describe variables. One-way analysis of variance was used to compare Tc and Dm means after single-impulse stimulation. Group membership (PAD patient or control) was set as an independent factor. A Tukey post hoc analysis was performed to test for additional differences between the groups, and R^2 values were computed to estimate effect size. To compare the Vc of the GM muscle of PAD patients and controls after multiple impulse stimulation, we performed a two-way analysis of variance. We divided the stimulation period into 10-s intervals and defined the presence or absence of claudication as two independent factors. A Tukey post hoc analysis was performed to further analyse possible differences in TMG parameters. The correlation between FT and ABI was tested with the Pearson correlation coefficient. All statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) software (SPSS Inc, Chicago, IL, USA). Statistical significance was defined at the $P < 0.05$ level unless otherwise noted.

Results

ABI measurements

The mean ABI values in PAD patients were 0.50 ± 0.16 for lower-ABI legs and 0.81 ± 0.16 for higher-ABI legs ($P < 0.001$). In controls, the mean ABI was 1.07 ± 0.10.

Single-impulse stimulation

The Dm of the GM muscle was not significantly different among the PAD patients' legs with lower ABI, the legs with higher ABI, and the legs of controls ($P = 0.361$), whereas the mean Tc was

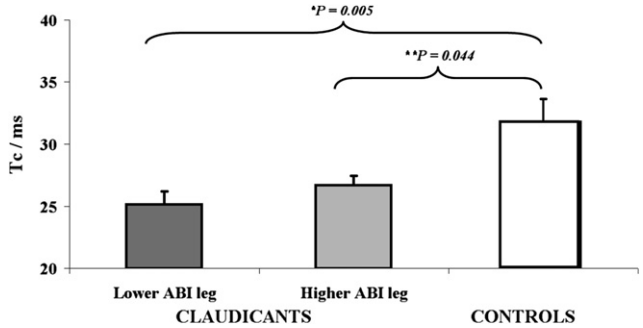

Figure 4. Graph shows the contraction time of gastrocnemius medialis muscles after single-impulse stimulation of the legs with lower and higher ABI in PAD patients and the legs of healthy controls. Tc = contraction time; ABI = ankle brachial index $^{*}P = 0.005$ between the legs of controls and the legs with lower ABI in PAD patients $^{**}P = 0.044$ between the legs of controls and the legs with higher ABI in PAD patients.

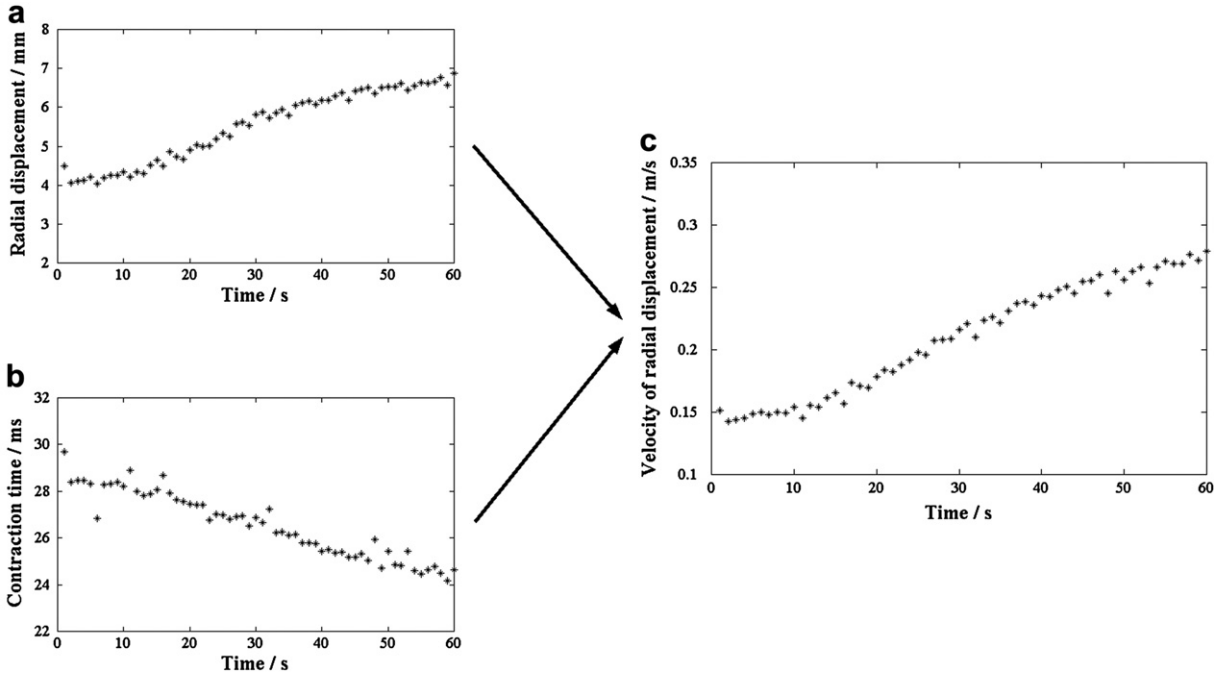

Figure 5. Typical result obtained with tensiomyography in a healthy individual by stimulating the gastrocnemius medialis muscle for 60 s with 1 Hz and supramaximal electrical impulses. a.) Maximal radial displacement b.) Contraction time and c.) Velocity of contraction (radial displacement).

