# Titelblad

**Titel:** The dynamics of motor unit behavior during dynamic contraction through fatigue.
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# Abstract

**Background:** Fatigue is a well-known ongoing process in sports and is proven to affect performance negatively. However, what happens on a neuromuscular level during fatigue is still an undiscovered area, and it has previously only been investigated during isometric contractions. The new approach of high-density electromyography (HD-EMG) has been used to unravel the potential mechanisms in terms of motor unit (MU) behavior during dynamic contractions leading to fatigue. Therefore, the purpose of this study was to evaluate the MU behavior during dynamic contractions leading to fatigue.

**Method:** Thirteen healthy individuals (10 males and 3 females, age: 28 ± 4 years, height: 176 ± 8 cm, body mass: 73 ± 6kg) participated in the experiment. The participants performed both isometric and dynamic protocols with an ankle dorsiflexion until exhaustion, where MU behavior was measured with HD-EMG matrixes placed on the tibialis anterior muscle. The data were then separated into three sectors across the fatiguing protocols (1: start, 2: middle, 3: end) and processed with a blind source separation algorithm. The mean- and peak discharge rates, number of MUs, recruitment duration, recruitment threshold, and the mean recruitment discharge rate were then statistically analyzed with the use of both one-way and two-way repeated measures ANOVA's with sectors and contraction types as the within-subjects factors.

**Results:** The main findings of this study were that no main effect was found across sectors for the mean- ($p = 0.558$) and peak discharge rate ($p = 0.096$). An interaction was observed ($p < 0.001$) for the number of motor units which decreased from sector 1 to sector 2 ($p = 0.019$) and sector 3 ($p = 0.001$) for the dynamic and isometric contractions. Further, a decrease from sector 2 to sector 3 was discovered ($p = 0.003$).

**Conclusion:** In conclusion, tendencies was observed for the mean discharge rate to decrease and then increase throughout muscle activity in the fatiguing protocol. Further, it can be concluded that the mean discharge rate for an eccentric contraction was lower compared to both a concentric and isometric contraction. It was also proven that the number of motor units decreased throughout fatigue.

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# 1.0 Introduction

Fatigue is a well-known phenomenon when discussing sports and physical activity. Muscular fatigue is commonly assumed to be a progressive factor that occurs from the start of a task and is commonly defined as a reduction in the ability to produce force (Enoka & Duchateau, 2008). Enoka and Duchateau (2008) explains this as the result of an impairment of different physiological processes which generates force as a means of contractile proteins. This means that muscular fatigue is not the specific moment in which the muscles stop their force production (i.e., becoming exhausted), it is an ongoing process.

Coutinho et al. (2018) explored the relation between muscular fatigue and performance in a five-a-side game of football. The players performed the five-a-side game under three different conditions; a) a control condition; b) a muscular fatigue condition, where participants performed repeated change of direction tasks before the game; c) and a mental fatigue condition, playing after completing a 30-minute Stroop color task (Coutinho et al., 2018). The study discovered that muscular fatigue negatively affected performance, where a 27% decrease in distance covered at high speeds was observed when comparing the muscular fatigue condition with the control condition. It is therefore imperative to investigate what happens during muscular contractions, specifically during fatigue, to improve athlete performance.

A week for athletes typically consists of a high physical load for longer periods of time at multiple times a week, which makes it important to improve individual stamina, whereas strength training is shown to be an effective tool for fatigue resistance (Salvador et al., 2009). Del Vecchio et al. (2019) investigated four weeks of strength training and the effect it had on MU behavior in the tibialis anterior muscle before and after ankle dorsiflexor strength training measured with HD-EMG signals recorded from two adhesive grids of 64 electrodes each. HD-EMG is a non-invasive technique to measure muscle activity with multiple electrodes, which allows for the identification of singular motor units by detecting the electrical impulses from action potentials (Drost et al., 2006). HD-EMG is relevant for investigating the impact fatigue has on individual motor units due to the previous mentioned ability to identify singular MUs and hereby the behavior of the MUs, meaning both specific motor unit discharge rate and recruitment. The study by Del Vecchio et al. (2019) was conducted on 25 recreationally active young men. The training protocol consisted of 40 ballistic contractions at maximum 75% of maximum voluntary contractions (MVC). After the ballistic contractions 30 sustained isometric ramp contractions were performed. It was discovered that a strength training intervention of 4 weeks leads to a decrease in MU recruitment threshold, and an increase in discharge rate during the plateau phase of the submaximal isometric contraction (Del Vecchio et al., 2019). The findings of Del Vecchio et al. (2019) regarding what happens with the development of MUs when strength training, gives precedence to investigate what happens with the MU behavior during muscular fatigue.

Martinez-Valdes et al. (2020) investigated MU behavior in relation to muscular fatigue during isometric knee extension on sixteen healthy and physically active men. To acquire data for the analysis a 64 grid HD-EMG were placed over both vastus medialis and lateralis muscles. Participants performed a ramp contraction at 50% MVC consisting of a 5 sec ramp-up, a 15 sec hold phase, followed by a 5 sec ramp-down. After the first test participants rested for 15 min, after which a contraction at 30% MVC was performed until exhaustion. The study found that during muscular fatigue the MU discharge rate of the two muscles decreased until ~40% of the total endurance time, followed by a continuous increase in discharge rate until task failure. Interestingly, subsequent research revealed that although discharge rates increased in a fatiguing task, peak discharge rates failed to match the frequency observed during the 50% MVC non-fatiguing task (Martinez-Valdes et al., 2020).

The studies above lack a comprehensive view of the MU behavior during dynamic contractions and the relation to fatigue. When measuring isometrically contracting muscles, an insight into the lengthening and shortening of the muscle fibers is not obtained, which causes insufficient discoveries, as muscle fibers often contract dynamically in daily tasks and physical activity such as sports. This is particularly important for the tibialis anterior muscle that plays a crucial role during the swing phase of both gait and running (Juneja & Hubbard, 2023). Therefore, Oliveira and Negro (2021) utilized HD-EMG as well to explore MU behavior not only during isometric contractions but also dynamic contractions in the tibialis anterior muscle. The dynamic contractions were performed at three different velocities and two different relative loads (5°/s, 10°/s, and 20°/s at 10% MVC and 25% MVC). Specifically, the study found that an increase in contraction velocity led to an increase of the discharge slope, along with recruitment and derecruitment discharge rates. Furthermore, they found that the recruitment angles were decreased during tests and that >92% of the MU recruited at 20°/s were active at both 5°/s and 10°/s, which suggests that MU discharge rate is therefore more likely to be responsible for controlling the muscle when performing dynamic contractions rather than recruitment. This means that it could be more important to increase the discharge rate for each MU rather than having a higher recruitment. Further, an intriguing finding was discovered when examining the mean discharge rate of MUs across both isometric and dynamic trials, revealing a notable level of correlation (5°/s: r = 0.53; 10°/s: r = 0.54; 20°/s: r = 0.55) (Oliveira & Negro, 2021).

Within the literature, it is commonly discussed and investigated how MUs behave during isometric contractions (Del Vecchio et al., 2019; Martinez-Valdes et al., 2020). However, it seems relevant to investigate if MUs behave comparably whether muscle contractions are performed isometrically or dynamically especially in relation to fatigue. Fatigue was described as an important factor in terms of high performance, which makes knowledge of MU behavior during dynamic contractions highly relevant (Coutinho et al., 2018).

