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Identification of muscular MRI biomarkers in Amyotrophic Lateral Sclerosis (ALS)

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Part 1: A pilot study assessing T1-weighted muscle MRI in Amyotrophic Lateral Sclerosis (ALS)

Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease usually assumed to target motor neurons (MNs). However, evidence of involvement of other cells types [1-5], also outside the central nervous system, has challenged this "neurocentric" view of ALS, which may thus be defined a "multi-systemic" disease [6]. Although muscle abnormalities in ALS are habitually considered secondary to MN damage [7], muscle, too, can be a primary target [8,9], with "dying back" degeneration of MNs occurring subsequently. In recent years, efforts to clarify the pathogenesis of ALS have focused on muscle tissue [10-14]. Muscle magnetic resonance imaging (MRI), shown to be useful to define prognostic biomarkers of ALS in the G93A-SOD1 mouse model [15], is an established diagnostic tool in inherited and acquired neuromuscular disorders [16,17]. In particular, T2-weighted (T2-w) images show acute pathological processes (e.g. oedema) [18], and T1-weighted (T1-w) sequences disclose chronic alterations (atrophy, fatty substitution) [18]. However, literature data on the role of muscle MRI in ALS are scarce and heterogeneous. Longitudinal studies in small cohorts [19,20] have evaluated, respectively, qualitative T1-w/T2-w images of the legs and volumetric T1-w images of the tongue, hands, and legs. Other authors [21] used T1-w and T2-w MRI to study nerve and muscle abnormalities in the upper and lower limbs of multifocal motor neuropathy (MMN) and ALS patients. Furthermore, brachial plexus and limb-girdle muscle MRI has been shown to play a pivotal role in the differential diagnosis between ALS and inflammatory conditions [22]. Recently, Jenkins et al. investigated the role of T2-w whole-body muscle MRI as a biomarker of denervation in MN disease [23].

In this study we compared T1-w images of hand, paraspinal and lower limb muscles in newly diagnosed ALS patients and in age-matched healthy controls (HCs) to look for evidence of muscular atrophy and remodelling (i.e. fatty substitution) and to relate the radiological findings to clinical and electromyographic (EMG) data.

Materials and Methods

Patients

In this cross-sectional pilot study, we enrolled ten right-handed patients newly diagnosed with probable or definite ALS, using the El Escorial criteria [24], between 1 January 2015 and 31 December 2015 at the C. Mondino National Neurological Institute. Exclusion criteria were inability to give informed consent, a contraindication to MRI, respiratory failure impairing ability to lie flat in the scanner. We scored clinical severity using the ALS Functional Rating Scale revised (ALS-FRSr) [25], and upper MN (UMN) involvement using the UMN burden score [26]. Nine age-matched healthy controls (HCs) were also recruited for MRI analysis. The institute's ethics committee approved the study and all subjects gave their written informed consent.

MRI data analysis

With subjects supine, a 1.5T MRI scanner (Philips Gyroscan, Koninklijke, The Netherlands) was used to obtain sequential axial T1-w images (slice thickness, 10 mm; interslice gap, 1 mm; repetition time (TR), 500 msec; echo time (TE), 15 msec; signal averages,1; voxel size,1,19x0,95x10) from the femoral head to the ankle, in order to study of the thigh and calf muscles. Axial T1-w (slice thickness, 10 mm; interslice gap, 0.3 mm; TR, 500 ms; TE, 8 msec; signal averages,4; voxel size,0.8x1,01x10 mm) and coronal T1-w images (slice thickness,5 mm; interslice gap, 1 mm; TR, 500 msec; TE, 15 msec; signal averages,3; voxel

size, 0.9x1,2x5 mm) were also obtained to study the paravertebral muscles from the intermeante dorsal to the sacral region. Finally, with subjects prone, hand muscles were studied with axial (slice thickness,3 mm; interslice gap, 5 mm; TR, 300 msec; TE, 10 msec; signal averages,4; voxel size, 1.19x1x3 mm) and coronal T1-w sequences (slice thickness,3 mm; interslice gap, 3 mm; TR, 300 msec; TE, 10 msec, signal averages,4; voxel size, 1.19x1x3 mm). Sequence parameters varied between individuals to ensure full anatomical coverage. Total scanning time was usually under 90 minutes.

Scans were examined by expert neuroradiologists, blinded to clinical data. They looked for normal and abnormal muscle bulk (atrophy – yes/no) and normal and abnormal signal intensity. Intrarater and inter-rater reproducibility was first confirmed by the two independent raters, who achieved coefficients of variation <5% for all regions of interest. Each muscle was assessed on T1-w sequences and graded for degree of fatty substitution using the Mercuri scale [27], i.e.:

- Normal: no traces of increased signal intensity in the muscle, graded 0;
- Mild: only traces of increased signal intensity in otherwise well preserved muscle, graded 1;
- Moderate: increased signal intensity in less than 50% of the muscle, graded 2;
- Severe: increased signal intensity in at least 50% of the muscle, graded 3.

EMG data analysis

We performed a semiautomatic quantitative analysis of the motor unit action potentials (MUAPs) in the first dorsal interosseus, thoracic paravertebral, and anterior tibial muscles, using Medelec Synergy SYN5-C (©Viasys Healthcare, Manor Way, Old Woking, Surrey UK). We recorded at least 10 MUAPs per muscle and considered their mean duration (msec).

Mean MUAP duration was considered normal (<10 msec), mild altered (10-12,5 msec), moderate altered (12,5-15 msec), severe altered (>15 msec) for the tibial anterior muscle, and normal (<9 msec), mild altered (9-11,25 msec), moderate altered (11,25-13,5 msec), severe altered (>13,5 msec) for first dorsal interosseus, and thoracic paravertebral muscles.

Statistical analysis

Data are reported as median, and IQR range for quantitative variables and percentages for categorical ones. A UMN burden score of 13 was used to stratify patients by UMN involvement, and clinically considered suitable for distinguish patients with mild, and severe UMN involvement; this cut-off was determined by the range and distribution of values, and following a clinical decision. To compare differences in MRI data among groups, chi square tests and non-parametric Mann-Whitney tests were performed. Spearman correlation coefficient was used to evaluate the association between MRI and ALSFRS-r items. P-values ≤0,05 were considered significant (two-sided). The statistical software STATA V.14 was used for the analysis.

Results

Ten patients (males= 6 and females=4; median age, 67,7 years, IQR 63,1-71,8) with definite (n=3), probable (n=4) or probable laboratory-supported (n=3) ALS, and nine age-matched HCs (males=6 and females=3; median age, 69,6 years, IQR 64,4-73,4) were recruited. Clinical and demographic data are summarized in Table 1.

Lower limb MRI was performed in all patients and HCs, paraspinal MRI in all patients and 8/9 HCs, right-hand MRI in 7/10 patients and 7/9 HCs, and left-hand MRI in 6/10 patients and 6/9 HCs (table 1 - supplementary data). Muscle atrophy was more frequent in patients than HCs (22,46% vs 0,72%, p<0,0001). Median rates of fatty substitution analysing muscles

by region and singly are reported in Table 2. No difference was found between right and left side. There are no statistically significant differences between patients and HCs for hand (p=0,142), thoracic paraspinal (p=0,100), gluteus maximus (p=0,482), anterior thigh (p=0,793), posterior thigh (p=0,664). We found statistically significant differences for iliopsoas (p=0,046), anterior calf (p=0,020), posterior calf (p=0,047). The Figures 1 and 2 show sample MRI scans.

Median rates of fatty substitution between spinal ALS patients and HCs were not significant for hand (p=0,081), thoracic paraspinal (p=0,079), iliopsoas (p=0,117), gluteus maximus (p=0,307), anterior thigh (p=0,462), posterior thigh (p=0,609). Hence, significant differences were found for anterior calf (p=0,009), and posterior calf (p=0,031). All the comparison of median rates of fatty substitution between bulbar ALS patients and HCs were not statistically significant except for iliopsoas ($p<0,05$). Median rates of fatty substitution between spinal and bulbar ALS patients were all not statistically significant. Same results were obtained after stratifying patients for UMN involvement ($p>0,05$, data not shown).

In all patients except one, mean duration of MUAPs (EMG analysis) was calculated for the first dorsal interosseus, thoracic paravertebral, anterior tibial muscles, and in all patients MRC scale (clinical muscles strength) was registered for the first dorsal interosseus and tibial anterior muscles (Table 3). ALS-FRSr items related to hand function, in particularly writing and handling a knife, were summarized and an inverse correlation with dominant hand MRI data was obtained (r= -0,7217).

Table 1. Clinical and demographic data of ALS patients (A, summary) and (B) for each patient.

A)

Data	
Gender, M/F	6/4
Age at onset, median in years (IQR)	66,5 (62-70)
Time from onset to diagnosis, median in months (IQR)	7 (5-11)
UMN burden score, median (IQR)	13 (8-14)

B)

Patient ID	Gender	Age at onset (years)	Site of onset	Type of involvement	UMN score	ALSFRSr
1	M	58	Spinal	UMN+LMN right leg	16	40
2	M	86	Bulbar	UMN+LMN tongue	13	43
3	M	78	Bulbar	UMN+LMN tongue	2	44
4	F	63	Spinal	LMN right leg	13	34
5	M	69	Spinal	LMN arms	14	48
6	M	67	Spinal	LMN left arm	13	44
7	F	68	Spinal	UMN right leg	16	33
8	F	71	Spinal	LMN left leg	1	38
9	M	66	Spinal	LMN arms	8	39
10	F	57	Spinal	LMN right arm	10	40

Abbreviations: M= male; F=female; ALS-FRSr= Amyotrophic Lateral Sclerosis Functional Rating Scale revised; UMN= upper motor neuron; LMN= lower motor neuron

Table 2. MRI data (degree of fatty substitution using the Mercuri scale) for patients and HCs.

Muscle *Meann (IQR)*	**Patients (n=10)**	**HCs (n=9)**	**P value**
Right hand	0 (0-0,5)	0 (0-0)	0,142
Right paraspinals	1 (1-2)	1 (0,5-0)	0,100
Right iliopsoas	1 (0-1)	0 (0-0)	0,046
Right gluteus maximus	1 (1-2)	1 (1-2)	0,482
Right anterior thigh	0,88 (0,5-1)	1 (0-1)	0,793
Right posterior thigh	1 (0,86-1)	1 (0-1)	0,664
Right anterior calf	0,875 (0,5-1)	0 (0-0,5)	0,020
Right posterior calf	1 (1-1,3)	0,67 (0,33-1)	0,047
Left hand	0 (0-0,5)	0 (0-0)	0,142
Left paraspinals	1 (1-2)	1 (0,5-1)	0,100
Left iliopsoas	1 (0-1)	0 (0-0)	0,046
Left gluteus maximus	1 (1-2)	1 (1-2)	0,482
Left anterior thigh	0,88 (0,5-1)	1 (0-1)	0,793
Left posterior thigh	1 (0,86-1)	1 (0-1)	0,664
Left anterior calf	0,875 (0,5-1)	0 (0,5-1)	0,020
Left posterior calf	1 (1-1,3)	0,67 (0,33-1)	0,047

Table 3. EMG (a) and clinical (b) data for patients.

a)

Patients (mean duration, msec)	**Right tibial anterior**	**Left tibial anterior**	**Right thoracic paraspinal**	**Left thoracic paraspinal**	**Right first dorsal interosseus**	**Left first dorsal interosseus**
1 (58)	Not done	15,02 (S)	12,8 (Mo)	Not done	9,95 (Mi)	Not done
2 (86)	11,93 (Mi)	15,84 (S)	10,52 (Mi)	11,72 (Mo)	7,73 (N)	13,44 (Mo)
3 (78)	13,75 (Mo)	13,93 (Mo)	10,61 (Mi)	13,15 (Mo)	12,55 (Mo)	12,3 (Mo)
4 (63)	12,37 (Mi)	Not done	15,04 (S)	Not done	10,56 (Mi)	Not done
5 (69)	14,5 (Mo)	Not done	Not done	12,94 (Mo)	15,8 (S)	Not done
7 (68)	>20 (S)	>20 (S)	15,57 (S)	14,04 (S)	10,7 (Mi)	11,06 (Mi)
8 (71)	14,63 (Mo)	11,95 (Mi)	15,8 (S)	16,01 (S)	10,68 (Mi)	12,57 (Mo)
9 (66)	15,89 (S)	12,05 (Mi)	13,67 (S)	16,47 (S)	13,89 (S)	>20 (S)
10 (57)	12,1 (Mi)	11,75 (Mi)	8,98 (N)	9,12 (Mi)	20,19 (S)	12,26 (Mo)

Legends: N = normal, Mi = mild altered, Mo = moderate altered, S = severe altered

b)

Patients (MRC)	**Right tibial anterior**	**Left tibial anterior**	**Right first dorsal interosseus**	**Left first dorsal interosseus**
1 (58)	3	5	3	4
2 (86)	5	5	5	5
3 (78)	5	5	5	5
4 (63)	5	5	5	5
5 (69)	5	5	5	3
6 (67)	3	4,5	5	5
7 (68)	0	3,5	5	5
8 (71)	4,5	3,5	3,5	3,5
9 (66)	5	5	3	3,5
10 (57)	5	5	3	3,5

Figure 1. MRI scans of healthy controls (a, c, e) and patients (b – patient #6, d – patient #9, f – patient #8): left hand axial T1-w (a, b – patient#6), paraspinal axial T1-w (c, d – patient #9), paraspinal coronal T1-w (e, f – patient #8).

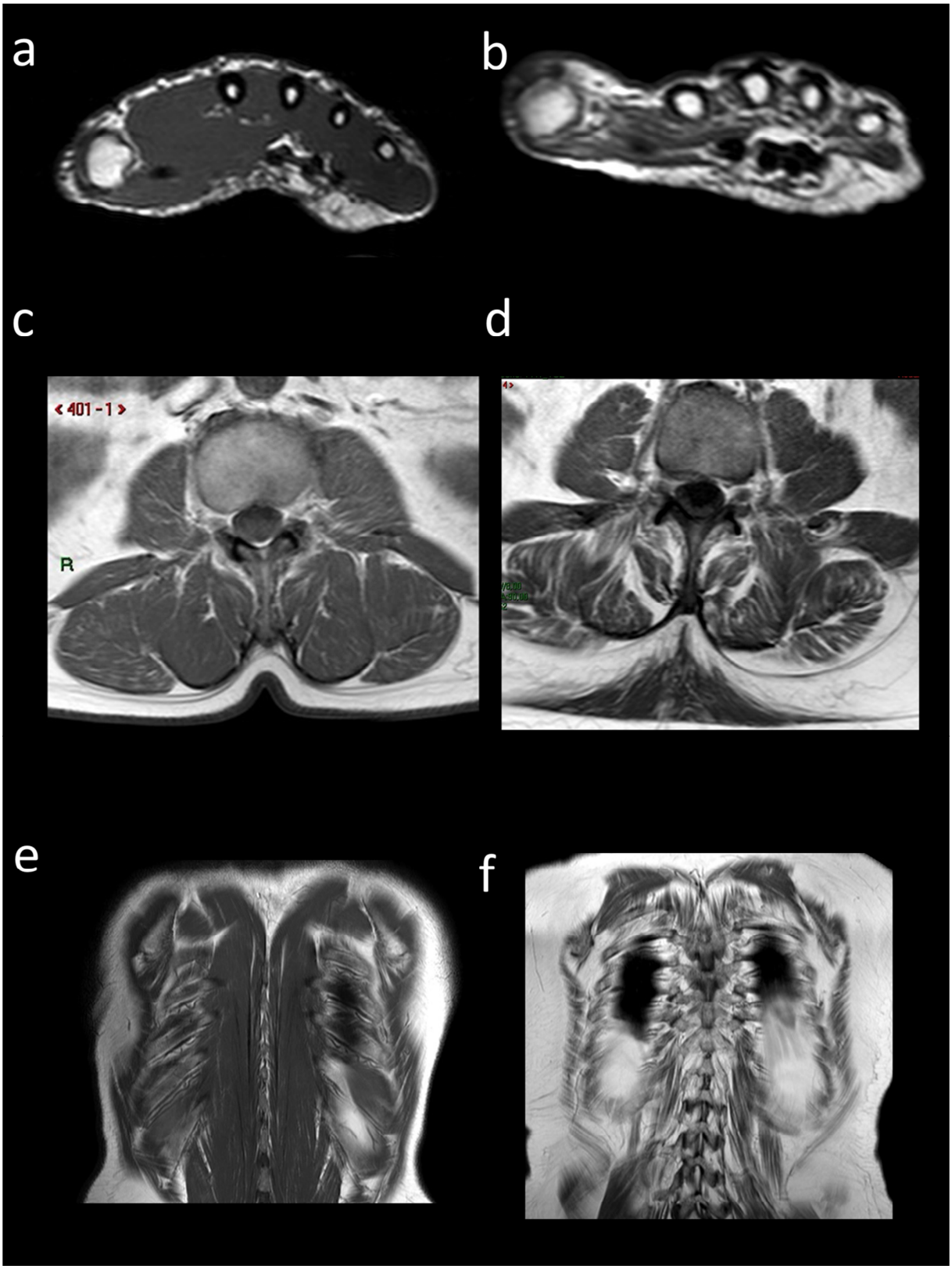
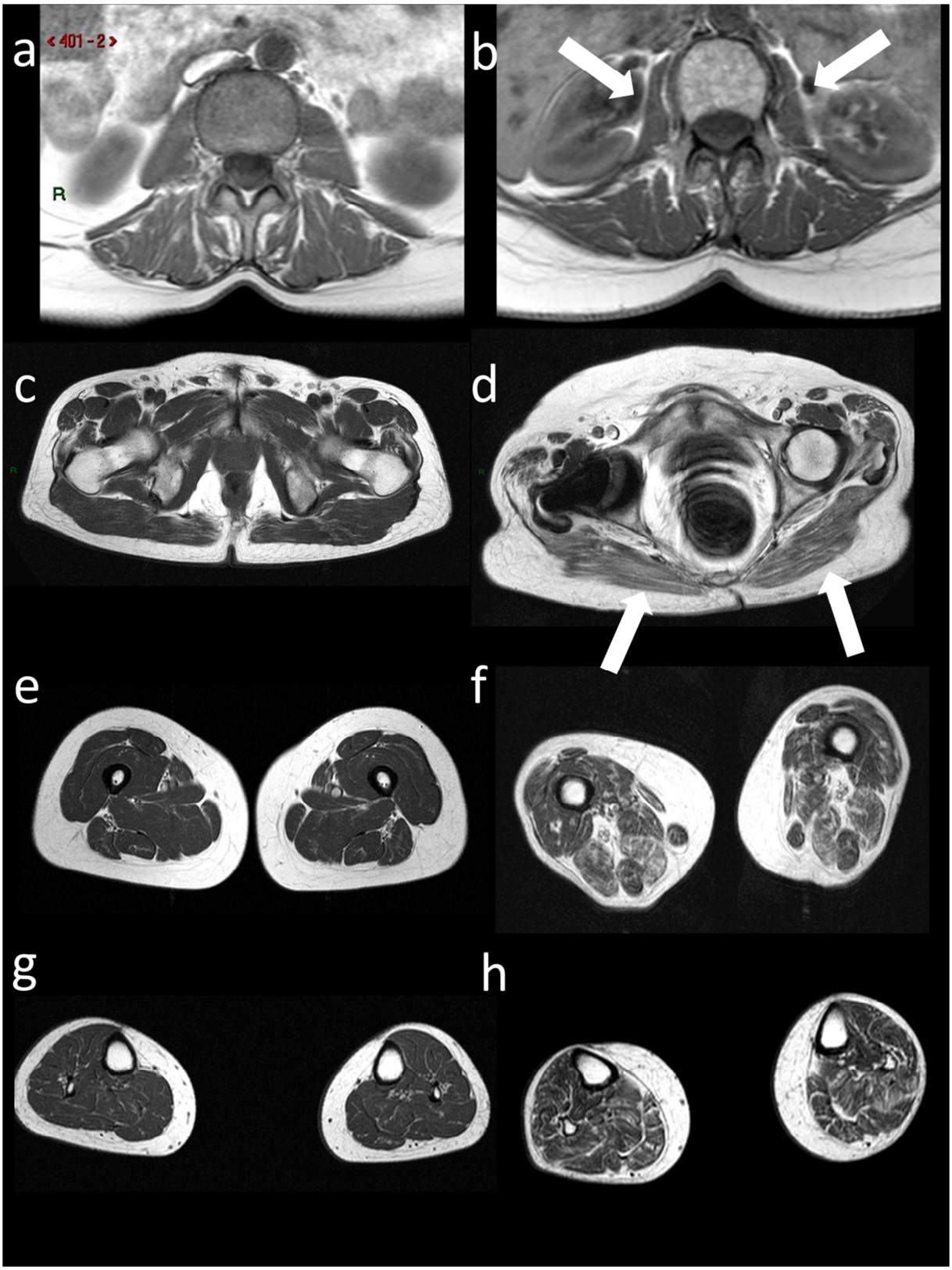