($F = 5.952$; $P = 0.005$) (Fig. 4). Tukey post hoc analysis revealed significant differences between the GM muscle Tc of PAD patients' legs with lower ABI and controls ($P = 0.005$), as well as between the PAD patients' legs with higher ABI and controls ($P = 0.044$). There were no significant differences between the higher-ABI and lower-ABI legs in PAD patients ($P = 0.674$).

Multiple-impulse stimulation

When the GM muscles of controls were stimulated with low-frequency (1-Hz) electrical impulses of supramaximal amplitude, an increase in Vc was typically observed throughout the stimulation period (Fig. 5c). A similar, near-sigmoid, curve was noticed for

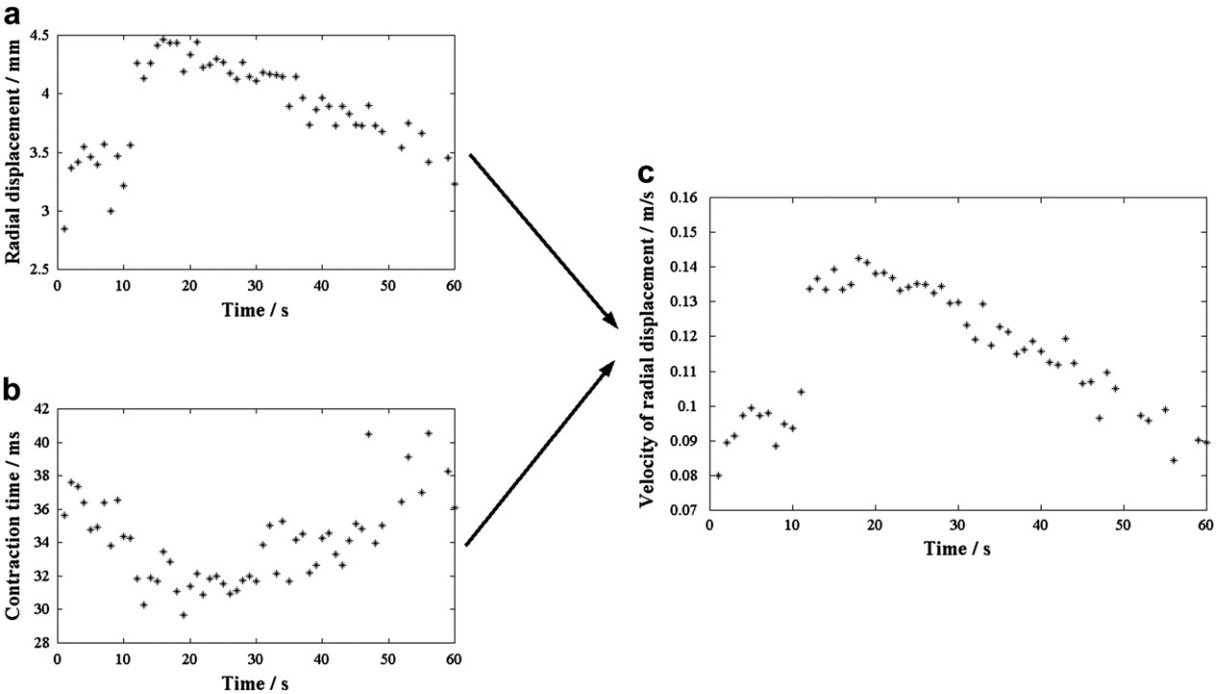

Figure 6. Typical result obtained with tensiomyography in a PAD patient by stimulating the gastrocnemius medialis muscle for 60 s with 1 Hz and supramaximal electrical impulses. a.) Maximal radial displacement b.) Contraction time and c.) Velocity of contraction (radial displacement).

the Dm values (Fig. 5a), whereas the Tc remained shortened throughout the stimulation period (Fig. 5b).

When the same stimulation protocol was applied to the GM muscles of PAD patients, a different velocity/time curve pattern emerged (Fig. 6). The radial displacement peaked earlier (after approximately 30 s) and was followed by a slow decrease towards its initial level (Fig. 6a). Inversely, the Tc shortened during the first 30 s and then slowly rose towards its initial level (Fig. 6b). As a result, the Vc rose during the first half of the stimulation period and then gradually diminished to its baseline value at the end of the stimulation period (Fig. 6c).

