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DIFFERENTIAL CONTRIBUTIONS OF ANKLE PLANTARFLEXORS DURING SUBMAXIMAL ISOMETRIC MUSCLE ACTION: A PET AND EMG STUDY

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ABSTRACT

The objective of this study was to investigate the relative contributions of superficial and deep ankle plantarflexors during repetitive submaximal isometric contractions using surface electromyography (SEMG) and high-resolution positron emission tomography (PET). Myoelectric signals during submaximal isometric exercise were obtained from both legs of twelve healthy volunteers (27 ± 4 yrs). A tracer ([18F]-FDG) was injected during the exercise and PET scanning was carried out immediately after the cessation of exercise protocol. The targeted muscles included the soleus, medial gastrocnemius (MG), lateral gastrocnemius (LG), and flexor hallucis longus (FHL). Despite the existence of considerable individual and bilateral differences in isometric MVC force production, muscle glucose uptake (GU) measured with PET and EMG of various plantarflexors were comparable bilaterally. In terms of %EMG MVC, FHL and MG displayed the highest activity (~34 %), while the activity of LG (~21 %) was the lowest. Cumulative EMG from all parts of the triceps surae (TS) muscle accounted for ~70 % of the total EMG signal. As for glucose uptake, the highest quantity was found in MG (2.4 ± 0.8 µmol/100g/min), whereas FHL (1.8 ± 0.6 µmol/100g/min) had the lowest uptake. Cumulative GU of triceps surae muscle constituted nearly 80 % of combined GU of all four plantarflexors. The findings of the current study provide valuable reference for studies where individual muscle contributions are estimated using models and simulations.

INTRODUCTION

Ankle plantarflexion in humans is brought about by activation of two different sets of muscles; superficial (triceps surae) and deep (e.g. flexor hallucis longus). The extent to which these two muscle groups contribute to a plantarflexion task varies in individuals. Relative activation of different plantarflexors during various tasks has been investigated using an in-situ buckle-type force transducer on the Achilles tendon (Gregor et al. 1991), estimations based on cadaver moment arm lengths and total muscle cross-sectional areas (van Zandwijk et al. 1998), recordings of tendon forces with in-vivo optic-fiber technique (Finni et al. 2000), and calculations of individual muscle contraction velocities using velocity-encoded cine phase-contrast MRI (Finni et al. 2006). The amount of reported triceps surae (TS) contribution to the total plantarflexion moment ranges from ~65% (Gregor et al. 1991) to ≥88% (van Zandwijk et al. 1998).

Previous studies have also established that the distribution of stress within different compartments of TS muscle is not homogenous. Cadaver studies have shown differences in the mediolateral forces within the Achilles tendon depending on how TS components were activated or loaded (Arndt et al. 1999a, Arndt et al. 1999b, Lersch et al. 2012), and calcaneal angle (Lersch et al. 2012). When all three muscles are loaded, lateral tendon forces are higher, while, on the contrary, medial forces tend to be greater when medial gastrocnemius is loaded alone (Arndt et al. 1999a). Similarly, significant differences in calcaneal moments can be seen when both gastrocnemii are loaded together compared to soleus alone (Arndt et al. 1999b). Furthermore, major inconsistency in soleus and gastrocnemius EMG, torque and force can stem from small changes in the relative muscle lengths due to alterations in the ankle and knee joint angles (Cresswell et al. 1995, Arndt et al. 1998).

Investigation of differential contributions of superficial and deep plantarflexors is challenging due to estimations and assumptions leading to uncertainties. While in-vivo studies are scarce, surface electromyography (SEMG) provides a convenient and non-invasive tool for studying skeletal muscle activation. Conventional bipolar SEMG has been widely used to measure the

electrical activity of skeletal muscles during various physical activities (Hermens et al. 2000). Despite the issues of optimal electrode positioning and signal reliability, SEMG provides valuable and consistent information which corresponds to the skeletal muscle work (Merletti & Conte 1997, Kleissen et al. 1997). SEMG can also be used to compare the contribution of individual TS components to the Achilles tendon force during functional tasks (Gregor et al. 1991).

