Masood, T., Kalliokoski, K. K., Bojsen-Møller, J., Magnusson, S. P., Finni, T. (2014). Plantarflexor muscle function in healthy and chronic Achilles tendon pain subjects evaluated by the use of EMG and PET imaging. *Clinical Biomechanics, 29*, 567-570.

Elsevier Editorial System(tm) for Clinical Biomechanics

Manuscript Draft

Manuscript Number: CLBI-D-13-00583R2

Title: "Plantarflexor Muscle Function in Healthy and Chronic Achilles Tendon Pain Subjects Evaluated by the Use of EMG and PET Imaging"

Article Type: Research Paper

Keywords: Biomechanics; Triceps surae; Achilles tendon; Glucose uptake; Tendinopathy; [18F]-Fluorodeoxyglucose

Corresponding Author: Mr. Tahir Masood, MSc

Corresponding Author's Institution: Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland

First Author: Tahir Masood, MSc

Order of Authors: Tahir Masood, MSc; Kari K Kalliokoski, PhD; Jens Bojsen-Møller , PhD; Peter Magnusson, DSc; Taija Finni , PhD

Abstract: ABSTRACT
Background: Achilles tendon pathologies may alter the coordinative strategies of synergistic calf muscles. We hypothesized that both surface electromyography and positron emission tomography would reveal differences between symptomatic and asymptomatic legs in Achilles tendinopathy patients and between healthy controls.
Methods: Eleven subjects with unilateral chronic Achilles tendon pain (28 yr) and eleven matched controls (28 yr) were studied for triceps surae and flexor hallucis longus muscle activity in response to repetitive isometric plantarflexion tasks performed at 30% of maximal voluntary contraction using surface electromyography and glucose uptake using positron emission tomography. Additionally, Achilles tendon glucose uptake was quantified.
Findings: Normalized myoelectric activity of soleus was higher ($P<0.05$) in the symptomatic leg versus the contralateral and control legs despite lower absolute force level maintained ($P<0.005$). Electromyography amplitude of flexor hallucis longus was also greater on the symptomatic side compared to the healthy leg ($P<0.05$). Both the symptomatic and asymptomatic legs tended to have higher glucose uptake compared to the control legs (overall effect size: 0.9 and 1.3, respectively). Achilles tendon glucose uptake was greater in both legs of the patient group ($P<0.05$) compared to controls. Maximal plantarflexion force was ~14% greater in the healthier leg compared to the injured leg in the patient group.
Interpretations: While the electromyography showed greater relative amplitude in the symptomatic leg, the results based on muscle glucose uptake suggested relatively similar behavior of both legs in the patient

group. Higher glucose uptake in the symptomatic Achilles tendon suggests a higher metabolic demand.

PLANTARFLEXOR MUSCLE FUNCTION IN HEALTHY AND CHRONIC ACHILLES TENDON PAIN SUBJECTS EVALUATED BY THE USE OF EMG AND PET IMAGING

*[1]Tahir Masood MSc,

[2]Kari Kalliokoski PhD, (Kari.Kalliokoski@tyks.fi)

[3,4]Jens Bojsen-Møller PhD, (jens.bojsen-moller@nih.no)

[4]S. Peter Magnusson DSc (spmagnusson@gmail.com)

[1]Taija Finni PhD (taija.finni@jyu.fi)

[1]Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland

[2]Turku PET Centre, University of Turku, Finland

[3] Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

[4]Institute of Sports Medicine Copenhagen, Dept. Ortopaedic Surgery M, Bispebjerg Hospital, and Center for Healthy Aging, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

*Corresponding author: tahir.masood@jyu.fi

Neuromuscular Research Center (NMRC)

Department of Biology of Physical Activity

Faculty of Sport and Health Sciences

P.O. Box 35 (VIV), 40014 University of Jyväskylä, FINLAND

Word count:

Abstract → 246

Main text → 4,068

Number of figures: 6

ABSTRACT

Background: Achilles tendon pathologies may alter the coordinative strategies of synergistic calf muscles. We hypothesized that both surface electromyography and positron emission tomography would reveal differences between symptomatic and asymptomatic legs in Achilles tendinopathy patients and between healthy controls.

Methods: Eleven subjects with unilateral chronic Achilles tendon pain (28 yr) and eleven matched controls (28 yr) were studied for triceps surae and flexor hallucis longus muscle activity in response to repetitive isometric plantarflexion tasks performed at 30% of maximal voluntary contraction using surface electromyography and glucose uptake using positron emission tomography. Additionally, Achilles tendon glucose uptake was quantified.

Findings: Normalized myoelectric activity of soleus was higher ($P<0.05$) in the symptomatic leg versus the contralateral and control legs despite lower absolute force level maintained ($P<0.005$). Electromyography amplitude of flexor hallucis longus was also greater on the symptomatic side compared to the healthy leg ($P<0.05$). Both the symptomatic and asymptomatic legs tended to have higher glucose uptake compared to the control legs (overall effect size: 0.9 and 1.3, respectively). Achilles tendon glucose uptake was greater in both legs of the patient group ($P<0.05$) compared to controls. Maximal plantarflexion force was ~14% greater in the healthier leg compared to the injured leg in the patient group.

Interpretations: While the electromyography showed greater relative amplitude in the symptomatic leg, the results based on muscle glucose uptake suggested relatively similar

47 behavior of both legs in the patient group. Higher glucose uptake in the symptomatic Achilles
48 tendon suggests a higher metabolic demand.

49

50 **Keywords**: Biomechanics; Triceps surae; Achilles tendon; Glucose uptake; Tendinopathy; [^{18}F]-
51 Fluorodeoxyglucose

INTRODUCTION

The Achilles tendon (AT) is among the strongest tendons in the human body (Józsa & Kannus 1997; Kvist, 1994). Despite its strength, the AT is susceptible to overuse injuries which, accompanied by tendon pain, impair function of the calf muscle-tendon unit (Silbernagel et al., 2006). Furthermore, it may affect the muscle activation strategies of the individual compartments of the triceps surae (TS) muscle group, and other ankle plantarflexors (Mafi et al., 2001; Roos et al., 2004).

Previous studies have established that the relative contribution within different compartments of the TS muscle group is inhomogeneous. Specifically, significant differences in the mediolateral forces within the Achilles tendon have been reported depending on how TS components were loaded (Arndt et al., 1999). The force contribution of various TS components also depends on muscle length since small changes in gastrocnemius length results in major changes in soleus and gastrocnemius electromyography (EMG), torque, and force (Arndt et al., 1998; Cresswell et al., 1995).

Past in-vivo studies have demonstrated considerable individual variation in the use of different plantarflexors in healthy individuals, displaying either low or high flexor hallucis longus (FHL) activation with corresponding tissue movement (Bojsen-Moller et al., 2010; Finni et al., 2003). Finni et al. (2006) showed that patients recovering from complete Achilles tendon rupture increased the contribution of FHL to motor tasks in their healthy and affected legs during rehabilitation. These observations lead the authors to hypothesize that this coordination strategy may contribute to Achilles tendon injuries since the FHL is an important deep

plantarflexor muscle (Klein et al. 1996). For this reason, and because the FHL is suitable for both PET and SEMG, we sought to further examine possible individual variations and the role of FHL during a plantarflexion task.