Because of substantial differences in peak Vc among the studied subjects, we normalised the subjects' Vcs to values between 0 and 1. Analysis of these normalised values revealed differences in mean Vc between the PAD patients and the controls (Fig. 7).

To test for changes in response over the stimulation period, we divided the stimulation period into the following intervals: 0–10 s, 11–20 s, 21–30 s, 31–40 s, 41–50 s and 51–60 s. Time interval was set as the first independent factor and the presence or absence of claudication as the second independent factor. The presence of claudication was associated with a significant time interval effect at $P < 0.001$ and an interaction effect at $P < 0.001$. In post hoc analysis, we found significant differences between Vc values at the first time interval and at all intervals after the 31st second (Fig. 7).

The average GM muscle FT was significantly lower in PAD patients (31.7 ± 11.7 s) than in controls (58.2 ± 0.7 s; $P < 0.001$). However, there was no significant correlation between the FT of the GM muscle and the PAD patients' baseline ABI value ($R = 0.21$; $P = 0.24$). The size of the illness effect on the GM muscle's time to fatigue was 37.85.

Discussion

This study demonstrates that the GM muscles of PAD patients possess different contraction characteristics compared to the GM muscles of healthy individuals. The Tc of the GM muscle was found to be significantly shorter in PAD patients, suggesting a predominance of type II fibres in this particular calf muscle. In PAD patients, no differences between lower-ABI legs and higher-ABI legs were observed in terms of Tc or Dm. Repetitive electrical stimulation of the GM muscles of healthy individuals resulted in a steady increase in Vc, whereas in PAD patients, Vc rose only in the first 30 s of stimulation before decreasing towards starting levels because of muscle fatigue. The time of onset of muscle fatigue was unrelated to the individual's ABI.

Decreased lower-limb arterial blood flow is the main clinical finding in patients with PAD. However, patients with similar lower-limb haemodynamic conditions show very different exercise tolerances.[3–5] Increased fatigability of the PAD patients' GM medialis muscles, found both in our study and in others,[19,20] supports the idea that besides a clear arterial insufficiency, patients with PAD have a specific PAD-related 'muscle insufficiency', which contributes to their lower-than-expected muscle performance and premature fatigue. Our data also suggest that calf muscle phenotype is altered in PAD patients so that the proportion of fatigue-prone type II fibres is increased.

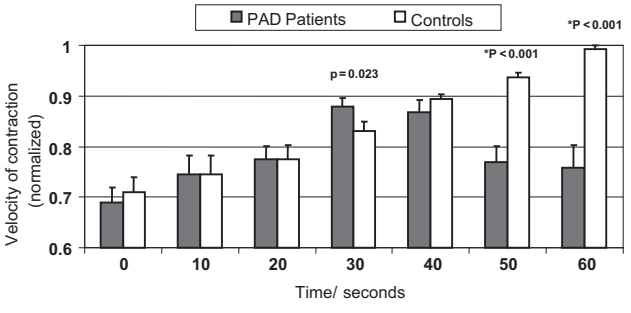

Figure 7. Graph shows changes in the velocity of contraction (normalized to maximal velocity and averaged across 10-s intervals) of the gastrocnemius medialis muscle in PAD patients and controls. The muscles were stimulated for 60 s with 1 Hz and supramaximal electrical impulses. *$P < 0.001$ for PAD patients versus controls.

To quantify muscle fatigue, volitional and non-volitional (electrically elicited) fatigue tests are used.[21] TMG, which was used in our study, is based on a transcutaneous electric stimulation and a synchronous measurement of specific contraction properties of the studied muscle Because supramaximal tetanic stimulation is not tolerated by some patients,[22] we used low-frequency electrical stimulation to trigger muscle fatigue. Stimulation of the GM muscle of healthy individuals for 1 min at the 1-Hz frequency used in our protocol resulted in an enhanced muscle output, which was probably due to the post-activation potentiation phenomenon.[23] This phenomenon is attributed to phosphorylation of myosin regulatory light chains, which makes actin and myosin more sensitive to myoplasmic Ca^{2+}. No such effect is seen at high stimulation frequencies, because the concentration of Ca^{2+} has already reached its 'saturation' level.[24] We observed this potentiation pattern also during the first half of the stimulation period in the GM muscle of PAD patients; afterwards, however, the amplitude and velocity of contraction started to decrease, marking the onset of muscle fatigue. We did not test different stimulation frequencies for triggering muscle fatigue; this should be done in the future to better define the ideal stimulation protocol.

