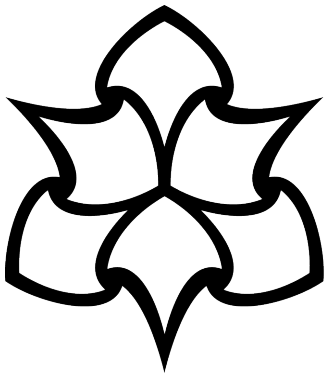

Frailty phenotype and frailty index are associated with distinct neuromuscular electrophysiological characteristics in men

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Running title: Neuromuscular electrophysiological characteristics in men

Abstract

Background.

The purpose of this study was to determine whether neuromuscular electrophysiological characteristics that are known to underlie sarcopenia are also associated with the more complex frailty syndrome.

Methods

Eighty-six men (mean (SD) age 74 (8) years) were classed as non-frail (robust), pre-frail or frail using criteria from the frailty phenotype (FP) and the frailty index (FI). The femoral nerve was maximally stimulated and the resulting compound muscle action potential amplitude (CMAP) was measured over the vastus lateralis (VL). Motor unit potential (MUP) size was assessed during voluntary contractions using intramuscular electromyography (iEMG). Logistic and negative binomial regression models determined relationships between FP and FI with CMAP and MUP sizes before and after adjustments for age and body mass index (BMI).

Results

Larger CMAP size was associated with a lower likelihood of frailty in fully adjusted models: a 1 standard deviation higher level in VL CMAP size was associated with a 0.4 (95% CI: 0.2-0.6, $p<0.01$) unit lower FI (40% of the FI range) and more than halving of the odds (OR: 0.43 (0.21-0.90)) of having a frail/pre-frail phenotype. Greater MUP size was also related to lower FI values using unadjusted and fully adjusted models. However, MUP size was not significantly related to FP in any model.

Conclusion

Smaller MUPs and a smaller CMAP were significantly associated with a higher likelihood of frailty, independent of age and BMI. These results relate neuromuscular electrophysiological characteristics to the complex frailty syndrome and identify motor unit remodelling as a possible contributing factor.

Keywords

Frailty, motor unit, muscle, electromyography

What is the central question of this study?

Human frailty is characterised by accumulated health complaints, including medical conditions, low physical and psychological function, as well as social components. It is currently unknown whether the condition is associated with neuromuscular changes detectable by electrophysiology obtained from voluntary and involuntary muscle contractions.

What is the main finding and its importance?

A higher likelihood of frailty was significantly associated with a smaller size of vastus lateralis motor unit potentials during voluntary contractions and smaller compound muscle action potentials generated by electrical stimulation. Importantly these associations were independent of age and BMI.

Introduction

Frailty is a major health, social and economic issue, especially in ageing populations with extended life expectancy. It is a leading cause of falls, fractures, immobility and loss of independence which results in increased healthcare costs and a higher risk of mortality (Fried *et al.*, 2001; Zaslavsky *et al.*, 2013). Frailty is most commonly classified by one, or both, of two sets of criteria: one based on the accumulation of deficits including medical conditions, physical function, psychological and social components to produce a "Frailty Index" (Rockwood *et al.*, 2005); and the other based on body size and physical function to give a 'Frailty Phenotype' (Fried *et al.*, 2001).

The major impacts of frailty are low physiological reserve and low physical function. These features are similar to those of sarcopenia and the main convergence of these two syndromes is on muscle mass and function (Cesari *et al.*, 2014). It follows therefore that underlying causes of sarcopenia might also contribute to frailty. Sarcopenia is due to a decrease in the cross sectional area (atrophy) of individual muscle fibres and a decrease in the numbers of muscle fibres (Mcphee *et al.*, 2018; Wilkinson *et al.*, 2018). These muscle changes are associated with declining numbers of motor neurons and functioning motor units (MUs) (Piasecki *et al.*, 2016*b*, 2016*c*, 2016*a*). A commonly used technique of assessing MU structure and function in humans is intramuscular electromyography (iEMG), which enables the detection and characterisation of individual motor unit potentials (MUPs) (Piasecki *et al.*, 2018*a*). This technique was used recently to show that sarcopenic individuals have smaller MUPs during voluntary muscle contractions and a smaller compound muscle action potential (CMAP) after transcutaneous electrical stimulation of the innervating nerve compared with healthy older adults (Piasecki *et al.*, 2018*b*). Electrophysiological characteristics such as the MUP and CMAP are often used to detect peripheral nerve disease progression (Maathuis *et al.*, 2013) because their utility is related more broadly to the neuromuscular system rather than being limited to sarcopenia. It remains unknown whether such MU changes occur with the progression of frailty.