One of the limitations of the SEMG method is that it provides information about muscle activity only from superficial regions, which may not represent the whole muscle (Knight & Kamen 2005). To study the whole muscle function, positron emission tomography (PET) enables non-invasive investigation of muscle glucose uptake as a measure of muscle activation (Nuutila & Kalliokoski 2000, Kalliokoski et al. 2007, Tashiro et al. 2008). Skeletal muscle glucose uptake is increased during exercise due to a coordinated rise in glucose delivery rate, surface membrane glucose uptake, and intracellular substrate flux through glycolysis (Rose & Richter 2005). Since skeletal muscle glucose uptake rises in parallel with exercise intensity, it reflects muscle's metabolic activity (Kemppainen et al. 2002). High resolution PET, combined with tracers like [^{18}F]-Fluorodeoxyglucose ([^{18}F]-FDG), has been employed to image and quantify glucose uptake as a result of exercise in healthy tendons (Kalliokoski et al. 2005, Bojsen-Møller et al. 2006) and skeletal muscles (Hargreaves 1998, Pappas et al. 2000, Fujimoto et al. 2000, Kemppainen et al. 2002, Hannukainen et al. 2005, Kalliokoski et al. 2007, Bojsen-Møller et al. 2010a, Rudroff et al. 2013).

Despite recent advances in PET imaging which enables assessment of muscle use during plantarflexion, no reports on the attempts to validate it against EMG have surfaced thus far. Although both SEMG and PET provide important insight into muscle behavior during exercise, no study to-date has incorporated them to investigate the behavior of ankle plantarflexors in voluntary contractions. Therefore, the purpose of this study was to evaluate muscle use during submaximal isometric contractions by both SEMG and PET. More specifically, we were interested whether the electrical and metabolic measures of muscle activity are comparable in describing the relative contribution of superficial (TS) and deep (FHL) plantarflexion muscles.

MATERIALS AND METHODS

Subjects

Twelve healthy volunteers were recruited for the study through public advertisements. These included eight males and four females with no history of a major leg injury over the past 12 months. The mean age, height, and body mass were 27 ± 4 (21 - 34) yrs, 174 ± 5 (163 - 180) cm, and 68 ± 6 (59 - 78) kg respectively. Informed written consent, following the explanation of the procedures and the risk involved, was obtained from all the participants before the testing. Approval for the study protocol was issued by the Ethics Committee of the Hospital District of South-Western Finland and conformed to the Declaration of Helsinki.

Experimental Protocol

Each subject took part in a series of tests at the Turku PET Centre, University of Turku, Finland. A diagram of the experimental design is given in Fig. 1. All components of the study were carried out on the same day for a subject. Participants were required to keep fasting for at least 8 hours prior to the PET scans. At the beginning, anthropometric measurements were obtained which included body mass, height, and leg length readings.

Subject preparation comprised shaving, abrading, and cleaning of skin for surface electromyography (SEMG), placement of electrodes on both legs, securing an electronic goniometer to the ankle, and a pregnancy test in the case of female subjects. In addition, catheters were inserted into the antecubital veins in both arms: one for venous blood sampling and the other for $[^{18}F]$-FDG tracer injection. This was followed by positioning of the subject in the exercise apparatus for force and SEMG measurements. Subjects were helped to familiarize themselves with the task by performing submaximal contractions with each leg. After a warm-up, maximal Voluntary Contraction (MVC) force during plantarflexion was recorded individually from both legs, and the highest of the three trials was selected for calculation of submaximal force target level that in turn was used during subsequent exercise.

Exercise protocol: The exercise protocol consisted of a set of five 5 sec unilateral "constant-force" submaximal, isometric contractions separated by a 5-second rest period. This was accomplished alternately, one leg at a time. Subjects performed the task while sitting on a seat placed on the floor with knees in full extension, hips flexed at right angle, and ankle in neutral position (Fig. 2). The target force level selected for this task was 30% of the MVC. Subjects received visual feedback of the plantarflexion force on a monitor in front of them. After 2 sets of warm up contractions for both legs, about 150 MBq of [^{18}F] FDG tracer was infused and subsequently 10 sets of 5-s isometric contractions were performed, five repetition each, for both legs. Thus the total time of exercise and rest before tracer injection was about 6-7 minutes and the submaximal exercise protocol lasted for about 15 minutes post-injection. Blood sampling for plasma radioactivity assessment was carried out repeatedly from the time of tracer injection until cessation of the PET scan.