It is currently known that MU discharge rate decreases during fatigue in isometric contractions (Martinez-Valdes et al., 2020), while Oliveira and Negro (2021) present in their study that an increase of velocity leads to an increase of discharge slope, recruitment- and derecruitment discharge rates performing dynamic contractions in the tibialis anterior muscle. Based on the results reported by Oliveira and Negro (2021) it seems possible to measure MU behavior during dynamic contractions performed until exhaustion. This leads to the main aim of the present study, to evaluate MU behavior during fatigue in dynamic contractions, as this aspect remains predominantly studied in the context of isometric contractions (Martinez-Valdes et al., 2020).

In the study by Oliveira and Negro (2021), the primary emphasis was placed on examining MU behavior across varying velocities, leading to the discovery of a correlation between discharge rates during isometric and dynamic contractions, which gives precedence to base hypotheses on the results of Martinez-Valdes (2021). The hypotheses are as follows: i) It was expected that the specific MU mean discharge rate would initially decrease, followed by an increase until exhaustion, ii) it was expected that the mean peak discharge rate would increase over the course of fatigue as well, and- iii) it was expected that the number of MUs recruited was lower towards the end of the trial when performing dynamic contractions.

# 2.0 Materials and Methods

## 2.1 Participants and Ethics

The study was conducted with thirteen healthy individuals (10 males and 3 females, means ± SD age: 28 ± 4 years; height: 176 ± 8 cm; body mass: 73 ± 6kg). A declaration of consent was signed by all participants which was approved by The North Denmark Region Committee on Health Research Ethics (N-20170081). The method was conducted in accordance with the Declaration of Helsinki (2004).

## 2.2 Study Design

The study was conducted on two different occasions, whereas the isometric fatigue protocol was performed on the first day and the dynamic fatigue protocol performed on the second day (Figure 1). During all tests visual feedback was provided on a screen with a marker to follow during the contractions.

In the present study an EMG-USB (OT Bioelettronica, Torino, Italy) was used to acquire data. A matrix consisting of 64 electrodes each with 8mm inter electrode distance was placed over the tibialis anterior muscle on the right leg. To establish proper alignment, the matrix line was drawn on the participants leg, from the lateral condyle of the tibia to the lower medial surface of the first cuneiform and the base of the first metatarsal. The muscular belly of the muscle was identified at approximately a third of the length. To ensure EMG accuracy, participants performed an ankle dorsiflexion while a palpation was conducted to locate the muscle.

The protocols were performed in a HUMAC Norm Chair (Humac Norm, CSMI), which made it possible to control the range of motion of which the dorsiflexion was performed. The participants performed the protocols with a knee angle of 90° whereas the ankle was 110°.

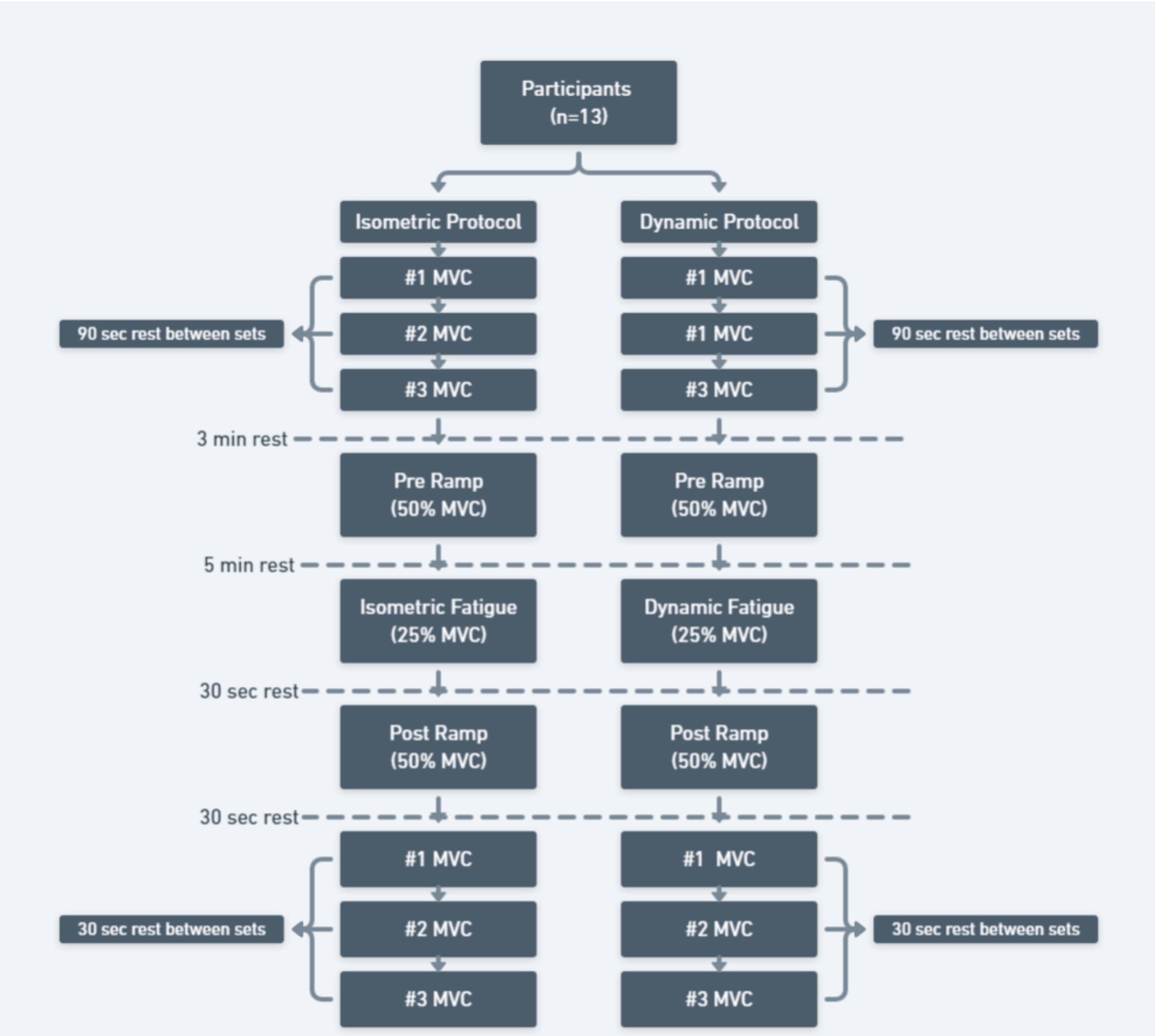


***Figure 1: Flowchart of the study design.*** *MVC = Maximal voluntary contraction. (n=13)*

## 2.3 Isometric and Dynamic Protocols

Both the isometric and dynamic protocols consisted of start measurements of three ankle dorsiflexion MVC at 100° with 90 seconds rest in between sets followed by 3 min rest (Figure 1). Additionally, both protocols consisted of a pre fatigue ramp test and a post fatigue ramp test, which consisted of a 5 second ramp up from 10% MVC/s, a 10 second isometric contraction at 50% MVC, followed by a 5 second ramp down at 10% MVC/s. After the pre fatigue ramp test and a 5-minute rest period the isometric fatigue protocol was conducted which consisted of a 5 second ramp up at 5% MVC/s, followed by an isometric contraction at 25% MVC until failure (Figure 2a). Failure in the isometric fatigue test was determined when the participant could not hold the contraction for more than 5 seconds within the 10% target range. Immediately after the test the participants performed the post fatigue ramp test and three MVC. The dynamic fatigue protocol was performed at 25% MVC and consisted of a 2 second ramp-up at 10°/s, a 2-second contraction with a knee flexion at 90°, followed by a 2 second ramp-down at 10°/s and a 2 second contraction with a knee flexion at 110° until failure (Figure 2b). Failure in the dynamic fatigue test was determined by the third error in the execution of the ramps, which implies failure to maintain the trace within a 10% range limit of the target MVC. Afterwards, the post fatigue ramp test was completed followed by three MVCs. However, for this specific study, only data from the isometric and dynamic fatigue protocols were used for further processing.