While muscle coordination can readily be assessed using surface EMG (SEMG), it only provides information about activity of the superficial muscles, which may not represent that of the whole muscle volume (Knight & Kamen 2005). Therefore, MRI and ultrasonography have been used to study tissue movement within the muscle (Bojsen-Moller et al., 2010; Finni et al. 2006; Huijing et al., 2011). Alternatively, positron emission tomography (PET) can be used to non-invasively investigate muscle glucose metabolism and thereby muscle metabolic activation (Nuutila & Kalliokoski 2000; Tashiro et al., 2008). High resolution PET has not only been employed to image and quantify glucose uptake as a result of exercise in skeletal muscles (Bojsen-Møller et al., 2010; Fujimoto et al., 2003; Hannukainen et al., 2005; Kemppainen et al., 2002; Kalliokoski et al., 2007; Rudroff et al., 2013), but also in healthy and injured tendons (Bojsen-Møller et al., 2006; Huang et al., 2006; Kalliokoski et al., 2005).

Since both SEMG and PET provide useful information regarding muscle-tendon behavior in health and disease, the overall purpose of this study was to investigate the electrical and metabolic activity patterns of various ankle plantarflexors in unilateral, chronic Achilles tendinopathy patients compared with healthy controls (CTRL). It was hypothesized that the relative contribution of different plantarflexors would differ between symptomatic (PAIN) and asymptomatic (NO-PAIN) legs, and further that it would differ from that of healthy controls. We also hypothesized that chronic tendon pain would lead to reduced maximal plantarflexion force

and glucose uptake, but similar SEMG, in the symptomatic leg in response to submaximal isometric exercise. In addition to muscle function, we also examined the Achilles tendon glucose uptake.

MATERIALS AND METHODS

Subjects

Tendon pain group: The target age range was 18-35 years. Other inclusion criteria included unilateral Achilles tendon pain for at least past 6 weeks (Bashford et al. 2008; Józsa & Kannus 1997) and absence of other major leg injury. Twenty Achilles tendon pain patients responded to the public recruitment advertisements for the study. After the screening process, eleven subjects - seven males and four females - were included in the study. The mean age (SD) of the participants was 28 (4) yr, height 174 (6) cm, and body mass 66 (6) kg. The subjects were physically active, recreational athletes (distance running, long jump, high jump, ice-hockey) who exercised on average 4.7 times a week the year prior to the study. Five patients had pain in the right Achilles tendon while the remaining six in the left leg. Average duration of symptoms at the time of baseline measurements was 9.8 (8) months (range: 2 - 25). Victorian Institute of Sports Assessment-Achilles (VISA-A) questionnaire, with a maximum possible score of 100, was used to measure the severity of Achilles tendinopathy (Robinson et al., 2001). Average VISA-A score in the subjects was 64 (18) (range: 27 - 86).

Healthy control group (CTRL): Eleven anthropometrically matched subjects, with no history of major leg injury or pain over the last year, were recruited by public announcements. The mean age, height, and body mass were 28 (4) yr, 173 (4) cm, and 67 (6) kg respectively. They reported to be physically active on average 2.4 times per week.

The study protocol was approved by the Ethics Committee of the Hospital District of South-Western Finland and conformed to the Declaration of Helsinki. All subjects gave informed written consent.

Experimental Protocol

Each subject took part in a series of tests, on a single day, at the Turku PET Centre, University of Turku, Finland. A schematic diagram of the experimental design is given in Fig. 1. Participants were required to fast for at least 8 hours prior to the PET scans. Before the study protocol, anthropometric measurements, such as body mass, height, and leg length, were obtained.

Subject preparation comprised shaving, abrading, and cleaning of skin for surface electromyography (SEMG), electrode placement on legs, securing an electronic goniometer to the ankle. In addition, catheters were inserted into the antecubital veins of both arms: one for venous blood sampling and the other for [^{18}F]-Fluorodeoxyglucose ([^{18}F]-FDG) tracer injection. Subsequently subjects were positioned in the exercise apparatus for force and SEMG measurements. Subjects were familiarized with the equipment and the task by performing submaximal contractions from each leg. Maximal Voluntary Contraction (MVC) of ankle plantarflexors was then recorded unilaterally and the highest of the three trials was used to determine the submaximal force target for each leg.

Exercise protocol: Subjects performed the task while sitting on a seat placed on the floor with knees in full extension and hips flexed at right angle (Fig. 2). Exercise protocol consisted of sets of five unilateral submaximal, isometric voluntary contractions, at 30% of respective MVC, lasting five seconds with a 5-second rest. Subjects were able to watch both the target force and the actual force exertion on a monitor display. Following two sets of warm up contractions for both legs, ~150 MBq of [^{18}F]-FDG tracer was infused after which the subjects performed 8 more sets with each leg, alternatingly. The total exercise and rest time before tracer injection was ~6-7 minutes followed by the post-injection exercise-rest of ~15 minutes. The subjects did not report pain or discomfort before, during, or immediately subsequent to the exercise protocol.

EMG from lower leg muscles was recorded during the exercise. At the cessation of the exercise protocol, subjects were moved to the PET scanner on a wheelchair and scanning started within 5 minutes. Repeated blood sampling for plasma radioactivity determination was started simultaneously with the tracer injection and continued until the end of the PET scan. Magnetic Resonance Imaging (MRI) was performed within an hour after the PET scanning.

EMG and Force Data 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. The electrodes on flexor hallucis longus (FHL) were placed after locating the muscle behind the medial malleolus by manual palpation. Furthermore, an indifferent electrode was

secured on the right medial malleolus to reduce noise signal. EMG data were detected online via EISA (bandwidth 10 Hz to 1 kHz per 3 dB) EMG detection system (model: 16-2, *University of Freiburg*, Germany) at a measurement frequency of 1000 Hz. Signal was pre-amplified with a factor of 200 by an integrated preamplifier in the cables. Analogue-to-digital conversion of EMG and force data was completed via *Power 1401* high-performance multi-channel data acquisition interface (*CED* Ltd., Cambridge, England). Compatible Signal 4.0 software (*CED* Ltd., Cambridge, England) was used to record, reduce, and analyze data. EMG signals from one set of five contractions were recorded separately for each leg from the beginning, middle, and end of the exercise protocol. That resulted in data comprising three sets of five 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 10Hz to remove noise signal and correct DC offset. Root Mean Square (RMS) amplitude of each muscle was calculated from a 3-second epoch during the middle of a 5-second submaximal isometric contraction. This RMS was normalized to the RMS amplitude from a 1-second time window during MVC. Additionally, SOL-to-FHL, MG-to-FHL, and LG-to-FHL submaximal EMG muscle ratios were computed by dividing the respective normalized RMS.

Plantarflexion Force: Isometric ankle plantarflexion force was measured by an in-house custom-built portable force transducer (*University of Jyväskylä*, Finland) (Fig. 2). The transducer plate was secured with steel chains, which 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. Force data recording was also done via Signal 4.0 (*CED* Ltd., Cambridge, England) in synch with EMG.