Figure 2. MRI scans of healthy controls (a, c, e, g) and patients (b – patient #10, d, f, h – patient #8): iliopsoas axial T1-w (a, b – patient #10), gluteus maximus axial T1-w (c, d – patient #8), thigh axial T1-w (e, f – patient #8), calf axial T1-w (g, h – patient #8).



Discussion and conclusions

Literature on muscle MRI in ALS is scarce and heterogeneous in terms of study type (longitudinal [19,20,23], cross-sectional [14,15]), region scanned (upper limbs [20-22], lower limbs [19-22], tongue [20], whole body [23]), type of images obtained (volumetric [19,20,22], qualitative T1-w/T2-w [19,22], quantitative T1-w/T2-w [19,21,22]), and use of controls (age-matched HCs [22], no age-matched HCs [19,20], other neurological controls [21]). To date, authors have mainly performed lower limb MRI with T1-w sequences. Bryan et al. [19] used T1-w and T2-w sequences to assess leg muscles over a longitudinal 4-month follow-up in 11 ALS patients and eight HCs. Visual inspection revealed abnormalities ("moth-eaten" appearance) on T1-w images in six patients, but mean muscle T1 time and volume were not different between patients and HCs. Instead, the ALS patients showed increased muscle T2 time, evaluated using quantitative analysis. In a longitudinal study, Jenkins et al. [20] evaluated possible atrophy of the thenar eminence, first dorsal interosseous, tibialis anterior and tongue using volumetric muscle 3T MRI in four ALS patients and 11 HCs. Progressive atrophy was found only in the thenar eminence and tibial anterior, and only in patients showing clinical progression. No data on subclinical atrophy were given. Clinical-radiological discordance in other muscles was suggested to reflect a contribution of UMN pathology. More recently, Staff et al. [21] studied the role of nerve and muscle 3T/1.5T MRI with T1-w and T2-w sequences in differential diagnosis between ALS and MMN. They showed upper and lower limb muscle MRI alterations in 57% of ALS patients (n=60), specifically atrophy in 35%, while MMN patients had no atrophy. Gerevini et al. [22] suggested that muscle and nerve root/plexus MRI might aid differential diagnosis between ALS and inflammatory neuropathies. They showed T1-w and T2-w abnormalities in the subscapular, supra- and infraspinatus muscles of all ALS patients (n=23). In particular, the supraspinatus was the most affected by T2-w alterations, while no difference in fatty

substitution or atrophy was found. Very recently, Jenkins et al. [23] proposed a very fast protocol for T2-w whole-body muscle MRI in order to longitudinally study the denervation in motor neuron disease. They did not focus on chronic muscle alterations that are pivotal in neurodegenerative disorders.

In our explorative study, we qualitatively assessed hand, paraspinal and lower limb muscles with T1-w MRI in 10 newly-diagnosed ALS patients and nine age-matched HCs. We selected that muscles because they belong to different regions (cervical, thoracic, lumbosacral) that are habitually evaluate in ALS patients in order to stage the disease using the El Escorial classification [24]. We didn't perform MRI for all subjects because the requested position was extremely uncomfortable during the exam (i.e. prone position for hands). We detected statistically significant differences between the two groups for iliopsoas (p=0,046), anterior calf (p=0,020), and posterior calf (p=0,047). The small range of values is due to the selection of patients with new diagnosis of ALS. No specific pattern of muscle involvement was found, as well as no asymmetry was detected. However, single muscle difference between left and right side was evident but it not emerged from the statistical analysis, probably due to a low sample size. We demonstrated that UMN damage is independent of muscle abnormalities, as no difference emerged on stratifying patients for UMN burden score.

Muscle analysis considering spinal ALS patients and HCs confirmed differences for anterior calf (p=0,009), and posterior calf (p=0,031). Furthermore, there is significant result for iliopsoas (p<0,05) between bulbar ALS patients and HCs. No difference emerged between bulbar and spinal ALS patients. In this subgroup analysis the disequilibrium of bulbar (n=2) and spinal (n=8) patients is pivotal to consider for the results, and it is necessary to increase the power size and improve the balance of recruitment to confirm these data. At the moment, it is possible to affirm that our spinal ALS patients often present distal lower limbs clinical

onset (i.e. Patrikios form). Moreover, iliopsoas, that is a spinal muscle, could be studied to support the diagnosis in bulbar patients.

Concerning the comparison of results between EMG and MRI, it is possible to underline a trend towards agreement was appreciable for first dorsal interosseus, paraspinal, and tibial anterior muscles. The level of muscle alterations is different between EMG and MRI, and the discrepancy was likely due to the greater sensitivity of EMG for the quantification of muscle damage is evident. Indeed, many moderate and severe alterations were identified using the EMG grading in muscles characterized by low grade of fatty substitution in MRI. MRI could, instead, be more useful for studying deeper muscles that are difficult to assess using EMG. Further data should be obtained increasing the sample size.

Finally, a trend towards agreement can be also appreciated for clinical aspects (MRC for first dorsal interosseus and tibial anterior muscles) and MRI. A concordance emerged between clinical and MRI data for the dominant hand. Indeed, low scores for specific ALSFRS-r items correspond to high level of MRI alterations. This region was tested because muscles assessed by MRI are selectively involved in writing and using a knife (both ALS-FRSr items).

Limitations of our explorative study included the low number of patients recruited, which reflects the rarity of ALS. To be able to collect significant data, and also take into account the clinical heterogeneity of ALS onset, we decided to assess numerous muscles in each patient. The full protocol included the evaluation of 68 muscles belonging to the cervical, thoracic and lumbar segments. We excluded bulbar muscles because the relevant literature data are not homogeneous [28], mainly for technical reasons. Finally, we could not perform quantitative assessment of muscle T1 time or volume because quantitative sequences require greater technical resources, and time-consuming post-acquisition processing [29]. The main technical problems we encountered were related to the coil at our disposal and to hand MRI: the prone

position was poorly tolerated both by patients and controls. For this reason, and also because since EMG data are sufficient to highlight abnormalities, we decided not to further analyse this region. In future studies we will consider to acquire more MRI data on specific regions, where is possible to provide significant data but poorly amenable to clinical and EMG assessment (e.g. the paraspinal muscles).

In conclusion, muscle T1-w MRI can distinguish ALS patients from HCs for specific regions (i.e. legs). MRI abnormalities could be found in pauci-symptomatic spinal muscles in bulbar-onset patients (i.e. iliopsoas). Paraspinal and leg muscles MRI may be a useful diagnostic tool in early ALS. From these preliminary results, we plan to conduct a longitudinal study in order to investigate more in deep the role of muscle through MRI measurements as a prognostic or predictive biomarker in ALS patients.

SUPPLEMENTARY MATERIALS

T1–weighted muscle MRI findings

A relative degree of severity was assigned to each muscle in each region, using the scoring system proposed by Mercuri: 0 = normal, 1 = mild, 2 = moderate, 3 = severe (see Methods section).
a atrophy, NA not available.
Hand: FDI first dorsal interosseus muscle, SDI second dorsal interosseus muscle, TDI third dorsal interosseus muscle, FoDI fourth dorsal interosseus muscle, ADM abductor digiti minimi muscle, FPI first palmar interosseus muscle, SPI second palmar interosseus muscle, TPI third palmar interosseus muscle, FDB flexor digitorum brevis muscle, TE thenar eminence
Paraspinal/pelvis: IP iliopsoas muscle, LD longissimus dorsi muscle, MF multifidus muscle, QL quadratus lumborum muscle, LaD latissimus dorsi muscle, GMa gluteus maximus muscle.
Thigh: anterior - VL vastus lateralis muscle, VI vastus intermedius muscle, VM vastus meanlis muscle, RF rectus femoris muscle; posterior - SA Sartorius muscle, GR gracilis muscle, AM adductor magnus muscle, BL biceps femoris long head muscle, ST semitendinosus muscle, SM semimembranosus muscle, AL adductor longus muscle.
Leg: anterior - TA tibialis anterior muscle, TP tibialis posterior muscle, PL peroneus longus muscle, EDL extensor digitorum longus; posterior - SO soleus muscle, GM meanl gastrocnemius muscle, GL lateral gastrocnemius muscle.

Patient ID (years)	**Right hand**										**Right paraspinal/pelvis**					
	FDI	**SDI**	**TDI**	**FoDI**	**ADM**	**FPI**	**SPI**	**TPI**	**FDB**	**TE**	**IP**	**LD**	**MF**	**QL**	**LaD**	**GMa**
1 (58)	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
2 (86)	0	0	0	0	0	0	0	0	0	0	1 a	1	1	1	1	1
3 (78)	0	0	0	0	0	0	0	0	0	0	1 a	1	1	1	1	1
4 (63)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
5 (69)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
6 (67)	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1
7 (68)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	2	2	2	2	2
8 (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1 a	2	2	2	2	3
9 (66)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1 a	2	2	2	2	3
10 (57)	2 a	2 a	2 a	2 a	2 a	2 a	2 a	2 a	2 a	1 a	0 a	1	1	1	1	1

Patient ID (years)	**Right thigh**											**Right leg**						
	VL	**VI**	**VM**	**RF**	**SA**	**GR**	**AM**	**BL**	**ST**	**SM**	**AL**	**TA**	**TP**	**PL**	**EDL**	**SO**	**GM**	**GL**
1 (58)	1	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1
2 (86)	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	1	0	0
3 (78)	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1	2	1	1
4 (63)	1 a	1 a	1 a	0 a	0 a	0 a	1 a	1 a	1 a	1 a	0 a	1 a	1 a	1 a	1 a	2 a	1 a	1 a
5 (69)	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
6 (67)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7 (68)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8 (71)	2 a	1 a	1 a	1 a	2 a	1 a	1 a	2 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	2 a	1 a	1 a
9 (66)	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	2 a	2 a	2 a
10 (57)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Patient ID (years)	Left hand										Left paraspinal/pelvis					
	FDI	SDI	TDI	FoDI	ADM	FPI	SPI	TPI	FDB	TE	IP	LD	MF	QL	LaD	GMa
1 (58)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	1	1	1	1	2
2 (86)	0	0	0	0	0	0	0	0	0	0	1 a	1	1	1	1	1
3 (78)	0	0	0	0	0	0	0	0	0	0	1 a	1	1	1	1	1
4 (63)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
5 (69)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
6 (67)	2 a	2 a	2 a	2 a	2 a	2 a	2 a	2 a	2 a	2 a	1	1	1	1	1	1
7 (68)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	2	2	2	2	2
8 (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1 a	2	2	2	2	3
9 (66)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1 a	2	2	2	2	3
10 (57)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1

Patient ID (years)	Left thigh											Left leg						
	VL	VI	VM	RF	SA	GR	AM	BL	ST	SM	AL	TA	TP	PL	EDL	SO	GM	GL
1 (58)	1	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1
2 (86)	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	3	2	2
3 (78)	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1	2	1	1
4 (63)	1 a	1 a	1 a	0 a	0 a	0 a	1 a	1 a	1 a	1 a	0 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a
5 (69)	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
6 (67)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7 (68)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8 (71)	2 a	1 a	1 a	1 a	2 a	1 a	1 a	2 a	1 a	3 a	1 a	1 a	1 a	1 a	1 a	2 a	1 a	1 a
9 (66)	1a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	1 a	2 a	2 a	2 a
10 (57)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Control ID (years)	Right hand										Right paraspinal/pelvis					
	FDI	SDI	TDI	FoDI	ADM	FPI	SPI	TPI	FDB	TE	IP	LD	MF	QL	LaD	GMa
1 (64)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
2 (71)	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
3 (64)	0	0	0	0	0	0	0	0	0	0	0a	2	2	1	1	1
4 (68)	0	0	0	0	0	0	0	0	0	0	0a	1	1	1	1	2
5 (74)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2
6 (69)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	1	1	1	1	0
7 (73)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8 (73)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2
9 (59)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Control ID (years)	Right thigh											Right leg						
	VL	VI	VM	RF	SA	GR	AM	BL	ST	SM	AL	TA	TP	PL	EDL	SO	GM	GL
1 (64)	1	1	1	1	1	1	1	1	2	1	1	0	0	1	0	1	1	0
2 (71)	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0
3 (64)	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1
4 (68)	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1
5 (74)	1	1	1	1	2	1	1	1	1	1	1	0	1	1	1	1	1	1
6 (69)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7 (73)	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	1	0
8 (73)	0	1	1	1	1	0	1	1	1	1	1	0	0	0	0	1	0	0
9 (59)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Control ID (years)	Left hand										Left paraspinal/pelvis					
	FDI	SDI	TDI	FoDI	ADM	FPI	SPI	TPI	FDB	TE	IP	LD	MF	QL	LaD	GMa
1 (64)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
2 (71)	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
3 (64)	0	0	0	0	0	0	0	0	0	0	0 a	2	2	1	1	1
4 (68)	0	0	0	0	0	0	0	0	0	0	0 a	1	1	1	1	2
5 (74)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2
6 (69)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	1	1	1	1	0
7 (73)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	1
8 (73)	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2
9 (59)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Control ID (years)	Left thigh											Left leg						
	VL	VI	VM	RF	SA	GR	AM	BL	ST	SM	AL	TA	TP	PL	EDL	SO	GM	GL
1 (64)	1	1	1	1	1	1	1	1	2	1	1	0	0	1	0	1	1	0
2 (71)	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0
3 (64)	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1
4 (68)	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1
5 (74)	1	1	1	1	2	1	1	1	1	1	1	0	1	1	1	1	1	1
6 (69)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7 (73)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
8 (73)	1	1	1	0	1	0	1	1	1	1	1	0	0	0	0	1	0	0
9 (59)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Part 2: MRI study of paraspinal muscles in patients with Amyotrophic Lateral Sclerosis (ALS)

Introduction

The paraspinal muscles are essential for the stabilization of the spine, the conservation of the correct posture and the fluency of the trunk movements [30]. Particularly, the multifidus muscle is medial and pivotal for the spinal balance, while erector spinae muscle, composed of longissimus dorsi and iliocostal, is lateral and involved in the other functions [30]. Paraspinal muscles' features habitually vary according to different variables (i.e. sex, body weight, physical activity) [30], but their evaluation can be helpful for the diagnosis of pathological conditions. Clinical evaluation globally helps for the classification of specific pattern (i.e. Pisa syndrome, camptocormia), while the electromyographic study is useful for superficial muscles at thoracic or lumbar level [31,32]. The analysis of single and profound muscles is not possible using those instruments. In the last years, MRI has become crucial for the study of paraspinal muscles in heterogeneous pathologies (i.e. inflammatory and congenital myopathies, radiculopathies) [33]. The evaluation of paravertebral muscles is essential during the diagnostic phase and follow up of patients affected by ALS, in order to demonstrate the involvement of thoracic domain and define the grade of disease [24]. In this context, the most useful diagnostic tool is electromyography, with the limitations cited above. Few data are available on muscle MRI of the paraspinal muscles in ALS [23-34] and are heterogeneous in terms of study typology, MRI scans, and results. Jenkins et al. demonstrated statistically significant difference of T2-w images of paraspinal muscles between healthy controls (n=22) and ALS patients (n=29) at baseline, while no difference during the longitudinal follow up (4 months) in ALS cohort [23]. In a recent pilot cross-sectional study our group evidenced no

difference between T1-w paraspinal muscles images of ALS patients (n=10) and healthy controls (n=9) [34].

To study the role of paraspinal MRI in the diagnosis of ALS, we compared T1-w images in newly diagnosed ALS patients, age-matched healthy controls, and patients affected by inflammatory myopathy and lumbar radiculopathy.

Materials and Methods

Patients

In this cross-sectional study, we enrolled fourteen patients newly diagnosed with probable or definite ALS, using the El Escorial criteria [4], between 1 January 2016 and 31 December 2018 at the C. Mondino National Neurological Institute. Exclusion criteria were inability to give informed consent, a contraindication to MRI, respiratory failure impairing ability to lie flat in the scanner. We scored clinical severity using the ALS Functional Rating Scale revised (ALS-FRSr) [7]. We also recruited for MRI analysis: - age-matched healthy controls (HCs); - patients with inflammatory myopathy; - patients affected by lumbar radiculopathy. The institute's ethics committee approved the study and all subjects gave their written informed consent.