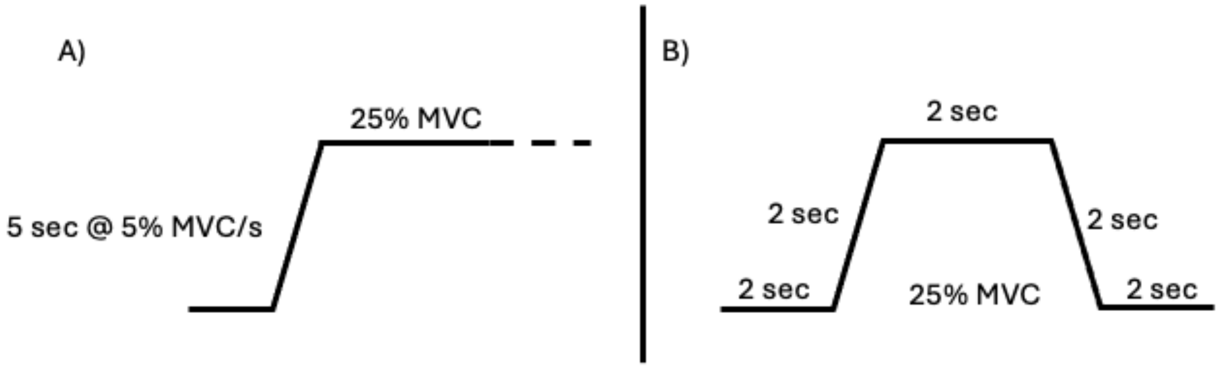


***Figure 2: Illustration of force produced, and the ramp performed in the isometric protocol (A) and the force produced and the ramps performed in the dynamic protocol (B).*** *MVC = Maximal voluntary contraction.*

## 2.4 Analytical Procedures and Calculations

For the analytical procedures the data were recorded with OTBiolab software, whereas it was later processed in MatLab (Version R2023b). The processing in MatLab consisted of using a blind source separation algorithm which have been validated on multichannel surface (Negro et al., 2016) and applied to studies in dynamic contractions (Kapelner et al., 2019; Oliveira & Negro, 2021). The blind source separation algorithm can identify individual MUs from the multi-channel signals (Negro et al., 2016). The process in MatLab consisted of multiple steps, where the first step consisted of selecting three sectors of approximately 60 seconds, for both the dynamic and isometric protocols (Figure 3); one sector at the beginning (low fatigue), one in the middle (middle fatigue), and one towards the end (high fatigue) of each test. This was done to both lower the processing time and to ensure the data represented fatigue over time. For the dynamic protocol, four cycles in each sector with the least fluctuations were chosen for further processing. Afterwards, the automatic decomposition was then performed, where signals were evaluated with the Silhouette measure (SIL), and only MUs with a SIL > 0.87 were included for the manual improvement of the signals. After the automatic decomposition, a manual cleaning of the motor unit pulse trains was processed manually with the assistance of an optimization procedure script. After the manual cleaning of the data two participants were excluded completely from the study due to lack of motor unit data. All the data were extracted into readable data, which were then converted into an Excel-document (Excel version 16.79.2 (Microsoft, Washington, USA)) and further analyzed in SPSS Statistics Version 29 (IBM, New York, USA).