Because neither SEMG, nor plantarflexion force showed decline during the exercise protocol, their average was taken to represent each muscle's electrical activity and force level.

Image Acquisition

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

MRI scanning was performed with *1.5 T Philips Intera MRI* (Philips Healthcare, Eindhoven, The Netherlands) for both legs. Lipid pills for anatomical reference were taped to the same anatomical landmarks as were used in the PET.

Image Analysis

After the PET images were corrected for decay, parametric fractional uptake rate (FUR) images were computed using the PET image data and the individual input function (plasma radioactivity data) as described previously (Fujimoto et al., 2003; Kemppainen et al., 2002). The regions of interest (ROIs) were then drawn, at an interval of 1 cm of muscle thickness, on the

196 transverse plane FUR images to include the whole individual muscle using *Carimas 2.0* software
197 (Turku PET Centre, University of Turku, Finland). All drawings were made by the same
198 investigator (TM) to avoid inter-observer differences. Glucose FUR values were obtained for
199 soleus, medial and lateral gastrocnemii, FHL, and the Achilles tendon. These values were further
200 converted to glucose uptake values using the following formula:

$$Glucose\ uptake\ (\mu\mathrm{mol} * 100\mathrm{g} - 1 * \mathrm{min} - 1) = \frac{FUR \times Plasma\ glucose}{Lumped\ constant \times Tissue\ density}$$

201 Plasma glucose level was obtained from the plasma sampling during the study. The lumped
202 constant is the value that takes into account differences in the uptake of glucose and ^{18}F-FDG
203 from the blood and it has been shown to be 1.2 for skeletal muscle (Kelley et al., 1999;
204 Peltoniemi et al., 2000). Tissue density was acquired from Report of the Task Group on
205 Reference Man (Snyder et al. 1975).

206 In order to examine the relative contribution of various plantarflexors, SOL-to-FHL, MG-to-FHL,
207 and LG-to-FHL muscle GU ratios were calculated, as in EMG. MRI images were used as an
208 anatomical reference to delineate the targeted muscles for ROIs drawings on PET images.

209 **Statistical analysis**

210 Normality of the data was explored using Shapiro-Wilk test, which revealed that some variables
211 had normal distribution, but not all. Mann–Whitney U test or independent samples T-test were
212 used accordingly to compare the skeletal muscle glucose uptake and electrical muscle activity
213 of the tendon-pain and healthy groups. Between-leg comparison within a group was analyzed
214 with either Wilcoxon signed rank test or paired samples T-test. *IBM SPSS 20.0* (*IBM Corporation*,

New York, USA) software was used for all statistical analyses. Alpha (α) level of significance was set at a *P* value of 0.05. The results are expressed as mean (SD) (standard deviation). Additionally, effect size (ES) was calculated for both within- and between-group comparisons.

RESULTS

Force: Maximal plantarflexion force was greater in NO-PAIN compared to PAIN [1250 (192) N vs. 1101 (176) N; $P<0.05$; ES = 0.8]. Since both control legs displayed similar force [1133 (236) N vs. 1129 (192) N], their average [1131 (191) N] was used for between-group comparisons.

The 30 % MVC target plantarflexion force used in the isometric exercise protocol was greater in NO-PAIN versus PAIN [369 (52) N vs. 325 (46) N; $P<0.005$; ES = 0.8]. Corresponding value for CTRL was 349 (56) N, which did not differ significantly from PAIN and NO-PAIN.

Electromyography

EMG during exercise: Within the tendinopathy subjects, EMG (%MVC) for soleus was higher in PAIN compared to NO-PAIN ($P<0.05$; ES = 1.7). Similarly, EMG magnitude in FHL was greater in PAIN ($P<0.05$; ES = 0.2). Soleus displayed greater activity in PAIN ($P<0.05$; ES = 1.4) compared to CRTL while FHL on the NO-PAIN side had lower ($P<0.05$; ES = 0.5) EMG level than CTRL (Fig. 3).

EMG ratios: During the submaximal exercise, MG-to-FHL ratio in PAIN was significantly smaller than NO-PAIN within the tendinopathy group ($P<0.05$; ES = 0.6). Other two ratios were similar across the legs. CTRL had smaller SOL-to-FHL ratio ($P<0.05$; ES = 1.7) than PAIN while MG-to-FHL ratio was significantly higher ($P<0.05$; ES = 0.5) in NO-PAIN compared to CTRL (Fig. 4).

Muscle-Tendon Glucose Uptake

Representative MRI and PET images from a patient in the study are shown in Fig. 5. In the patient group, all three triceps surae muscles showed a tendency towards a lower glucose uptake in NO-PAIN compared to NO-PAIN (Fig. 6), while the opposite was seen for FHL. Achilles tendon GU rate was identical across the two legs.

Both PAIN and NO-PAIN tended to have greater muscle GU rate compared to CTRL. In the case of Achilles tendon, both PAIN (ES = 1.2) and NO-PAIN (ES = 1.0) had significantly higher GU rate than CTRL (P<0.05). Glucose uptake rate ratios between the superficial and deep plantarflexors revealed no significant differences between legs or groups.

DISCUSSION

In chronic Achilles tendinopathy patients, the stronger NO-PAIN leg exercised with less relative EMG than the PAIN leg during the submaximal exercise. Muscle glucose uptake rate tended to be higher in the patient legs compared to the control legs. The overall EMG findings showed a greater relative activity in the symptomatic leg versus the asymptomatic leg, which corresponded to our hypothesis. On the other hand, the GU results suggested similar behavior between the two patient legs, which contradicted our hypothesis. These dissimilar findings by the two fundamentally different methodologies are further discussed below.

In line with our hypothesis, the maximal isometric plantarflexion force was significantly greater in NO-PAIN compared to PAIN. We also expected a similar discrepancy between PAIN and CTRL but no difference was evident. One explanation may be that the subjects in the

255 patient group were physically more active and had therefore about 10 % stronger legs than
256 CTRL despite being age-weight-height matched. For obvious reasons, the differences in the
257 MVC were also reflected in the target force levels for the submaximal isometric exercise.

258 Unsurprisingly, FHL demonstrated greater relative myoelectric activity in the
259 symptomatic leg versus the asymptomatic leg. It was expected that reduced triceps surae input
260 due to an injured Achilles tendon would lead to a compensatory rise in the activity of
261 corresponding secondary plantarflexors, such as the FHL. Surprisingly, however, soleus in the
262 symptomatic leg was also found to have more activity (~67 %) than its asymptomatic
263 counterpart, and the mechanism for this increase remains unknown. However, it must be noted
264 that SOL-to-FHL ratio was *not* significantly different between the two patient legs due to a
265 concomitant rise in the activity of both muscles in the symptomatic leg. The soleus muscle also
266 happened to be the most active of the four muscles in the symptomatic leg whereas the MG
267 was the most active in the asymptomatic leg. Additionally, the soleus muscle in the
268 symptomatic leg showed more relative activity than healthy controls despite the other three
269 muscles being comparable in their activity levels. In contrast, all triceps surae components of
270 the asymptomatic leg behaved in a similar manner to that of controls. This scenario, except for
271 the soleus, is in accordance with our hypothesis that EMG activity of triceps surae components
272 in PAIN will be similar to that of NO-PAIN and CTRL. Similarly, the assumption that TS muscles of
273 NO-PAIN in the tendon pain group would behave similarly to the CTRL was also found to be
274 true.

