be useful in establishing fatigue status of skeletal muscle without exacerbating the functional decrement of the muscle.

**Key words:** muscle contractile properties, maximal voluntary contraction, TMG, passive muscle tension, peripheral fatigue

## Introduction

Muscle fatigue is characterised by a decrease in the external force or torque generating capacity,[1] and/or by impairment in peak power output.[2] The manifestation and magnitude of this reduced function depends upon multiple factors including the muscle contraction mode,[1] the nature of the fatigue protocol[3] and the source of the fatigue.[4] Fatigue-related alterations of skeletal muscle can be observed, amongst other factors, by changes in its contractile and mechanical properties.

Since fatigue is a condition that affects both athletic performance and clinical mobility, the need for a valid monitor of muscle response is important to enable optimal management of athletes and patients. In situations of muscle fatigue, or indeed musculoskeletal injury, it is impractical to assess muscle function through a measure which makes use of voluntary efforts (i.e. MVC), due to centrally mediated inhibition.[5] Furthermore, the potential for aggravation of any damage to the musculoskeletal unit cannot be ruled out. Having been developed over the last 15 years, tensiomyography (TMG) is a portable and non-invasive means of measuring muscle response through combined use of sub-maximal (below voluntary maximal activation) electrical stimulus and a digital displacement sensor,[6-8] similar



Human Kinetics, 1607 N Market St, Champaign, IL 61825

to that used in mechanomyography.[9] TMG records spatial and temporal parameters of the radial displacement of the muscle belly in response to electrical stimuli[10] and is reliable within[11] and between days.[12] Furthermore, TMG has also demonstrated good long-term stability following fatigue[13] and has displayed significant interclass correlation coefficient with decline and recovery of maximal voluntary contraction (MVC) following exercise-induced muscle damage.[14] **In particular muscle displacement (Dm) and contraction time (Tc) have shown greatest stability**.[12]

TMG has successfully detected fatigue-associated changes following ultra-endurance triathlon,[15] and resistance exercise.[16] However, these studies report inconsistent results in the fatigue-induced alteration of the TMG parameters, perhaps due to the vast differences in the fatigue protocols administered and the different muscles measured. Furthermore, previous studies have failed to relate the TMG alterations to any valid functional measure, such as maximal voluntary contraction (MVC) or passive muscle tension (PMT), which leaves the physiological interpretation of the TMG data open to question. Therefore, in order to effectively provide meaningful validation of TMG measurement to local fatigue it is important to overcome this limitation. In practical terms, sub-maximal TMG could offer an attractive measure for sport and medical practitioners in their assessment of muscle response and status following fatigue based activity without necessitating voluntary contractile effort.

Accordingly, the aim of the present investigation was to evaluate peripheral fatigue-induced alterations in mechanical and contractile properties of the Gastrocnemius muscle, as measured by TMG. MVC and PMT were measured before and after intervention, to quantify the extent of muscle fatigue, and allow us to better interpret changes in TMG response; to

our knowledge this has not been previously reported. **It was hypothesised that a reduction in size and velocity of muscle displacement would indicate muscle fatigue in line with impairments in muscle function (decreased MVC) and elevated muscle stiffness (increased PMT). The findings of this study could help to establish TMG as a non-invasive alternative to quantify muscle fatigue**.

## Methods

*Participants*

Twenty-one healthy males with a mean (± SD) age, height, and mass of 21.3 ± 3.4 years, 182.0 ± 6.1cm, and 79.5 ± 10.0kg, volunteered and gave their written informed consent to participate in this study. **All participants were recreationally active and free from injury. Females were excluded from the study in order to maintain cohort homogeneity**. The study was performed in accordance with the principles outlined in the *Declaration of Helsinki* and was approved by the local research ethics committee.