MRI data analysis

With subjects supine, a 1.5T MRI scanner (Philips Gyroscan, Koninklijke, The Netherlands) was used to obtain sequential axial T1-w (slice thickness, 10 mm; interslice gap, 0.3 mm; TR, 500 ms; TE, 8 msec; signal averages,4; voxel size,0.8x1,01x10 mm) and coronal T1-w images (slice thickness,5 mm; interslice gap, 1 mm; TR, 500 msec; TE, 15 msec; signal averages,3; voxel size, 0.9x1,2x5 mm) of the paravertebral muscles from the intermediate

dorsal to the sacral region. Sequence parameters varied between individuals to ensure full anatomical coverage. Total scanning time was usually under 30 minutes.

Scans were examined by expert neuroradiologists, blinded to clinical data. They looked for normal and abnormal signal intensity. Each muscle was assessed on T1-w sequences and graded for degree of fatty substitution using the Mercuri scale [8], i.e.:

- Stage 0: normal appearance;
- Stage 1: early moth-eaten appearance, with scattered small areas of increased density on the T1 MR sequence;
- Stage 2a: late moth-eaten appearance, with numerous discrete areas of increased density (MRI) with beginning confluence, comprising less than 30% of the volume of the individual muscle;
- Stage 2b: late moth-eaten appearance, with numerous discrete areas of increased density (MRI) with beginning confluence, comprising 30–60% of the volume of the individual muscle;
- Stage 3: washed-out appearance, fuzzy appearance due to confluent areas of increased density (MRI) with muscle still present at the periphery;
- Stage 4: end-stage appearance, muscle replaced by increased density (MRI) connective tissue and fat, with only a rim of fascia and neurovascular structures distinguishable.

Statistical analysis

Data are reported as mean, and range for quantitative variables and percentages for categorical ones. To compare differences in MRI data among groups, chi square tests and non-parametric Kruskall-Wallis tests were performed. To test intra and inter-rater

reproducibility Cohen's Kappa was performed. The statistical software STATA V.14 was used for the analysis.

Results

Fourteen patients (males=7 and females=7; mean age, 61,3 years, range 29-79) with definite (n=2), or probable (n=12) ALS, and eleven age-matched HCs (males=8 and females=3; mean age, 66,3 years, range 48-76) were recruited. Ten patients (males=5 and females=5; mean age 46 years, range 26-62) with inflammatory myopathy, and nineteen (males=13 and females=6; mean age 61,4, range 44-75) with lumbar radiculopathy were also recruited as pathological controls. Clinical and demographic data are summarized in Table 1.

Table 1. Clinical and demographic data of ALS patients (A, summary) and (B) for each patient.

A)

Data	
Gender, M/F	7/7
Age at onset, mean in years (range)	61,3 (29-79)
Time from onset to diagnosis, mean in months (range)	12 (3-26)
ALSFRSr at MRI, mean (range)	41,2 (33-47)

B)

Patient ID	Gender	Age at onset (years)	Site of onset	ALSFRSr at MRI
1	M	29	Spinal	47
2	F	52	Spinal	45
3	F	39	Spinal	38
4	M	74	Spinal	44
5	F	65	Bulbar	40
6	M	64	Spinal	33
7	F	55	Spinal	39
8	F	76	Bulbar	42
9	M	73	Bulbar	44
10	F	64	Spinal	41
11	M	56	Spinal	32
12	M	69	Spinal	46
13	F	63	Bulbar	44
14	M	79	Spinal	42

Paraspinal MRI was performed in all patients and controls, and each muscle was graded using the Mercuri scale (Table 2). Intrarater and inter-rater reproducibility was first confirmed by the two independent raters, who achieved coefficients of variation <5% for all regions of interest. To test intra and inter-rater reproducibility Cohen's Kappa was performed for each muscle: multifidus ($k=0,935$; $p<0,05$), longissimus dorsi ($k=0,919$; $p<0,05$), iliocostal ($k=0,963$; $p<0,05$), iliopsoas ($k=0,957$; $p<0,05$).

Table 2. T1–weighted muscle MRI findings. A relative degree of severity was assigned to each muscle in each region, using the scoring system proposed by Mercuri (see Methods section).

Patient ID (years)	**Paraspinal/pelvis**			
	MF	**LD**	**IC**	**IP**
1 (29)	0	0	0	0
2 (52)	1	0	0	0
3 (39)	2a	1	1	0
4 (74)	1	1	2a	0
5 (65)	2a	2a	2a	0
6 (64)	0	0	0	0
7 (55)	2a	1	1	0
8 (76)	2b	2b	2b	0
9 (73)	2a	2a	2a	1
10 (64)	2a	1	2a	0
11 (56)	2a	1	2a	0
12 (69)	2a	1	1	0
13 (63)	2a	1	1	0
14 (79)	1	1	1	0

HC ID (years)	**Paraspinal/pelvis**			
	MF	**LD**	**IC**	**IP**
1 (56)	1	1	1	0
2 (69)	1	1	1	0
3 (72)	2a	2a	2a	0
4 (67)	1	1	1	0
5 (48)	1	2a	2a	0
6 (76)	1	1	1	0
7 (75)	1	1	2a	1
8 (61)	1	1	1	0
9 (76)	2a	2a	2a	1
10 (63)	2a	1	2a	0
11 (67)	2a	2a	2a	1

IM ID (years)	Paraspinal/pelvis			
	MF	LD	IC	IP
1 (29)	0	0	0	0
2 (62)	1	1	1	0
3 (60)	2b	1	2a	1
4 (24)	1	1	1	0
5 (49)	2a	2a	2a	1
6 (39)	2a	1	2a	0
7 (42)	2a	2a	2a	0
8 (49)	1	1	2a	0
9 (53)	3	3	3	0
10 (53)	2a	2a	2a	0

LR ID (years)	Paraspinal/pelvis			
	MF	LD	IC	IP
1 (57)	2a	1	2a	0
2 (51)	1	0	1	0
3 (44)	2a	1	1	0
4 (63)	2a	2a	2a	1
5 (71)	2a	2a	2a	1
6 (66)	2b	2a	2a	1
7 (63)	2a	1	1	0
8 (64)	2b	2a	2a	1
9 (68)	1	1	2a	1
10 (57)	1	1	1	0
11 (64)	1	1	2a	0
12 (51)	1	1	2a	0
13 (60)	1	1	2a	0
14 (70)	2b	2a	2b	1
15 (75)	2b	2b	2b	1
16 (64)	2a	1	1	1
17 (67)	2b	2a	2a	0
18 (62)	1	1	2a	0
19 (50)	2a	1	2a	1

HC=healthy control, IM=inflammatory myopathy, LR=lumbar radiculopathy

Paraspinal/pelvis: MF multifidus muscle, LD longissimus dorsi muscle, IC iliocostal muscle, IP iliopsoas muscle.

No difference was found between right and left side. There were no statistically significant differences between groups for multifidus (p=0,1646), longissimus dorsi (p=0,8958), iliocostal (p=0,376), while there was a statistically significant difference for iliopsoas (p=0,003) between ALS patients and patients with radiculopathy, where the latter group had more serious involvement. After stratification of ALS patients for spinal and bulbar onset, we

considered five groups, and we found statistically significant differences between bulbar ALS and spinal ALS for longissimus dorsi (p=0,0216), while no difference between groups for multifidus (p=0,1441), iliocostal (p=0,06553), and iliopsoas (p=0,08132). The Figures 1 and 2 show MRI scans.

Figure 1. MRI scans of a patient: paraspinal axial T1-w.

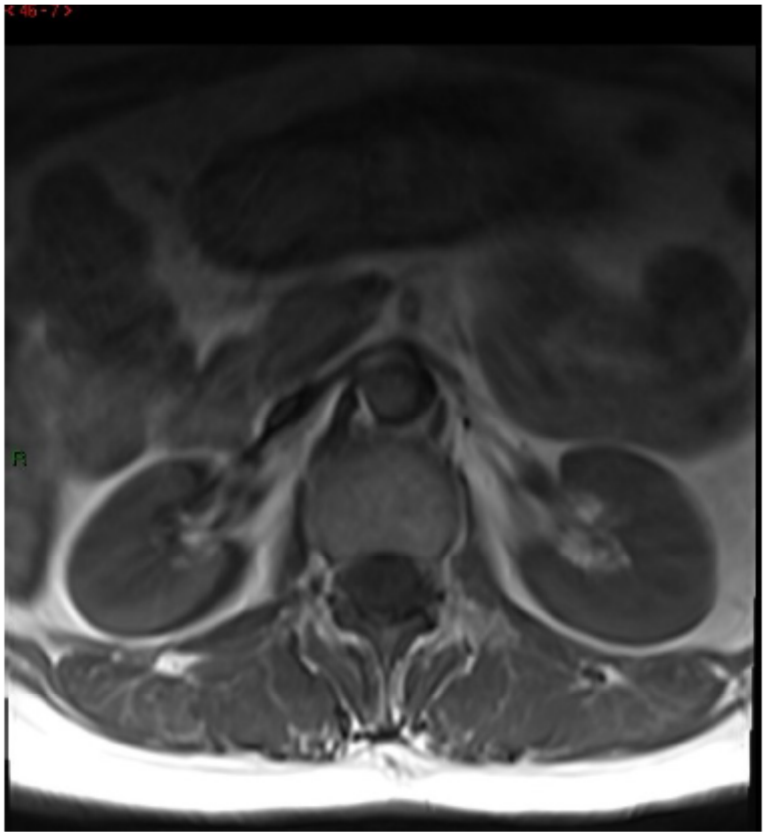
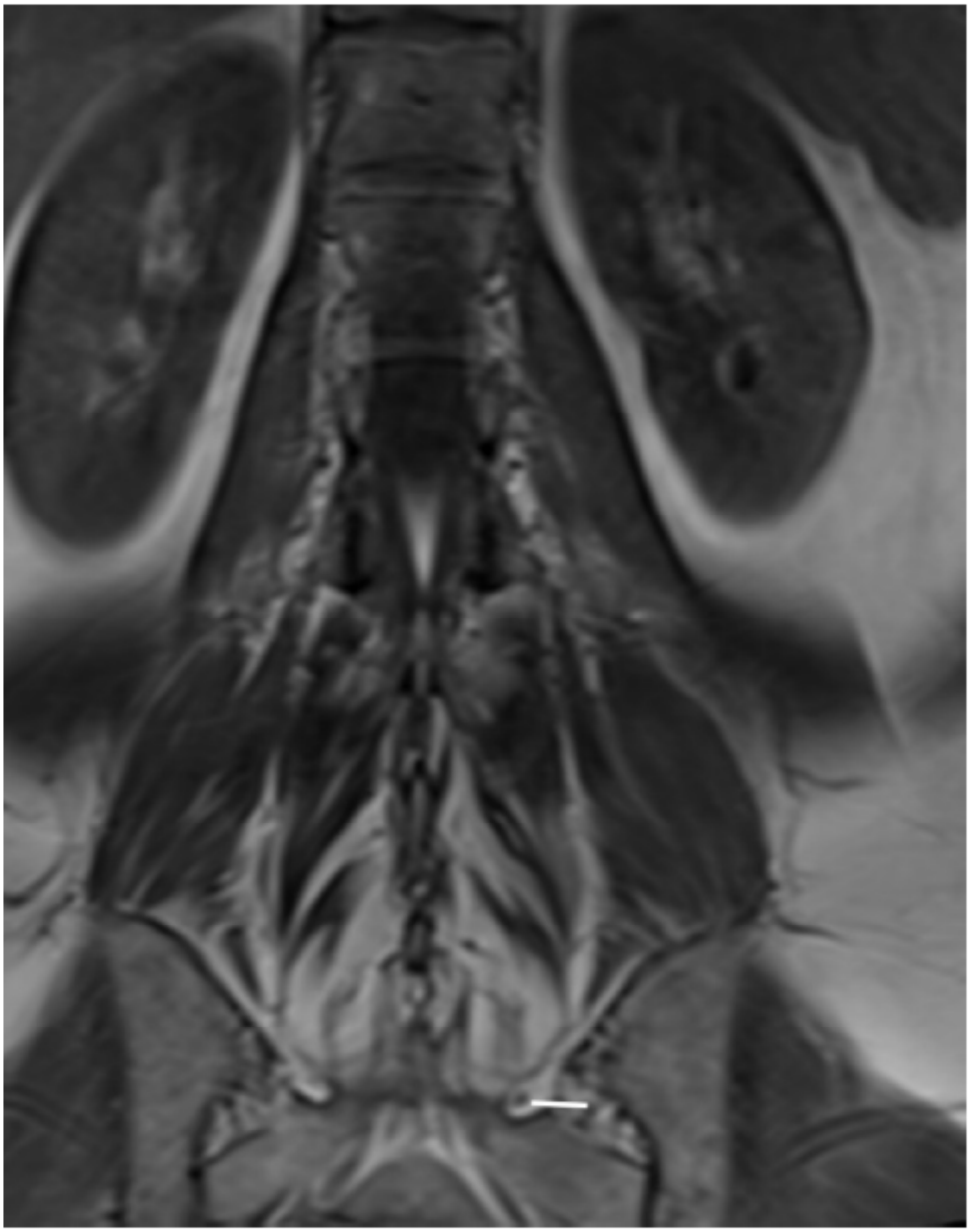


Figure 2. MRI scans of a patient: paraspinal coronal T1-w.



Discussion and conclusions

Literature on paraspinal MRI in ALS is very scarce and heterogeneous in terms of study type (longitudinal [5], cross-sectional [6]), and type of images obtained (qualitative T1-w [6] and T2-w [5]). In the two cited studies, age-matched healthy controls [5, 6] but no pathological controls were used. Jenkins et al. [5] proposed a very fast protocol for T2-w whole-body

muscle MRI in order to longitudinally study the denervation in motor neuron disease. They demonstrated statistically significant difference of T2-w images of paraspinal muscles between healthy controls (n=22) and ALS patients (n=29) at baseline, while no difference during the longitudinal follow up (4 months) in ALS cohort [5]. They did not performed T1-w scans for chronic muscle alterations. In our explorative study [6], we evidenced no difference between T1-w paraspinal muscles images of newly-diagnosed ALS patients (n=10) and healthy controls (n=9).

In the present study, we decided to study the difference between T1-w paraspinal MRI alterations of ALS patients and different groups of controls. We focused our attention on paraspinal muscles because of their importance to stage ALS using El Escorial criteria. The evaluation of paraspinal muscles is habitually made by EMG at thoracic level, but MRI allows to perform a larger evaluation including profound muscles and different levels. We also included the study of iliopsoas even though it is not a proper paraspinal muscles but has similar features and functions. For the study, we performed T1-w scans in order to compare chronic alterations in ALS patients and patients with chronic pathologies characterized by different pathogenesis, such as inflammatory myopathy and lumbar radiculopathy. We did not detect statistically significant differences between groups for multifidus, longissimus dorsi and iliocostal. Patients with radiculopathy have alterations of iliopsoas more than ALS patients ($p<0,05$). Iliopsoas muscle is innervated by motor fibres of lumbar plexus (L2-L4), that are often involved in degenerative radicular pathology. Interestingly, we previously found significant difference for iliopsoas ($p<0,05$) between bulbar ALS patients and HCs [6] but we have not confirmed this data in the present study. For those considerations, the study of iliopsoas in a larger cohort of patients could be appealing in order to clarify its involvement. We stratified ALS patients for different onset (spinal vs bulbar) and detected statistically significant difference between spinal ALS and bulbar ALS for longissimus dorsi. This data

confirms that the pathological process seems to be limited to the specific region in bulbar patients. In addition there is also a trend to significance between spinal ALS and other groups for longissimus dorsi, that becomes a candidate to be a diagnostic ALS biomarker. Furthermore, we observed a trend to significance also for other muscles (multifidus p=0,1441, iliocostal p=0,06553, and iliopsoas p=0,08132) after stratification of ALS patients. Paraspinal MRI could help to distinguish spinal ALS patients from other pathologies in the diagnostic phase. These data must be confirmed in a larger cohort.

We did not performed T2-w, that are crucial for looking at acute alterations in muscles, and quantitative assessment of muscle T1 time or volume, that need many technical resources and are time-consuming. Those are limitations of the study, and further studies should consider a full comprehensive protocol with qualitative and quantitative T1-w and T2-w images in order to evaluate acute and chronic muscular alterations. Other limitation of our explorative study is the low number of patients recruited, which reflects the rarity of ALS.

In conclusion, paraspinal T1-w MRI could help to distinguish spinal ALS patients from healthy and pathological controls. In particular, study of longissimus dorsi can play the role of diagnostic ALS biomarker. To confirm these preliminary results, we plan to conduct a study in a larger cohort of patients, adding a longitudinally follow up in order to investigate the role of paraspinal MRI as a prognostic biomarker in ALS patients.

Part 3: A longitudinal study of lower limb muscles in Amyotrophic Lateral Sclerosis (ALS) patients using quantitative MRI

Introduction

For many years qualitative analyses of MRI scans were performed in order to study acute and chronic muscle alterations. Principal limit of qualitative analysis is related to the intra and inter-rater reproducibility, that is habitually confirmed by two independent evaluations by different raters who achieved coefficients of variation <5% for the regions of interest. In the last years different scans and automatic/semi-automatic programmes of post-processing analysis have been improved in order to perform detailed quantitative evaluation and to minimize operator-dependent factors. T2-w multi-echo was frequently used to study acute alterations of muscles in animal models [35]. Recently, Water T2 substitutes T2 mapping and Global T2 in humans [36-38], because of its advantage in reducing interference caused by fatty substitution. Literature on muscle MRI in ALS patients is poor of quantitative [19,21,22] studies, and only Bryan et. al [19] in 1999 performed a longitudinal study assessing quantitative evaluation of MRI scans. They followed ALS patients and healthy controls from the baseline MRI to the 4-month follow up MRI scans (three-dimensional volume gradient-echo) of lower limb muscles. Very recently, Klickovic et al. [39] proposed a qualitative and quantitative MRI analysis of bulbar and lower limb muscles in order to differentiate ALS and spinal bulbar muscular atrophy (SBMA).