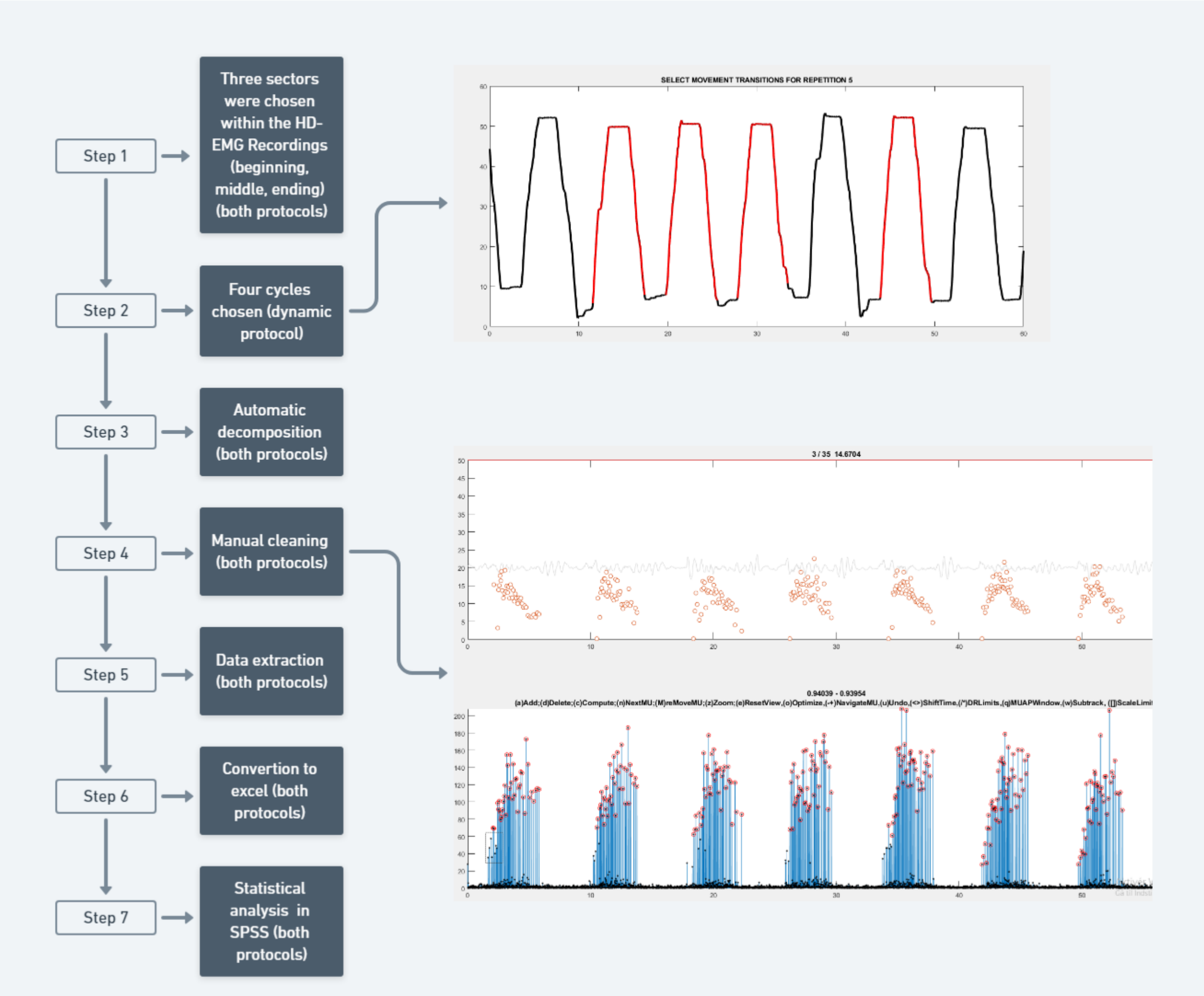


***Figure 3: Flowchart of the analytical procedures with illustrations of the manual data cleaning.***

## 2.5 Statistics

All statistical tests were completed using SPSS Statistics Version 29 (IBM, New York, USA). Test of normality was performed before further analysis was completed to ensure normally distributed data. Considering the relatively small sample size (n < 50) the Shapiro-Wilks test was used to evaluate the normality of the data. All data used in the statistical analysis were proven to be normally distributed.

All coefficients of variation variables (force and position) were tested as an effect of time in relation to fatigue and were tested using two-way repeated measures ANOVA, with the factors being contraction type (concentric, isometric, and eccentric; all dynamic) and sectors (sector 1, sector 2, and sector 3).

All variables regarding dynamic contractions (mean discharge rate concentric; mean discharge rate eccentric; peak discharge rate; number of MUs; recruitment discharge rate; recruitment threshold; recruitment duration) and isometric contractions (mean discharge rate; peak discharge rate: number of MUs) were tested as an effect over time (3 sectors) in relation to fatigue. This gave precedence to use the two-way repeated measures ANOVA for mean discharge rate due to several contraction types (mean discharge rate concentric, mean discharge rate eccentric, and mean discharge rate for the isometric protocol) across all three sectors. Furthermore, both the peak discharge rate and number of MUs were tested with the two-way repeated measures ANOVA, whereof the protocols (dynamic and isometric) being one factor, while sectors being the other.

In relation to the recruitment, only data regarding the dynamic protocol was tested statistically in terms of the recruitment discharge rate, recruitment threshold, and recruitment duration, which means multiple one-way repeated measures ANOVA was performed.

Due to lack of sufficient data four participants were excluded from the statistical analysis regarding the coefficient of variation for force and positioning (n=7). For the variable regarding mean discharge rate five participants were excluded (n=6). For the peak discharge rat*e* 4 participants were excluded (n=7). In relation to the mean recruitment discharge rate and recruitment duration four participants were excluded (n=7). Furthermore, six participants were excluded for the recruitment threshold variable (n=5).

# 3.0 Results

## 3.1 Coefficient of variation for force production and positioning

Data regarding coefficient of variation for force production during the dynamic protocol showed no interaction between contraction types (concentric, isometric, and eccentric) and sectors ($F(4,24) = 0.204$, $p = 0.934$). Furthermore, a main effect was found across the sectors ($F(2,12) = 4.241$, $p = 0.04$). However, the pairwise comparison showed no significant main effect due to the Bonferroni correction (all $p$'s $> 0.107$). For the contraction types a main effect was found ($F(2,12) = 25.158$, $p < 0.001$). The pairwise comparisons showed that the force coefficient of variation in the isometric contraction phase was significantly lower than both the concentric ($p < 0.001$) and eccentric contraction types ($p = 0.007$) across all sectors (Figure 4A).

For data concerning the coefficient of variation for the positioning during the dynamic protocol, no interaction between contraction types and sectors was discovered ($F(4,24) = 0.111$, $p = 0.977$). Further, a main effect was discovered across sectors ($F(2,12) = 4.562$, $p = 0.034$). After performing a pairwise comparison it was discovered that the position coefficient of variation in sector 1 was significantly lower than sector 3 ($p = 0.004$) across all contraction types. No main effect was discovered for the contraction types ($F(2,12) = 1.204$, $p = 0.334$) (Figure 4B).

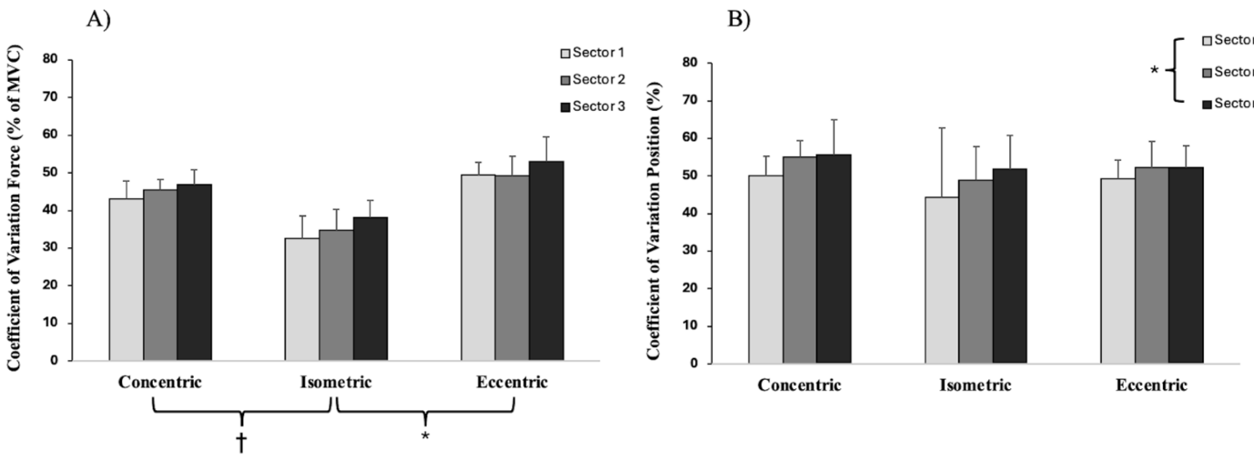


***Figure 4: Coefficient of variation for force (A) and position (B) during all phases in the dynamic protocol across all sectors*** *(n=7). Results are presented as mean ± SD. † = Significant difference of p < 0.001. * = Significant difference of p < 0.05.*

## 3.2 Mean discharge rate for motor units

A two-way ANOVA with repeated measures found no significant interaction for the mean discharge rate for MUs ($F(1.60,8.01) = 3.867$, $p = 0.073$). Furthermore, no main effect was discovered within sectors ($F(1.04, 5,17) = 0.407$, $p = 0.558$). However, focusing on contraction types a main effect was found ($F(2, 10) = 112.612$, $p < 0.001$). The pairwise comparison with Bonferroni correction proved that the behavior of MUs in the concentric contraction throughout the dynamic protocol had a significantly higher mean discharge rate compared to both the eccentric contraction ($p < 0.001$) and the isometric contraction within the isometric protocol ($p = 0.001$) across all sectors. It was also discovered that the mean MU discharge rate for the eccentric contraction was significantly lower compared to the mean MU discharge rate for the isometric contraction in the isometric protocol ($p = 0.002$) (Figure 5).