*Design*

Mechanical and contractile properties of the right Gastrocnemius Medialis (GM) were monitored using TMG (BMC Ltd, Ljubljana). **GM is one of the propulsive muscles, fundamental to different types of human locomotion and is located superficially, making it clearly measurable by TMG**. Participants were also tested for PMT and MVC of the right plantar flexors. Testing was carried out on two occasions, one week apart, as illustrated in figure 1. Measurements were taken at a number of time points before and after either the control or fatigue intervention, according to the following order: TMG and PMT

(measurement 1, M1), warm-up, TMG and PMT + MVC (M2), either control or fatigue intervention in random order, TMG and PMT + MVC (M3). Both TMG and PMT measurements were recorded three minutes after the warm-up, and after the control or fatigue intervention, to limit the effects of post activation potentiation in the GM muscle.[18] Participants reported to the laboratory on the morning of each experimental trial in a fasted and rested state. Twenty-four hour dietary intake records were completed on the day preceding each trial, and participants were instructed to replicate their dietary intake before each visit.

*Warm-up*

Participants warmed up by cycling at a low intensity (75 Watts) on an electromagnetically braked cycle ergometer (Lode Ergometer, Netherlands) for 5 minutes at a cadence between 80 and 90 rpm.

*TMG protocol*

**TMG measurements were performed exactly as described by Ditroilo et al (2013).[13] Briefly, participants lay in a prone position on a padded bench. A foam pad, placed slightly proximal to the ankle joint, supported a knee flexion angle of around 5°. The digital displacement transducer (TMG–BMC Ltd, Ljubljana) was then positioned perpendicular to the muscle belly of the right GM with an initial pressure of 1.5 x $10^{-2}$ N/mm$^2$, controlled by consistently retracting the spring-loaded transducer probe to 50% of its length**. This measuring position was selected by first manually palpating the GM to locate the thickest part of the muscle and then later, if needed, the position was slightly adjusted to obtain the





Human Kinetics, 1607 N Market St, Champaign, IL 61825

highest mechanical response with the least amount of co-activation when externally stimulated; co-activation was typically identified by a second peak in the TMG response curve. Once the appropriate position was obtained, it was marked with a permanent marker pen to ensure exact uniformity when the sensor was repositioned for subsequent measurements. The centre point of each of the 2 stimulating electrodes (5cm$^2$) (Axelgaard, USA) was located approximately half way from the position of the sensor (~5cm) to the start of the respective GM proximal distal tendons. After each measurement these electrodes were left in place and unplugged to avoid any possible changes in muscle response via alterations in surface electrodes distance.[10] A single 1ms wide stimulation pulse was delivered, which applied initial current amplitude of 20mA. **This amplitude was progressively increased by 10mA increments until maximal response was obtained, i.e. no further displacement of the muscle belly could be produced as identified by a plateau in the twitch response curves**. In order to minimize the effects of fatigue and potentiation, rest periods of 10 seconds were allowed between each stimulation pulse. Typical maximal responses were observed at amplitude between 40 and 70mA and only the output data for that particular stimulation intensity were used for analysis. Figure 2 shows a typical TMG displacement/ time curve before and after the administration of the fatigue protocol. Output parameters were extracted and analysed from each maximal twitch response:[10] *Displacement* (Dm), the extent of maximal radial deformation (mm) of the muscle belly during contraction; *Contraction velocity* (Vc), the rate (mm·s$^{-1}$) of contraction between 10% and 90% of maximal displacement. **Raw data were extracted from the TMG software and Vc was calculated according to the formula: [Vc = Dm80/Tc] where Tc = contraction time between 10% and 90% of peak radial displacement of the muscle belly; Dm80 = the radial displacement occurring during the time period of Tc.**[19] Muscle contraction time (Tc) has

been widely reported in previous studies,[10,15-16] as the temporal change from 10%-90% of muscle Dm, providing a value relative to the spatial characteristics of each muscle. However, when assessing intramuscular alterations, i.e. pre- and post- fatigue, the significance of calculating Tc in this manner should be questioned. **Indeed, in the absence of signal latency, it is possible that a decrease in Dm could associate with a decrease in Tc, when calculated as described above. Apparent decreases in Tc, suggesting a faster twitch response, could be reported simply as a result of reduced overall muscle contraction (Dm)**. It was therefore proposed that assessment of Vc could provide greater insight, when monitoring the fatigue status of a muscle.