Since low muscle mass and weakness are defining features of sarcopenia and also core components of the frailty phenotype, we hypothesised that a small CMAP and/or MUPs would be associated with the more complex frailty syndrome. It is important to determine the causes of muscle deficits in frailty because they can contribute to slowness, low physical activity levels and perceptions of fatigue within the definition of the frailty phenotype (Fried *et al.*, 2001) and they represent potentially modifiable components in the manifestation of frailty. Therefore, we aimed to determine the association between electrophysiological characteristics of MUs in a large limb muscle (CMAP and MUPs) and the frailty phenotype and frailty index measures in older men.

Methods

Ethical approval

Ethical approval for the study was obtained from the National Research Ethics Service Committee Northwest (15/NW/0426) and conformed to the standards set by the latest *Declaration of Helsinki*, except for registration in a database. Written informed consent was obtained from each participant. All volunteers were living independently, but still the capacity of the frail older adults to consent was assessed by a single clinician experienced in general and geriatric medicine. This process considered volunteers' ability to comprehend, retain and weigh up the information provided and screening for the exclusion criteria.

Participants

A total of 114 men aged 65-90 years were recruited from the Greater Manchester area between 2014-2016. The participants were recruited from the local universities' databases, National Health Service general practices and secondary care, including outpatient departments, day hospitals and community physiotherapy centres. The study was also made open to the general public through poster and newspaper advertisements. Only men were recruited to avoid the additional confounding factors of sex differences in muscle size, differences in fluctuating hormones known to influence muscle plasticity, such as androgens, and sex differences in subcutaneous adipose tissue thickness that would affect CMAP signal attenuation. Exclusion criteria included: individuals who lack capacity to consent for the study and comply with the protocol (including those who have a legal guardian); body mass index (BMI) < 18 kg m^{-2} or >35 kg m^{-2}; history of cachexia or malnutrition; institutionalised (e.g. living in a nursing home); presence of co-morbidity [specifically: neurological disorders (stroke resulting in reduced mobility, Parkinson's disease, dementia, motor neuron disease); cancer diagnosis (excluding non-fatal cancers, e.g. skin cancer, stable prostate cancer, and other stable cancers with a good prognosis); communicable disease such as HIV/AIDS or hepatitis; heart failure (breathless at rest or when walking < 100 m); NYHA III or IV]; permanent pacemaker *in situ* (an exclusion for magnetic resonance scanning only); implantable cardioverter-defibrillator (ICD) *in situ*; myocardial infarction within the last 6 months, uncontrolled angina, peripheral arterial disease (if this limits function to walking < 100 m), deep vein thrombosis within the last 3 months; severe chronic obstructive pulmonary disease or asthma (causing shortness of breath after a few minutes of walking or with changing clothing (MRC shortness of breath scale grades 4 or 5); coagulation disorder or use of anticoagulants (e.g. warfarin, sinthrome, dabigatran, rivaroxaban, apixaban, low-molecular-weight heparin) that could cause excessive bleeding or bruising; lower limb or vertebral fracture within the previous year; hip/knee and/or spinal stenosis surgery during the last 12 months; physical limitation and pain due to conditions that conflict with study procedures; amputation of part of a lower limb; and non-fluent speakers of the English language].

The participants completed questionnaires, physical tests, neuromuscular assessments, magnetic resonance and dual-energy X-ray absorptiometry imaging.

Assessments

Questionnaires

Each participant completed a questionnaire on health, lifestyle and medical history including medication.