In the present study we compared quantitative images (6-point Dixon GRE and multi-echo TSE T2-w) of lower limb muscles in ALS patients at different timepoints to look for evidence of disease progression and to relate the radiological findings to clinical data.

Materials and methods

Patients

In this longitudinal study, we enrolled fifteen patients diagnosed with possible, probable or definite ALS using the El Escorial Criteria [24]. We recruited patients who attended the motor neuron disease clinic at the C. Mondino National Neurological Institute. In addition, 7 healthy controls were recruited. We scored clinical severity using the ALS Functional Rating Scale revised (ALS-FRSr) [25]. For the clinical-radiological correlation the sub-item of the score indicating lower limbs motor function was used. All participants underwent full assessment including medical history and an evaluation of the clinical and neurologic conditions. Patients were clinically evaluated at every time point (baseline, T1 after 6 months, and T2 after 12 months). Exclusion criteria for all participants were concomitant neuromuscular diseases, inability to give informed consent, respiratory failure impairing ability to lie flat in the scanner and safety-related MRI contraindications. The institute's ethics committee approved the study and all subjects gave their written informed consent.

MRI data acquisition

Magnetic resonance imaging was performed on a 3T Skyra whole-body scanner (Siemens Healthcare, Erlangen, Germany), using a 32-channel spine coil. Quantitative fat fraction mapping images were obtained with the 6-point Dixon gradient-echo technique (Voxel size: 1.0×1.0×5.0 mm, Slice thickness: 5.00 mm, TR: 35.0 ms, TE: 1.73 ms). Multi-echo TSE T2-weighted 17 echoes images were also acquired (Voxel size: 1.2×1.2×10.0 mm, Slice thickness: 10.0 mm, TR: 4100.0 ms, TE: 10.90 ms). Thighs and calves axial images were obtained bilaterally and Vit E capsules were used as markers in order to obtain reproducible longitudinal data as regards sequences positioning. Total scanning time was usually under 60 minutes.

MRI data analysis

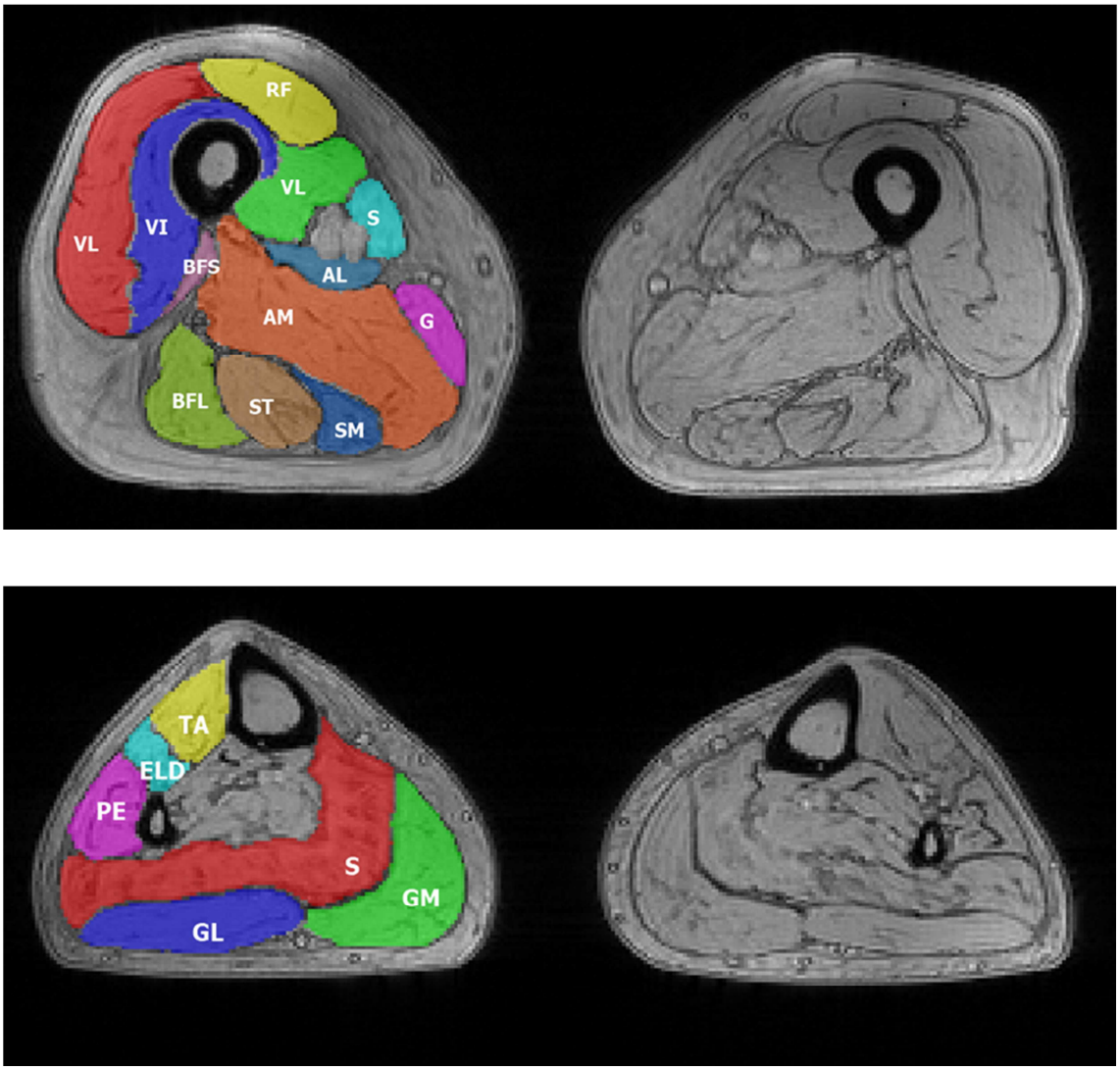
After MRI acquisition, DICOM images were converted in NIfTI. A single operator blinded to clinical data outlined the main thigh and calf muscles using the open-source software ITK-SNAP. Each muscle was outlined as a ROI (region of interest). We evaluated 12 thigh muscles (vastus lateralis (VL), vastus intermedius (VI), and vastus meanlis (VM) rectus femoris (RF), adductor magnus (AM), adductor longus (AL), long head of biceps femoris (BFL), short head of biceps femoris (BFS), semitendinosus (ST), semimembranosus (SM), sartorius (S), and gracilis (G)) and 6 calf muscles (gastrocnemium lateralis (GL), gastrocnemius medialis (GM), soleus (So), extensor digitorum longus (EDL) and peroneus (Pe)), bilaterally (Figure 1). Muscle regions of interest were drawn by an expert operator without including non-muscular tissue as fat tissue, fascia or blood vessels; muscle perimeter was outlined 2 mm within the borders of the perimuscular fascia. ROIs were initially outlined on the Multi-echo images and in particular on the first echo, then registered on the gradient-echo Dixon images using linear and non-linear transformations. The ROIs registered on the gradient echo images were subsequently revised by the same operator using ITK-SNAP in order to correct errors that may have occurred in the registration process. These last regions were used to calculate the mean fat fraction value for every muscle bilaterally. Accordingly, Global T2 value was calculated from the ROIs on the Multi-echo images. The Water T2 value for every ROI was extracted from the Global T2 value through a specific algorithm taking into account the fat fraction value previously calculated for the correspondent ROI.

Statistical analysis

Data are reported as mean, range and standard deviation for quantitative variables and percentages for categorical ones. To compare differences in MRI data among groups and timepoints, Kruskall-Wallis and Wilcoxon tests were performed. Spearman correlation

coefficient was used to evaluate the association between MRI and ALSFRS-r items. P-values ≤0,05 were considered significant (two-sided). The statistical software STATA V.14 was used for the analysis.

igure 1. ROIs for thigh (a) and calf (b) muscles.



Results

Fifteen patients (males= 8 and females=7; mean age 63,73±14,10) with definite (n=3), probable (n=4) or probable laboratory-supported (n=3) ALS, and seven HCs (males=4 and females=3; mean age 54,71±7,02) were recruited. All patients were affected by sporadic ALS. Mean diagnostic delay was 12,6 months (±8,88), mean ALS-FRSr score at the diagnosis was

43,4/48±2,41. Mean ALS-FRSr scored was 41,53/48±4,41 with a lower limbs sub-score of 6,87/±1,77 at baseline. Mean ALS-FRSr score decreased over time reaching 36,08/48±5,55, with a lower limbs sub-score of 5,58/8±2,47 at the last time point. We have been able to obtain data at three time points only from a limited number of patients (see supplementary). We analysed patients with almost two evaluation for the longitudinal study (73,34%). Inability to come for the radiological and clinical evaluation was due to the worsening of the clinical condition, or death caused by disease progression. The average time between two sequential follow-ups was 209 days; specifically 218 days for patients and 192 days for healthy controls. Clinical and demographic data are summarized in Table 1.

Table 1. Clinical and demographic data.

Patients		
Female		47%
Male		53%
Age at onset (years)		62,20±14,12
Age at t0 (years)		63,73±14,10
ALSFRSr at diagnosis		43,4/48±2,41
ALSFRSr at t0		41,53±4,41
ALSFRSr at the last time point		36,08±5,55
Lower limbs ALSFRSr sub-score at t0		6,87±1,77
Lower limbs ALSFRSr sub-score at last t.p.		5,58±2,47
Diagnostic delay		12,6±8,88
El Escorial category	Definite	6,67%
	Probable	80%
	Possible	13,33%
Treatment with Riluzole		100%
Type of onset	Bulbar	26,67%
	Spinal	73,33%
Side of onset (if spinal onset)	Left side	54,55%
	Right side	27,27%
	Bilateral	18,18%
Healthy controls		
Age at t0		51,13±12,05

We obtained Water T2 and Fat Fraction values for every left and right muscle, and an average value of the two sides. Data were extracted from the images at baseline and at subsequent time points.

At baseline, we found statistically significant differences between patients and healthy controls in Water T2 values, with higher values in patients than controls, in almost all the calf muscles and VL, VI, AM, RF and BFS ($p<0,05$) bilaterally. Water T2 values were also significantly higher in patients than controls in left VM and left BFL ($p<0,05$). There were no statistically significant differences in the fat fraction between patients and controls at baseline in all the calf muscles and all thigh muscles besides VL, BFS bilaterally and right S.

The same analysis performed at T1/T2 did not yield any statistical difference between patients and controls in Water T2 values in all calf muscles and most of thigh muscles with the exception of VL, VI and VM when considering average values between the two sides ($p<0,05$). With regards to fat fraction, we found statistically significant differences with higher values of fat fraction in patients than controls in right SO, GL, GM and left PE in the calf, VL, VI, VM in the thigh ($p<0,05$). Tables 2-3 show the results in a schematic view.

Clinical-radiological correlation analysis did not show any statistically significant correlation between lower limbs ALSFRSr sub-score and fat fraction values. We found statistically significant correlation between lower limbs ALSFRSr sub-score and Water T2 values a few of the analysed muscles.

Table 2. Statistical significance in Water T2 and fat fraction of thigh muscles in patients vs controls at baseline and at T1/T2

THIGH				
Muscolo	**WaterT2 t0**	**WaterT2 t1/t2**	**FF t0**	**FF t1/t2**
Vastus lateralis R/L average	0,005363	0,043880	0,028960	0,004489
Vastus lateralis L	0,008208	0,090840	0,101400	0,006985
Vastus lateralis R	0,005363	0,211500	0,034360	0,010570
Vastus meanlis R/L average	0,129600	0,018790	0,065360	0,043880
Vastus meanlis L	0,037570	0,090840	0,075970	0,079230
Vastus meanlis R	0,417600	0,051220	0,075970	0,037420
Vastus intermedius R/L average	0,005363	0,043880	0,116500	0,037420
Vastus intermedius L	0,004305	0,133500	0,219200	0,090840
Vastus intermedius R	0,005363	0,117900	0,172200	0,031740
Rectus femori R/L average	0,018200	0,449100	0,151800	0,068820
Rectus femori L	0,061760	0,525400	0,101400	0,133500
Rectus femori R	0,012330	0,565800	0,339300	0,133500
Sartorius R/L average	0,274600	0,347000	0,151800	0,486500
Sartorius L	0,378200	0,607500	0,412800	0,525400
Sartorius R	0,148400	0,379300	0,047780	0,413400
Gracilis R/L average	0,192200	0,379300	0,274800	0,211500
Gracilis L	0,549100	0,260400	0,732900	0,260400
Gracilis R	0,072250	0,880100	0,133200	0,260400
Adductor magnus R/L average	0,018200	0,189600	0,245900	0,117900
Adductor magnus L	0,012330	0,079230	0,682200	0,079230
Adductor magnus R	0,018200	0,525400	0,133200	0,117900
Semimembranosus R/L average	0,052560	0,694700	0,075970	0,449100
Semimembranosus L	0,084160	0,880100	0,065360	0,260400
Semimembranosus R	0,052560	0,449100	0,116500	0,413400
Semitendinosus R/L average	0,097610	0,927900	0,452800	0,260400
Semitendinosus L	0,148400	0,287500	0,682200	0,287500
Semitendinosus R	0,169300	0,607500	0,452800	0,449100
Long head of biceps femori R/L average	0,052560	0,975900	0,494900	0,235100
Long head of biceps femori L	0,018200	0,694700	0,682200	0,211500
Long head of biceps femori R	0,097610	0,832800	0,339300	0,260400
Short head of biceps femori R/L average	0,026390	0,379300	0,024300	0,832800
Short head of biceps femori L	0,015020	0,287500	0,011560	0,975900
Short head of biceps femori R	0,097610	0,518000	0,133200	0,880100
Adductor longus R/L average	0,274600	0,832800	0,116500	0,133500
Adductor longus L	0,169300	0,880100	0,632800	0,260400
Adductor longus R	0,341300	0,785900	0,087950	0,211500

Table 3. Statistical significance in Water T2 and fat fraction of calf muscles in patients vs controls at baseline and at T1/T2.

CALF				
Muscle	**WaterT2 t0**	**WaterT2 t1/t2**	**FF t0**	**FF t1/t2**
Soleus R/L average	0,001846	0,755300	0,192200	0,051860
Soleus L	0,001846	0,442800	0,192200	0,143200
Soleus R	0,008123	0,977400	0,169300	0,020490
Gastrocnemium meanlis R/L average	0,023970	0,887400	0,341300	0,012090
Gastrocnemium meanlis L	0,029270	0,442800	0,192200	0,038720
Gastrocnemium meanlis R	0,019520	0,712500	0,459200	0,044900
Gastrocnemium lateralis R/L average	0,005069	0,477600	0,860100	0,038720
Gastrocnemium lateralis L	0,003093	0,798700	0,971900	0,012090
Gastrocnemium lateralis R	0,035560	0,712500	0,860100	0,059660
Tibialis anterior R/L average	0,003093	0,842800	0,549100	0,143200
Tibialis anterior L	0,002396	0,932300	0,860100	0,197800
Tibialis anterior R	0,006435	0,932300	0,503100	0,078020
Extensor digitorum longus R/L average	0,006435	0,842800	0,378200	0,143200
Extensor digitorum longus L	0,001414	0,842800	0,378200	0,143200
Extensor digitorum longus R	0,019520	0,798700	0,274600	0,265700
Peroneus R/L average	0,002396	0,670700	0,061760	0,051860
Peroneus L	0,003971	0,842800	0,097610	0,318600
Peroneus R	0,003971	0,409500	0,112700	0,020490

Discussion and conclusions

Precedent experience of quantitative muscle MRI in ALS patients is limited, in particular for longitudinal study. Bryan et al. [19] used T1-w and T2-w sequences to assess leg muscles over a longitudinal 4-month follow-up in eleven ALS patients and eight HCs. Visual inspection revealed abnormalities (“moth-eaten” appearance) on T1-w images in six patients, but mean muscle T1 time and volume were not different between patients and HCs. Instead, the ALS patients showed increased muscle T2 time, evaluated using quantitative analysis. In

their conclusion, authors declared that T2 relaxation time was the best indicator of motor neuron dysfunction. This study was very pioneering, and the design should be replicated assessing new radiological techniques. More recently, Klickovic et al. [39] studied bulbar and lower limb muscles in ALS (n=21) patients, spinal bulbar muscular atrophy (SBMA) patients (n=21) and healthy controls (n=16) using 3-point Dixon, semiquantitative T1-w and short tau inversion recovery (STIR) scans. They demonstrated that quantitative imaging revealed significant fat infiltration in bulbar and limb muscles in SBMA compared to controls, identifying a characteristic pattern of muscle involvement. In ALS, semiquantitative STIR imaging detected marked hyperintensities in lower limb muscles, distinguishing ALS from SBMA and controls. The results of this study are very intriguing, but lacks the longitudinal follow up.

In our study, we quantitatively assessed lower limb muscles with 6-point Dixon GRE and multi-echo TSE T2 scans obtaining Water T2 and Fat Fraction values in 15 newly-diagnosed ALS patients and seven HCs. We obtained scans at baseline and during the follow up, but some patients were lost for the disease progression and their inability to perform MRI. We detected statistically significant differences between ALS patients and controls at baseline for many muscles of thigh and calf using Water T2 value that shows acute muscular alterations. The difference between ALS patients and controls wasn’t significant at the other timepoints, likely due to the extinguishing of denervation. Inversely, we didn’t find statistically significant difference between patients and controls in Fat Fraction of many muscles at baseline but the difference was significant for many of them at the other timepoints. These data reflect the chronic process of fatty substitution, that is typical of ALS muscles. No specific pattern of muscle involvement was found, while asymmetry was detected in many muscles of the same patients (see supplementary materials). In the other paper [34] no asymmetry was detected due to the use of qualitative analysis. We didn’t perform a

stratification for spinal and bulbar onset but the alterations cited above were present in bulbar patients too. Muscle MRI using quantitative analysis can be very useful to show disease activity in muscles that are clinically normal. This element should be used to confirm the diagnosis and allow patients to be early enrolled in clinical trials.

A concordance didn't emerge between clinical and MRI results. In particular, specific ALSFRS-r items for lower limbs did not correspond to high level of Fat Fraction. These data are likely due to the poor number of patients in the study, and new analysis should be done in a larger cohort. Indeed, Klickovic et al. found a correlation between clinical and radiological data [39], using the same approach as we did.

Limitations of our explorative study included the low number of patients recruited, which reflects the rarity of ALS. Furthermore, patients had difficulty to repeat MRI during the follow up for the progression of the disease. Shorter time between scans could be rethink but it is likely that MRI doesn't significantly change in less than 6 month. Moreover, it could be interesting add a cohort of pathological controls, in order to clarify the specificity of the analysis, and to discover specific pattern of muscular involvement.