**Maximal voluntary contraction (MVC) protocol**

**Plantar flexor isometric MVC was performed in an isokinetic dynamometer (Kin-Com, Chattanooga Group Inc., USA). The participant had their right foot fastened securely into the plantar flexion attachment and was also held in place using two securely fastened shoulder straps and a lap belt. A 90˚ ankle angle to the tibia was ensured for each subject (figure 3). Following two sub-maximal warm-up sets, participants each performed a 5-s MVC of the right plantar flexors. Three trials of the MVC were completed with 60s recovery between attempts. Participants were verbally motivated to ensure the greatest possible effort for the duration of all attempts.**

*Passive muscle tension (PMT) protocol*

**Measurements of PMT of the right plantar flexors were made on the same isokinetic dynamometer, with a set-up identical to the MVC protocol (figure 3). Participants were**

158 **instructed to completely relax once in position, and the mean passive force of the ankle**
159 **flexed at 90° was recorded during a period of 15s, as a measure of passive muscle tension**
160 **in the plantar flexors in a static position**.[20] A single measure was taken to determine PMT,
161 as subsequent stretching of the ankle joint would cause an accumulative stretch effect. **An**
162 **intra-session reliability, as measured by the intraclass correlation coefficient, ≥ 0.80 has**
163 **been previously reported for this type of measurement**.[21]

164 *Fatigue protocol*

165 **The fatigue intervention used in the current investigation differs from previous studies in**
166 **this area[15,16] in a number of key ways. Firstly, fatigue was induced locally with a low**
167 **frequency stimulation that will necessitate a prolonged recovery, compared to higher**
168 **frequency fatigue.[17] Secondly, as motor unit discharge rarely exceeds 30Hz during**
169 **voluntary contraction,[17] low frequency stimulus can be considered a more functionally**
170 **relevant intervention. Finally, as TMG is a passive and peripheral measurement it will**
171 **minimise confounding variables such as the variability of central control factors**. Whilst
172 remaining secured in the same position as for PMT the participants received the fatigue
173 intervention, which consisted of a 5 minute electrical stimulation of the right GM, to evoke
174 fatigue. The stimulation protocol involved a train of 15 electrical pulses (1 every 100ms)
175 with a 1 second gap before the start of each subsequent train. The protocol lasted 5 minutes
176 and participants were asked to endure the maximum current they could, to ensure fatigue
177 (~110 mA). The control intervention consisted of the same positioning but receiving no
178 stimulation for a period of 5 minutes to account for the effect of time. Also in the same
179 position, with the ankle placed at 90°, isometric MVC of the plantar flexors was measured,
180 before and after both intervention and control, to assess whether fatigue occurred. Each

participant performed three 5 second MVCs, with 60 seconds recovery between attempts. Participants were provided with consistent verbal motivation to ensure maximal effort throughout.

*Statistical Analysis*

All data are presented as mean ± SD. **After testing for assumption of normality of the dependent variables and log-transforming where necessary (i.e. when not normally distributed)**, a 3 (measurements: before warm-up, M1; after warm-up, M2; after intervention, M3) x 2 (condition: control and fatigue intervention) ANOVA with repeated measures on both factors was used to detect differences in PMT and TMG parameters as a result of the fatigue/ control protocol. Where a significant F value was found a Tukey post hoc test was used to identify where any significant difference occurred. Paired *t-test* was conducted to compare the pre- / post-fatigue MVC difference between the control and fatigue intervention. Effect size (ES) was also calculated using eta-squared (η2) and interpreted as small (0.01), moderate (0.06) or large (0.14).[22] The percentage differences between control and fatigue intervention were also calculated and interpreted based on the minimum detectable change as reported in a previous reliability study.[13] An alpha level of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using Statistica version 10 (Statsoft LTD, Bedford, UK).