Immediately after the exercise bout, the subject was moved to the PET scanner on a wheelchair in order to minimize additional use of leg musculature. Magnetic Resonance Imaging (MRI) was performed after the PET scanning.

EMG and Force Acquisition and Analyses

Electromyographic data were recorded using conventional bipolar SEMG electrodes from both legs. Silver-Silver Chloride Ambu Blue Sensor N electrodes (*Ambu* A/S, Ballerup, Denmark) with an inter-electrode distance of 22 mm were placed, according to the SENIAM recommendations (Hermens et al. 1999), over soleus, medial gastrocnemius (MG), and lateral gastrocnemius (LG) muscles. For flexor hallucis longus (FHL), a pair of electrodes was placed after locating the muscle behind the medial malleolus by manual palpation. Furthermore, a reference electrode was secured on the medial malleolus of right ankle. EMG data was sampled via EISA (bandwidth 10 Hz to 1 kHz per 3 dB), a 10-channel EMG detection system (model: 16-2) (*University of Freiburg*, Freiburg im Breisgau, Germany) at a sampling frequency of 1000 Hz). The signal was pre-amplified with a factor of 200 by an integrated preamplifier in the shielded cables. Analogue-to-digital conversion of EMG and force data was done through a *Power1401* high-

performance multi-channel data acquisition interface (*Cambridge Electronic Design* Ltd., Cambridge, England). Compatible Signal 4.0 (*Cambridge Electronic Design* Ltd., Cambridge, England) computer software was used for data recording, data reduction, and further analyses. EMG signals from one set of 5 contractions were recorded separately for each leg from the beginning, middle, and end of the exercise protocol. That resulted in data comprising 3 sets of 5 submaximal isometric contractions each for either leg.

EMG data was differentiated by high-pass filtering (second order Butterworth filter, 12dB/octave) with a cutoff frequency of 10 Hz to remove noise signal and correct DC offset. The Root Mean Square (RMS) amplitude of each muscle was calculated from a 3-second epoch during the middle of intermittent submaximal isometric contractions. This EMG RMS was normalized to the RMS amplitude assessed from a 1-second time window during the MVC and is denoted in the text as %EMG MVC. The occurrence of fatigue was checked by comparing the RMS from the beginning, middle, and end of the exercise protocol. Additionally, Sol-to-FHL, MG-to-FHL, and LG-to-FHL EMG muscle ratios during the submaximal exercise were computed by dividing the respective normalized RMS muscle values. This was done to establish the relative contributions of primary plantarflexors (triceps surae) to a secondary plantarflexor (FHL). These calculations were based on the assumption that all plantarflexors were maximally activated during MVC. Furthermore, cumulative EMG was computed by adding up electrical activities of all four muscles, which was eventually used as the 100% value in the estimation of the relative contribution from individual muscles to the isometric task.

Plantarflexion Force: Isometric ankle plantarflexion force was measured by an in-house custom-built portable force transducer (*University of Jyväskylä*, Jyväskylä, Finland) (Fig. 2). The transducer plate, mounted on a plastic stand, was secured in place by use of steel chains. The chains, one on each side, were connected to the seat-back creating a rigid frame. Mean absolute ankle plantarflexion force during the submaximal isometric contractions was calculated along with the MVC force. The force signal was recorded via Signal 4.0 (*Cambridge Electronic Design* Ltd., Cambridge, England) in synch with EMG at a sampling frequency of 1000 Hz.

Image Acquisition

PET: The participants were positioned supine in the scanner with radioactive markers on lateral malleoli and medial femoral condyles to enable subsequent alignment of PET and MRI images. A *CTI-Siemens ECAT EXACT HR$^+$* (*Siemens*, Knoxville, TN, USA) PET scanner was used. The legs of the subject were scanned in four adjacent regions covering the whole leg region from toes to lower thigh. The emission scan of each region lasted for approximately 5 minutes and was followed with a transmission scan lasting about 2 minutes per region. Altogether the scan of the legs with transition time between the regions took approximately 32 minutes.