Anthropometry measures

Body mass (kg) and height (m) were measured using calibrated scales and stadiometry. Total body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, version EnCore 10.50.086) with participants lying supine with legs and arms fully extended. Appendicular lean mass with appendicular bone mineral content (BMC) removed was normalised to height (Piasecki *et al.*, 2018*b*).

Assessment of physical function, activity and frailty

Physical function was assessed objectively using the Short Physical Performance Battery (SPPB) which included an assessment of four-meter walking speed, standing balance and chair stands. The 'Timed up and go' (TUG) was also performed, where participants were invited to stand from a seated position and walk 3 metres forward around a cone as quickly as possible, returning to their original seating position. The time from the command "three, two, one, go" until the participant returned to his seated position was recorded.

Grip strength was measured using a handgrip dynamometer (JAMAR). Participants were instructed to squeeze the handle as hard as possible for 3 seconds and the maximum contraction force (in kg) was recorded. This was repeated twice for each hand, alternating between right and left with 30 seconds rest between trials.

Frailty measures

Frailty was characterised by the two commonly used approaches: the frailty phenotype (FP) and the frailty index (FI).

We adapted the FP from the Cardiovascular Health Study (Fried *et al.*, 2001) based on five criteria: sarcopenia (appendicular lean mass/(height)^2 $\leq$ 7.26 kg/m^2), exhaustion (answer "most of the time" or "a moderate amount of time" to question "I felt that everything I did was an effort" or "I could not get going"), slowness (4 metre walk: slowest 20% by height), weakness (grip strength: lowest 20% by BMI) and low activity (< 150 mins/week spent on physical activity). Individuals meeting one or more of these criteria were classed as either 'frail' or 'pre-frail' (combined as a group) and those with none of these criteria were classed

as ‘robust’. This was done because of the relatively small number of men in the frail category leading to a limited ability to adjust regression models for relevant covariates.

The FI comprised 37 health deficits (symptoms and signs, functional impairments) which accumulate with age and are associated with adverse health outcomes. The deficit variables were derived from questionnaire data as well as scores from physical performance tests and self-reported co-morbidities. The FI was created using a standardised procedure (Searle *et al.*, 2008) and calculated as a ratio between number of deficits present in an individual to total number of deficits possible. Binary variables were coded as 0 or 1 in the case of absence or presence of a deficit, respectively. The intermediate response (e.g. sometimes/maybe) was coded with a value of 0.5. Continuous variables were dichotomised based on the distribution of participants’ scores; cut-off points were set at the worst performing 10^{th} centile. Individuals with over 20% of missing data on relevant deficits were excluded from the analysis.

Electromyography (EMG)

All EMG data were collected from around the motor point of the VL which was identified as the area of muscle providing the largest twitch from a percutaneous electrical stimulus applied by a cathode probe at 400 V, pulse width of 50 μs and current of around 8 mA, (DS7AH Digitimer, Welwyn Garden City, Hertfordshire, UK). A self-adhesive anode electrode (Dermatrode, Farmadomo, NL) was placed over the right gluteus. The motor point was typically located around 210-220 mm from the lateral femoral condyle, central on the transverse plain.

The CMAP was recorded at the VL motor point by surface EMG after percutaneous femoral nerve stimulation. With the anode remaining on the right gluteus, the cathode was placed over the femoral nerve approximately halfway between the anterior superior iliac spine and the pubis tubercle, proximal to the groin crease. The stimulator was set at 400 V with 50 μs pulse width and the current was increased through successive stimulations until the recorded potential (M-wave) plateaued, at which point the current was increased by approximately 30 mA to ensure supramaximal stimulation for the CMAP (usually at 100-150 mA). The reference electrode was placed over the patella tendon (disposable self-adhering Ag-AgCl electrodes; 95mm^2, Ambu Neuroline, Baltorpbakken, Ballerup, Denmark) and a common ground was placed over the patella (Ambu Neuroline Ground).