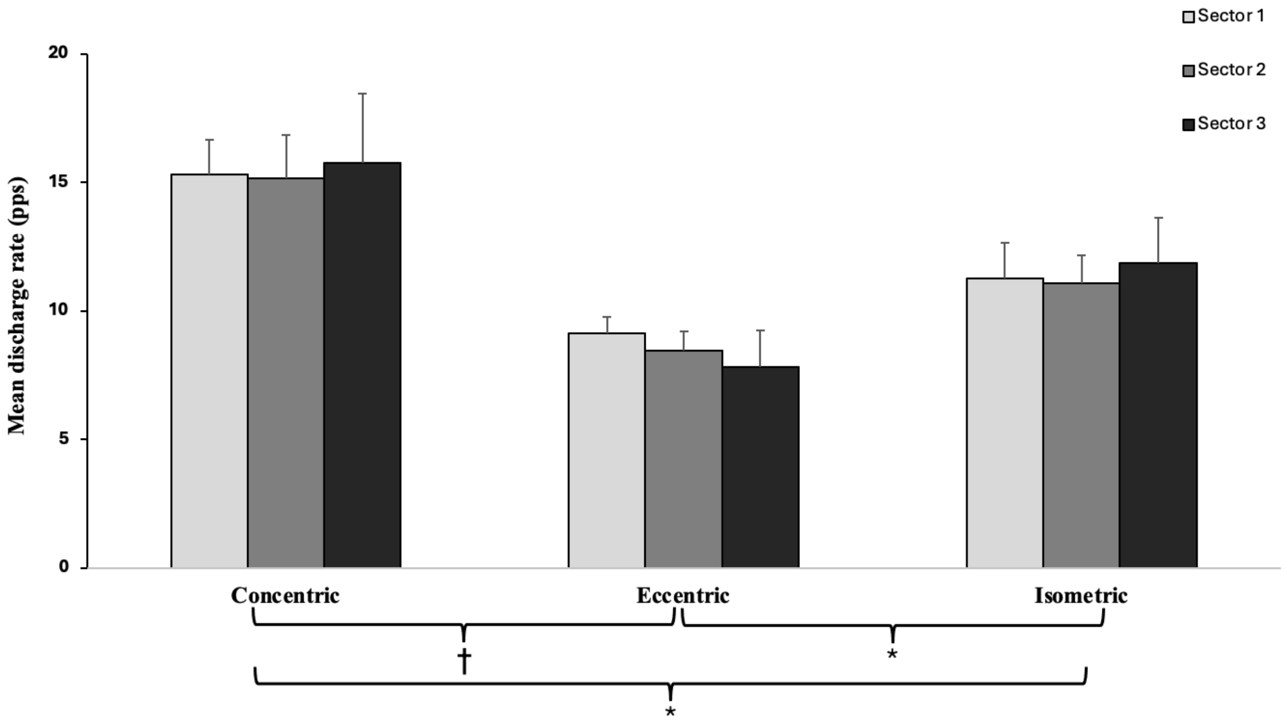


***Figure 5: Mean discharge rate for motor units during concentric and eccentric phase for the dynamic protocol and for the isometric protocol across all three sectors*** *(n=6). Results are presented as mean ± SD. † = Significant difference of p < 0.001. * = Significant difference of p < 0.05. DR = discharge rate*

## 3.3 Peak discharge rate for motor units

Regarding the peak discharge rate of MUs, no interaction was discovered between the two protocols ($F(2,12) = 3.495$, $p = 0.64$). Furthermore, no main effect was found within sectors ($F(1.05,6.32) = 2.863$, $p = 0.096$). However, a main effect was observed between the protocols ($F(1,6) = 77.712$, $p < 0.001$). The dynamic protocol presented a significantly higher MU peak than the isometric protocol ($p < 0.001$) across all sectors (Figure 6).

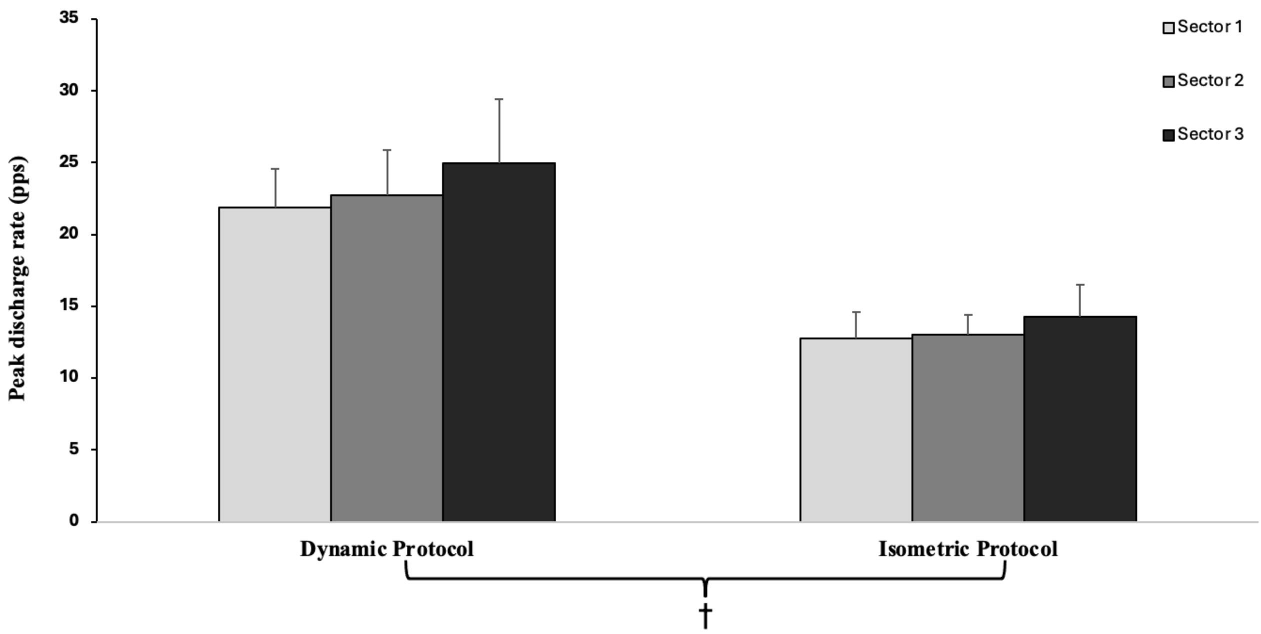


***Figure 6: Peak discharge rate for motor units during dynamic contractions and isometric contractions across all sectors*** *(n=7). Results are presented as mean ± SD. † = Significant difference of p < 0.001.*

## 3.4 Motor unit recruitment

Data regarding the number of MUs that were recruited showed an interaction between sectors and protocols after the performance of a two-way repeated measures ANOVA ($F(2,20) = 11.312$, $p < 0.001$). The simple main effects analyses revealed that within the dynamic protocol, the number of MUs within the first sector was significantly higher than in sector 2 ($p = 0.019$) and 3 ($p = 0.001$). In addition, the muscle contraction performed within sector 2 of the dynamic protocol, it was observed that the number of MUs was significantly higher than in sector 3 ($p = 0.003$). For the isometric protocol, the number of MUs in sector 1 was significantly higher compared to sector 2 and 3 (both $p$'s < 0.001). It was also discovered that the muscle contractions performed in sector 2 had recruited significantly more MUs than in sector 3 ($p < 0.001$). Finally, it was observed that the number of MUs was significantly higher in both sector 1 ($p < 0.001$) and sector 2 when performing the isometric protocol compared with the dynamic protocol ($p = 0.033$) (Figure 7).