Very few studies have explored the effect of low-frequency electrical stimulation on the fatigability of calf muscles in PAD patients. Cole et al.[25] measured the plantar flexion torque in six healthy young adults during 5 min of transcutaneous electrical stimulation of the triceps surae under different thigh cuff occlusion pressures. They found that with undisturbed lower-leg circulation, the generated force steadily rose over the stimulating period, while complete ischaemia of the leg caused a 47% drop in the generated force. The authors, therefore, concluded that low-force twitch contractions of calf muscles are extremely sensitive to impaired blood perfusion.

Anderson and colleagues[26] studied changes in plantar flexion torque in 30 patients during 5 min of electrically evoked twitch contractions of calf muscles A fifth of the patients could not complete the procedure because of unbearable discomfort. In patients who finished the test, twitch torque had declined to 73% ± 30% of the initial value after 5 min. The stimulation protocol used in our study was better tolerated by patients (we had no dropouts among the 34 participants) and was seemingly more sensitive, because muscle fatigue was observed after only 30 s of stimulation. However, different types of subjects and different end points were measured in the two studies, so no firm conclusions can be drawn.

Muscle fatigue is the most common clinical symptom of PAD, but it is also the leading symptom in a variety of other chronic diseases. In similar studies that used electrically induced contractions, premature muscle fatigue was found in the calf muscles of patients with chronic heart failure[27] and in the thigh muscles of patients with chronic obstructive pulmonary disease.[28] Multiple mechanisms for this increased fatigability were suggested, including peripheral neuropathy, disuse atrophy, fibre type shift, skeletal muscle myopathy, changes related to hypoxia and systemic inflammation.[29] These alterations could have affected also the studied muscles in our PAD patients and this could explain why we did not find any differences in Tc and Dm between the lower-ABI and higher-ABI legs in PAD patients. Hiatt et al.[30] and Jansson

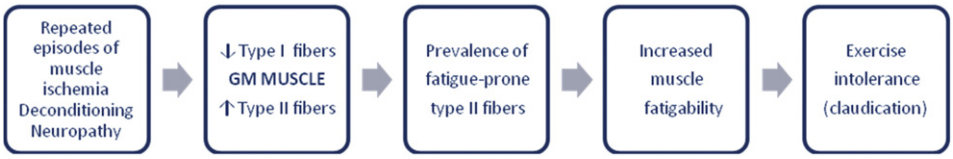

Figure 8. Proposed biomechanical changes in the gastrocnemius muscles of claudicants.

et al.[31] also did not find any differences in the distribution of muscle fibre types, fibre dimension, capillary density or myoglobin content in biopsy samples from the GM muscles of PAD patients whose legs had significantly different ABI values. However, the lack of differences between individuals' left and right legs in the parameters measured in our study could be also the result of the inability of single-impulse stimulation to detect discrete biomechanical differences between the legs. Future studies of PAD patients should include repetitive impulse stimulation protocols for both legs.

It was shown in previous studies[13,32] that the time of contraction measured with TMG correlates well ($r = 0.88–0.93$) with the proportion of type I fibres in biopsy samples. Therefore, the shorter Tcs and increased fatigability found in our PAD patients during repetitive GM muscle stimulation indicate a predominance of type II (anaerobic and fatigue-prone) fibres in those patients' GM muscles. These results agree with those of McGuigan et al.[33] and Ambrosio et al.,[34] who found that the GM muscles of PAD patients have decreased expression of MHC1 isoforms (characteristic for type I, fatigue-resistant fibres) and increased expression of MHC 2b and MHC 2x isoforms (characteristic for type IIb and IIx fibres, respectively). They concluded that repeated episodes of ischaemia trigger a shift from type I to type II fibres, the last being subject to earlier energy depletion and muscle fatigue (Fig. 8). The impact of PAD on muscle phenotype is clearly an important area of research, and there is a need to establish what effect these pathophysiologic alterations might have on exercise performance.

This study has some limitations: the number of patients studied is relatively low, and the patients were heterogeneous in terms of sex, age, ABI and the level of the diseased arterial segment. In addition, conditions other than PAD could have influenced leg performance.[35]

Conclusions

In this study, the GM muscles of PAD patients were shown to contract faster and fatigue earlier than those of healthy controls. This increased fatigability was independent of severity of patients' PAD (as indicated by the patients' ABI), which confirmed the lack of relationship between leg arterial blood flow and muscle performance.

A shift in the fibre-type composition of the GM muscle from fatigue-resistant (type I) to fatigue-prone (type II) fibres could explain greater fatigability in PAD patients. But it is probable that a complex interaction between a variety of systemic (e.g., neuropathy, myopathy, disuse and smoking) and local factors (e.g., reduced blood flow, hypoxia, reperfusion injury, oxidative stress, muscle atrophy, metabolic and histomorphologic changes) determines the level of muscle insufficiency. Calf muscle biomechanical function could be a novel parameter to consider when therapies to improve walking distance are tested.

Funding

None.

Conflict of Interest

None.

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