MUPs were recorded using a 25 mm disposable concentric needle electrode with a recording area of 0.07 mm^2 (Model N53153; Teca, Hawthorne, NY, USA). The same ground electrode was shared with the surface EMG. All iEMG signals were sampled at 25 kHz and bandpass filtered at 10 Hz to 10 kHz (CED 1902 amplifier; Cambridge Electronics Design Ltd). With the participant sitting relaxed, the needle electrode was inserted into the VL muscle at the motor point to a depth of around 1-2 cm. The participant then performed a sustained voluntary isometric contraction at 25% of their maximum effort and held it for 12-15

seconds with real time feedback from a visual display. The needle was positioned to ensure sharp rise times of potentials. In between contractions the needle was repositioned using combinations of 180-degree needle rotations and needle withdrawals of around 3 mm. This was repeated until a minimum of six recordings from spatially distinct areas had been obtained.

EMG analysis

All CMAP and MUP data were recorded using Spike2 software (Version 8.1, Cambridge Electronic Designs) and stored for offline analysis. The amplitude (size) of the CMAP was defined as the amplitude of the negative peak (upward deflection) of the M-wave. Intramuscular EMG signals were decomposed using custom written DQEMG software (Stashuk, 1999; Piasecki *et al.*, 2016*c*, 2018*a*). Individual MUPs were isolated and MUP area (size) was taken as the total area under the curve within the MUP duration, as previously described (Piasecki *et al.*, 2016*c*). The MUPs sampled from any single participant were collated to calculate the mean MUP size for that individual. A minimum of 8 MUPs per participant were required for inclusion for further analysis.

Statistical analysis

Descriptive statistics are presented as the mean ± standard deviation (SD) or n (%). Statistical significance of between-group differences was assessed using analysis of variance.

Frailty phenotype models: For the purpose of analysis, individuals who were pre-frail or frail were combined in a single frail/pre-frail group. Logistic regression models were fitted to determine relationships between each predictor (MUP or CMAP) and binary outcome (frail/pre-frail vs. robust). Each predictor was considered as an untransformed value standardised as a Z score [(raw score – mean)/standard deviation] to allow comparison of results between the predictors. The models were then further adjusted for age and body mass index as these were significantly correlated with the predictors. The results were displayed as odds ratios (OR with 95% confidence intervals) for prevalent frailty associated with z-score differences in either MUP or CMAP predictors.

Frailty index models: In view of the significant right skewing of the FI variable (a count variable), the relationship between MUP or CMAP predictors and FI was assessed using a negative binomial regression model. The FI variable was rescaled and converted to a 0-37 count scale where ‘0’ represented no deficits and ‘37’ represented the maximum number of deficits. As in the FP analysis, predictor variables in this model were also standardised as Z-scores and presented as standardised beta coefficients with 95% confidence intervals. The models were adjusted for age and BMI.

To assess whether the associations between CMAP and MUP predictors with frailty phenotype were different in prefrail and frail men, we performed an exploratory multinomial regression analysis using a 3-tier (robust, prefrail, frail) FP as an outcome.

All analyses were performed using STATA 13 SE software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Results

Out of 114 men who participated in the study, 86 with complete data necessary to determine frailty phenotype and frailty index remained in the analytical sample (Table 1). The clinical characteristics of participants who remained in the study were not significantly different compared to those who were excluded (n=28) apart from a higher prevalence of smoking in excluded participants.

The mean (SD) age of the men was 74 (6) years and BMI 26.1 (4.0) kg/m^2. The prevalence of cardiovascular disease was 22% and diabetes 14%. There was a low prevalence of smoking (5%) and over half of the participants were taking three or more medications for medical conditions (the most common groups of drugs being statins, antiplatelets and proton pump inhibitors) (Table 1).

The mean (range) frailty index was 0.18 (0–0.69). Classifying participants according to frailty phenotype identified 28 as robust, 40 as pre-frail and 18 as frail.

Motor unit potential and CMAP data are available for 74 and 79 of the participants, respectively. The mean (SD) number of MUPs sampled was 20 (9) per participant. The mean MUP size was 1445 (706) μVms and the CMAP amplitude was 6946 (2781) μV. Men in the lowest tertile of MUP size (having the smallest MUPs) were older and more likely to be smokers and to have cardiovascular disease compared to participants with larger MUPs (Table 2). Men in the lowest tertile of CMAP size were older and had higher BMI and a higher prevalence of CVD and diabetes compared with those with larger CMAPs (Table 2). Representative images of CMAPs and MUPs are shown in Figure 1.