In conclusion, quantitative muscle MRI analysis allows to distinguish ALS patients from HCs for specific regions (i.e. legs) at baseline and during the follow up. MRI pattern traces the pathological process of ALS, demonstrating denervation in initial phase and fatty substitution in chronic phase. Hence, muscle MRI can be useful as prognostic biomarker. MRI abnormalities could be found in pauci-symptomatic spinal muscles in bulbar-onset patients From these preliminary results, we plan to conduct a longitudinal study with a larger cohort in order to confirm muscle MRI measurements as a prognostic or predictive biomarker in ALS patients.

SUPPLEMENTARY MATERIALS

Thigh: anterior - VL vastus lateralis muscle, VI vastus intermedius muscle, VM vastus meanlis muscle, RF rectus femoris muscle; posterior - SA Sartorius muscle, GR gracilis muscle, AM adductor magnus muscle, BFL/BFS biceps femoris long and short head muscle, ST semitendinosus muscle, SM semimembranosus muscle, AL adductor longus muscle.
Leg: anterior - TA tibialis anterior muscle, TP tibialis posterior muscle, PE peroneus muscle, EDL extensor digitorum longus; posterior - SO soleus muscle, GM meanl gastrocnemius muscle, GL lateral gastrocnemius muscle.

Table 1. Calf FF patients

	Time point	SO mean	SO left	SO right	GM mean	GM left	GM right	GL mean	GL left	GL right
Patient 1	T0	0,078253	0,087722	0,069434	0,099480	0,105011	0,093851	0,106223	0,101039	0,118926
Patient 1	T1	0,104125	0,103531	0,104550	0,111805	0,119959	0,102703	0,121534	0,117255	0,137972
Patient 2	T0	0,045469	0,036073	0,054890	0,034666	0,031944	0,036954	0,026401	0,024111	0,028951
Patient 2	T2	0,112825	0,065746	0,163952	0,076365	0,059342	0,118119	0,096987	0,068611	0,135122
Patient 3	T0	0,140142	0,140712	0,139760	0,089711	0,071182	0,107776	0,055243	0,053788	0,058110
Patient 3	T1	0,147456	0,131315	0,162899	0,110596	0,090382	0,125063	0,071766	0,076967	0,065471
Patient 3	T2	0,191726	0,168193	0,215672	0,142308	0,094346	0,187413	0,115516	0,098480	0,133701
Patient 4	T0	0,121130	0,126629	0,115998	0,154584	0,144140	0,168762	0,130906	0,089546	0,220487
Patient 4	T1	0,104106	0,109024	0,100423	0,141314	0,120458	0,164764	0,094708	0,077522	0,153488
Patient 4	T2	0,186758	0,192976	0,180130	0,174291	0,126605	0,236109	0,194892	0,105776	0,359276
Patient 5	T0	0,077055	0,090188	0,067312	0,071974	0,084782	0,063558	0,066581	0,079584	0,053948
Patient 5	T1	0,101338	0,108633	0,093512	0,081306	0,095150	0,070532	0,070264	0,090812	0,049667
Patient 5	T2	0,147593	0,131380	0,163579	0,112349	0,119463	0,105757	0,097681	0,095264	0,099834
Patient 6	T0	0,068277	0,053243	0,078995	0,098511	0,057802	0,145442	0,158937	0,095540	0,225729
Patient 6	T1	0,066353	0,067863	0,065066	0,102074	0,075304	0,122979	0,104407	0,063580	0,142209
Patient 6	T2	0,077366	0,080395	0,074639	0,129284	0,129526	0,129027	0,118278	0,071168	0,166661
Patient 7	T0	0,091362	0,106115	0,077993	0,058367	0,088594	0,043332	0,085176	0,064199	0,095869
Patient 7	T2	0,140012	0,156946	0,128567	0,197675	0,286302	0,141487	0,307130	0,411297	0,241675
Patient 8	T0	0,196131	0,233270	0,162819	0,282203	0,207147	0,341535	0,171604	0,183645	0,161888
Patient 8	T1	0,205066	0,226413	0,184919	0,294629	0,274062	0,315700	0,330139	0,345169	0,316568
Patient 9	T0	0,134655	0,123533	0,143233	0,081610	0,087899	0,078801	0,060260	0,065104	0,054052
Patient 9	T2	0,147433	0,129535	0,164577	0,099619	0,099338	0,099852	0,088403	0,085353	0,092499
Patient 10	T0	0,255255	0,175112	0,339317	0,187276	0,143485	0,249799	0,278644	0,262225	0,295201
Patient 11	T0	0,137015	0,163456	0,123194	0,144707	0,192969	0,095391	0,075456	0,075457	0,075454
Patient 12	T0	0,067038	0,073774	0,059257	0,095391	0,100964	0,090311	0,060827	0,079282	0,039124
Patient 13	T0	0,156564	0,136200	0,175578	0,085969	0,076692	0,095877	0,068777	0,065082	0,071752
Patient 13	T1	0,280140	0,237096	0,319075	0,169587	0,160463	0,177760	0,147324	0,097293	0,189905
Patient 14	T0	0,102921	0,066358	0,135001	0,088708	0,060795	0,114534	0,127433	0,056981	0,168982

Patient 14	T1	0,082040	0,083416	0,080788	0,095610	0,096080	0,095142	0,086472	0,087294	0,085771
Patient 15	T0	0,112209	0,127652	0,095957	0,104528	0,204498	0,068911	0,057551	0,071643	0,049735
Patient 15	T1	0,161618	0,169510	0,153476	0,173045	0,234834	0,123223	0,141309	0,184313	0,110311

	Time point	**TA mean**	**TA left**	**TA right**	**EDL mean**	**EDL left**	**EDL right**	**PE mean**	**PE left**	**PE right**
Patient 1	T0	0,029920	0,037586	0,021360	0,026517	0,031056	0,023201	0,066944	0,086805	0,050617
Patient 1	T1	0,031913	0,038981	0,022812	0,034029	0,040869	0,027183	0,083653	0,092809	0,069860
Patient 2	T0	0,029324	0,025989	0,034153	0,044728	0,032525	0,053799	0,038426	0,033953	0,043285
Patient 2	T2	0,072387	0,051212	0,101242	0,112711	0,076815	0,151259	0,108105	0,078527	0,158261
Patient 3	T0	0,051705	0,057201	0,045467	0,091298	0,109070	0,068684	0,105736	0,106158	0,105264
Patient 3	T1	0,041364	0,038611	0,045500	0,087669	0,097866	0,080203	0,102818	0,095121	0,108459
Patient 3	T2	0,069811	0,080086	0,055757	0,120631	0,142285	0,103469	0,209453	0,164254	0,246531
Patient 4	T0	0,079270	0,069206	0,090154	0,100164	0,116514	0,081787	0,166980	0,163943	0,169349
Patient 4	T1	0,101826	0,104340	0,098491	0,103533	0,150981	0,093172	0,158995	0,183067	0,109138
Patient 4	T2	0,088427	0,073997	0,103079	0,113321	0,122553	0,105101	0,190728	0,159750	0,220659
Patient 5	T0	0,038671	0,042388	0,034763	0,090388	0,113765	0,063201	0,125058	0,143528	0,105510
Patient 5	T1	0,037226	0,038481	0,036264	0,090422	0,096091	0,085330	0,141679	0,151149	0,129007
Patient 5	T2	0,054442	0,053493	0,055330	0,134780	0,132520	0,136533	0,168153	0,177510	0,155587
Patient 6	T0	0,055535	0,062250	0,048317	0,083937	0,096933	0,068894	0,142771	0,203947	0,096142
Patient 6	T1	0,057551	0,081975	0,032944	0,068557	0,097535	0,054921	0,087848	0,097130	0,079663
Patient 6	T2	0,090422	0,106231	0,069784	0,097058	0,127220	0,079076	0,143617	0,151274	0,137162
Patient 7	T0	0,103360	0,119615	0,091404	0,171243	0,187549	0,161352	0,154896	0,155116	0,154677
Patient 7	T2	0,168682	0,187842	0,146961	0,270282	0,314180	0,237863	0,213640	0,218467	0,208206
Patient 8	T0	0,077069	0,089159	0,064198	0,165704	0,169972	0,161599	0,168524	0,302454	0,100651
Patient 8	T1	0,124361	0,160137	0,088629	0,194869	0,271875	0,135542	0,305044	0,437344	0,206565
Patient 9	T0	0,056048	0,056006	0,056101	0,116267	0,121237	0,108433	0,096815	0,086739	0,109877
Patient 9	T2	0,069185	0,066456	0,071911	0,115253	0,109058	0,120339	0,109797	0,105766	0,114361
Patient 10	T0	0,152457	0,115486	0,197847	0,210470	0,129148	0,303232	0,344108	0,218744	0,484949
Patient 11	T0	0,123946	0,141208	0,108253	0,237933	0,189133	0,300043	0,289202	0,332080	0,255260
Patient 12	T0	0,040090	0,033405	0,048673	0,062956	0,066002	0,060181	0,078966	0,081298	0,077443

Patient 13	T0	0,099802	0,079774	0,127330	0,167625	0,159655	0,174427	0,129531	0,145665	0,115095
Patient 13	T1	0,142963	0,113929	0,170238	0,238419	0,233252	0,242566	0,223458	0,227699	0,219784
Patient 14	T0	0,040530	0,039548	0,042064	0,079734	0,064184	0,123792	0,138227	0,109226	0,169677
Patient 14	T1	0,046840	0,045634	0,048376	0,106644	0,117545	0,093660	0,132819	0,135672	0,129307
Patient 15	T0	0,094756	0,112980	0,066663	0,120520	0,121876	0,118321	0,182656	0,187044	0,174910
Patient 15	T1	0,116275	0,129592	0,101257	0,154116	0,163379	0,144140	0,190043	0,218940	0,159504

Table 2. Calf FF controls

	Time point	**SO mean**	**SO left**	**SO right**	**GM mean**	**GM left**	**GM right**	**GL mean**	**GL left**	**GL right**
Control 1	T0	0,093797	0,102613	0,084774	0,093300	0,097786	0,089387	0,080096	0,069085	0,089670
Control 1	T2	0,108010	0,105936	0,110085	0,082538	0,088959	0,076390	0,135510	0,147907	0,125003
Control 2	T0	0,105755	0,108300	0,103788	0,483985	0,120744	0,742743	0,077159	0,091492	0,066861
Control 2	T2	0,113743	0,122942	0,104809	0,400902	0,191517	0,604584	0,144366	0,161099	0,129637
Control 3	T0	0,057318	0,062214	0,052968	0,055649	0,056965	0,054521	0,054590	0,050354	0,060456
Control 3	T2	0,064820	0,064564	0,065068	0,070026	0,067435	0,072408	0,116569	0,100364	0,130450
Control 4	T0	0,068316	0,069774	0,066732	0,055570	0,049984	0,062022	0,103643	0,074456	0,135000
Control 4	T1	0,068350	0,065067	0,072459	0,110675	0,109129	0,112196	0,102888	0,091676	0,113314
Control 4	T2	0,067198	0,058406	0,075480	0,057979	0,051092	0,066280	0,044954	0,037367	0,050421
Control 5	T0	0,110297	0,103216	0,117077	0,128143	0,131780	0,124361	0,127550	0,139997	0,109483
Control 5	T1	0,077067	0,083424	0,070955	0,118091	0,117504	0,118654	0,170500	0,182047	0,155095
Control 6	T0	0,104871	0,095109	0,112458	0,078424	0,069435	0,086306	0,141353	0,189047	0,083045
Control 6	T1	0,107588	0,096442	0,116625	0,072103	0,068589	0,074639	0,051290	0,048836	0,053498
Control 7	T0	0,071107	0,074680	0,067473	0,056589	0,049971	0,061588	0,046770	0,043108	0,050178
	Time point	**TA mean**	**TA left**	**TA right**	**EDL mean**	**EDL left**	**EDL d**	**PE mean**	**PE left**	**PE right**
Control 1	T0	0,075285	0,070952	0,082515	0,077850	0,095320	0,061103	0,087292	0,087717	0,086708
Control 1	T2	0,066283	0,066778	0,065809	0,083308	0,086817	0,080563	0,094205	0,099587	0,089387
Control 2	T0	0,050466	0,056628	0,045015	0,125304	0,140835	0,109694	0,083686	0,088427	0,075569
Control 2	T2	0,065193	0,071319	0,058407	0,126546	0,145002	0,105985	0,091138	0,099673	0,079998
Control 3	T0	0,052531	0,062526	0,042868	0,069623	0,094768	0,051676	0,069541	0,072005	0,067494
Control 3	T2	0,059676	0,068379	0,050351	0,084976	0,103049	0,070130	0,080355	0,081844	0,078925

Control 4	T0	0,036995	0,044041	0,027864	0,050579	0,081337	0,032232	0,092260	0,103228	0,080217
Control 4	T1	0,037956	0,045242	0,029562	0,044149	0,049884	0,039169	0,077100	0,098151	0,057809
Control 4	T2	0,036584	0,040069	0,033006	0,042716	0,047291	0,038966	0,080956	0,082316	0,080094
Control 5	T0	0,083392	0,093877	0,072026	0,148276	0,156760	0,142948	0,142299	0,158467	0,125245
Control 5	T1	0,076801	0,083666	0,068773	0,157776	0,177254	0,145828	0,134414	0,159632	0,109988
Control 6	T0	0,095951	0,077660	0,117172	0,128818	0,077466	0,189917	0,073720	0,076367	0,070478
Control 6	T1	0,065530	0,050419	0,080578	0,106107	0,085256	0,136949	0,079598	0,083634	0,074861
Control 7	T0	0,032799	0,036731	0,028417	0,045077	0,059887	0,036411	0,123092	0,124119	0,121689

Table 3. Calf Water T2 patients

	Time point	SO mean	SO left	SO right	GM mean	GM left	GM right	GL mean	GL left	GL right
Patient 1	T0	44,627334	44,804460	44,417443	49,825004	48,550998	51,053699	50,591419	42,206686	54,097762
Patient 1	T1	46,318276	46,094072	46,566047	48,821437	48,722889	48,914671	48,313233	47,169651	49,058135
Patient 2	T0	48,131711	52,754134	41,955597	44,775557	49,575224	39,396363	42,874471	45,328989	38,685427
Patient 2	T2	50,466825	55,610230	46,086857	47,519375	47,620333	47,462950	49,048008	53,399709	46,489591
Patient 3	T0	52,205856	52,877130	51,282386	46,825624	48,111947	45,611944	57,425091	58,474984	56,316275
Patient 3	T1	48,637491	50,315236	46,956913	46,881438	47,062878	46,654395	46,732658	47,366243	46,066415
Patient 3	T2	47,290055	47,806414	46,757224	47,354543	47,338811	47,370646	46,216556	46,234112	46,201633
Patient 4	T0	47,842458	47,573137	48,112551	52,045412	54,474119	50,126600	52,745020	58,336683	50,485762
Patient 4	T1	45,620164	45,899690	45,233481	46,560558	48,163265	45,158189	46,095389	47,208456	45,868073
Patient 4	T2	46,203045	46,225610	46,181068	45,879317	46,001017	45,774204	47,835670	51,861229	46,076601
Patient 5	T0	47,035345	46,710171	47,400487	50,601328	50,897736	50,090619	49,100168	47,514994	51,686503
Patient 5	T1	46,566164	46,716599	46,420891	51,406790	51,369347	51,454824	45,657491	46,695215	44,582371
Patient 5	T2	46,951806	47,331244	46,579288	50,753024	50,901667	50,638783	49,544815	49,763101	49,354805
Patient 6	T0	43,095128	43,286321	42,884971	44,391262	46,557131	42,506611	48,613065	55,184255	43,944062
Patient 6	T1	44,197257	43,690277	44,787367	45,676574	46,317047	44,920193	46,766506	48,598215	45,314145
Patient 6	T2	42,503277	42,165623	42,843553	45,234108	46,247083	44,185047	45,551980	49,200787	41,714058
Patient 7	T0	53,822343	51,924473	55,987204	63,008383	61,832077	65,194035	61,663831	62,184979	60,266550
Patient 7	T2	51,972044	53,327964	50,522015	55,508950	56,790210	53,452189	47,954612	50,835537	44,130705
Patient 8	T0	46,717918	44,672405	48,703269	48,044122	48,403576	47,593341	48,961710	49,572185	48,297369

Patient 8	T1	47,822183	46,512845	49,320892	49,963697	47,780335	52,384013	50,364128	48,370333	53,452557
Patient 9	T0	45,483518	45,879700	45,143136	44,902443	46,726116	42,205901	41,807545	41,873001	41,775544
Patient 9	T2	47,412784	47,836501	46,937695	44,759077	45,887360	43,107270	43,175594	44,567021	42,218291
Patient 10	T0	60,509916	65,233235	56,290003	57,513373	60,002248	55,705908	59,247457	64,746060	53,694945
Patient 11	T0	52,875898	51,096864	55,264417	59,424687	49,194544	69,722729	53,739810	53,352840	53,899866
Patient 12	T0	52,865895	53,381512	52,413046	55,621724	54,286137	57,044491	55,689596	54,246557	57,161303
Patient 13	T0	53,508359	55,834482	50,928476	57,412094	60,323339	54,453830	54,049843	57,395901	50,022596
Patient 13	T1	55,156339	56,374562	53,764948	60,127655	61,581285	58,353015	56,371794	54,765704	58,108108
Patient 14	T0	45,816234	45,103382	46,603069	47,235145	44,218443	50,488873	42,664322	43,725524	41,198851
Patient 14	T1	47,852987	45,915371	50,051946	49,039937	45,554942	52,607416	45,108507	44,585427	45,700673
Patient 15	T0	55,072231	53,314535	57,262484	62,963173	62,897822	63,207147	58,670274	54,111735	63,669963
Patient 15	T1	58,555877	57,225902	59,835977	64,207794	64,715403	63,325996	66,124372	65,047861	67,604098

