

1 **Title:** Short-term neuromuscular electrical stimulation training of the tibialis anterior did not
2 improve strength and motor function in facioscapulohumeral muscular dystrophy patients

3

4 **Authors:** Aude-Clémence M. Doix^{1,4}, Karin Roeleveld⁴, Jérémy Garcia³, Pauline Lahaut³,
5 Véronique Tanant³, Manuella Fournier-Mehouas^{1,3}, Claude Desnuelle^{2,3}, Serge S. Colson¹ &
6 Sabrina Sacconi^{2,3}

7

8 **Affiliations:**

9 ¹ Université Côte d'Azur, LAMHES, France

10 ² Université Côte d'Azur, CNRS, INSERM, IRCAN, France

11 ³ Université Côte d'Azur, CHU, France

12 ⁴ Norwegian University of Science and Technology (NTNU), Department of Neuroscience,
13 Trondheim, Norway

14

15 **Correspondence**

16 Sabrina Sacconi, Université Côte d'Azur (UCA)

17 Peripheral Nervous System, Muscle & ALS Department, Pasteur 2 Hospital, Nice, France

18 Institute for Research on Cancer and Aging INSERM, U1081, CNRS, UMR 7284, (IRCAN);

19 Faculty of Medicine, F-06100, Nice, France

20 Email: sacconi.s@chu-nice.fr, sacconi@unice.fr

21

22 **Author Disclosures:**

23 Competing Interests: The authors have declared that no competing interests exist.

24 Funding or grants: This project was supported by Nice University Hospital (10AOI04) and by
25 the bilateral researcher exchange program Aurora, financed by the Norwegian Research
26 Council and the French Ministry of Foreign affairs (Grant number: 27407SG).

27

28 **Title:** Short-term neuromuscular electrical stimulation training of the tibialis anterior did not
29 improve strength and motor function in facioscapulohumeral muscular dystrophy patients

30

31 **ABSTRACT**

32 **Objective:** To investigate the effects on motor function, muscle strength and endurance of
33 short term neuromuscular electrical stimulation (NMES) training of the *tibialis anterior* (TA)
34 muscles in patients with facioscapulohumeral muscular dystrophy type 1 (FSHD1) in
35 comparison with healthy controls.

36 **Design:** This prospective study included ten patients with FSHD1 and ten healthy participants
37 (HP). Maximal voluntary isometric contraction (MVC) of ankle dorsiflexion (DF) and a 2-
38 minute sustained DF MVC with surface electromyography recordings (sEMG) of the TA and
39 the soleus muscles were measured and motor function clinical tests were performed before
40 and after the training period.

41 **Results:** No significant short term training effect was found in any of the investigated
42 variables for either group, although a tendency towards an increase was noted for the manual
43 muscle testing of the FSHD1. Patients with FSHD1 showed lower MVC force and lower
44 maximal TA sEMG amplitude than HP. During the 2-minute sustained MVC, the percentage
45 of force loss was lower for the FSHD1 patients, suggesting that they were experiencing a
46 lower amount of muscle fatigue compared to the HP group.

47 **Conclusion:** The present NMES protocol was not strenuous enough and/or the parameters of
48 stimulation were not adequate to improve dorsiflexion strength, muscle endurance and motor
49 function in FSHD1 patients and HP.

50

51

52 **KEY WORDS:** Isometric strength; Muscle endurance; Electromyography; Neuromuscular
53 disorder
54