Multivariate regression analysis showed that higher CMAP size was associated with a lower likelihood of frailty as assessed by both frailty phenotype and frailty index in models with, and without, adjusting for age and BMI (Table 3). For example, in fully adjusted models, a 1 SD higher level in VL CMAP size was associated with a 0.4 unit lower FI (40% of the full FI range) and more than halving of the odds (OR: 0.43) of having a frail/pre-frail phenotype.

Similarly, higher MUP size was also related to lower frailty index values in unadjusted and fully adjusted models. However, higher MUP size was not significantly related to frailty phenotype in any model (Table 3).

Further adjustment for diabetes did not significantly alter the results (data not shown).In the secondary analysis applying frailty phenotype, we found that MUP size was negatively related to risk of frailty but not prefrailty. This association was independent of age, BMI and diabetes when entered individually. CMAP size was negatively related to risk of frailty in all models, whereas the association with prefrailty was significant only in unadjusted and BMI adjusted models (Table 4).
We also observed significant differences in MUP size and CMAP across frailty categories (Table 5).

Discussion

This study presents two novel findings. First, frail older men defined by both the frailty index and the frailty phenotype were more likely to have a smaller electrically-evoked CMAP. Secondly, frail older men defined by the frailty index were more likely to have smaller MUPs during voluntary muscle contractions held at 25% MVC. These significant relationships were independent of the possible confounders of age and BMI. Our observations relate frailty to distinct neuromuscular electrophysiological characteristics. Secondary analysis suggested that smaller MUPs are features of frailty but not prefrailty, as assessed by FP.

The maximal CMAP represents the summation of all action potentials from all muscle fibres within the recording range of the surface electrode after being activated at the same time. Age-related decreases in CMAP size have been reported for a number of muscles, including the VL (Piasecki *et al.*, 2016*c,* 2019), tibialis anterior (McNeil *et al.*, 2005; Piasecki *et al.*, 2016*a*), biceps brachii (Power *et al.*, 2012) and soleus (Dalton *et al.*, 2008) based on comparisons of young and old participants. Although CMAP size invariably decreases with advancing older age, the association between CMAP size and frailty is not simply explained by ageing *per se* because it remained significant after adjusting for age. Interestingly, CMAP size has been used to track clinical progression of motor neuron or muscle disorders, including ALS (Mori *et al.*, 2016) and spinal muscular atrophy (Lewelt *et al.*, 2010), but we show that it has the potential to provide novel insights into the multi-morbidity frailty syndrome defined by a progressive loss of physiological reserve. Clearly, the CMAP measured after stimulation of a peripheral motor nerve to induce muscle excitation is directly related to transmission along the nerve axons and at the neuromuscular junctions, and transmission along muscle fibres. As this pathway defines peripheral control of muscle contractions, CMAP size was expected to be associated with the frailty phenotype which strongly considers physical function (Fried *et al.*, 2001). The fact that CMAP size was also associated with the frailty index, which sums a number of clinically-relevant ‘deficits’ (Searle *et al.*, 2008), may reflect more broadly a range of morbidities common to the criteria used to determine the frailty index and frailty phenotype.

CMAPs are relatively easy to measure, but their limitation is that they provide relatively little information about the extent of changes to MU number or sizes. Motor unit numbers decline with advancing older age and some of the surviving MUs increase in size to compensate (Hepple & Rice, 2016; Piasecki *et al.*, 2016*b*). However, we recently showed that the expansion of MU size was absent in people with clinically-relevant low muscle mass and weakness (sarcopenia) (Piasecki *et al.*, 2018*b*). This finding is extended in the present study beyond sarcopenia by showing that smaller MUPs, indicative of smaller MUs (Zalewska & Hausmanowa-Petrusewicz, 1999), are related to the complex condition of the frailty syndrome assessed by the frailty index. The failure to expand MU size in frailty may be due to low physical activity levels or to an unfavourable nerve or muscle tissue milieu that does not support reinnervation because of chronic low-grade inflammation, oxidative stress, or changing hormone levels (Hepple & Rice, 2016; Wilkinson *et al.*, 2018), but very little is currently known about these mechanisms.