MRI: In addition, MRI scanning was performed with *1.5 T Philips Intera MRI* (Philips Healthcare, Eindhoven, The Netherlands) for both legs for specific determination of muscle locations. Lipid pills were taped to the same anatomical landmarks as used for radioactive markers in PET scanning for later superimposition of the two data images.

Image Assessment

The obtained PET images were corrected for decay and parametric fractional uptake rate (FUR) images were computed using the PET image data and the individual input function (plasma radioactivity data) as described previously (Kemppainen et al. 2002, Fujimoto et al. 2003). The regions of interest (ROIs) were drawn to include the whole individual muscle on the transverse plane FUR images using *Carimas 2.0* software (*Turku PET Centre, University of Turku*, Turku, Finland). ROIs were drawn on every fourth cross-sectional PET image planes, which were separated by approximately 1 cm from each other, and by the same investigator to avoid inter-observer differences. FUR values were obtained for soleus, medial and lateral gastrocnemii, and FHL. These values were then further converted to glucose uptake values using the following formula:

$$Glucose\ uptake\ (\mu mol/100g/min) = \frac{FUR\ \text{x}\ Plasma\ glucose}{Lumped\ Constant}$$

Plasma glucose level was obtained from the plasma sampling during the study. Lumped constant is the value that takes into account differences in the uptake of glucose and ^{18}F-FDG from the blood and it has been shown to be 1.2 for skeletal muscle (Kelley et al. 1999, Peltoniemi et al. 2000).

In order to examine the relative contribution of various plantarflexors, Sol-to-FHL, MG-to-FHL, and LG-to-FHL muscle GU ratios were calculated, as was done for EMG. MRI images were used as an anatomical reference to delineate the targeted muscles while regions of interest were drawn on PET images. Similar to EMG, glucose uptake rate values from triceps surae and FHL were added together and the resultant sum was taken as the 100% to estimate the relative contribution of individual muscles during the exercise protocol.

Statistical analysis

IBM SPSS 20.0 (*IBM Corporation*, New York, USA) software was used for the statistical treatment of the data. Normality of the data was checked by using Shapiro-Wilk test which revealed that most of the data was not distributed normally. Therefore, a non-parametric test, Wilcoxon signed-rank test, was used to compare the skeletal muscle glucose uptake and electrical muscle activity of the two legs. Additionally, correlations between various muscles (Pearson's *r*) and parameters (Spearman's rank) were sought. Alpha (α) level of significance was set at *p* value 0.05. The results are expressed as mean ± S.D (standard deviation).

RESULTS

Plantarflexion force: The mean MVC from right and left legs was 1133 ± 236 N vs. 1129 ± 192 N, respectively. The 30% MVC target plantarflexion force maintained in the intermittent isometric exercise protocol was 352 ± 71 N vs. 347 ± 55 N.

Electromyography

Neither plantarflexion force, nor SEMG, of the intermittent, voluntary, submaximal isometric exercise protocol showed any signs of fatigue in terms of changes in the RMS (Fig. 3). Therefore, the mean value of recordings from the beginning, middle, and end was taken to represent each muscle's electrical activity.

The highest MVC-normalized RMS activity was evident in FHL (34.05 ± 25% EMG MVC) and MG (33.25 ± 12% EMG MVC), while LG (20.69 ± 12% EMG MVC) displayed the lowest activity. Combined RMS of the three TS components constituted 69 ± 13% of the cumulative EMG while FHL accounted for the remaining 31 ± 13% (Fig. 4A). The most active component of TS relative to FHL was MG in both right (1.3 ± 0.5) and left legs (1.2 ± 1.2) without significant differences between the ratios (Fig. 5).