In the past, behavior of the TS muscle EMG during submaximal isometric contractions has been reported in the literature. EMG (%MVC) of soleus, MG, and LG at the beginning of a sustained unilateral submaximal (40 %MVC) isometric exercise was shown to be ~43%, ~33%, and ~28% respectively in older healthy male subjects (Mademli & Arampatzis, 2005). In dynamic exercises Kinugasa et al. (2005) reported that EMG activities were 49% (Sol), 64-88 % (MG), and 57 % (LG) 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). In our much younger healthy controls, values were ~26 % (Sol), ~33 % (MG), and ~21 % (LG). On the other hand, muscles in the injured legs of our patient group yielded the values of ~38 % (Sol), ~32 %, (MG), and ~18 % (LG).

Contrary to the findings using SEMG, plantarflexor muscle GU rate showed no significant differences in either absolute or relative terms. It was expected that the glucose uptake of the TS muscle of PAIN would be appreciably lower than CTRL. However, CTRL had a tendency for lower GU rate than both PAIN and NO-PAIN. In the tendon pain group, despite a greater absolute force in NO-PAIN, no significant differences in GU rate was observed between the two legs although there was a trend towards a higher uptake in NO-PAIN triceps surae. In the case of FHL, the GU rate was expected to be higher in PAIN as an indicator of a compensatory increment in the contribution from the deep plantarflexors. Although the trend of glucose uptake behavior of all muscles was in line with our expectations, the differences were not significant for our hypothesis to be accepted.

While there are no previous studies on the glucose uptake behavior of various plantarflexors, under submaximal isometric conditions, in Achilles tendinopathy patients, a study on quadriceps femoris muscle reported GU of 7.5 µmol*100g^{-1}*min^{-1} in cycling exercise [91 W) at 30% VO_{2max} (Kemppainen et al., 2002). This 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^{-1}*min^{-1} using the same protocol as Kemppainen et al. at (77 W). Yet another study, comparing GU response to exercise in trained and untrained men, reported a quadriceps femoris GU of ~5 µmol*100g^{-1}*min^{-1} in untrained men during cycle ergometry at 30% VO_{2max} (Fujimoto et al., 2003).

In the Achilles tendon itself, GU rate was significantly lower than that of muscles' in both groups ranging from 1.0 to 1.5 µmol*100g^{-1}*min^{-1} in CTRL and PAIN respectively. In the Achilles tendinopathy patients, the tendon GU rate in *both* legs was significantly higher than in the control group. While the higher GU in the asymptomatic tendon can be explained by greater force transmission, the higher metabolic activity in the symptomatic tendon is more difficult to explain. Achilles tendinopathy is known to be associated with increased cell count (Pingel et al. 2012) which might be responsible for the observed higher glucose uptake. Although inflammation could theoretically have a role in the finding, most histological (Movin et al., 1997), biochemical (Alfredson et al., 2001), and microdialysis (Alfredson et al., 1999) studies have concluded that inflammation is absent in Achilles tendinopathy. Even though microdialysis technique has demonstrated a lack of appreciable GU in the peritendinous tissue of healthy Achilles tendons (Langberg et al., 1999), an FDG-PET case study has shown an abnormally high Achilles tendon glucose uptake in the case of Achilles tendinopathy (Huang et

318 al., 2006), which corroborates the findings of the present study. The underlying mechanism for
319 the higher metabolic demand in the symptomatic Achilles tendinopathy is unknown, but may
320 relate to an increase in cell density.

321 Previously, Hannukainen et al. (2005) published the Achilles tendon GU of well under 1
322 µmol*100g^{-1}*min^{-1} during cycle ergometry at 30% VO_{2max} and it stayed constant as the exercise
323 intensity was increased to 75% VO_{2max}. However, it must be noted that while the plantarflexion
324 task in the present study caused direct strain to Achilles tendon, cycling used by Hannukainen
325 and colleagues has only a limited impact on Achilles tendon. A case study involving a 30-year
326 old male reported a 2-fold increase in Achilles tendon glucose uptake, as a result of exercise,
327 compared to the resting contralateral leg (Kalliokoski et al., 2007). A comparable increase was
328 also observed in glucose uptake in patellar and quadriceps tendons during one-leg dynamic
329 knee extension exercise protocol (Kalliokoski et al. 2005).

330 We examined the relative contributions of primary plantarflexors (triceps surae) to a
331 secondary plantarflexor (FHL) by calculating SOL-to-FHL, MG-to-FHL, and LG-to-FHL muscle
332 ratios. Regarding muscle GU ratios, there were no differences observed across legs or groups.
333 Concerning EMG, the ratios were closest to 1 signifying balanced contribution between deep
334 and superficial plantarflexors in healthy controls. However, in patients there was no systematic
335 pattern in this parameter although significant difference was observed between legs in MG-to-
336 FHL ratio. This was due to a significantly lower FHL activity in NO-PAIN. While comparing the
337 two groups, PAIN had significantly greater SOL-to-FHL ratio than CTRL. This finding contradicts
338 with the observations based on muscle displacement between healthy and Achilles tendon

injury patients. Finni et al. (2006) reported that SOL-to-FHL muscle peak displacement ratios were greater in healthy compared to the injured without differences between the legs within the patients. It should be noted that different methodological factors can play a role in these two contradictory findings. While EMG ratios are affected by uncertainty of muscle activation during MVC, the muscle displacement ratios are influenced by muscle architecture and mechanics. What are similar to both studies are the large individual differences between the subjects potentially suggesting a wide range of individual coordinative strategies within the plantarflexors.

Limitations of the study: Assumption made in this study that all compartments of TS muscle were fully activated when MVC was recorded warrants caution while making inferences based on the findings. Similarly, contractions at 30% MVC level do not imply that each muscle was activated at 30% of its capacity. The EMG results reported here are normalized to the EMG RMS during the MVC and, thus, only represent the relative activation of the muscles. The same argument applies to the muscle EMG ratios. Furthermore, although the exercise protocol could theoretically be performed during the PET scan, we chose to do the exercise before the PET scan. This same protocol has been recently applied in several other experiments (Fujimoto et al., 2003; Hannukainen et al., 2005; Kemppainen et al., 2002).

In conclusion, although surface EMG revealed major inter-leg and group differences in the use of triceps surae muscle, glucose uptake, reflecting the function of the entire muscle, did not manifest significant differences between healthy subjects and chronic Achilles tendon pain patients. In the case of Achilles tendon, both legs in the patient group displayed significantly

higher GU compared to the healthy tendons. These differential results from local and global measures call attention to the complexity of muscle-tendon function in three-dimensional space.

ACKNOWLEDGMENTS

The project was funded by research grants from Academy of Finland, Ministry of Education and Culture (TF) and Finnish Cultural Foundation (KK).

REFERENCES

Alfredson, H., Forsgren, S., Thorsen, K., Fahlström, M., Johansson, H., Lorentzon, R., 2001. Glutamate NMDAR1 receptors localised to nerves in human Achilles tendons. Implications for treatment? *Knee Surgery, Sports Traumatology, Arthroscopy,* **9**(2), 123-126.