In a study reported by Syrjälä and colleagues (Syrjälä *et al.*, 2000) older men with frequent falls tended to have lower amplitude motor evoked potentials in the legs when compared to older men without frequent falls. Although we do not know if the 'fallers' were frail, it is well known that frail older people have increased risk of falls (de Vries *et al.*, 2013). Lower amplitude motor evoked potentials in frequent fallers could have been explained by smaller MUs, although that possibility was not investigated by Syrjälä and colleagues (Syrjälä *et al.*, 2000).

The main strength of the present study is that this study is the first to relate the electrophysiological measurements, CMAP and MUP sizes, to the presence of frailty. We studied frail and pre-frail men alongside robust men using advanced recently developed techniques for electrophysiological characterisation of MUs. Although the sample size may be considered small in comparison to epidemiological studies of frailty, it is large for this type of detailed physiological profiling. A main limitation is the cross-sectional study design which prevents conclusions about causality. Secondly, 25% of the men who agreed to participate in the study did not have complete data on frailty status and were excluded from this analysis. This issue highlights some of the difficulties encountered when studying frail elderly participants (unable or declining to participate in all elements of a research programme). However, as we have shown, those excluded from analysis did not differ from those included in the analysis and we expect that the data presented here are representative of older men in the UK. Thirdly, we used DXA-measured low muscle mass rather than unintentional weight loss in the frailty phenotype. We took this action because DXA-measured low muscle mass can be more directly measured; it is more relevant to our study and is more likely to be chronic rather than a feature of recent illness. We consider this to be a significant improvement on the original criteria but this approach to classifying frailty has not been validated and therefore may be considered a limitation. However, in the absence of consensus regarding measurement of frailty, we believe that our frailty phenotype is a valid construct and we believe that it is more comprehensive than many other frailty assessment tools, often largely

based only on questionnaire-derived information. Finally, our study was limited to men and therefore generalisability to women is unknown.

Conclusion

We have shown that older men with small CMAPs and MUPs have a higher likelihood of frailty and that these relationships were independent of age and BMI. These novel findings implicate aberrant neuromuscular structure and function involving MU remodelling to the complex frailty syndrome.

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Conflict of interest

We have no conflicts of interest to disclose.

Author contributions

All experiments were conducted at Manchester Metropolitan University, UK. All authors contributed to the conception or design of the work, acquisition or analysis and interpretation of data for the work, drafting the work and revising it critically for important intellectual content. All authors had final approval of the submitted version for publication and agree to be accountable for all aspects of the work.

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Tables

Table 1. Clinical characteristics of the study participants

Characteristics		mean (SD)/ n(%)
N		86
Age, years		74 (5)
BMI, kg/m^2		26.1 (4.1)
Grip Strength (kg)		37.7 (8.1)
Current smoker, n (%)		4 (5)
Alcohol excess (≥14 unit/week), n (%)		31 (39)
Respiratory disease, n (%)		14 (16)
Cardiovascular disease, n (%)		19 (22)
Diabetes, n (%)		12 (14)
Osteo-/Rheumatoid arthritis, n (%)		28 (33)
Taking ≥3 medications, n (%)		41 (52)
Frailty index		0.18 (0.17)
Frailty phenotype: robust		28 (33)
	prefrail	40 (47)
	frail	18 (21)
Sarcopenia, n (%)		48 (56)
Exhaustion, n (%)		15 (17)
Low activity, n (%)		18 (21)
Weakness, n (%)		19 (22)
Slowness, n (%)		18 (21)
MUP area, µV .ms		1445 (706)
CMAP amplitude, µV		6947 (2782)

Data are displayed as mean (SD) or n (%).

BMI, body mass index; CMAP, compound muscle action potential; MUP, motor unit potential;

N, number

Table 2. Clinical characteristics of the study participants stratified according to tertiles of MUP and CMAP in vastus lateralis.