Muscle-Tendon Glucose Uptake

Representative MRI and PET images from a subject in the study are shown in Fig. 6. Mean bilateral muscle and tendon glucose uptake was very similar. The unit for all values reported here is: μmol/100g/min. The highest uptake was present in MG in both right and left legs (2.4 ± 0.8 and 2.6 ± 0.8 respectively). Both soleus and LG displayed similar GU rate (right: 2.1 ± 0.6, left: 2.1 ± 0.6 vs. right: 2.1 ± 0.8, left: 2.1 ± 0.6). The lowest uptake was observed in FHL (right: 1.8 ± 0.6, left: 1.8 ± 0.6). Combined data from the two legs revealed that collective TS muscle GU accounted for 79% (SD: 4) of overall GU by all four investigated plantarflexors (Fig. 4B).

Similarly to EMG, no differences in the relative muscle GU were found between the legs. All TS components were shown to be considerably more active compared to FHL. MG-to-FHL ratio was (~1.4 ± 0.3) while both soleus and LG had very similar ratio with FHL (~1.2 ± 0.3) (Fig. 5B).

There was a significant positive correlation (*P*<0.005) among the GU of all muscles but no association was evident in the case of SEMG.

DISCUSSION

This study revealed using surface EMG that the triceps surae contribution to overall plantarflexion force is about 70 % while muscle glucose uptake suggests about 10 % greater role for TS muscle group. The findings of the current study can provide valuable reference for studies where individual contributions are estimated based on models and simulations.

The results revealed heterogeneous contribution from individual TS muscle compartments during intermittent isometric exercise with 30 % MVC. In terms of SEMG, activity of soleus was 24 % EMG MVC, MG 33 % EMG MVC, and LG 21 % EMG MVC. A previous study has shown that mean EMG of soleus, MG, and LG at the beginning of a sustained, unilateral submaximal (40% MVC) isometric exercise was ~43 % EMG MVC, ~33 % EMG MVC, and ~28 % EMG MVC respectively, in older healthy male subjects (Mademli & Arampatzis 2005). In dynamic exercises Kinugasa et al. (2005) reported that EMG activity in MG was 64-88 % EMG MVC, soleus 49 % EMG MVC, and LG 57 % EMG MVC in repetitive, single leg calf-raise exercise. In the same study, MRI technique revealed that only ~46 % of MG, in terms of muscle volume, was activated compared to ~35 % in the case of soleus and LG (Kinugasa et al. 2005). The differences between these studies may stem from different exercise modes, subject groups, and differences in protocols. In the past, EMG of both gastrocnemius and soleus has been shown to increase significantly over time during sustained unilateral submaximal isometric exercise at 10-40 % MVC (McLean & Goudy 2004, Löscher et al. 2008, Pereira et al. 2011). However, the present protocol applied intermittent contractions, instead of sustained, resulting in no significant change in the EMG RMS during the exercise protocol.

Similar to the results for EMG, the MG had the highest GU (~2.5 µmol/100g/min). Soleus and LG displayed a very similar GU behavior (~2.1 µmol/100g/min) while FHL had the lowest GU among all muscles. In the past, Kemppainen et al (2002) measured muscle glucose

uptake per unit area of mass for quadriceps femoris muscle under both resting and exercising conditions. Resting muscle GU was found to be very low and it increased significantly with an increase in the exercise (cycle ergometry) intensity (30%, 55%, and 75% VO_{2max}.). At 30% $VO_{2max,}$ (91 ± 24 W) the uptake of 7.5 µmol/100g/min was reported which is more than twice the GU of plantarflexors in our study. Another study (Hannukainen et al. 2005) reported the values of ~4.5 µmol/100g/min using the same protocol as Kemppainen et al, at (76.9 ± 15.6 W). Yet another study, comparing GU response to exercise in trained and untrained men, reported quadriceps femoris GU of ~5 µmol/100g/min in untrained men during cycle ergometry at 30% VO_{2max} (Fujimoto et al. 2003). Reasons for discrepancy in GU values in submaximal exercise may include the use of different exercise modalities and examined muscle groups. Most other studies are incomparable to the present because of either a different exercise device/intensity (Oi et al. 2003, Kalliokoski et al. 2005, Reinking & Osman 2009) or a different quantifier was used to report the glucose uptake [SUV (Rudroff et al. 2013) or 'glucose uptake index' (Bojsen-Moller et al. 2006)]. Some studies used exercise for the whole body (Fujimoto et al. 1996, Kilgore & Watson 1998) or only upper extremities (Pappas et al. 2001).