	Time point	**TA mean**	**TA left**	**TA right**	**EDL mean**	**EDL left**	**EDL d**	**PE mean**	**PE left**	**PE right**
Patient 1	T0	38,986484	37,823734	40,095219	38,987369	38,372408	39,675093	42,671759	41,566077	43,988794
Patient 1	T1	38,497395	37,337106	39,401813	39,441754	38,936921	39,922690	42,497594	41,374589	43,650869
Patient 2	T0	43,919598	49,320262	39,979048	45,551759	48,169919	42,033606	49,917990	54,546683	45,713299
Patient 2	T2	50,154343	52,801559	48,263475	50,180905	51,001376	49,516360	50,412001	56,593494	46,381910
Patient 3	T0	39,778027	40,437811	39,171598	44,182678	44,190596	44,173039	46,668809	46,698777	46,637170
Patient 3	T1	39,474582	39,770524	39,268532	42,709449	41,584558	43,588013	44,152667	44,491984	43,753844
Patient 3	T2	43,408485	42,692197	43,938843	45,145397	45,998364	43,910461	42,602885	45,156032	40,395926
Patient 4	T0	48,595876	52,043004	44,954238	50,325767	50,889025	49,426236	54,777177	53,501913	56,347841
Patient 4	T1	44,405826	45,730927	43,397680	47,578077	48,000379	47,163596	47,519084	49,783354	46,530920
Patient 4	T2	44,231144	45,011455	43,500521	45,336215	45,172726	45,503914	49,373479	48,443914	50,268895
Patient 5	T0	44,320286	44,928535	43,739842	48,079719	47,437186	48,674212	51,970054	51,840806	52,079493
Patient 5	T1	40,629691	41,095749	40,102347	43,844032	45,597330	41,982003	47,400267	47,821502	47,061369
Patient 5	T2	41,037071	41,118406	40,957730	44,187273	44,605094	43,803829	49,417639	49,586249	49,299978
Patient 6	T0	43,666390	41,778492	44,948929	48,007760	46,038526	49,656422	47,802419	43,849246	50,550614
Patient 6	T1	41,543697	40,838977	42,212091	43,891748	42,398591	45,990824	44,589732	42,823514	46,510655
Patient 6	T2	40,825404	39,921950	41,427706	41,417192	40,558586	42,610510	42,852065	43,701666	41,682325
Patient 7	T0	58,203628	53,670520	64,107635	67,986456	66,650600	69,457399	56,615114	51,781635	62,153476

Patient 7	T2	51,812253	50,019467	54,396659	53,184306	53,831452	52,511421	48,582329	49,463105	47,882619
Patient 8	T0	45,645116	42,139379	49,424280	46,156725	44,153840	48,427854	49,295457	45,967534	55,466654
Patient 8	T1	48,045069	44,742115	50,908799	49,546061	45,835619	54,032045	51,790838	49,295948	55,619731
Patient 9	T0	42,265662	43,086046	41,604971	42,922771	43,167375	42,764569	41,442254	41,542900	41,355986
Patient 9	T2	41,804847	41,325676	42,220276	43,422489	43,232615	43,659831	44,070102	44,241753	43,950489
Patient 10	T0	54,974389	55,954506	54,164351	50,813463	52,638191	49,650450	57,222157	60,436967	55,068547
Patient 11	T0	52,643598	50,593709	54,799974	57,712984	56,261052	58,946345	55,806089	52,614051	60,594147
Patient 12	T0	46,151186	46,036635	46,242827	48,785747	48,913278	48,661270	47,386083	47,157871	47,738693
Patient 13	T0	48,137225	51,113493	45,752086	48,319243	49,036787	47,561193	50,119708	52,036862	47,811751
Patient 13	T1	55,063331	56,876172	53,393773	48,407893	47,194903	49,868698	51,897801	49,058772	54,712355
Patient 14	T0	44,696537	42,421597	46,278601	46,664013	44,705345	47,614543	47,102968	46,433109	47,772826
Patient 14	T1	46,080402	46,623116	45,708902	47,497044	47,072203	48,035176	48,848559	48,958224	48,746510
Patient 15	T0	55,162194	50,293402	58,350909	50,183489	48,598397	51,344843	55,971446	50,690708	60,780689
Patient 15	T1	45,919308	50,507168	42,467385	50,630833	54,010050	46,731736	57,885964	56,770262	58,990123

Table 4. Calf Water T2 controls

	Time point	SO mean	SO left	SO right	GM mean	GM left	GM right	GL mean	GL left	GL right
Control 1	T0	43,237203	42,921110	43,508173	43,181281	42,881619	43,516488	42,658792	42,004568	43,371312
Control 1	T2	41,386964	41,273346	41,501407	41,863331	40,323376	43,667744	40,718050	39,362705	42,203938
Control 2	T0	45,381772	45,102368	45,726388	63,508284	73,326830	50,416889	53,118258	50,915040	56,078832
Control 2	T2	43,162958	42,916110	43,407931	45,848149	46,848386	44,801149	42,894733	42,669754	43,140533
Control 3	T0	44,061959	44,370382	43,710869	43,603638	44,764944	42,045200	37,928392	36,820947	38,930366
Control 3	T2	41,101386	40,747720	41,467481	40,531217	39,650148	41,539325	42,854271	43,702604	41,767088
Control 4	T0	41,081044	41,625220	40,517434	40,976941	41,749871	40,178176	38,737383	39,599832	38,094799
Control 4	T1	39,646152	40,139275	39,161163	38,829583	38,950103	38,700826	37,698690	37,499131	37,912103
Control 4	T2	40,155976	40,406836	39,880444	39,013300	38,923588	39,104361	38,089251	38,140274	38,044663
Control 5	T0	44,275165	43,914112	44,606852	44,706716	44,452580	45,004785	41,785293	41,608259	41,980116
Control 5	T1	43,421648	43,240421	43,611553	41,836901	41,737826	41,931814	39,591801	39,181308	39,973935
Control 6	T0	41,633885	41,933100	41,260673	38,551103	39,330839	37,604576	37,989102	38,282282	37,695095

Control 6	T1	42,899539	43,332842	42,385861	40,441258	40,041383	40,955076	38,775557	37,895282	39,666172
	Time point	**TA mean**	**TA left**	**TA right**	**EDL mean**	**EDL left**	**EDL d**	**PE mean**	**PE left**	**PE right**
Control 1	T0	39,047074	38,901883	39,143424	39,353445	38,817119	40,093491	39,591126	38,834171	40,429083
Control 1	T2	38,022731	37,705803	38,367527	39,058516	39,424083	38,497724	39,009130	38,895816	39,097790
Control 2	T0	39,233869	38,443594	40,098150	44,468863	42,524767	46,015304	44,305570	42,967194	45,111638
Control 2	T2	40,544978	40,085304	40,903633	44,113381	43,121859	45,454031	42,098252	43,226221	41,198299
Control 3	T0	40,593835	39,923379	41,226694	43,208040	41,532663	45,340338	42,733837	42,145209	43,293517
Control 3	T2	36,194305	37,013893	35,538634	36,797462	39,219173	33,799155	36,255796	37,950912	34,769500
Control 4	T0	37,724786	36,928588	38,341975	38,966198	38,193342	40,026720	40,746722	39,813442	41,903750
Control 4	T1	37,410885	36,859758	37,929144	37,451508	37,126904	37,869804	38,786692	38,003012	39,688554
Control 4	T2	37,766625	37,043849	38,524003	37,912653	37,458032	38,555301	39,719957	38,969039	40,514361
Control 5	T0	42,583589	41,571267	43,447336	44,683789	42,896572	46,707623	46,639729	46,385320	46,848742
Control 5	T1	40,857401	39,263241	42,288503	42,380784	41,403435	43,224628	42,283609	41,096798	43,170261
Control 6	T0	38,899372	38,806533	38,986851	39,101253	37,892251	40,495796	39,194791	39,011725	39,342810
Control 6	T1	40,130055	39,382163	40,852816	41,429035	41,244756	41,629899	41,348836	42,195993	40,500612

Table 5. Thigh FF patients

	Time point	VL mean	VL left	VL right	VM mean	VM left	VM right	VI mean	VI left	VI right
Patient 1	T0	0,095349	0,100442	0,090466	0,085009	0,093346	0,077840	0,073892	0,078294	0,070421
Patient 1	T1	0,106633	0,098503	0,114347	0,072712	0,083833	0,062781	0,063346	0,066404	0,060915
Patient 2	T0	0,078826	0,045234	0,120592	0,051525	0,057634	0,039330	0,037904	0,037783	0,038078
Patient 2	T2	0,111325	0,083315	0,158659	0,070391	0,071450	0,068005	0,070283	0,065097	0,081018
Patient 3	T0	0,076819	0,076476	0,077123	0,139693	0,179106	0,103519	0,075357	0,082942	0,067641
Patient 3	T1	0,074511	0,076150	0,072925	0,136964	0,175191	0,104799	0,076235	0,080628	0,071767
Patient 3	T2	0,128998	0,123901	0,133753	0,170084	0,222912	0,124752	0,109368	0,113855	0,104607
Patient 4	T0	0,071701	0,075899	0,067868	0,051203	0,061969	0,039528	0,049533	0,057230	0,042457
Patient 4	T1	0,074061	0,073742	0,074381	0,049602	0,061610	0,038744	0,046883	0,054994	0,039288
Patient 4	T2	0,113936	0,101820	0,125715	0,064247	0,080492	0,051109	0,052365	0,056448	0,048852
Patient 5	T0	0,065945	0,076716	0,056721	0,076905	0,089327	0,065127	0,064451	0,082816	0,047172
Patient 5	T1	0,097302	0,091566	0,102143	0,081936	0,093899	0,070723	0,073258	0,081020	0,066609
Patient 5	T2	0,109912	0,118965	0,101713	0,121968	0,146157	0,098135	0,084550	0,087643	0,081782
Patient 6	T0	0,068564	0,084483	0,052660	0,052812	0,064799	0,041796	0,048200	0,062838	0,034517
Patient 6	T1	0,069774	0,072953	0,066989	0,050806	0,066525	0,035445	0,049088	0,063547	0,036798
Patient 6	T2	0,079268	0,086484	0,072810	0,060430	0,077227	0,046047	0,057750	0,071979	0,044216
Patient 7	T2	0,158239	0,186118	0,133882	0,101854	0,098766	0,105288	0,117251	0,122287	0,111765
Patient 8	T0	0,182331	0,157993	0,205912	0,096520	0,110076	0,084426	0,111064	0,098782	0,121857
Patient 8	T1	0,219160	0,210530	0,227546	0,096855	0,109738	0,087749	0,124527	0,122416	0,126599
Patient 9	T0	0,070243	0,058346	0,083543	0,070102	0,070803	0,069194	0,059690	0,053249	0,066404
Patient 9	T2	0,099202	0,094796	0,104539	0,142950	0,149128	0,134348	0,079858	0,074350	0,085607
Patient 10	T0	0,160899	0,154911	0,167269	0,140949	0,143764	0,138395	0,099195	0,099385	0,098965
Patient 11	T0	0,115591	0,124944	0,106690	0,150026	0,188670	0,117077	0,120607	0,140982	0,097930
Patient 12	T0	0,109009	0,078504	0,137227	0,063370	0,061583	0,065209	0,109025	0,070308	0,140793
Patient 13	T0	0,083104	0,072683	0,094800	0,060244	0,064287	0,056038	0,068091	0,068491	0,067573
Patient 13	T1	0,117108	0,114077	0,121398	0,116892	0,136912	0,093403	0,094421	0,086805	0,104266
Patient 14	T0	0,079966	0,083697	0,076396	0,085816	0,117080	0,054188	0,054206	0,057233	0,050980
Patient 14	T1	0,142834	0,129637	0,155307	0,099322	0,108183	0,090848	0,078565	0,077291	0,079587
Patient 15	T0	0,043201	0,037499	0,048220	0,063755	0,069880	0,058197	0,036303	0,037873	0,034834

Patient 15	T1	0,068769	0,059494	0,076525	0,075548	0,078116	0,072819	0,055618	0,061879	0,050002

	Time point	**RF mean**	**RF left**	**RF right**	**SA mean**	**SA left**	**SA right**	**GR mean**	**GR left**	**GR right**
Patient 1	T0	0,093828	0,093826	0,093830	0,168887	0,146781	0,192948	0,113708	0,099969	0,127535
Patient 1	T1	0,049567	0,061129	0,037717	0,164542	0,157357	0,171108	0,090162	0,093338	0,086771
Patient 2	T0	0,081129	0,105249	0,053645	0,132545	0,130873	0,134284	0,065667	0,063048	0,068020
Patient 2	T2	0,115887	0,130813	0,096357	0,193787	0,226255	0,154217	0,100775	0,094423	0,108561
Patient 3	T0	0,129689	0,154548	0,103736	0,223935	0,300357	0,151492	0,143400	0,140385	0,145651
Patient 3	T1	0,118335	0,119762	0,116863	0,236669	0,268635	0,210531	0,204342	0,237905	0,171535
Patient 3	T2	0,129277	0,128637	0,129890	0,273074	0,349993	0,205180	0,191374	0,218846	0,166744
Patient 4	T0	0,063528	0,062829	0,064259	0,172410	0,212325	0,124711	0,107502	0,112820	0,101089
Patient 4	T1	0,059745	0,060740	0,058522	0,157395	0,183943	0,121633	0,096273	0,101895	0,089873
Patient 4	T2	0,098789	0,126823	0,069530	0,146906	0,162652	0,130835	0,116784	0,108059	0,125527
Patient 5	T0	0,098605	0,081715	0,111210	0,139463	0,139767	0,139195	0,077501	0,089979	0,063550
Patient 5	T1	0,170180	0,092233	0,259347	0,133069	0,159558	0,109240	0,084833	0,090593	0,077629
Patient 5	T2	0,124226	0,113479	0,132877	0,130950	0,139576	0,122487	0,065777	0,066455	0,065048
Patient 6	T0	0,077709	0,106041	0,045302	0,159705	0,141439	0,177212	0,060311	0,068126	0,052596
Patient 6	T1	0,113171	0,171622	0,054114	0,143711	0,121464	0,163414	0,058943	0,064455	0,053640
Patient 6	T2	0,106777	0,141433	0,066950	0,137322	0,142945	0,131745	0,089711	0,101927	0,077376
Patient 7	T2	0,106263	0,156614	0,063324	0,198450	0,202935	0,194295	0,112831	0,100150	0,122413
Patient 8	T0	0,112478	0,117974	0,105029	0,281870	0,350088	0,224617	0,170855	0,173134	0,168599
Patient 8	T1	0,121971	0,119103	0,124973	0,245784	0,298088	0,199243	0,168442	0,153005	0,184473
Patient 9	T0	0,050342	0,051792	0,048938	0,196931	0,220173	0,172994	0,176919	0,199571	0,150540
Patient 9	T2	0,139728	0,158074	0,119325	0,202603	0,211470	0,194684	0,195023	0,170325	0,223867
Patient 10	T0	0,118996	0,137435	0,100713	0,236647	0,281033	0,195043	0,142891	0,166465	0,122547
Patient 11	T0	0,086309	0,117020	0,061850	0,259260	0,299594	0,213570	0,116227	0,119136	0,113600
Patient 12	T0	0,117527	0,079974	0,148276	0,158906	0,156500	0,161576	0,163537	0,229835	0,134176
Patient 13	T0	0,108068	0,172302	0,048791	0,102927	0,098524	0,107713	0,100422	0,103394	0,097383
Patient 13	T1	0,125902	0,162475	0,085841	0,177414	0,172485	0,183398	0,145879	0,146598	0,145124
Patient 14	T0	0,113436	0,152888	0,075819	0,265936	0,299239	0,236457	0,132044	0,127453	0,136214
Patient 14	T1	0,162560	0,167869	0,158739	0,288301	0,292404	0,285276	0,296784	0,314598	0,279206

Patient 15	T0	0,057666	0,045701	0,070520	0,130128	0,136510	0,124524	0,112413	0,092575	0,130138
Patient 15	T1	0,071158	0,061439	0,081049	0,157626	0,146358	0,172796	0,206430	0,246410	0,168997

	Time point	**AM mean**	**AM left**	**AM right**	**SM mean**	**SM left**	**SM right**	**ST mean**	**ST left**	**ST right**
Patient 1	T0	0,107063	0,104829	0,108858	0,171349	0,173756	0,167371	0,119098	0,115819	0,122472
Patient 1	T1	0,113222	0,121081	0,107219	0,165841	0,175739	0,152256	0,113111	0,109960	0,116289
Patient 2	T0	0,051115	0,052901	0,049215	0,094768	0,108014	0,084528	0,081023	0,079653	0,082462
Patient 2	T2	0,068791	0,064059	0,074884	0,111771	0,098360	0,122911	0,097016	0,087048	0,108963
Patient 3	T0	0,131608	0,134161	0,128864	0,120089	0,123724	0,116466	0,130885	0,121367	0,140196
Patient 3	T1	0,134105	0,131554	0,136728	0,116024	0,128893	0,101849	0,131915	0,128807	0,134735
Patient 3	T2	0,140517	0,134305	0,147047	0,164111	0,168572	0,159803	0,166504	0,149620	0,183719
Patient 4	T0	0,097986	0,112106	0,086328	0,131465	0,128251	0,133222	0,096406	0,092992	0,099479
Patient 4	T1	0,094334	0,103128	0,086463	0,126864	0,124872	0,127993	0,084332	0,076442	0,091520
Patient 4	T2	0,106501	0,113427	0,100814	0,144244	0,123973	0,155822	0,104497	0,098103	0,109869
Patient 5	T0	0,102294	0,106232	0,098010	0,119697	0,160630	0,090601	0,082506	0,100033	0,066592
Patient 5	T1	0,130606	0,129139	0,131949	0,269495	0,324432	0,217060	0,107950	0,107930	0,107967
Patient 5	T2	0,115158	0,114770	0,115537	0,147117	0,141461	0,151771	0,097563	0,101581	0,094146
Patient 6	T0	0,055392	0,063179	0,045145	0,087293	0,097000	0,077964	0,083747	0,090878	0,076502
Patient 6	T1	0,049308	0,050413	0,048027	0,087148	0,093207	0,080103	0,083394	0,088892	0,078132
Patient 6	T2	0,072383	0,077669	0,065614	0,122455	0,145351	0,102254	0,086158	0,088959	0,083361
Patient 7	T2	0,123479	0,117439	0,129554	0,175974	0,182055	0,170765	0,126663	0,141850	0,114995
Patient 8	T0	0,127432	0,150889	0,108707	0,210935	0,156874	0,345231	0,243692	0,247058	0,240561
Patient 8	T1	0,147601	0,167574	0,129120	0,417685	0,455869	0,388999	0,259107	0,282812	0,238007
Patient 9	T0	0,157089	0,125739	0,192178	0,218556	0,275362	0,167697	0,147058	0,135562	0,158909
Patient 9	T2	0,188579	0,143972	0,246129	0,191209	0,219536	0,172360	0,153406	0,146726	0,161422
Patient 10	T0	0,144720	0,146322	0,142834	0,374835	0,486635	0,312261	0,127147	0,123330	0,131826
Patient 11	T0	0,178768	0,194220	0,160748	0,264541	0,293974	0,241447	0,132246	0,151639	0,114994
Patient 12	T0	0,080478	0,078670	0,082387	0,125362	0,141716	0,107858	0,133429	0,132923	0,134016
Patient 13	T0	0,084612	0,072307	0,095848	0,178460	0,145665	0,246385	0,122932	0,125131	0,120697
Patient 13	T1	0,137166	0,137921	0,136417	0,175862	0,156264	0,219002	0,141694	0,160035	0,123709
Patient 14	T0	0,104771	0,107409	0,102571	0,234275	0,174893	0,319771	0,145522	0,134789	0,155140