	T1 MUP	T2 MUP	T3 MUP	p-value	T1 CMAP	T2 CMAP	T3 CMAP	p-value
N	**25**	**25**	**24**		**27**	**26**	**26**	
Age, years	**76±6**	**71±5.0[a]**	**73±6.0**	**0.006**	**78±7**	**72±5[a]**	**71±4[a]**	**<.001**
BMI, kg/m2	26.8±3.8	26.2±5.1	24.4±2.8[a]	0.123	**28.3±4.4**	**24.9±3.5[a]**	**24.4±3.0[a]**	**<.001**
Smoking, n (%)	**3 (12%)**	**0**	**0**	**0.047**	2 (7%)	1 (4%)	1 (4%)	0.791
Frequent Alcohol, n (%)	8 (36%)	8 (37%)	9 (41%)	0.949	8 (31%)	9 (41%)	11 (44%)	0.597
Diabetes, n (%)	5 (20%)	2 (8%)	1 (4%)	0.081	**9 (33%)**	**1 (4%)**	**0**	**<.001**
Cardiovascular disease, n (%)	**9 (36%)**	**4 (16%)**	**2 (8%)**	**0.044**	**14 (52%)**	**3 (12%)**	**0**	**<.001**

CMAP, compound muscle action potential; MUP, motor unit potential; T, tertile

[a] the value significantly different to T1 value

Following Bonferroni correction for multiple group comparisons, significant p value set at ≤ 0.017

Table 3. Unadjusted and multivariable-adjusted cross-sectional relationships of motor unit characteristics with frailty measures

Predictor	Model and covariates	Frailty index			Frailty phenotype		
		β	95% CI	p-value	OR	95% CI	p-value
MUP Size	1. Unadjusted	**-0.15**	**-0.23, -0.07**	**<.001**	0.83	0.67, 1.02	0.071
	2. BMI	**-0.13**	**-0.20, -0.05**	**0.002**	0.82	0.66, 1.02	0.081
	3. BMI + Age	**-0.10**	**-0.18, -0.02**	**0.013**	0.82	0.65, 1.03	0.095
CMAP Size	1. Unadjusted	**-0.57**	**-0.74, -0.40**	**<.001**	**0.40**	**0.21, 0.74**	**0.003**
	2. BMI	**-0.51**	**-0.70, -0.33**	**<.001**	**0.34**	**0.17, 0.68**	**0.002**
	3. BMI + Age	**-0.40**	**-0.61, -0.20**	**<.001**	**0.43**	**0.21, 0.90**	**0.026**

Negative β means that the 1SD increase in predictor is associated with improvement of frailty status, whereas positive β means that the predictor is associated with worsening of frailty status. An OR <1 means that a 1SD higher value of the predictor is associated with a lower risk of prefrailty or frailty whereas an OR >1 means that a 1SD higher value of the predictor is associated with a higher risk of prefrailty or frailty

Table 4. Relative risk of prefrailty or frailty in relation to a 1 SD higher level MUP or CMAP compared to robust participants

Predictor	Model and covariates	Frailty Phenotype					
		Prefrail			Frail		
		RRR	95% CI	p-value	RRR	95% CI	p-value
VL MUP	Unadjusted	0.91	0.74, 1.13	0.398	**0.44**	**0.26, 0.76**	**0.003**
	+ BMI	0.90	0.72, 1.13	0.371	**0.44**	**0.25, 0.77**	**0.004**
	+ Age	0.91	0.72, 1.13	0.392	**0.52**	**0.31, 0.87**	**0.014**
	+ Diabetes	0.91	0.74, 1.14	0.426	**0.48**	**0.27, 0.82**	**0.008**
VL CMAP	Unadjusted	**0.50**	**0.26, 0.97**	**0.041**	**0.17**	**0.07, 0.42**	**<0.001**
	+ BMI	**0.43**	**0.21, 0.89**	**0.023**	**0.15**	**0.05, 0.40**	**<0.001**
	+ Age	0.59	0.29, 1.20	0.146	**0.28**	**0.11, 0.73**	**0.010**
	+ Diabetes	0.52	0.26, 1.05	0.069	**0.19**	**0.07, 0.50**	**0.001**

RRR - relative risk ratio. A significant RRR <1 for frail means that a 1SD higher value in the predictor is associated with a lower risk of frailty compared to the robust group. A significant RRR <1 for prefrail means that a 1SD higher value in the predictor is associated with a lower risk of prefrailty compared to the robust group.