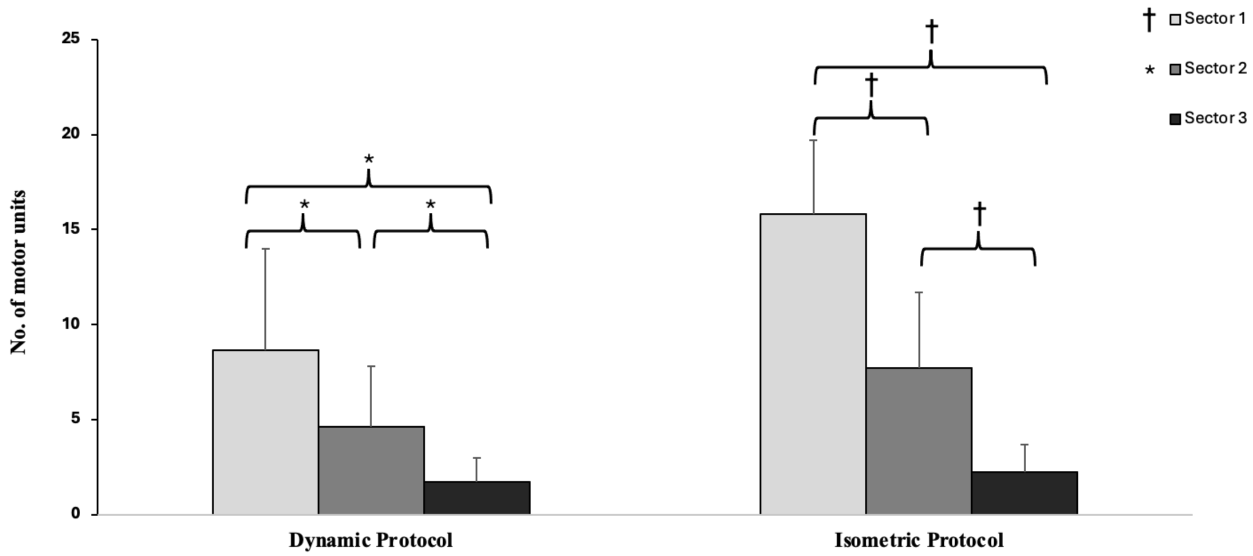


***Figure 7: Number of motor units recruited across all sectors in both the dynamic and isometric protocol*** *(n=11). Results are presented as mean ± SD. † = Significant difference of p < 0.001.*

## 3.5 Recruitment in the dynamic protocol

Data regarding mean recruitment discharge rate was tested with a one-way repeated measures ANOVA, which did not show any main effect across sectors ($F(2,12) = 0.520$, $p = 0.608$) (Figure 8A). Furthermore, a one-way repeated measures ANOVA was also performed on recruitment threshold data, of which no main effect was observed across sectors ($F(2,8) = 0.860$, $p = 0.459$) (Figure 8B). Finally, no main effect across sectors was found using the one-way repeated measures ANOVA for the recruitment duration data ($F(2,12) = 1.345$, $p = 0.297$) (Figure 8C).

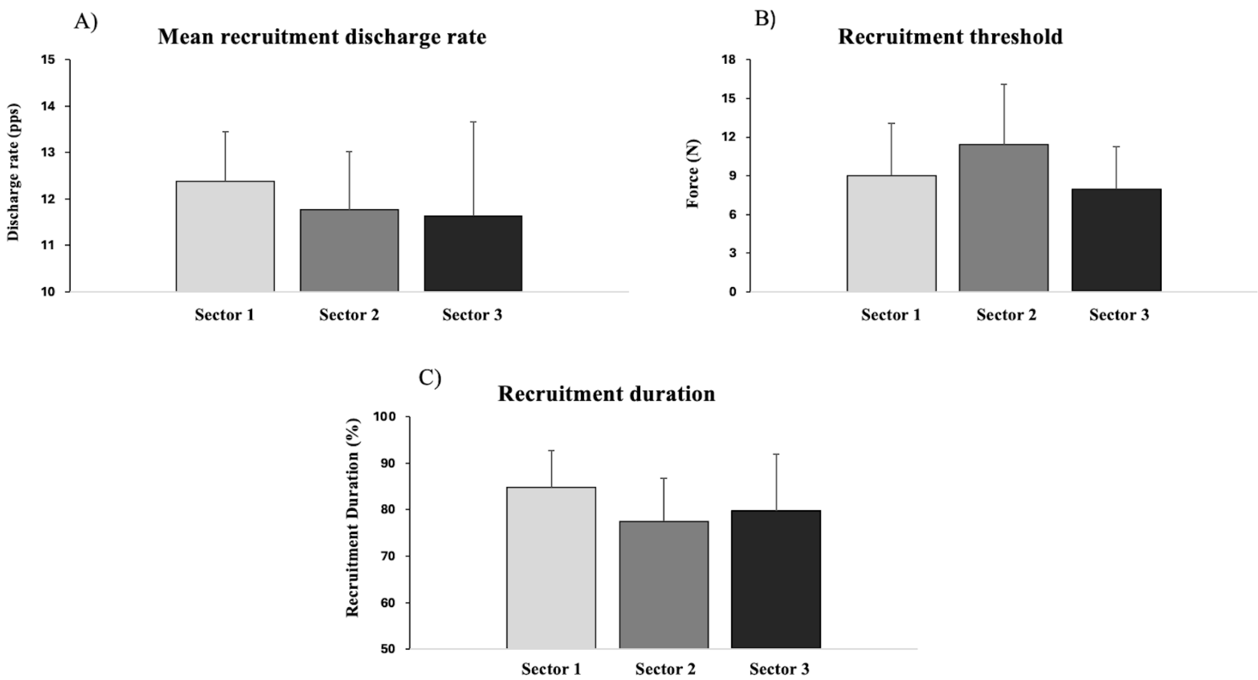


***Figure 8: Mean recruitment discharge rate (A)*** *(n=7)*, ***mean recruitment threshold (B)*** *(n=5)*, ***and mean recruitment duration (C)*** *(n=7) for the dynamic protocol across all sectors. Results are presented as mean ± SD.*

# 4.0 Discussion

## 4.1 Study aim and hypotheses

The main purpose of this study was to clarify MU behavior during fatigue in dynamic contractions to exhaustion. Additionally, the results regarding dynamic contractions were compared to data from an exclusively isometric protocol to further clarify the differences in MU behavior between the two contraction types over the course of fatigue.

To ensure that the dynamic protocol was performed correctly, statistical analysis of the coefficient of variation for both force and position were performed. No interaction was discovered, which ensures that the participants performed the dynamic protocol without a significant amount of fluctuation throughout the duration of the protocol. However, a pairwise comparison proved that the position of the foot varied with a significant amount from the first sector to the last sector, which implies that the participants have performed the dynamic protocol until exhaustion as it is previously shown that position control suffers when working under exhaustion (Dietz et al., 1994).