We calculated Sol-to-FHL, MG-to-FHL, and LG-to-FHL ratios of EMG and GU to further quantify the relative role of deep and superficial plantarflexor muscles. Considerable individual variations in activation strategies were observed. For instance, EMG activity of soleus ranged from as low as 1/5th of, to as high as nearly twice the activity of FHL (Fig. 5A). The ratios based on GU displayed slightly smaller individual variability. Based on GU, the TS-FHL ratios generally exceeding 1 denoting that every TS component had taken up more glucose than flexor hallucis longus. For example, both Sol-to-FHL and MG-to-FHL ratios ranged from 0.8 to ~2. Previously, muscle ratios in terms of peak muscle displacement, during submaximal isometric contractions, have been investigated using MRI (Finni et al. 2006). Finni and colleagues showed that Sol-to-FHL muscle displacement ratios displayed a large discrepancy between healthy subjects, ranging from 0.6 to 9.6 at 20% MVC and 1.1 to 4.7 at 40% MVC. Similarly, clear differences in the contribution of deep plantarflexors among the subjects were present in another study (Bojsen-Moller et al. 2010b). The individual behavior in plantarflexor

coordination may potentially play a role when injury occurs or have functional significance during rehabilitation from Achilles tendon injury (Finni et al. 2006).

Cumulative TS muscle GU accounted for 79 % of the total GU of all four muscles. In the past, muscle moment investigations have established the input of TS to the residual plantarflexion moment to be 65% on average while pedaling at power outputs of 90, 180, and 270W (Gregor et al. 1991). In another study, Arndt et al (1998) found that directly measured AT moment was 121% of the resultant plantarflexion moment, under the forefoot, during isometric plantarflexion at 25% MVC with nearly straight knee. This exceptionally high contribution, as explained by the authors, was due to opposing moment from the antagonistic dorsiflexors. Findings based on cadaver moment-arm length data and muscle cross-sectional area had suggested that TS components were responsible for at least ~88% of total plantarflexion moment (van Zandwijk et al. 1998). The present study by having only FHL as a representative of the entire deep muscle compartment suggested ~70 - 80% contribution from TS during submaximal task based on EMG and GU, respectively.

Even though there were some similarities between the findings from the two methods, there was no real correlation observed between the two methods. This is consistent with Rudroff et al. (2013) who concluded that PET was more sensitive, than SEMG, in revealing modulation of physical activity in thigh muscles during fatiguing contractions of knee extensor muscles at 25 % MVC force. In the current study, PET findings compared to SEMG are evident in figure 5. Although the exercise protocol could theoretically be performed during the PET scan, we chose to do the exercise before the PET scan. Such a protocol has been recently applied in several other experiments (Kemppainen et al. 2002, Fujimoto et al. 2003, Hannukainen et al. 2005) made possible by the properties of [^{18}F]-FDG that enables entrapment into the cells. As there is some tracer still available in the plasma to be taken up by the cells after the cessation of exercise, it may affect the measure of glucose uptake during exercise. However, this effect has previously been estimated to be small (Kemppainen et al. 2002) and most probably in line with the relative usage of the muscles during the preceding exercise. Thus, the lapse between

the cessation of exercise and image acquisition in our study is likely not to have affected the data.

As expected, the mean maximal isometric force production was found to be similar in right and left legs, even though significant bilateral and inter-individual variability existed. The submaximal force levels maintained during the exercise protocol were high enough to reveal appreciable muscle GU and low enough to enable the completion of the task without the onset of fatigue. One assumption made in this study is that all compartments of TS muscle were fully activated when MVC was recorded. Therefore, caution must be observed while making inferences based on the findings. The SEMG results reported here are normalized to the EMG RMS during the MVC and, thus, only represent the relative activation of the muscles. It has been suggested in the literature that it is better to use M-wave, rather than % EMG MVC, for the normalization of SEMG (Arabadzhiev et al. 2010) due to the factors influencing the myoelectric signal, such as cross talk, amplitude cancellation, etc. (Farina et al. 2004).