## Results

*TMG parameters*

**Dm demonstrated a fatigue-associated alteration. A significant main effect for 'condition' (F=7.2, p=0.002, $\eta^2$ = 0.27) was documented for Dm, along with a post-hoc difference at M3 demonstrating that the fatigue condition was significantly lower than control condition (3.3 ± 1.2 vs 4.0 ± 1.4 mm, p=0.031; figure 4), with a percentage difference of 17.7%. No significant difference was found for any of the factors or their interaction for Vc, which exhibited 121.8 ± 43.2 vs 124.7 ± 45.5 mm·s$^{-1}$ at M1, 121.3 ± 45.7 vs 124.9 ± 44.7 mm·s$^{-1}$ at M2, 131.3 ± 44.6 vs 139.8 ± 50.6 mm·s$^{-1}$ at M3.**

*MVC and PMT*

**Plantar flexor isometric MVC exhibited a significant interaction 'condition x measurement' (F=12.4, p=0.001, $\eta^2$ = 0.91) with post-hoc analysis showing a significant decline following the fatigue intervention (-122.6 ± 104 N; p<0.001) but not following control (-25.7 ± 71.3 N, p=0.115). The PMT exhibited a significant interaction 'condition x measurement' (F=5.9, p=0.005, $\eta^2$ = 0.23). The post-hoc analysis revealed at M3 that fatigue caused significantly more tension than control (139.8 ± 54.3 vs. 111.3 ± 44.6 N, p=0.007; figure 5), with a percentage difference of 20.4%.**

## Discussion

This study was designed to evaluate the validity of TMG, as a sub-maximal assessment method, to detect local muscular fatigue, against functional physiological measures. Fatigue of the GM was achieved, as evidenced by the significant decline in peak force (MVC), which was absent following the control condition. This alteration in functional capacity of the muscle was associated with a significant decline in TMG Dm, similar to previous studies

following dynamic fatigue.[16,23] In addition, plantar flexor PMT increased following the fatigue intervention suggesting that the GM skeletal muscle-tendon unit became stiffer. Despite these alterations, muscle twitch Vc appeared to remain unaffected by fatigue.

When considering the physiological effects of fatigue there are a number of important variables to examine. We have previously demonstrated that during fatigued voluntary contractions muscle fibre conduction velocity declines due to a reduction in extracellular pH.[24] It is likely that this occurs due to a pH driven alteration of the Na+ and K+ gradient across the sarcolemma[25] and impairs action potential propagation. Therefore, during TMG measurement the electrical stimulus applied to the surface of the fatigued muscle should result in a slowing down of the action potentials propagated to reduce Ca2+ release and subsequent excitation-contraction (E-C) coupling. Low-frequency fatigue, as characterized by a disproportionate reduction in force at lower stimulation frequencies, has been associated with E-C uncoupling.[26] It has been suggested that E-C uncoupling is attributable to, amongst other factors, impaired Ca2+ transport via Ryanodine receptor channels in the triadic compartment.[27] Furthermore, other contributing factors will be from increased Pi which can push the cross-bridge into a low force generating status[28] and may also cause actin and myosin to detach.[29] These altered characteristics of muscle function will inevitably impair its force generation capacity, as shown by the significant decline in MVC.