55 INTRODUCTION

56 With a European prevalence of 4/100,000, the facioscapulohumeral muscular dystrophy
57 (FSHD) is the most common inherited muscular dystrophy disease. The FSHD is genetically
58 heterogeneous and two types of FSHD (*i.e.*, FSHD1, 95% of patients and FSHD2, 5% of
59 patients) have been identified.¹ Independently of the type of FSHD (*i.e.*, 1 or 2), the disease is
60 characterized by a progressive asymmetric muscle weakness and atrophy usually spreading
61 from facial to shoulder girdle, arms, abdominal and lower limb muscles.² In addition to
62 muscle weakness, fatigue and pain are the two other most frequently reported symptoms. In
63 particular, severe fatigue, a major burden in daily life activities, is reported by 61% of patients
64 with FSHD³ conducting to a sedentary lifestyle through a reduced level of physical activity.⁴
65 The reduced level of muscle strength has been identified as a key factor in explaining low
66 level of physical activity and high experienced fatigue.⁵ In patients with FSHD1, *tibialis*
67 *anterior* muscles can be affected in earlier stages of the disease than other lower limb muscles
68 ^{6,7} and this decline in *tibialis anterior* function is frequently considered as the first disabling
69 symptom.⁸ Since the *tibialis anterior* has a strong functional role in gait and balance, both its
70 weakness and fatigue may lead to a loss of mobility and increase the risk of falling.⁴ Since no
71 therapeutic treatments are yet available for FSHD,² it is of interest to propose alternative
72 procedures to moderate the progressive loss of strength, endurance and muscle function.
73 Aerobic exercises have been proposed to improve muscle function in patients with FSHD, but
74 some studies failed to show improvements on strength of such training, even though no
75 deleterious effects were reported.^{9,10} Neuromuscular electrical stimulation (NMES) is another
76 type of exercise broadly used in rehabilitation settings.¹¹ When NMES training was performed
77 on patients suffering from disabling forms of muscular dystrophy, such as Duchenne and
78 Becker dystrophies, tolerance and efficacy were shown to maintain or even improve muscle
79 strength.¹²⁻¹⁵ Comparable results in the *tibialis anterior* and the quadriceps muscles were

80 reported in a group of mixed patients with neuromuscular disorders including patients with
81 FSHD.¹⁶ More recently, NMES training, performed on shoulder girdle and knee extensor
82 muscles, was found to be safe and effective in improving strength and muscle function in
83 patients with FSHD1.¹⁷ The two studies that have investigated the NMES training programs
84 in FSHD^{16,17} involved long training periods of 14 and 5 months respectively. Although
85 beneficial effects of short term (less than 8 weeks) NMES training programs on muscle
86 strength and/or endurance in healthy participants^{18,19} or patients with muscular dystrophy¹²
87 were found, such programs have not been implemented in patients with FSHD. Therefore, the
88 objective of this study was to investigate the effect of a bilateral 8-week NMES training on
89 the *tibialis anterior* muscle in adults with FSHD1. It was hypothesized to observe muscle
90 strength and endurance gains in dorsiflexion as well as improved motor function in patients
91 with FSHD1.

92

93 **METHODS**

94

95 **Participants**

96 Ten adults with FSHD1 (mean \pm standard deviation (SD): 5 females and 5 males; age $62.3 \pm$
97 10.2 year; height: 168.5 ± 12.8 cm; body mass: 73.7 ± 15.2 kg) and 10 healthy participants
98 (HP) age matched (7 females and 3 males; age 56 ± 4.8 year; height: 171.5 ± 9.01 cm; body
99 mass: 74.8 ± 12.4 kg) volunteered to take part in the study and written informed consent was
100 obtained from all participants. The study was carried out according to the Declaration of
101 Helsinki and approved by the local Institutional Human Ethics Committee (CPP10.067). The
102 trial was declared (NCT00821548).

103 Adults patients diagnosed with FSHD1 were recruited from the outpatient record of the
104 physical medicine and rehabilitation department at the hospital and were included into the

105 study according to the following criteria: number of 4q35 D4Z4 <11 repeats (mean of the
106 group 6.89 ± 1.37 units), no mutation on *SMCHD1* gene ; muscle weakness of ankle
107 dorsiflexion from 2 to 4 at least on one leg, assessed by manual muscle testing (MMT).²⁰
108 Exclusion criteria comprised previous NMES training of the lower-limb; history of cancer,
109 joints pathologies, or collagenopathies, parturient, or breast-feeding woman or simultaneous
110 participation to another research study.