Alfredson, H., Thorsen, K., Lorentzon, R., 1999. In situ microdialysis in tendon tissue: high levels of glutamate, but not prostaglandin E2 in chronic Achilles tendon pain. *Knee surgery, sports traumatology, arthroscopy,* **7**(6), 378-381.

Arndt, A., Komi, P., Brüggemann, G., Lukkariniemi, J., 1998. Individual muscle contributions to the in vivo Achilles tendon force. *Clin Biomech,* **13**(7), 532-541.

Arndt, A., Brüggemann, G., Koebke, J., Segesser, B., 1999. Asymmetrical loading of the human triceps surae: I. Mediolateral force differences in the Achilles tendon. *Foot & Ankle International,* **20**(7), 444-449.

Bashford, G.R., Tomsen, N., Arya, S., Burnfield, J.M., & Kulig, K., 2008. Tendinopathy discrimination by use of spatial frequency parameters in ultrasound B-mode images. *IEEE Transactions on Medical Imaging* **27**(5), 608-615.

Bojsen-Møller, J., Kalliokoski, K.K., Seppänen, M., Kjaer, M., Magnusson, S., 2006. Low-intensity tensile loading increases intratendinous glucose uptake in the Achilles tendon. *J Appl Physiol,* **101**(1), 196-201.

Bojsen-Møller, J., Losnegard, T., Kemppainen, J., Viljanen, T., Kalliokoski, K.K., Hallén, J., 2010. Muscle use during double poling evaluated by positron emission tomography. *J Appl Physiol,* **109**(6), 1895-1903.

Cresswell, A.G., Löscher, W., Thorstensson, A., 1995. Influence of gastrocnemius muscle length on triceps surae torque development and electromyographic activity in man. *Experimental Brain Research,* **105**(2), 283-290.

Finni, T., Hodgson, J.A., Lai, A.M., Edgerton, V.R., Sinha, S., 2006. Muscle synergism during isometric plantarflexion in Achilles tendon rupture patients and in normal subjects revealed by velocity-encoded cine phase-contrast MRI. *Clin Biomech,* **21**(1), 67-74.

Finni, T., Hodgson, J.A., Lai, A.M., Edgerton, V.R., Sinha, S., 2003. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. *J Appl Physiol,* **95**(2), 829-837.

Fujimoto, T., Kemppainen, J., Kalliokoski, K.K., Nuutila, P., Ito, M., Knuuti, J., 2003. Skeletal muscle glucose uptake response to exercise in trained and untrained men. *Med Sci Sports Exerc,* **35**(5), 777-783.

Hannukainen, J., Kalliokoski, K., Nuutila, P., Fujimoto, T., Kemppainen, J., Viljanen, T., et al., 2005. In vivo measurements of glucose uptake in human Achilles tendon during different exercise intensities. *Int J Sports Med,* **26**(09), 727-731.

Hermens, H.J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., et al., 1999. *European recommendations for surface electromyography.* Roessingh Research and Development The Netherlands.

Huang, S.S., Jian, Q.Y., Chamroonrat, W., Alavi, A., Zhuang, H., 2006. Achilles tendonitis detected by FDG-PET. *Clin Nucl Med,* **31**(3), 147-148.

Huijing, P.A., Yaman, A., Ozturk, C., Yucesoy, C.A., 2011. Effects of knee joint angle on global and local strains within human triceps surae muscle: MRI analysis indicating in vivo myofascial force transmission between synergistic muscles. *Surgical and radiologic anatomy,* **33**(10), 869-879.

Józsa, L.G., Kannus, P., 1997. *Human tendons: anatomy, physiology, and pathology.* Human Kinetics Champaign, IL.

Kalliokoski, K.K., Bojsen-Møller, J., Seppänen, M., Johansson, J., Kjaer, M., Teräs, M., et al., 2007. Contraction-induced [18F]-fluoro-deoxy-glucose uptake can be measured in human calf muscle using high-resolution PET. *Clinical physiology and functional imaging,* **27**(4), 239-241.

418 Kalliokoski, K.K., Langberg, H., Ryberg, A.K., Scheede-Bergdahl, C., Doessing, S., Kjaer, A., et al.,
419 2005. The effect of dynamic knee-extension exercise on patellar tendon and quadriceps femoris
420 muscle glucose uptake in humans studied by positron emission tomography. *J Appl Physiol,*
421 **99**(3), 1189-1192.

422 Kelley, D.E., Williams, K.V., Price, J.C., Goodpaster, B., 1999. Determination of the lumped
423 constant for [18F] fluorodeoxyglucose in human skeletal muscle. *Journal of nuclear medicine:*
424 *official publication, Society of Nuclear Medicine,* **40**(11), 1798-1804.

425 Kemppainen, J., Fujimoto, T., Kalliokoski, K.K., Viljanen, T., Nuutila, P., Knuuti, J., 2002.
426 Myocardial and skeletal muscle glucose uptake during exercise in humans. *J Physiol,* **542**(2),
427 403-412.

428 Kinugasa, R., Kawakami, Y., Fukunaga, T., 2005. Muscle activation and its distribution within
429 human triceps surae muscles. *J Appl Physiol,* **99**(3), 1149-1156.

430 Klein, P., Mattys, S., Rooze, M., 1996. Moment arm length variations of selected muscles acting
431 on talocrural and subtalar joints during movement: An In vitro study. *Journal of Biomechanics,*
432 **29**(1), 21-30.

433 Knight, C., Kamen, G., 2005. Superficial motor units are larger than deeper motor units in
434 human vastus lateralis muscle. *Muscle & nerve,* **31**(4), 475-480.

435 Kvist, M., 1994. Achilles tendon injuries in athletes. *Sports Medicine,* **18**(3), 173-201.

436 Langberg, H., Skovgaard, D., Karamouzis, M., Bülow, J., Kjær, M., 1999. Metabolism and
437 inflammatory mediators in the peritendinous space measured by microdialysis during
438 intermittent isometric exercise in humans. *J Physiol,* **515**(3), 919-927.

439 Mademli, L., Arampatzis, A., 2005. Behaviour of the human gastrocnemius muscle architecture
440 during submaximal isometric fatigue. *Eur J Appl Physiol,* **94**(5-6), 611-617.

441 Mafi, N., Lorentzon, R., Alfredson, H., 2001. Superior short-term results with eccentric calf
442 muscle training compared to concentric training in a randomized prospective multicenter study
443 on patients with chronic Achilles tendinosis. *Knee Surgery, Sports Traumatology, Arthroscopy,*
444 **9**(1), 42-47.

445 Movin, T., Gad, A., Reinholt, F.P., Rolf, C., 1997. Tendon pathology in long-standing achillodynia:
446 biopsy findings in 40 patients. *Acta Orthopaedica,* **68**(2), 170-175.

447 Nuutila, P., Kalliokoski, K., 2000. Use of positron emission tomography in the assessment of
448 skeletal muscle and tendon metabolism and perfusion. *Scand J Med Sci Sports,* **10**(6), 346-350.