Table 5. Motor unit characteristics and frailty index across frailty phenotype categories

Parameter	Frailty status			
	Robust	Prefrail	Frail	p value
N	28	40	18	
VL CMAP, mV	8301.6±2199.1	7006.8±2607.4	4360.3±2435.8[ab]	<.001
VL MUP, ms/mV	1645.5±514.7	1491.4±802.6	907.4±525.2[ab]	<.001
Frailty Index	0.08±0.11	0.15±0.11	0.39±0.16[ab]	<.001

[a] significantly different compared to robust; b, significantly different compared to prefrail

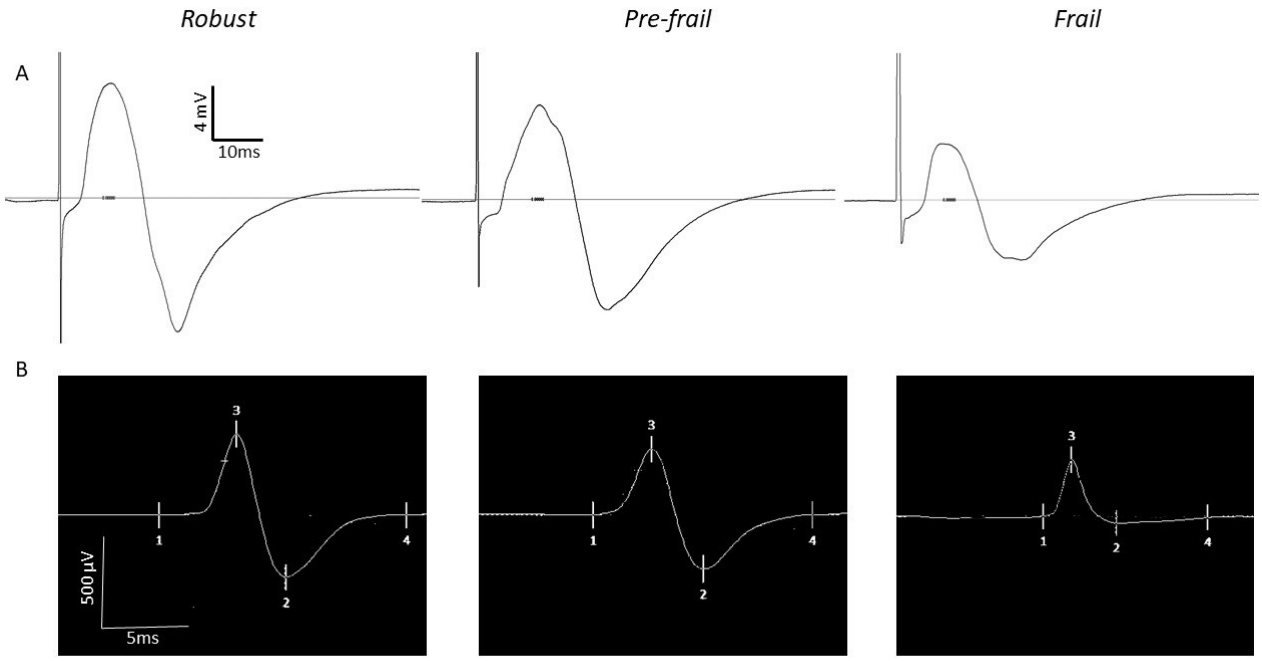

Figure 1. Representative images of A) CMAP and B) MUP. CMAP negative peak amplitudes displayed here; robust 8632 = µV, pre-frail = 7112 µV, frail = 4215 µV. MUP areas displayed here; robust = 1632 µV.ms, pre-frail = 1471 µV.ms, frail = 915 µV.ms. Numbers indicate; 1 MUP onset, 2 negative peak, 3 positive peak, 4 MUP end.