**It has been reported previously that a stiffer muscle, as we have evidenced here by the rise in PMT (figure 5), will be associated with a reduced TMG Dm measurement.[8] In contrast to the current findings, Garcia-Manso et al[15] showed an *increase* in Biceps Femoris TMG Dm associated with fatigue following an ironman triathlon. The precise reasons for this disparity are unclear; however Morin, Tomazin, Edouard, & Millet[30]**

**showed a small decline in whole leg stiffness during a running task, following a 24-hour marathon. These authors postulated that central fatigue would have been apparent which would have been linked to altered peripheral feedback from muscle afferents triggered from cytokines. This, we suggest, may be why an increase in TMG Dm was observed following an ironman triathlon when a decline has been reported with other types of fatigue from far shorter contractile/ exercise durations.** Other studies have also demonstrated alterations in Dm alongside muscle architectural changes. Firstly, Pisot et al,[8] showed that following 35 days of bed rest, TMG Dm increased alongside the reduction in muscle thickness which the authors suggested would have contributed to reduced muscle stiffness. Secondly, we previously demonstrated[31] that altering the length of the muscle will determine the magnitude of TMG parameters, such that longer muscle length, as achieved through altered joint angle, results in reduced Dm. Thirdly, although not relating the decline in TMG Dm to muscle stiffness changes, other studies[16,23] have also demonstrated a decline in TMG Dm following fatigue, suggesting that this is an important parameter when assessing the muscle status in this regard.

In the present study we observed decreases in TMG Dm without significant alterations in Vc. Given previously described reductions in action potential propagation and muscle fibre conduction velocity, associated with fatigue,[24] it may have been expected that TMG Vc would be observed to decline post-fatigue, in concurrence with Dm. It is plausible that the lack of significant alteration in Vc is due to the high degree of inter-individual variability associated with the measurement. **Indeed, changes between measurements (M1, M2, M3) ranged from about -25% to +25% between participants**. The comparably low amplitude of the electrical stimulation used to elicit the peak TMG response, may perhaps render these





Human Kinetics, 1607 N Market St, Champaign, IL 61825

data difficult to compare to existing conduction velocity findings. As such, it may be inappropriate to consider alterations in the speed/ time component of the TMG response, when assessing muscle fatigue, with the focus instead being placed on spatial alterations (Dm), which we have shown here to be indicative of increased muscle stiffness.

As with any type of physiological measurement there will be a degree of variability. We have previously accounted for this variability with TMG measured under different muscle conditions[13] and shown Dm to be well within acceptable limits. Analogous to this is establishing minimal detectable change so practitioners and researchers can be confident that the given magnitude of observed change following any intervention is real and physiologically significant. We have demonstrated in this study that the fatigue-altered Dm parameter (17.7%) clearly exceeds the minimal detectable change thresholds of 15.1%.[13] Furthermore, the effect size for the data presented in this study, as described by Cohen,[22] is "large" suggesting that this particular TMG measure is sufficiently sensitive to adequately detect local muscular fatigue. **Nonetheless, a number of limitations must be considered. Current findings can only be applied to a healthy, young male population. It remains to be seen whether TMG measurements are sufficiently sensitive to detect fatigue associated changes in alternative cohorts. Additionally, GM was selected for investigation as its anatomical position facilitates measurement using TMG. Muscles which are not located superficially, but may still be of interest, are not measureable using the methods described herein**.

**Conclusion**

This is the first study to demonstrate that TMG was effective in detecting local muscular fatigue in the GM. We propose that this response was directly related to increased stiffness

of the muscle from impaired contractile capacity. It should be emphasised that, when assessing local muscular fatigue, Dm of the muscle is a valid measure, **however it remains to be seen whether TMG has the sensitivity to detect any changes in Vc in a different context**. The current findings have important implications for researchers and practitioners seeking to establish fatigue status of skeletal muscle, with implications for prevention of **over-training injuries** in sports-related activities. Given the **non-invasive and sub-maximal** nature of this type of measurement, TMG can be used to determine local muscular fatigue in patients who may be unable to exert the maximal effort required for voluntary muscle function assessments. **Additionally, TMG measurements are exempt from the bias of volitional effort and motivation, facilitating the incorporation of the procedure into existing programmes.[32] Furthermore, TMG could be utilised regularly, as a monitoring tool, without fear of detriment to muscle function**.