449 Peltoniemi, P., Lönnroth, P., Laine, H., Oikonen, V., Tolvanen, T., Grönroos, T., et al., 2000.
450 Lumped constant for [18F] fluorodeoxyglucose in skeletal muscles of obese and nonobese
451 humans. *American Journal of Physiology-Endocrinology and Metabolism,* **279**(5), E1122-E1130.

452 Pingel, J., Fredberg, U., Qvortrup, K., Larsen, J.O., Schjerling, P., Heinemeier, K., et al., 2012.
453 Local biochemical and morphological differences in human Achilles tendinopathy: a case
454 control study. *BMC Musculoskeletal Disorders*, **13**(1), 53.

455 Robinson, J., Cook, J.L., Purdam, C., Visentini, P., Ross, J., Maffulli, N., et al., 2001. The VISA-A
456 questionnaire: a valid and reliable index of the clinical severity of Achilles tendinopathy. *Br J*
457 *Sports Med,* **35**(5), 335-341.

458 Roos, E.M., Engström, M., Lagerquist, A., Söderberg, B., 2004. Clinical improvement after 6
459 weeks of eccentric exercise in patients with mid-portion Achilles tendinopathy - a randomized
460 trial with 1-year follow-up. *Scand J Med Sci Sports,* **14**(5), 286-295.

461 Rudroff, T., Kalliokoski, K.K., Block, D.E., Gould, J.R., Klingensmith, W.C., Enoka, R.M., 2013.
462 PET/CT imaging of age-and task-associated differences in muscle activity during fatiguing
463 contractions. *J Appl Physiol,* **114**(9), 1211-1219.

464 Silbernagel, K.G., Gustavsson, A., Thomeé, R., Karlsson, J., 2006. Evaluation of lower leg function
465 in patients with Achilles tendinopathy. *Knee Surgery, Sports Traumatology, Arthroscopy,* **14**(11),
466 1207-1217.

467 Snyder, W.S., Cook, M.J., Nasset, E.S., Karhausen, L., Tipton, I., 1975. Report of the Task Group
468 on Reference Man, Chapter 2 gross and elemental content of reference man. *Annals of the*
469 *ICRP/ICRP Publication,* **23**, 273-334.

470 Tashiro, M., Itoh, M., Fujimoto, T., Masud, M.M., Watanuki, S., Yanai, K., 2008. Application of
471 positron emission tomography to neuroimaging in sports sciences. *Methods,* **45**(4), 300-306.

FIGURES LEGENDS:

Figure 1. Schematic diagram of experimental protocol. After subject preparation and warm-up exercise, radioactive tracer ([^{18}F]-Fluorodeoxyglucose) was administered followed by isometric submaximal exercise protocol. Positron emission tomography was performed after the exercise which preceded magnetic resonance imaging.

Figure 2. Experimental setup. A 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 data acquisition device and an electronic goniometer around the right ankle.

Figure 3. Comparison of EMG (%MVC) values during submaximal isometric contractions for both study groups. (Sol = Soleus, MG = Medial Gastrocnemius, LG = Lateral Gastrocnemius, FHL = Flexor Hallucis Longus)

***** Significant difference between PAIN and CTRL (P<0.05).

Significant difference between NO-PAIN and CTRL (P<0.05).

! Significant difference between PAIN and NO-PAIN (P<0.05).

Figure 4. Comparison of selected plantarflexor muscle EMG ratios during submaximal isometric contractions for both study groups. (TS = Triceps Surae, MG = Medial Gastrocnemius, Sol = Soleus, LG = Lateral Gastrocnemius, FHL = Flexor Hallucis Longus)

***** Significant difference between PAIN and CTRL (P<0.05).

Significant difference between NO-PAIN and CTRL (P<0.05).

! Significant difference between PAIN and NO-PAIN (P<0.05).

Figure 5. Images from a tendon pain patient: **A**) an axial MRI section with region of interest (ROI) drawing on triceps surae muscle of the left leg, **B**) same image superimposed with PET sinograms.

Figure 6. Muscle glucose uptake comparison for Tendon pain group and Healthy control group. (Sol = Soleus, MG = Medial Gastrocnemius, LG = Lateral Gastrocnemius, FHL = Flexor Hallucis Longus, AT = Achilles Tendon)

***** Significant difference between PAIN and CTRL ($P<0.05$).

Significant difference between NO-PAIN and CTRL ($P<0.05$).

FIGURES:

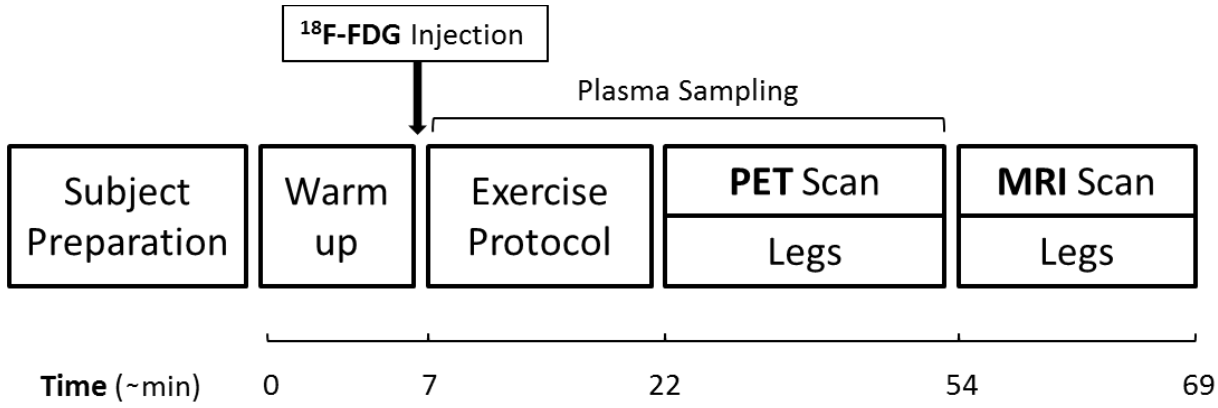

Figure 1

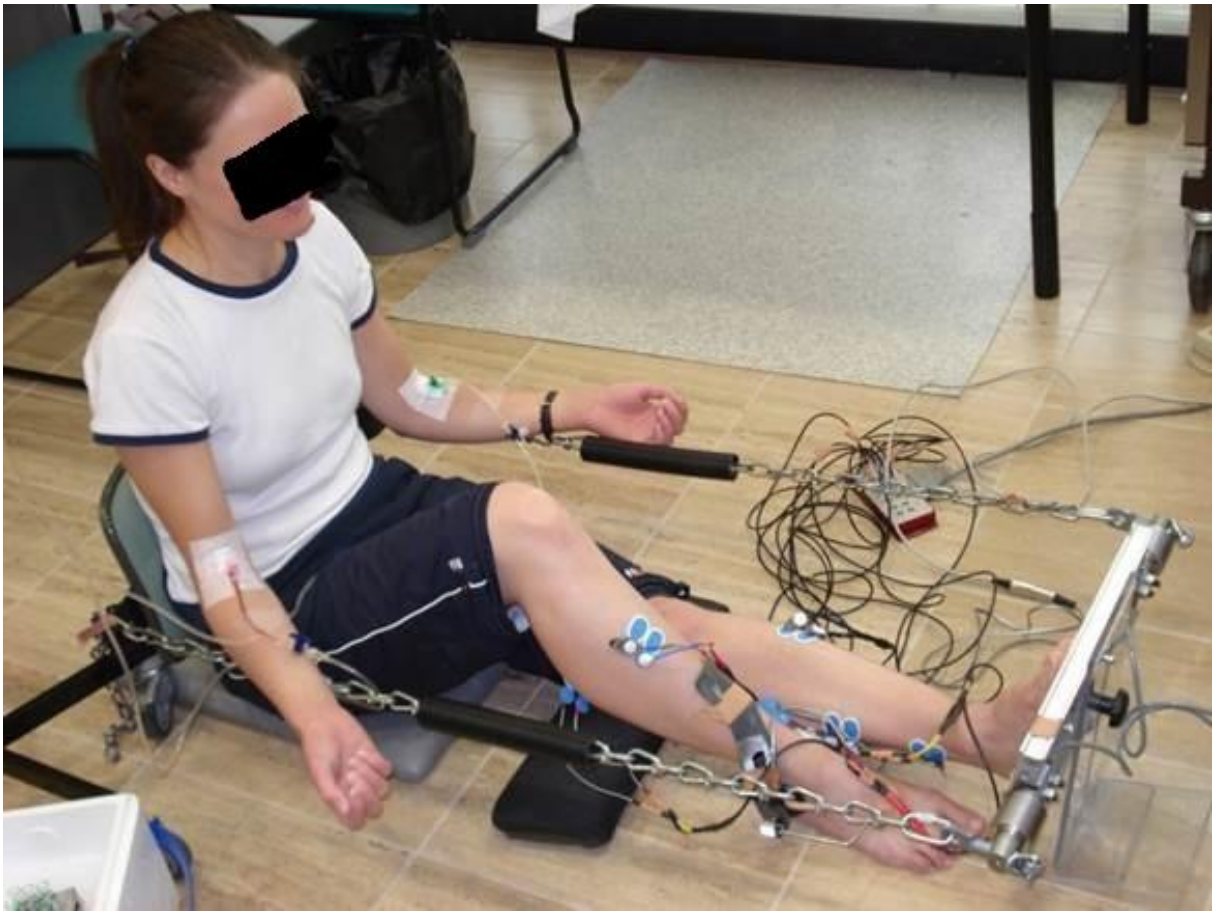

Figure 2

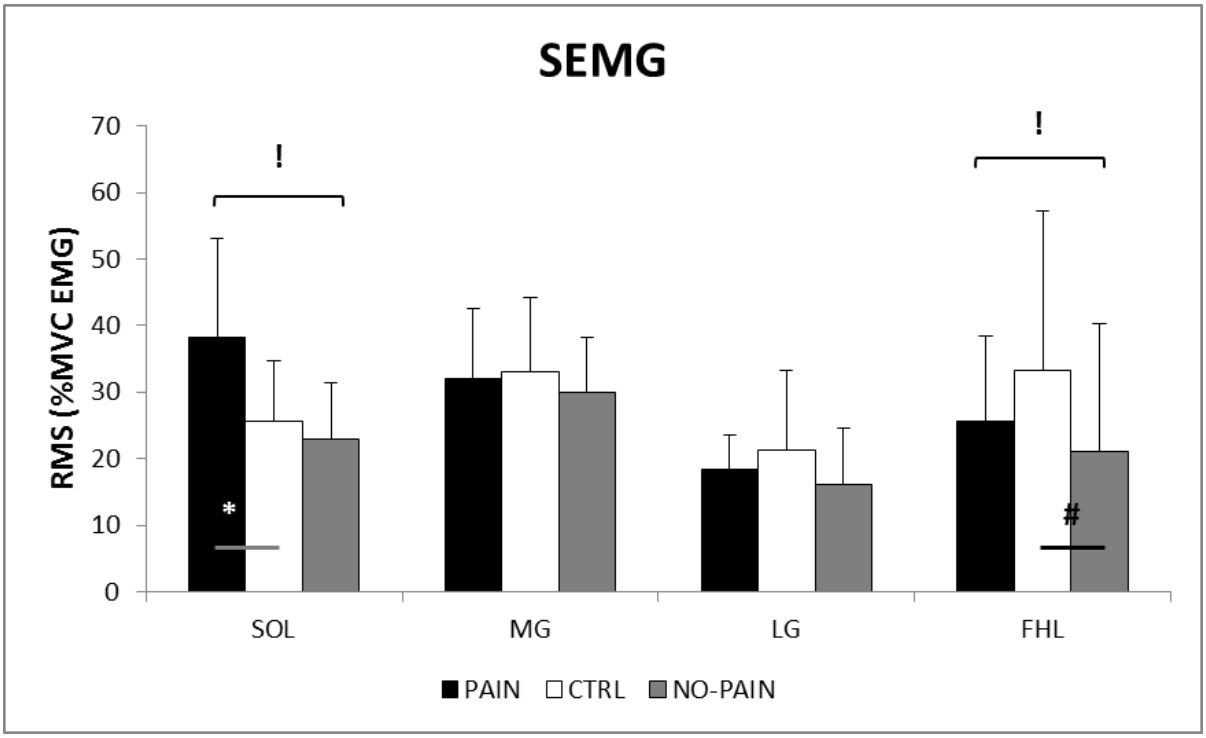
SEMG
RMS (%MVC EMG)
70
60
50
40
30
20
10
0
!
!
*
#
SOL
MG
LG
FHL
PAIN
CTRL
NO-PAIN