It was hypothesized in relation to the studies of Oliveira and Negro (2021) and Martinez-Valdes et al. (2020) that the mean MU discharge rate would decrease from sector 1 to sector 2 followed by an increase to sector 3. No main effect was found within the three sectors, which means that the hypothesis was rejected. An important factor as to why no interactions were observed could be because of the inclusion of both continuous and intermittent MUs in the statistical analysis. Mixing both MU types can be a relevant factor, because continuous MUs might have a different pattern of behavior under different circumstances such as contraction type and activation duration in comparison to intermittent MUs. In the review by Enoka and Stuart (1992), it is discussed that changes in contraction types, might change which MUs is responsible for the force contributed to the specific muscle contraction. The results for the mean discharge rate of MUs show tendencies as the hypothesized pattern, for both the concentric contraction while performing the dynamic protocol and for the isometric protocol, but not for the eccentric contraction. It is well known that eccentric force is greater than concentric force (Doss & Karpovich, 1965), which could prove as an important factor when comparing the two contraction types at the same absolute force. Furthermore, it was previously shown that the total endurance for the same absolute force is greater in eccentric force compared with concentric force (Carrasco et al., 1999). Nuzzo et al. (2023) have also proven that the difference in this specific relationship increases by 0.2% for every 1°/s increase in velocity, which makes the relative load difference even higher in the present study. These differences in the concentric and eccentric force, might explain the reason for the MUs discharge rate continuous decrease during the eccentric contraction, more specifically the muscle might not be close to fatigue during the eccentric contraction.

The previous mentioned relationship might explain the reason for the rejection of the second hypothesis regarding MU peak discharge rate, which were based on the findings of Del Vecchio et al. (2019). Del Vecchio et al. (2019) showed that a training period of four weeks decreased MU recruitment threshold values and increased the discharge rate, whereas the peak discharge rate should increase as well due to the decrease in the number of MUs recruited over time. In the present study data for peak discharge rate is only represented across the whole dynamic protocol, which makes it difficult to differentiate between the concentric and eccentric contraction. However, the continuous decrease in mean MU discharge rate for the eccentric contraction might influence the peak discharge rate, which meant no main effect could be observed. Even though no main effect was found, a tendency for an increased peak discharge rate over the course of fatigue could still be observed within the statistical analysis for both the dynamic contractions as well as for the isometric contractions. These results indicate a higher discharge rate in general for MUs in a dynamic protocol when keeping the muscle activated until exhaustion, which could be explained by several factors such as specificity to the exercise or task. In addition, it could be explained that dynamic muscle contractions might differentiate too much from isometric contractions on a neuromuscular basis which can make it difficult to compare the two types of muscle contractions even in terms of MU behavior (James et al., 2024). The falsification of the second hypothesis, can also be a result of insufficient data in terms of what appears to be a low sample size (n=7), which could have an impact on the results presented in the present analysis.

Based on the findings of both Del Vecchio et al. (2019) and Oliveira and Negro (2021), the third hypothesis suggests that the number of MUs recruited was lower towards the end of the trial when performing dynamic contractions. The results previously presented showed a significant difference for the within factor in all sectors, which means that the hypothesis was accepted. Not only did the present study find that the number of MUs recruited decreased across all sectors in both the dynamic and isometric protocol. Since the dynamic muscle contractions performed in the trials were utilized at the same amount of force throughout the testing, it suggests that the number of MUs recruited throughout the dynamic activation of the tibialis anterior muscle is a minor influential factor than the discharge rate in terms of keeping the muscle going at a constant pace and load. This agrees with the conclusion of the study by Oliveira and Negro (2021), which found that >92% of MUs recruited was to be found within all three velocities performed in their trials indicating that no additional MUs were recruited. These results suggest that the discharge rate of the single MU is a more substantial contributor for controlling dynamic muscle contractions against a constant load. Additionally, notable correlations were found between the mean discharge rate across muscle contraction types (isometric and dynamic compared) over the course of all three velocities (Oliveira & Negro, 2021). This evidence gives reason to believe that the number of MUs recruited would decrease as well, when performing muscle contractions isometrically until exhaustion, which was discovered in the present study as well.

Further, the training intervention study by Del Vecchio et al. (2019) also shows that the absolute number of MUs recruited declined, while the mean discharge rate was increased when tested at the same relative load after four weeks of strength training, which might be a result of a decreased MU recruitment threshold and increased muscle resilience. In contrast to the present study, Del Vecchio et al. (2019) did not investigate MU behavior in relation to fatigue, which therefore makes it difficult to draw exact comparisons to the present study. However, it does appear that a decrease of MU recruitment threshold has an influence on the amount of MUs recruited when tested at a constant load both isometrically and dynamically (Del Vecchio et al., 2019; Oliveira & Negro, 2021). This assumption also seems to be the case in other studies regarding MU behavior and fatigue (Carpentier et al., 2001). Carpentier et al. (2001) investigated singular MU behavior in relation to fatigue as well, for which they utilized a different EMG approach in terms of extracting data with a selective electrode inserted into the muscle by a hypodermic needle.

Carpentier et al. (2001) divided MUs into high- (>25% MVC) and low threshold (<25% MVC) units and found that the high threshold MUs had a decrease in activation threshold, whereas the low threshold MUs either had no change or an increase in activation threshold. These findings may contribute to the understanding of MUs that has been considered in the present study, since the dynamic protocol is performed at 25% MVC, and therefore can be considered high threshold MUs. As Carpentier et al. (2001) investigated singular MUs, the overall dropout rate of MUs were not able to be detected. Nevertheless, since there are indications in the study that high threshold MUs had a decrease in threshold activation, it can be inferred that a decrease in the number of MUs recruited could possibly be observed as fatigue progresses. This suggests similar tendencies, in regards of a decrease in MU recruitment threshold, as seen in the literature (Del Vecchio et al., 2019; Oliveira & Negro, 2021). However, it should be mentioned that both the musculature and the test method used in the Carpentier et al. (2001) are not similar to the literature or the present study.

## 4.2 Strengths and limitations

Several methodological considerations that have been included in the present study are considered to be strong, such as the blind source separation algorithm, which have been extensively validated (Negro et al., 2016). In addition, HD-EMG has been used to obtain multiple MU action potentials and is considered a particular strength of this study in terms of application speed and signal quality obtained (Stegeman et al., 2012). Further, it is a non-invasive approach, which is an advantage for comfortability of the participants, and it ensures reliability and reproducibility for testing the same specific area of a given muscle group in contrast to the invasive EMG-technique using a hypodermic needle (Stegeman et al., 2012). Moreover, both the methodological applications have previously been used to investigate dynamic contractions, which further increases the validity of data obtained (Kapelner et al., 2019; Oliveira & Negro, 2021). However, HD-EMG can be problematic regarding the non-invasive application. Several steps must be taken into consideration, such as for example removal of hair, cleaning of skin, and the adhesive nature of the HD-EMG, which can make it hard to reposition the grids on to the surface of the skin and hereby interfere with the signals obtained (Stegeman et al., 2012).