**Acknowledgements**

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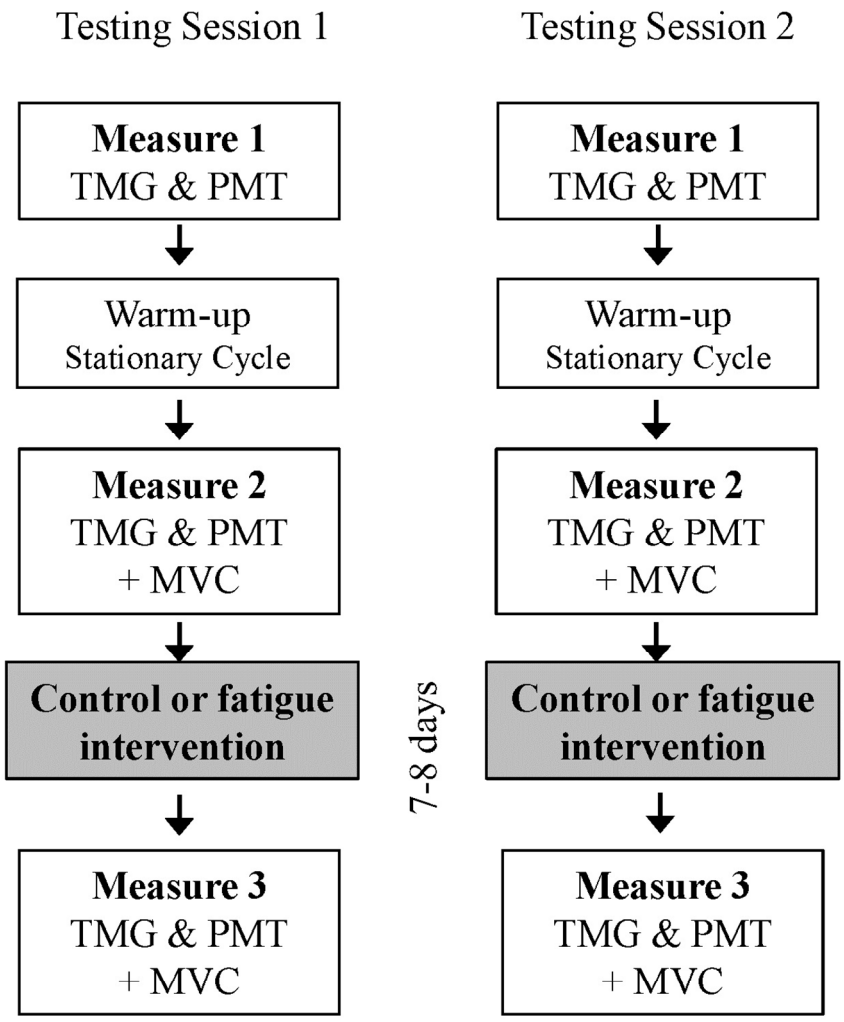


Figure 1. Schematic representation of the research design. TMG = Tensiomyography; PMT = passive muscle tension.
120x150mm (300 x 300 DPI)