Figure 3

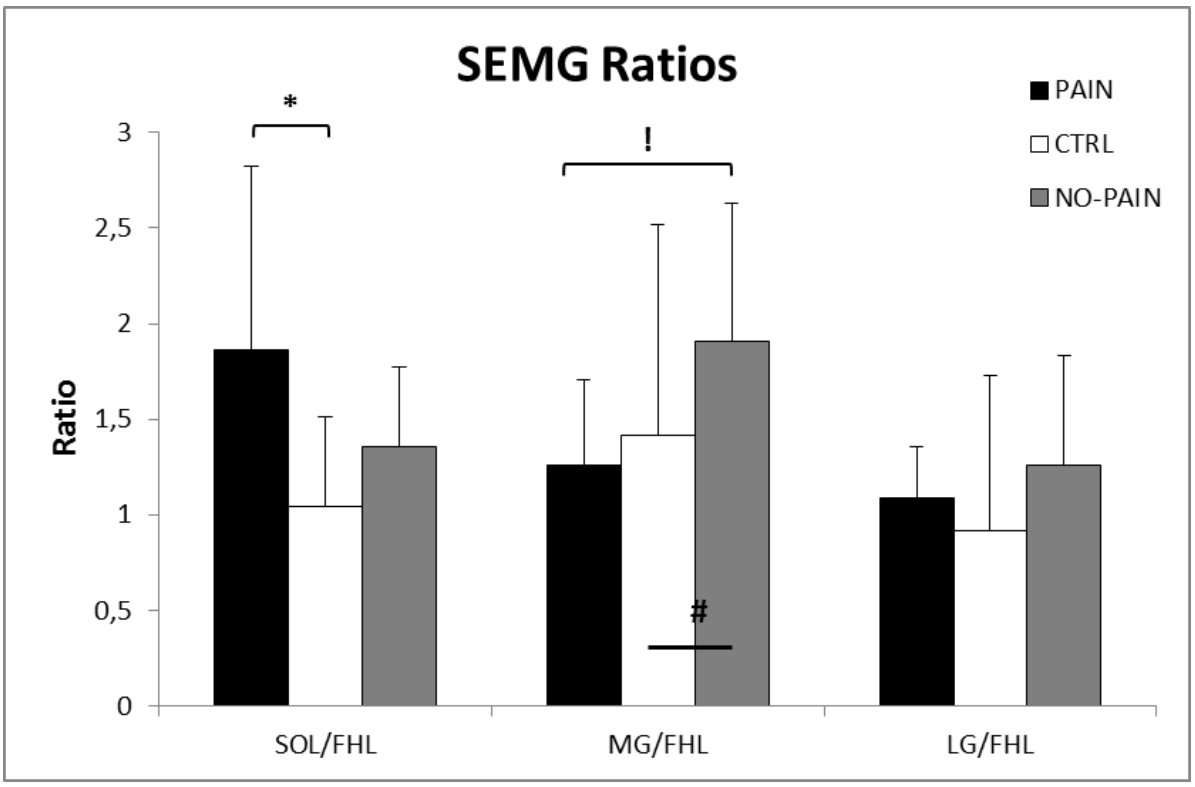
SEMG Ratios
PAIN
CTRL
NO-PAIN
Ratio
3
2,5
2
1,5
1
0,5
0
*
!
#
SOL/FHL
MG/FHL
LG/FHL

Figure 4

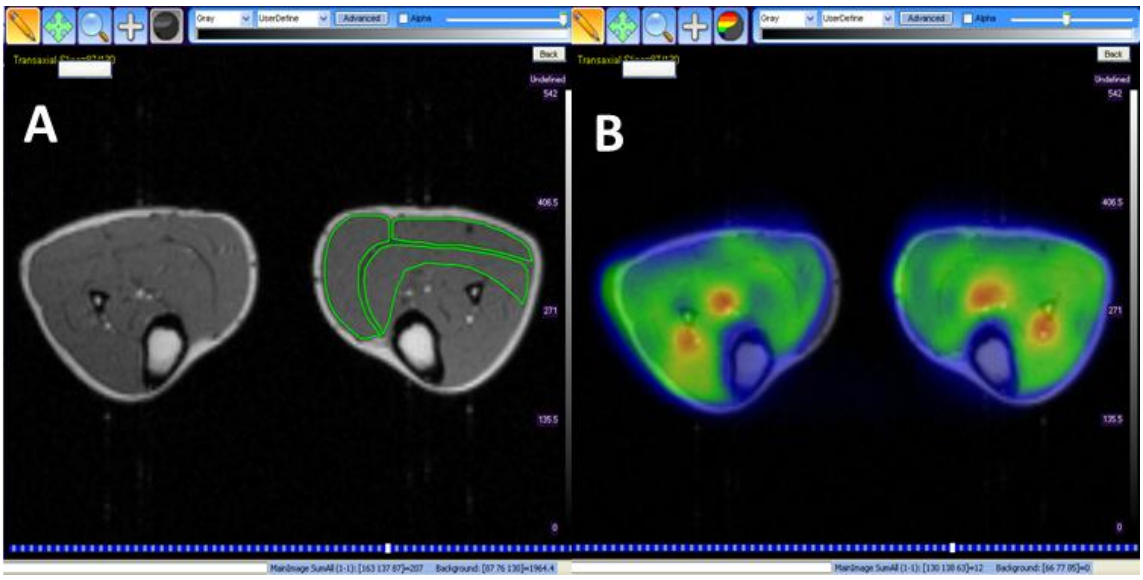

Figure 5

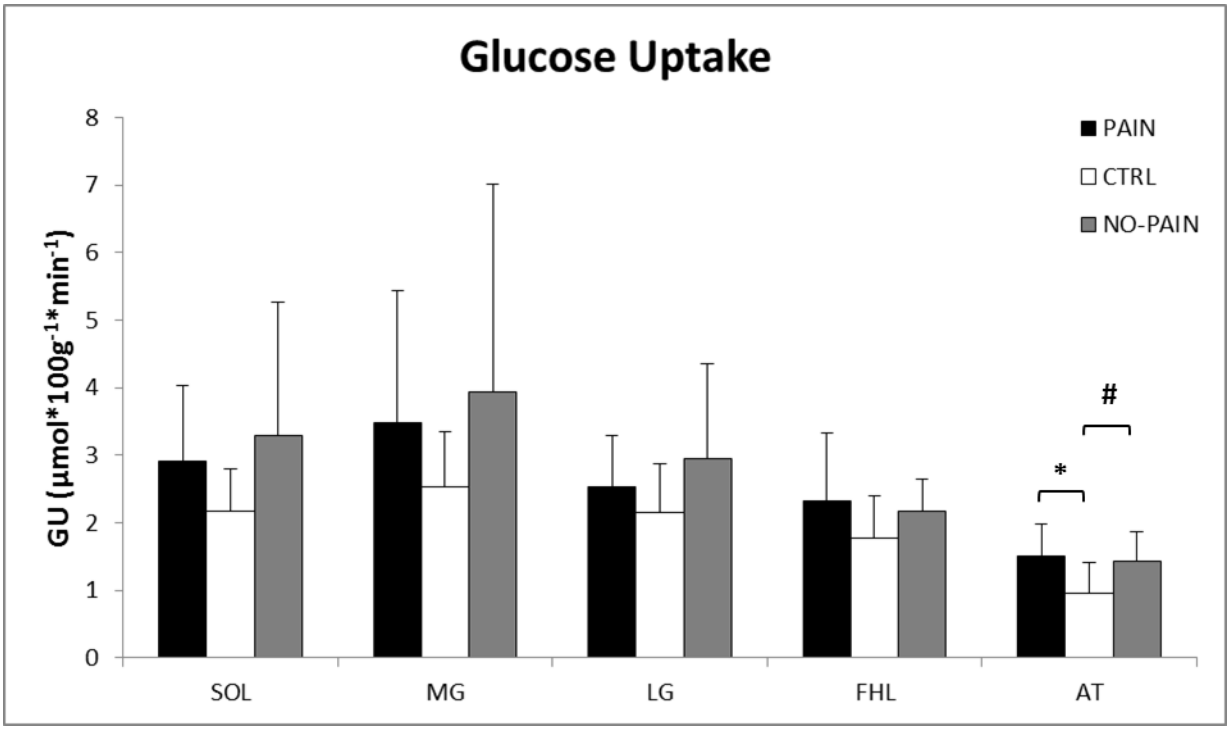
Glucose Uptake
GU (µmol*100g-1*min-1)
0
1
2
3
4
5
6
7
8
PAIN
CTRL
NO-PAIN
*
#
SOL
MG
LG
FHL
AT

Figure 6