Patient 14	T1	0,155129	0,152907	0,156835	0,289217	0,298718	0,270316	0,204490	0,223821	0,185379
Patient 15	T0	0,052093	0,048440	0,056019	0,068028	0,076295	0,060982	0,062487	0,066044	0,058994
Patient 15	T1	0,088041	0,094582	0,080300	0,100999	0,122012	0,086365	0,097476	0,105272	0,089680

	Time point	**BFL mean**	**BFL left**	**BFL right**	**BFS mean**	**BFS left**	**BFS right**	**AL mean**	**AL left**	**AL right**
Patient 1	T0	0,132972	0,138199	0,126021	0,110903	0,129501	0,091808	0,088491	0,092465	0,084292
Patient 1	T1	0,154340	0,166909	0,139827	0,166868	0,123656	0,233584	0,067283	0,071903	0,062953
Patient 2	T0	0,212394	0,176790	0,250380	0,644151	0,587258	0,710438	0,057470	0,061542	0,053932
Patient 2	T2	0,104276	0,080316	0,133126	0,246490	0,564276	0,176281	0,095742	0,113457	0,076725
Patient 3	T0	0,103292	0,090453	0,115566	0,226161	0,223703	0,230066	0,158480	0,167700	0,148144
Patient 3	T1	0,127436	0,106799	0,146180	0,213818	0,193033	0,246630	0,172670	0,187713	0,154047
Patient 3	T2	0,158116	0,154555	0,161296	0,242731	0,273385	0,208793	0,242212	0,241231	0,243264
Patient 4	T0	0,101331	0,100518	0,102045	0,201589	0,227267	0,157749	0,074540	0,089640	0,059307
Patient 4	T1	0,101579	0,118311	0,086881	0,147430	0,207833	0,100663	0,081379	0,088667	0,073132
Patient 4	T2	0,100582	0,107477	0,094515	0,104527	0,145426	0,076534	0,097863	0,097714	0,098019
Patient 5	T0	0,096999	0,103783	0,090275	0,207552	0,300501	0,109103	0,158913	0,222979	0,105738
Patient 5	T1	0,111250	0,111508	0,111010	0,510776	0,534834	0,475937	0,095322	0,130632	0,066394
Patient 5	T2	0,105317	0,107980	0,102627	0,159431	0,178859	0,139383	0,181334	0,319858	0,094932
Patient 6	T0	0,079982	0,080232	0,079693	0,080264	0,092571	0,062141	0,050442	0,053199	0,047233
Patient 6	T1	0,081424	0,073181	0,090977	0,065860	0,073782	0,057080	0,057327	0,054796	0,060010
Patient 6	T2	0,090999	0,096016	0,085795	0,161407	0,190591	0,117950	0,059547	0,070536	0,046571
Patient 7	T2	0,181399	0,190452	0,168357	0,280606	0,283818	0,275225	0,123024	0,125084	0,120811
Patient 8	T0	0,288249	0,281373	0,292432	0,800525	0,809070	0,791739	0,156767	0,170711	0,140329
Patient 8	T1	0,241305	0,278518	0,209878	0,542855	0,653986	0,443331	0,190588	0,188474	0,192835
Patient 9	T0	0,082025	0,084260	0,079753	0,334246	0,371778	0,294863	0,138151	0,143080	0,133591
Patient 9	T2	0,098769	0,097972	0,099698	0,204885	0,235647	0,182746	0,260568	0,251130	0,267740
Patient 10	T0	0,125836	0,114156	0,146616	0,483505	0,538226	0,424194	0,134501	0,149707	0,122891
Patient 11	T0	0,152924	0,175818	0,127174	0,230068	0,268388	0,196444	0,242944	0,247247	0,238749
Patient 12	T0	0,246303	0,131076	0,326390	0,389833	0,374260	0,409812	0,085206	0,090285	0,078190
Patient 13	T0	0,123710	0,107473	0,143164	0,196797	0,172964	0,226737	0,084381	0,086657	0,082263
Patient 13	T1	0,141645	0,135860	0,148449	0,386862	0,468451	0,291504	0,134296	0,137416	0,130718

Patient 14	T0	0,116321	0,126463	0,106317	0,672446	0,640170	0,727614	0,122187	0,122580	0,121757
Patient 14	T1	0,181372	0,165980	0,198047	0,189680	0,173413	0,198908	0,166147	0,179495	0,154752
Patient 15	T0	0,058598	0,046419	0,070473	0,060028	0,070596	0,046796	0,089719	0,089851	0,089564
Patient 15	T1	0,131664	0,134193	0,129396	0,208229	0,289494	0,141555	0,112320	0,084661	0,142240

Table 6. Thigh FF controls

	Time point	**VL mean**	**VL left**	**VL right**	**VM mean**	**VM left**	**VM right**	**VI mean**	**VI left**	**VI right**
Control 1	T0	0,066322	0,073478	0,059024	0,061165	0,071546	0,049640	0,044987	0,050617	0,039932
Control 1	T2	0,084782	0,092487	0,076556	0,075141	0,089886	0,058226	0,059384	0,066843	0,051946
Control 2	T0	0,075964	0,075648	0,076234	0,068135	0,083702	0,053761	0,058332	0,059021	0,057548
Control 2	T2	0,106676	0,094258	0,118286	0,067819	0,075148	0,061051	0,066465	0,067857	0,065102
Control 3	T0	0,069018	0,074882	0,063764	0,050324	0,050081	0,050534	0,055813	0,058456	0,053247
Control 3	T2	0,114295	0,096847	0,129469	0,061698	0,068957	0,054889	0,071197	0,054869	0,088260
Control 4	T0	0,042856	0,042890	0,042820	0,037652	0,048830	0,030361	0,037412	0,043672	0,032284
Control 4	T1	0,040726	0,046666	0,036567	0,041966	0,047939	0,036184	0,033964	0,040951	0,029012
Control 4	T2	0,048518	0,049644	0,047539	0,047183	0,057642	0,037558	0,042985	0,052356	0,036457
Control 5	T0	0,112514	0,118693	0,106059	0,101246	0,123809	0,074212	0,090023	0,092407	0,087395
Control 5	T1	0,113372	0,115852	0,110655	0,097646	0,108895	0,083540	0,097612	0,110270	0,083907
Control 6	T0	0,051383	0,057906	0,045316	0,065628	0,065446	0,065755	0,049901	0,048035	0,051815
Control 6	T1	0,055308	0,061281	0,049762	0,060184	0,062504	0,058144	0,052857	0,051891	0,053766
Control 7	T0	0,062081	0,067102	0,057091	0,051824	0,056231	0,045264	0,063790	0,080635	0,048727
	Time point	**RF mean**	**RF left**	**RF right**	**SA mean**	**SA left**	**SA right**	**GR mean**	**GR left**	**GR right**
Control 1	T0	0,059238	0,066220	0,053268	0,135865	0,133522	0,137927	0,090813	0,097886	0,084801
Control 1	T2	0,063170	0,064774	0,061868	0,140255	0,131492	0,147962	0,109617	0,115544	0,104656
Control 2	T0	0,136990	0,099674	0,170695	0,173784	0,192163	0,156355	0,099543	0,104924	0,094622
Control 2	T2	0,070771	0,073681	0,068065	0,128650	0,146053	0,108210	0,094791	0,099762	0,090113
Control 3	T0	0,073694	0,101874	0,049847	0,145464	0,162864	0,129147	0,129973	0,170544	0,084000
Control 3	T2	0,077611	0,102064	0,055979	0,165546	0,150727	0,180306	0,169025	0,209019	0,129352
Control 4	T0	0,041659	0,048060	0,036486	0,083080	0,104211	0,068242	0,067642	0,068009	0,067311
Control 4	T1	0,047389	0,062000	0,034364	0,083987	0,087622	0,080567	0,059906	0,063183	0,056535
Control 4	T2	0,050661	0,053479	0,047745	0,095887	0,105922	0,086966	0,082952	0,098511	0,077333
Control 5	T0	0,113228	0,120996	0,106211	0,275363	0,324179	0,208209	0,204290	0,211774	0,195180

Control 5	T1	0,127707	0,128150	0,127272	0,254110	0,273706	0,229767	0,185115	0,188869	0,181211
Control 6	T0	0,071782	0,069197	0,074083	0,134989	0,172393	0,093314	0,085408	0,086542	0,084272
Control 6	T1	0,069088	0,072306	0,066174	0,112236	0,112215	0,112256	0,081887	0,091259	0,072086
Control 7	T0	0,030963	0,031748	0,030368	0,091254	0,085638	0,095911	0,094752	0,104851	0,081108

	Time point	**AM mean**	**AM left**	**AM right**	**SM mean**	**SM left**	**SM right**	**ST mean**	**ST left**	**ST right**
Control 1	T0	0,097529	0,118619	0,076224	0,148676	0,168486	0,130060	0,137763	0,137352	0,138160
Control 1	T2	0,186483	0,198049	0,175332	0,131586	0,126418	0,137029	0,081416	0,089943	0,073107
Control 2	T0	0,074567	0,069383	0,079990	0,098427	0,087805	0,111247	0,088797	0,092819	0,084443
Control 2	T2	0,078493	0,068547	0,089531	0,102375	0,087139	0,121227	0,090678	0,090957	0,090392
Control 3	T0	0,086998	0,113065	0,061857	0,135976	0,146972	0,123609	0,131394	0,126032	0,136969
Control 3	T2	0,106380	0,140002	0,073564	0,162208	0,194697	0,130280	0,131067	0,135710	0,126373
Control 4	T0	0,053874	0,055881	0,052428	0,064423	0,053627	0,076613	0,082340	0,084187	0,079918
Control 4	T1	0,056835	0,066915	0,047622	0,058658	0,057259	0,060050	0,071368	0,073738	0,068329
Control 4	T2	0,070152	0,083373	0,059329	0,076007	0,071601	0,080122	0,085054	0,078759	0,095518
Control 5	T0	0,178363	0,178244	0,178472	0,189011	0,164669	0,230342	0,197270	0,208079	0,187119
Control 5	T1	0,192957	0,193793	0,192182	0,142476	0,133314	0,154240	0,205249	0,205577	0,204937
Control 6	T0	0,087111	0,086895	0,087278	0,110150	0,099280	0,124015	0,096330	0,103343	0,089097
Control 6	T1	0,086443	0,089942	0,083773	0,100707	0,095918	0,106752	0,095941	0,100260	0,090975
Control 7	T0	0,076863	0,087261	0,069547	0,064359	0,067878	0,060911	0,065152	0,067665	0,062872
	Time point	**BFL mean**	**BFL left**	**BFL right**	**BFS mean**	**BFS left**	**BFS right**	**AL mean**	**AL left**	**AL right**
Control 1	T0	0,098922	0,103706	0,094525	0,123822	0,124503	0,123001	0,087965	0,099560	0,076516
Control 1	T2	0,095878	0,089123	0,102281	0,117681	0,142021	0,096082	-	-	-
Control 2	T0	0,068139	0,069690	0,066518	0,070249	0,064561	0,076197	0,132364	0,127538	0,136624
Control 2	T2	0,077567	0,077703	0,077409	0,086367	0,074616	0,098359	0,136607	0,148398	0,124692
Control 3	T0	0,136380	0,158300	0,111985	0,175771	0,080161	0,295782	0,066853	0,088836	0,041137
Control 3	T2	0,140300	0,151417	0,128796	0,160726	0,115097	0,208533	0,071423	0,089797	0,051767
Control 4	T0	0,073876	0,067172	0,084076	0,079880	0,082248	0,076355	0,071535	0,104830	0,041956
Control 4	T1	0,073910	0,068617	0,080895	0,078827	0,083243	0,072686	0,051371	0,054793	0,047091
Control 4	T2	0,107963	0,092784	0,122234	0,039384	0,041272	0,038027	0,057312	0,067264	0,054240
Control 5	T0	0,149444	0,155593	0,142061	0,458283	0,447237	0,468820	0,144469	0,161665	0,126220
Control 5	T1	0,145008	0,145867	0,144012	0,226990	0,235459	0,215935	0,125970	0,126585	0,125322
Control 6	T0	0,141036	0,138082	0,144077	0,096756	0,094241	0,097789	0,069489	0,056762	0,080217

Control 6	T1	0,135625	0,139479	0,131619	0,129453	0,112596	0,146058	0,067463	0,065757	0,068919
Control 7	T0	0,078800	0,079732	0,077876	0,063410	0,083392	0,056911	0,059337	0,187408	0,037256

Table 7. Thigh Water T2 patients

	Time point	VL mean	VL left	VL right	VM mean	VM left	VM right	VI mean	VI left	VI right
Patient 1	T0	42,314349	42,833253	41,703733	41,016767	41,886458	39,996290	41,455564	41,427922	41,491955
Patient 1	T1	44,990343	44,420043	45,595941	43,087324	42,350146	44,275574	42,750024	42,035322	43,672532
Patient 2	T0	42,133630	42,525834	41,816746	39,279399	37,343114	40,203734	41,848397	42,917879	41,047603
Patient 2	T2	48,221992	53,007325	45,219333	44,199366	47,769328	42,586847	46,574153	49,611860	44,970011
Patient 3	T0	45,369637	45,030755	45,780708	45,035833	44,318198	45,627697	44,061796	42,322013	45,393467
Patient 3	T1	43,448342	42,638398	44,367198	40,999437	39,916186	42,057314	42,056338	41,872493	42,298590
Patient 3	T2	43,937989	44,517769	43,361115	42,420226	42,227038	42,765635	43,785253	45,084102	42,504556
Patient 4	T0	43,213622	42,977593	43,447257	41,501081	41,775484	41,131203	42,672780	42,157479	43,347718
Patient 4	T1	43,594343	41,675374	45,465956	38,001283	40,875872	35,372586	40,791290	40,023828	41,830264
Patient 4	T2	44,352043	43,708166	44,992842	42,782799	42,625352	42,976346	43,576100	42,913718	44,234128
Patient 5	T0	44,575368	44,163439	45,047460	42,956075	42,041065	45,081208	43,122515	42,801453	43,321527
Patient 5	T1	42,973057	42,525680	43,487225	43,830450	43,418869	44,112058	42,477839	41,947589	43,160817
Patient 5	T2	43,826553	42,615905	45,202400	44,008820	41,313522	46,936192	43,072168	41,879367	44,517210
Patient 6	T0	42,776465	41,592853	43,972652	41,174436	40,622535	41,795076	42,502699	41,513600	43,776284
Patient 6	T1	40,752333	40,115891	41,428846	42,687410	42,982902	42,157783	40,566594	38,812043	41,883060
Patient 6	T2	42,972133	43,254011	42,688930	43,626225	43,082427	44,386861	44,163018	45,012017	43,155046
Patient 7	T0	47,193926	46,499546	48,123312	47,167192	46,110143	48,913105	48,917558	49,140678	48,663355
Patient 7	T2	47,428158	46,829268	48,150349	47,362252	45,729600	48,771254	49,862933	49,815676	49,908863
Patient 8	T0	46,159845	44,533192	47,619990	43,720960	42,861948	44,656841	46,650143	45,505348	48,191154
Patient 8	T1	47,555136	45,761089	49,257279	45,204067	44,859195	45,781435	46,595149	45,767298	47,490000
Patient 9	T0	42,858144	44,164797	41,826943	42,691629	43,271566	42,118655	42,867830	43,698777	42,177003
Patient 9	T2	43,247912	44,500014	42,318148	43,244434	42,083877	44,190924	43,781908	43,320404	44,241663
Patient 10	T0	47,731380	45,129275	50,109167	43,390673	43,266952	43,536615	45,670748	45,320272	45,975566
Patient 11	T0	46,782576	46,415549	47,226679	44,734727	41,596587	48,989740	48,063049	45,997721	49,759381
Patient 12	T0	44,747201	44,020985	45,703756	41,744181	41,126497	42,359551	42,319792	39,595224	45,445876
Patient 13	T0	41,681755	44,164745	39,718226	38,880434	40,442606	37,411286	41,998211	44,437274	40,021529
Patient 14	T0	45,245344	44,858354	45,733530	42,668277	42,456982	42,944425	44,455980	44,550579	44,344019
Patient 14	T1	45,721919	46,186141	45,239322	43,605904	43,243633	44,001203	44,244481	44,263970	44,211952

Patient 15	T0	42,551630	42,142910	43,027246	40,575329	40,148606	41,056164	42,525938	42,126549	42,999797
Patient 15	T1	46,549460	45,149688	47,932622	45,691882	42,955875	48,319889	49,524929	48,107550	51,133314