The present study reports novel data on the relative contribution of superficial and deep plantarflexor muscles during an isometric task using SEMG and high-resolution PET. The findings from surface EMG suggest that the triceps surae contribution to plantarflexion force is about 70 %. On the other hand, examination of muscle glucose uptake behavior, using high-resolution PET, indicated 10 % greater role for TS muscle group in plantar flexor efforts. These findings provide reference values for future research.

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FIGURE LEGENDS:

Figure 1. Schematic diagram of the experimental protocol.

Figure 2. Experimental setup. The subject is pressing with her left foot against the force transducer during a submaximal contraction. Intravenous catheters can be seen on both arms for blood sampling and tracer injection. Also visible are some of the SEMG electrodes connected to the acquisition device and an electronic goniometer around the right ankle.

Figure 3. Examples of raw recordings of force and EMG of Soleus (SOL) Medial gastrocnemius (MG), and Lateral Gastrocnemius (LG) at the beginning, middle, and end of intermittent, submaximal isometric contractions from the right leg of a subject.

Figure 4. Relative contributions of examined ankle plantarflexors under submaximal contracting conditions based on surface EMG (A) and high-resolution PET (B). Values represent the percent contribution of each muscle to the cumulative EMG or GU.
* Significant difference between the methods within a given muscle * ($P<0.05$), ** ($P<0.01$), *** ($P<0.005$)

Figure 5. Comparison of triceps surae -to-flexor hallusis longus muscle ratios calculated using EMG (A) and glucose uptake (B) during submaximal isometric contractions for both legs. Each individual leg is represented by dot and mean values by horizontal line. (MG = Medial Gastrocnemius, Sol = Soleus, LG = Lateral Gastrocnemius, FHL = Flexor Hallucis Longus) (Y-axis is the same for both A and B)