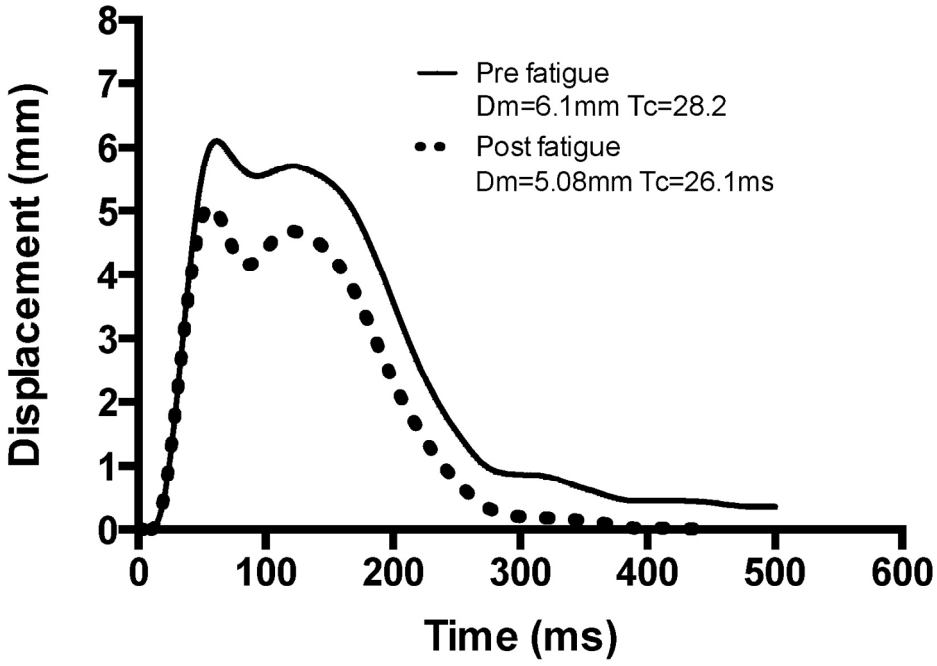


Figure 2. Typical displacement/ time curve of the tensiomyographic signal before and after the administration of the fatigue protocol. Dm = muscle displacement; Tc = contraction time.
150x110mm (300 x 300 DPI)

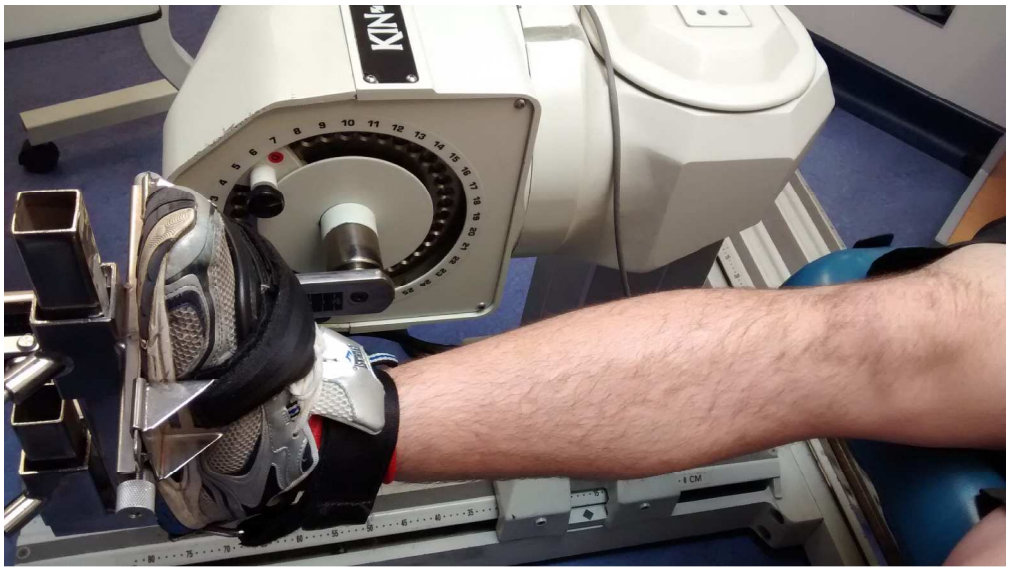


Figure 3. Isokinetic dynamometer setup for PMT and MVC assessment. Ankle flexed at 90° relative to the tibia.
342x192mm (300 x 300 DPI)