	Time point	**RF mean**	**RF left**	**RF right**	**SA mean**	**SA left**	**SA right**	**GR mean**	**GR left**	**GR right**
Patient 1	T0	37,612929	38,036612	37,192168	43,022655	44,425846	41,611974	41,896694	40,728035	43,307046
Patient 1	T1	40,201559	39,510145	40,863129	50,774647	52,449169	48,889904	43,488113	43,838404	43,241206
Patient 2	T0	38,822807	37,183147	40,228849	34,772535	28,874561	39,982412	40,520786	39,461369	41,517884
Patient 2	T2	43,126118	45,315548	41,655686	45,955529	45,685268	46,141577	47,996974	49,849246	46,754732
Patient 3	T0	43,908474	43,239497	44,763601	49,111463	45,721099	54,380653	47,066289	47,396357	46,598015
Patient 3	T1	41,595219	42,073354	40,767884	43,367794	43,123618	43,985094	41,624921	46,398328	38,514508
Patient 3	T2	37,551959	37,114913	37,973333	42,195314	38,332380	44,796563	39,044556	38,748393	39,442525
Patient 4	T0	42,020246	42,655234	41,337515	49,094231	48,748899	49,490649	48,153431	50,691474	45,309883
Patient 4	T1	42,308955	41,459624	43,267519	45,414093	45,468342	45,357877	43,595229	43,475377	43,685344
Patient 4	T2	41,515517	41,432917	41,584891	49,858202	45,586394	54,391550	48,500000	51,836683	45,997487
Patient 5	T0	46,018021	47,352526	44,461099	47,197063	46,182699	48,017343	42,747886	42,458299	42,964253
Patient 5	T1	43,104859	43,590702	42,526920	43,139369	43,550459	42,551467	38,740420	38,245614	39,281070
Patient 5	T2	40,453693	40,489404	40,403102	43,358492	41,556443	44,789907	41,443550	42,065223	40,939982
Patient 6	T0	40,694560	39,376622	42,006780	49,757004	50,898679	48,120603	41,545944	42,071448	41,038879
Patient 6	T1	36,804848	35,773405	37,968277	40,650637	39,128156	42,021711	39,466971	39,981070	38,885123
Patient 6	T2	40,011515	39,610831	40,349448	44,546158	44,683322	44,429376	39,794807	39,280371	40,413075
Patient 7	T0	40,691127	38,819230	42,085727	50,916664	52,085427	49,823794	46,937673	46,976057	46,890808
Patient 7	T2	43,177563	42,235294	44,317674	55,226517	57,724176	52,529045	48,442695	50,267030	46,323807
Patient 8	T0	40,474050	41,435700	39,673146	46,629416	43,313341	49,484925	43,561158	41,923905	45,378110
Patient 8	T1	43,282195	39,940833	46,231156	55,085303	53,378769	58,375008	45,057068	46,501110	43,901835
Patient 9	T0	39,983196	39,892871	40,069174	45,094063	44,345609	45,857715	42,409459	42,903805	41,986351
Patient 9	T2	41,107351	40,723825	41,474235	46,853091	45,973798	48,003569	44,587702	44,527505	44,635946
Patient 10	T0	42,071140	41,539870	42,615059	46,579927	45,380025	47,874202	46,687437	48,485565	44,815916
Patient 11	T0	44,488786	43,858552	45,301668	43,658826	34,685403	54,842121	42,304263	40,936562	43,570024
Patient 12	T0	42,202890	40,784644	43,946321	49,962055	47,883990	52,049566	44,465013	45,746916	42,061444
Patient 13	T0	39,192919	39,913901	38,375633	41,295208	44,849505	38,323081	37,937633	32,430882	42,838464
Patient 14	T0	43,074098	42,449986	43,929562	47,167416	45,778894	48,379928	45,931443	45,579146	46,251713
Patient 14	T1	41,636258	41,781287	41,461890	50,027114	46,354500	53,347560	46,942479	47,571521	46,337401

Patient 15	T0	39,114510	32,259966	40,550518	43,204788	39,117105	44,922485	40,920909	41,108281	40,201005
Patient 15	T1	43,703105	43,774587	43,637265	44,190545	40,066169	48,393232	44,834955	44,904781	44,754809

	Time point	**AM mean**	**AM left**	**AM right**	**SM mean**	**SM left**	**SM right**	**ST mean**	**ST left**	**ST right**
Patient 1	T0	45,405894	43,910381	47,295840	45,653775	45,473858	45,775484	40,999090	40,343202	41,688954
Patient 1	T1	44,742240	45,125531	44,212909	49,419685	48,826227	49,889934	43,647646	42,983710	44,345891
Patient 2	T0	40,717836	41,306327	40,247429	41,550375	41,507538	41,612060	41,558048	43,024758	39,992516
Patient 2	T2	45,334965	47,693351	43,579663	45,432161	46,938452	42,442061	41,863320	43,051969	40,693186
Patient 3	T0	45,242554	46,025110	44,499149	47,047423	48,332672	45,831736	48,308985	48,475668	48,123300
Patient 3	T1	42,521574	43,448600	41,641243	42,014288	41,905130	42,146734	41,984142	42,268770	41,603381
Patient 3	T2	41,943882	43,168553	40,741923	41,949558	42,819842	41,185154	42,915628	44,341736	41,451085
Patient 4	T0	45,154757	44,202716	46,385392	45,040201	45,648543	43,832596	43,158769	42,578518	43,810279
Patient 4	T1	42,689659	42,240817	43,185082	40,356211	41,497931	38,818847	40,622109	41,767794	39,400451
Patient 4	T2	45,708560	46,114343	45,240607	43,610528	43,698900	43,385029	43,360249	43,617383	43,100061
Patient 5	T0	44,731402	44,274876	45,145832	47,490246	47,141649	48,077052	43,072357	41,797019	44,135643
Patient 5	T1	43,107616	43,216482	43,004128	43,847274	42,255345	45,804020	40,798834	40,011589	42,072097
Patient 5	T2	45,432242	44,826896	45,990938	46,243102	47,274971	44,365979	41,176399	41,295221	41,031675
Patient 6	T0	44,278797	43,457336	44,915626	47,672532	46,974274	48,624232	45,601265	45,513397	45,691320
Patient 6	T1	42,297369	43,128926	41,599407	44,436537	43,892058	44,878729	42,038829	41,707894	42,391775
Patient 6	T2	43,283671	42,286278	44,057019	43,453956	42,820199	44,051291	42,165390	42,672356	41,685350
Patient 7	T0	47,963092	48,082585	47,815065	52,459509	53,515477	51,507538	44,749572	41,041987	47,701005
Patient 7	T2	46,116248	46,575014	45,633860	48,008934	46,831937	49,185930	44,831237	43,374273	46,775815
Patient 8	T0	42,950867	41,416457	44,911425	43,428141	40,659821	44,652590	43,529116	42,608090	44,269357
Patient 8	T1	44,583462	45,691139	43,384627	40,835058	42,343719	38,980147	43,676790	44,445434	42,852498
Patient 9	T0	44,049673	44,979179	43,303007	42,008277	41,678115	42,598237	40,834302	41,340367	40,388610
Patient 9	T2	45,436196	47,462760	43,890394	43,044469	43,776985	41,813292	41,355638	42,625333	40,228783
Patient 10	T0	44,596826	45,024314	44,198314	43,673423	46,446518	40,276382	41,977975	42,540803	41,482686
Patient 11	T0	44,997176	44,456767	45,528154	54,712709	54,916771	54,550137	43,160429	41,796634	44,146878
Patient 12	T0	46,046442	46,607611	45,453620	49,704316	49,617786	49,768244	46,073257	48,737304	44,736494
Patient 13	T0	41,113676	40,672111	41,602478	43,678435	46,496808	41,802899	41,680212	43,452480	39,956654
Patient 14	T0	44,027714	43,582609	44,582247	46,360874	43,107538	47,694209	45,083073	44,600782	45,539981
Patient 14	T1	43,009770	43,116510	42,877964	42,460435	43,108647	42,094237	43,778121	44,797517	42,660075

Patient 15	T0	44,091963	42,229828	45,899209	42,136953	41,765885	42,488606	39,841629	40,091785	39,611437
Patient 15	T1	46,894020	45,337198	48,503528	47,180346	45,076150	50,361108	43,233498	42,923830	43,491179

	Time point	**BFL mean**	**BFL left**	**BFL right**	**BFS mean**	**BFS left**	**BFS right**	**AL mean**	**AL left**	**AL right**
Patient 1	T0	45,179320	44,066486	45,907720	42,219071	39,151178	45,402010	37,519061	35,867139	39,249829
Patient 1	T1	47,293294	46,611074	47,841446	49,411055	47,614543	50,900846	42,568231	42,532570	42,605322
Patient 2	T0	47,649323	52,235581	43,787905	39,082077	43,434413	31,193467	40,477187	40,447103	40,542786
Patient 2	T2	43,701486	43,595902	43,761932	44,654853	43,259153	45,109267	44,357306	45,023924	43,795825
Patient 3	T0	48,395647	48,115846	48,637195	47,621106	50,988275	43,668342	51,244471	49,899682	53,627420
Patient 3	T1	45,126726	45,850851	44,502324	43,046140	44,619650	41,291071	52,072993	53,277624	50,208433
Patient 3	T2	46,127729	46,901392	45,227090	41,416242	42,079629	40,762905	41,707692	44,063173	39,585865
Patient 4	T0	45,174733	43,766219	46,637840	48,786791	48,164737	49,539804	43,746648	42,930816	44,647401
Patient 4	T1	42,821351	43,204872	42,473986	44,971632	42,667885	47,593138	43,408368	44,419394	42,088087
Patient 4	T2	44,986382	45,513210	44,429214	42,919040	42,780205	43,216080	45,175137	46,159237	44,034000
Patient 5	T0	44,805572	43,909332	45,944758	52,290193	50,825439	56,261307	50,718718	47,314259	54,989451
Patient 5	T1	41,185116	41,970631	40,671719	44,041949	43,164977	44,965901	42,247037	41,693505	42,909623
Patient 5	T2	42,423645	42,342364	42,497977	43,364584	44,701284	41,321703	42,844691	41,136405	45,638538
Patient 6	T0	45,465271	45,901295	45,117728	45,228708	43,113568	46,807170	41,223686	41,791526	40,675946
Patient 6	T1	44,018527	43,899934	44,099279	40,313385	40,877859	39,859956	40,433043	41,616981	39,195980
Patient 6	T2	43,650504	44,422111	42,939446	43,150126	41,293970	46,243719	40,917585	40,983642	40,861965
Patient 7	T0	52,909780	52,324032	54,001284	66,062571	72,548457	52,442211	50,148452	48,934010	51,369090
Patient 7	T2	50,279879	50,921079	49,902574	41,402324	41,457286	41,353828	48,742971	49,758583	47,590333
Patient 8	T0	44,366768	44,648570	44,079955	42,189982	42,264657	41,992315	45,352794	45,551486	45,144389
Patient 8	T1	45,941016	46,645612	44,968432	49,778623	53,094886	44,534299	52,293902	53,364469	51,525984
Patient 9	T0	40,897872	41,242386	40,592640	49,196558	50,317486	43,668342	48,431837	49,328761	47,145150
Patient 9	T2	41,460278	41,018318	41,829292	45,644788	45,030980	46,698492	46,113494	46,345003	45,922420
Patient 10	T0	44,717738	46,539200	43,832205	45,625349	43,356316	47,126401	44,791186	43,122467	46,630486
Patient 11	T0	47,216734	47,601049	46,691324	48,290576	46,269442	53,949749	51,279513	50,876965	51,632692
Patient 12	T0	55,434508	62,712558	51,758539	44,954460	40,999113	49,437186	46,721734	48,632533	44,574198
Patient 13	T0	41,821346	43,520170	40,346715	47,838109	54,922572	39,857910	44,654101	39,040201	50,282812
Patient 14	T0	45,189103	45,061672	45,314633	46,103398	44,752609	56,137832	44,359521	45,121405	43,496052
Patient 14	T1	44,853691	45,261767	44,515648	44,349333	46,371859	42,182340	45,611128	45,893673	45,263380

Patient 15	T0	42,855832	42,325746	43,313267	43,474515	43,034136	44,248515	45,759442	46,404751	44,702235
Patient 15	T1	48,270892	47,085427	49,481942	47,794171	48,000442	47,242093	44,578000	43,168177	45,636604

Table 8. Thigh Water T2 controls

	Time point	VL mean	VL left	VL right	VM mean	VM left	VM right	VI mean	VI left	VI right
Control 1	T0	39,002536	38,509378	39,459938	41,755529	41,244290	42,400533	40,180038	39,853007	40,515067
Control 1	T2	39,486748	38,912563	39,956428	39,831303	39,444008	40,171400	40,111449	39,755420	40,445525
Control 2	T0	42,729433	42,509738	42,976130	42,040407	41,038448	43,173750	42,440240	41,953949	42,859025
Control 2	T2	44,653177	44,307659	44,995641	43,164875	41,520065	45,072287	44,518212	44,217280	44,852342
Control 3	T0	40,128994	40,048001	40,211381	41,545904	39,886683	43,600666	39,274180	38,742835	39,862883
Control 3	T2	39,733797	39,131352	40,367861	39,078111	39,016028	39,138100	39,437158	38,909512	39,942273
Control 4	T0	37,455085	37,171812	37,750550	38,224070	38,073755	38,418738	37,663125	37,565274	37,792794
Control 4	T1	36,548393	36,178868	37,022983	37,142970	37,098853	37,197896	36,653396	36,480694	36,871105
Control 4	T2	37,245567	37,047768	37,459953	37,948324	37,588532	38,378927	37,451044	37,199760	37,744758
Control 5	T0	44,995921	45,433803	44,599644	42,806331	42,661622	42,949465	43,387994	43,059338	43,686783
Control 5	T1	42,343967	42,847948	41,881870	41,295511	41,506688	41,108413	42,586577	42,913595	42,292883
Control 6	T0	39,577149	39,073184	40,127410	39,590814	38,809729	40,696573	39,696525	39,375953	40,032676
Control 6	T1	40,393568	40,218627	40,566223	40,892773	40,367164	41,590149	41,079534	40,522069	41,668912
	Time point	**RF mean**	**RF left**	**RF right**	**SA mean**	**SA left**	**SA right**	**GR mean**	**GR left**	**GR right**
Control 1	T0	38,089645	37,263264	39,097667	44,500874	43,912358	44,881393	42,935104	42,861499	43,014668
Control 1	T2	38,023451	38,053248	37,993107	42,860558	43,348780	42,390627	40,391455	43,004474	37,310433
Control 2	T0	38,862934	38,640861	39,119019	45,320682	43,566316	46,667056	41,610957	40,826113	42,453936
Control 2	T2	43,173564	41,633770	45,052306	46,695612	44,584384	48,965183	44,640742	43,930405	45,271901
Control 3	T0	39,249400	39,057552	39,490674	47,107049	46,843544	47,460218	41,413416	42,773049	39,949197
Control 3	T2	39,431374	39,654564	39,163190	44,790710	46,409718	42,793609	39,108131	39,137794	39,082858
Control 4	T0	35,221635	35,064790	35,403959	40,194188	40,217909	40,166237	40,705246	42,656552	38,446220
Control 4	T1	35,083072	34,655310	35,543097	39,098611	38,601059	39,653071	38,706262	38,871864	38,532571
Control 4	T2	35,963480	35,239225	36,707623	38,117547	37,969248	38,230697	37,558698	37,210543	37,934588
Control 5	T0	42,899228	43,021342	42,756869	50,933439	48,464125	52,795927	50,048394	48,638782	51,304119
Control 5	T1	41,262339	40,989696	41,559706	42,105796	41,697597	42,338503	40,882122	41,651267	40,192118
Control 6	T0	38,157771	38,208364	38,096424	40,986936	40,819231	41,146314	38,729955	37,895281	39,530819
Control 6	T1	36,815838	36,079726	37,668726	40,201526	39,774458	40,795450	39,757667	39,177178	40,337170

	Time point	AM mean	AM left	AM right	SM mean	SM left	SM right	ST mean	ST left	ST right
Control 1	T0	40,691230	40,478529	40,909758	42,641465	43,244391	41,905516	42,632870	42,735459	42,520041
Control 1	T2	44,306900	44,877150	43,711207	39,966561	40,425958	39,558208	37,211354	36,620387	37,848625
Control 2	T0	41,953665	42,281581	41,623651	43,411773	44,972158	42,031672	41,940261	42,603057	41,559943
Control 2	T2	43,263512	44,453979	42,170056	41,112646	41,411502	40,862828	42,420154	42,964667	42,118069
Control 3	T0	41,122803	40,545651	41,810519	45,323584	44,123889	46,361999	41,288075	40,950248	41,643986
Control 3	T2	40,387568	39,859725	40,958795	41,466026	41,163639	41,844537	39,626815	39,332005	39,928783
Control 4	T0	39,332991	39,024291	39,727272	39,293193	39,596737	38,977857	38,293057	38,263593	38,317972
Control 4	T1	38,348249	38,404612	38,285142	37,312967	37,414921	37,198349	36,704585	36,951997	36,469161
Control 4	T2	38,389371	38,366124	38,415451	39,362420	39,719187	38,972870	37,329577	37,125238	37,488279
Control 5	T0	46,126340	45,348683	46,899687	48,293224	48,898338	47,674378	49,027902	48,396566	49,787495
Control 5	T1	43,651562	43,225871	44,050362	42,123450	42,594337	41,638128	41,686262	41,477459	41,896936
Control 6	T0	40,875586	40,961253	40,763203	40,521049	40,661811	40,393926	39,161671	38,400068	39,970085
Control 6	T1	42,898394	42,672296	43,186054	42,639898	42,719934	42,575418	40,459633	39,966862	40,931921
	Time point	**BFL mean**	**BFL left**	**BFL right**	**BFS mean**	**BFS left**	**BFS right**	**AL mean**	**AL left**	**AL right**
Control 1	T0	41,917846	41,884991	41,951619	40,459440	38,639627	41,152702	45,049133	41,722458	46,505254
Control 1	T2	40,483398	40,922695	40,044101	39,955380	39,392217	40,767522	-	-	-
Control 2	T0	42,000671	42,422025	41,513995	38,557890	36,936244	39,778894	46,310619	47,928619	44,848084
Control 2	T2	42,078042	42,926384	41,117841	40,812318	40,731758	40,912811	47,081530	45,203289	49,275581
Control 3	T0	46,016416	44,518460	47,382642	42,321525	38,823664	44,320303	46,292099	45,446551	46,858659
Control 3	T2	40,837123	41,141636	40,537984	40,807886	38,370424	42,172864	40,638049	40,587146	40,681462
Control 4	T0	40,636777	41,519046	39,958796	40,793430	41,192362	40,367221	40,370656	39,774849	40,930342
Control 4	T1	39,118319	39,524263	38,784497	38,498414	37,536132	39,480245	38,438707	38,239946	38,612098
Control 4	T2	39,463094	39,016492	39,900463	37,130599	36,013675	38,606389	36,956747	36,436923	38,814177
Control 5	T0	46,295470	45,870897	46,690632	56,860653	55,010346	58,573561	45,282422	45,058090	45,546686
Control 5	T1	43,735236	42,164044	44,970985	42,454315	41,226625	43,584716	42,305060	42,068250	42,581280

Control 6	T0	41,478278	42,197447	40,761496	39,491206	39,359121	39,761665	41,457945	41,743343	41,082841
Control 6	T1	43,663216	44,265415	43,124182	41,740727	41,287095	42,276839	43,192202	43,597057	42,736901

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