Figure 6. Exemplary images from a subject in the study. MRI images from A) coronal (posterior), B, axial, and C) sagittal sections and **D,E,F**) the same images superimposed with PET sinograms. Region of interest drawings are visible on the triceps surae muscle of the left leg in **B** and one calibration marker is visible on right lateral malleolus in **D**).
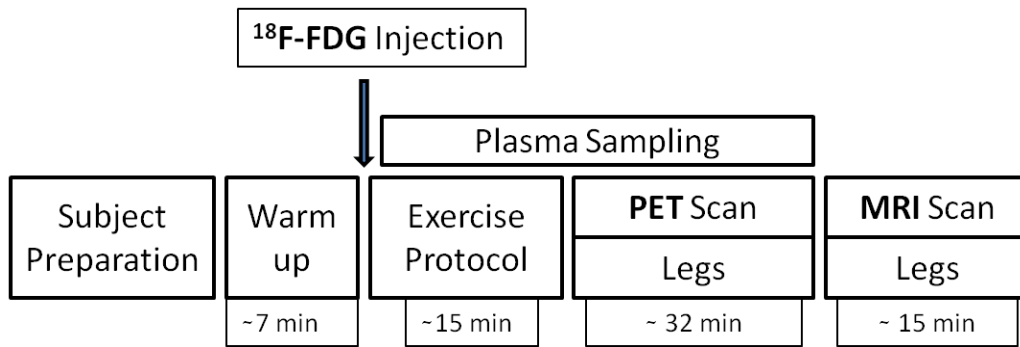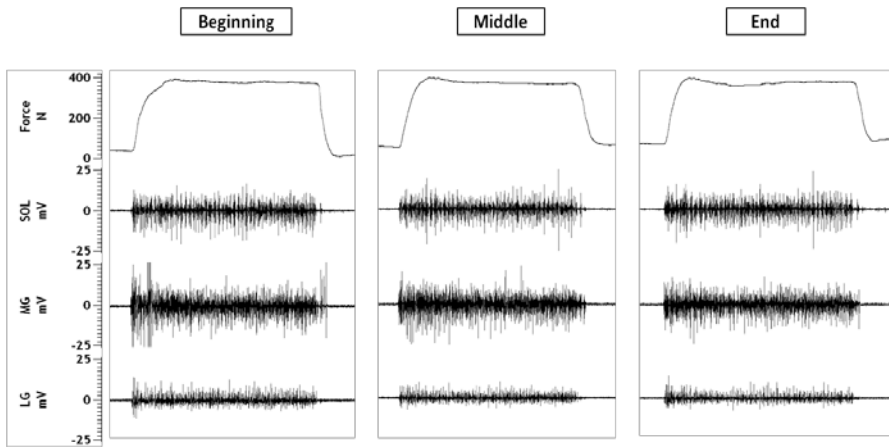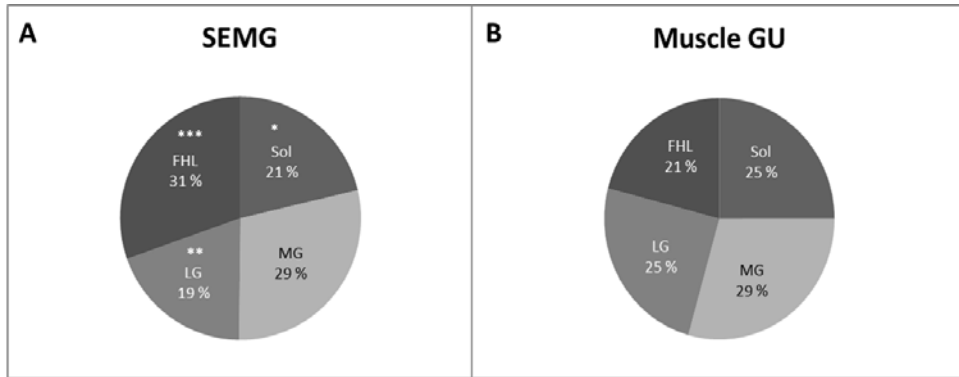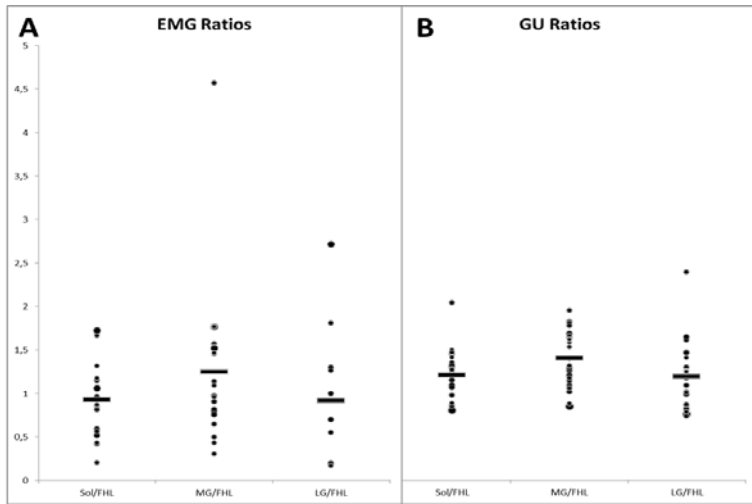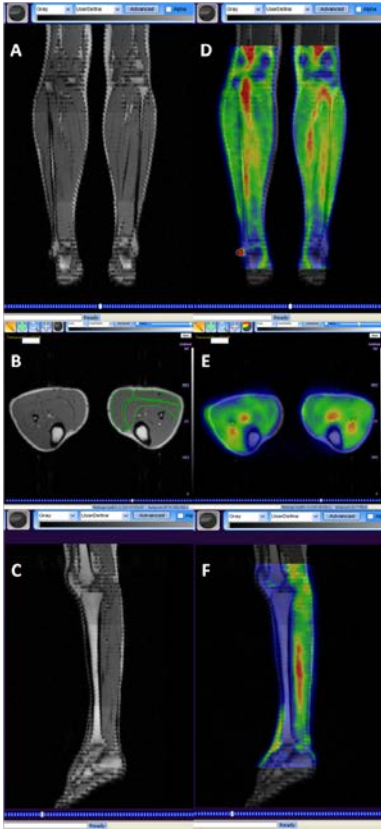
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