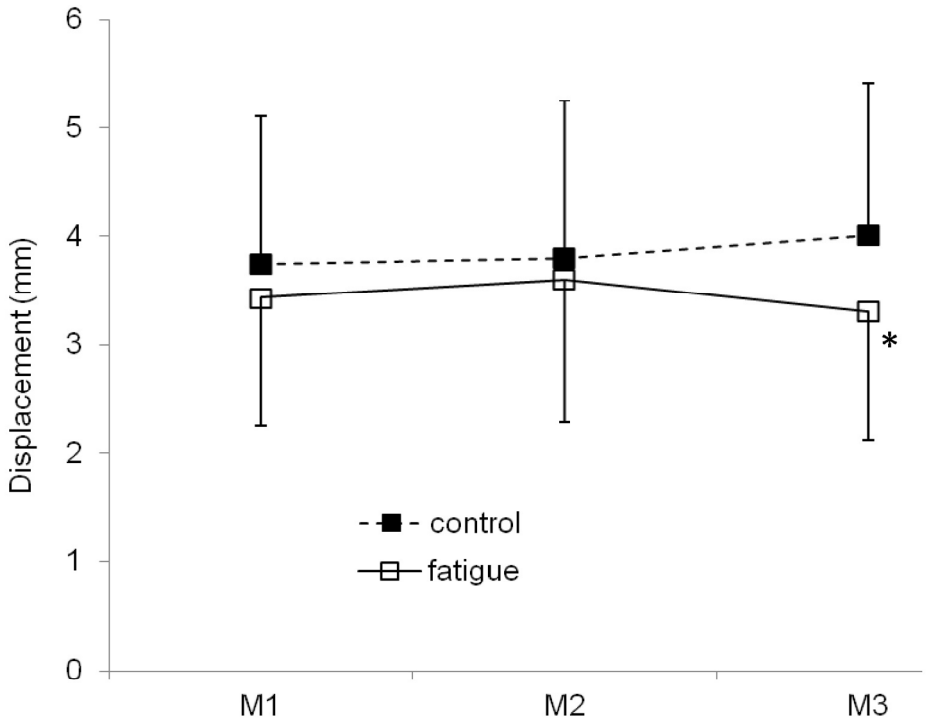


Figure 4. Average values (+ SD) of passive muscle tension as assessed on the isokinetic dynamometer at the three measurement points. * = significant different from 'control' at M3, $p < 0.01$.
442x383mm (96 x 96 DPI)

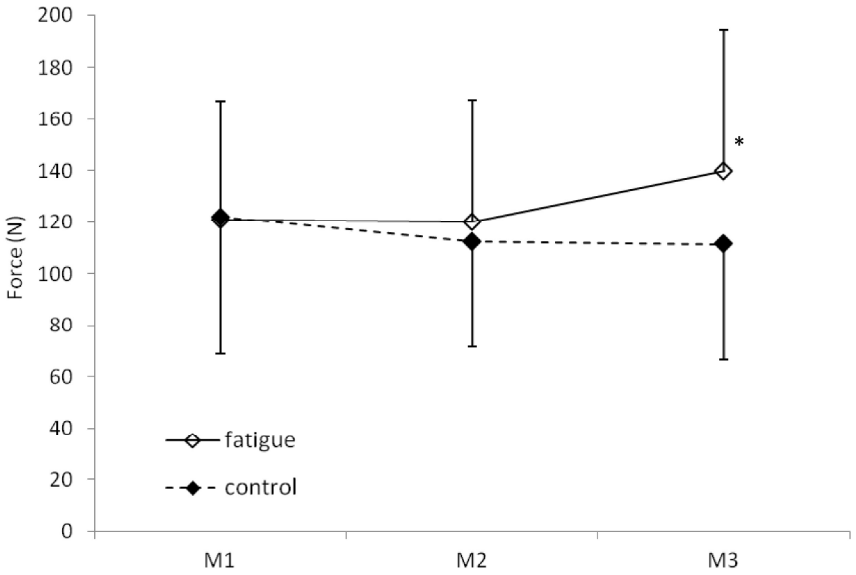


Figure 5. Average values (+ SD) of muscle displacement as assessed by tensiomyography at the three measurement points. * = significant different from 'control' at M3, $p < 0.05$.
254x190mm (300 x 300 DPI)