In respect to the statistical analysis of the present study, thirteen participants contributed with data of which only eleven participants had sufficient data after the manual cleaning and could thus be included in further analysis. However, of these eleven participants not all had sufficient data across all sectors, which meant a further exclusion from multiple statistical analyses. This lowered the sample size in multiple analyses, which lowered both the power and validity of these results and might have negatively influenced potential interactions. With a greater sample size, the present study might have seen significant interactions regarding the increased MU discharge rate both for mean and peak values.

**In conclusion**, the present study demonstrated that MU behavior for dynamic contractions show tendencies to that of isometric contractions, meaning that the MU mean discharge rate lowers until approximately halfway through the endurance period, followed by an increase until exhaustion. However, regarding the hypotheses of the present study it is rejected because no significant interactions were observed in the case of the mean MU discharge rate in relation to fatigue. Furthermore, no interactions were observed in relation to MU peak discharge rate, meaning the second hypothesis was also rejected, where a tendency for an increase in peak discharge rate was observed. Finally, the third hypothesis was confirmed on the grounds that the number of MUs recruited was lower across all sectors, meaning a significantly lower number of MUs were recruited at the end of the endurance period. Additionally, it was observed that the mean discharge rate for MUs was significantly lower in the eccentric contraction compared to both the concentric and the isometric contraction (isometric protocol), which implies that eccentric contractions are more resilient to fatiguing tasks and therefore behave differently on a neuromuscular basis.

# 5.0 Perspectives

The results derived from the present study provide further knowledge of MU behavior, specifically during fatiguing dynamic contractions. Therefore, the results give additional insight of MU behavior during fatigue. However, mainly tendencies are discovered within this study, therefore an extended investigation including a larger sample is required to be able to confirm these tendencies. These findings make it possible for coaches and sports scientists to design training protocols that optimize performance and delay the onset of fatigue, which can be achieved by targeting specific contraction types (concentric, eccentric, and isometric). As eccentric contractions are shown to be more resilient to fatigue. Therefore, it would be beneficial to use the obtained knowledge regarding MU patterns within these contraction types. Furthermore, the research can be used to tailor specific rehabilitation programs considering the different MU behavior in both isometric and dynamic contractions to enhance recovery outcomes.

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# 7.0 Literature

[1] Carrasco, D. I., Delp, M. D., & Ray, C. A. (1999). Effect of concentric and eccentric muscle actions on muscle sympathetic nerve activity. *Journal of Applied Physiology*, *86*(2), 558–563. https://doi.org/10.1152/jappl.1999.86.2.558

[2] Carpentier, A., Duchateau, J., & Hainaut, K. (2001). Motor unit behaviour and contractile changes during fatigue in the human first dorsal interosseus. *Journal of Physiology*, *534*(3), 903–912. https://doi.org/10.1111/j.1469-7793.2001.00903.x

[3] Coutinho, D., Gonçalves, B., Wong, D. P., Travassos, B., Coutts, A. J., & Sampaio, J. (2018). Exploring the effects of mental and muscular fatigue in soccer players' performance. *Human Movement Science*, *58*, 287–296. https://doi.org/10.1016/j.humov.2018.03.004

[4] Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R. M., Felici, F., & Farina, D. (2019). The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *Journal of Physiology*, *597*(7), 1873–1887. https://doi.org/10.1113/jp277250

[5] Dietz, V., Discher, M., & Trippel, M. (1994). Task-dependent modulation of short- and long-latency electromyographic responses in upper limb muscles. *Electroencephalography and Clinical Neurophysiology. Evoked Potentials*, *93*(1), 49–56. https://doi.org/10.1016/0168-5597(94)90091-4

[6] Doss, W. S., & Karpovich, P. V. (1965). A comparison of concentric, eccentric, and isometric strength of elbow flexors. *Journal of Applied Physiology*, *20*(2), 351–353. https://doi.org/10.1152/jappl.1965.20.2.351

[7] Drost, G., Stegeman, D. F., Van Engelen, B., & Zwarts, M. J. (2006). Clinical applications of high-density surface EMG: A systematic review. *Journal of Electromyography and Kinesiology*, *16*(6), 586–602. https://doi.org/10.1016/j.jelekin.2006.09.005

[8] Enoka, R. M., & Duchateau, J. (2008). Muscle fatigue: what, why and how it influences muscle function. *Journal of Physiology*, *586*(1), 11–23. https://doi.org/10.1113/jphysiol.2007.139477

[9] Enoka, R. M., & Stuart, D. G. (1992). Neurobiology of muscle fatigue. *Journal of Applied Physiology*, *72*(5), 1631–1648. https://doi.org/10.1152/jappl.1992.72.5.1631

[10] James, L. P., Weakley, J., Comfort, P., & Huynh, M. (2024). The Relationship between Isometric and dynamic Strength following Resistance Training: A Systematic Review, Meta-Analysis, and Level of Agreement. *International Journal of Sports Physiology and Performance*, *19*(1), 2–12. https://doi.org/10.1123/ijspp.2023-0066

[11] Kapelner, T., Vujaklija, I., Jiang, N., Negro, F., Aszmann, O. C., PríNcipe, J. C., & Farina, D. (2019). Predicting wrist kinematics from motor unit discharge timings for the control of active prostheses. *Journal of Neuroengineering and Rehabilitation*, *16*(1). https://doi.org/10.1186/s12984-019-0516-x

[12] Martinez-Valdes, E., Negro, F., Falla, D., Dideriksen, J. L., Heckman, C. J., & Farina, D. (2020). Inability to increase the neural drive to muscle is associated with task failure during submaximal contractions. *Journal of Neurophysiology*, *124*(4), 1110–1121. https://doi.org/10.1152/jn.00447.2020

[13] Negro, F., Muceli, S., Castronovo, A. M., Holobar, A., & Farina, D. (2016). Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *Journal of Neural Engineering*, *13*(2), 026027. https://doi.org/10.1088/1741-2560/13/2/026027

[14] Nuzzo, J. L., Pinto, M. D., Nosaka, K., & Steele, J. (2023). The Eccentric:Concentric strength ratio of human skeletal muscle in vivo: meta-analysis of the influences of sex, age,

joint action, and velocity. *Sports Medicine*, *53*(6), 1125–1136. https://doi.org/10.1007/s40279-023-01851-y

[15] Oliveira, A. S., & Negro, F. (2021). Neural control of matched motor units during muscle shortening and lengthening at increasing velocities. *Journal of Applied Physiology*, *130*(6), 1798–1813. https://doi.org/10.1152/japplphysiol.00043.2021

[16] Salvador, E. P., Dias, R. M. R., Gurjão, A. L. D., Avelar, A., Pinto, L. G., & Cyrino, E. S. (2009). Effect of eight weeks of strength training on fatigue resistance in men and women. *Isokinetics and Exercise Science*, *17*(2), 101–106. https://doi.org/10.3233/ies-2009-0340

[17] Stegeman, D. F., Kleine, B. U., Lapatki, B. G., & Van Dijk, J. P. (2012). High-density Surface EMG: Techniques and applications at a motor unit level. *Biocybernetics and Biomedical Engineering*, *32*(3), 3–27. https://doi.org/10.1016/s0208-5216(12)70039-6

[18] Juneja, P., & Hubbard, J. B. (2023). Anatomy, Bony Pelvis and Lower Limb: Tibialis Anterior Muscles. In *StatPearls*. StatPearls Publishing.