111

112 **Neuromuscular electrical stimulation training**

113 Bilateral neuromuscular electrical stimulation (NMES) training sessions of the *tibialis*
114 *anterior* muscles were performed with a Compex (Rehab 400, Cefar-Compex, DJO France
115 SAS, Mouguerre, France) portable battery-powered stimulator. Participants either exercised at
116 home or were trained by one of the experimenters or a physiotherapist. All healthy
117 participants as well as four patients with FSHD1 carried out their training sessions at home. In
118 the case participants trained at home, a weekly appointment was set-up with one of the
119 experimenters to provide feedback and to control the quality of the training. During these
120 training sessions participants were seated (hips, knees and ankles angles at 90°) with their feet
121 fixed. During the training sessions, the participants were instructed to place comfortably their
122 feet under a heavy-weighted object so that the feet would be firmly stuck and would not move
123 during the contractions. The participants were simultaneously stimulated bilaterally with self-
124 adhesive electrodes (2 mm thick) made of elastomer (5 cm x 5 cm) that were positioned as
125 follows: the positive electrode was placed on the superior part of the muscle, whereas the
126 negative electrode was placed on the medial part of the muscle, over the muscle bulk.
127 The NMES training program lasted for 8 weeks, with 3 sessions a week. Each session was
128 composed of a 2-minute warm-up, followed by the 20-minute working out session, and
129 finishing with 3 minutes of relaxation. The NMES program consisted in isometric

130 contractions of 9s (rise time: 1.5s; steady tetanic stimulation time: 6s; fall time: 1.5s) followed
131 by a pause lasting 7 seconds (duty cycle: 56.25%) at 35 Hz and with a 200 μ s pulse-width.
132 These stimulation parameters were chosen accordingly to previous successful NMES using
133 low-frequency protocols in patients with neuromuscular disorders to increase muscle strength.
134 ¹²⁻¹⁷ Participants were encouraged to increase stimulation intensity progressively every 5
135 minutes throughout each session up to individual tolerance threshold (i.e., discomfort/pain)
136 since strength gains would be dependent on the stimulation intensity.^{21,22} As individual
137 tolerance threshold varied among participants, they were instructed to increase progressively
138 stimulation intensity during the warm-up period to ensure a visible muscle contraction.
139 However, since the feet were secured, no joint movement was induced. Moreover, during
140 each of the training sessions, the participants or the physiotherapist, according to the training
141 modality (i.e., supervised or at home) had to fill-out a questionnaire consisting in reporting the
142 following: date and time of the training session; the mean intensity of the neuromuscular
143 electrical stimulation delivered; self-evaluation of their perceived fatigue after the training
144 session; the discomfort related to the NMES delivered during training sessions. Visual
145 analogue scales (VAS) were used to score perceived fatigue and discomfort. A score of 0 mm
146 indicated no fatigue or no discomfort and 100 mm indicated unbearable fatigue or maximum
147 discomfort.

148

149 **Study design**

150 To disclose the effect of the 8-week NMES training, at pre and post training, the participants
151 had first a blood sample collection, followed by a clinical examination and neuromuscular
152 tests. Following this, they answered a questionnaire during a 30 min period of rest and then
153 performed a 6-min walk test (6MWT).

154

155 *Blood samples collection*

156 To establish tolerance to NMES training, Plasma Creatine Kinase (CK) was measured after
157 the fourth week (W4), and once randomly during the training in addition to before and after
158 the 8-week training period (W8). Blood samples were collected and analyzed at the hospital.
159 The first and last CK measurements were determined at rest, whereas the 4-week and the
160 random test were performed within two hours following the NMES training. Plasma CK
161 activity was determined spectrophotometrically by an automatic analyzer using a test kit
162 (Roche/Hitachi Automated Clinical Chemistry Analyzer, Modular P-800, Roche Diagnostics,
163 Meylan, France). The CK activity was considered as a biological marker of training-induced
164 damage for each participant.

165

166 *Clinical examination*

167 Muscle function and strength of the TA were assessed manually by a physiotherapist, by
168 manual muscle testing - MMT.²⁰ Depending on the amplitude of the ankle dorsiflexion,
169 without extension of the hallux, scores were ranked from 0 where the muscle is no longer
170 capable of force production to 5 representing the absence of muscle impairment.

171 The Motor Function Measurement (MFM) assessed the functional capacity of daily life
172 activities: standing still, weight transfers, sitting, proximal and distal motor ability of muscles,
173 walking, standing up, raising up arms, stepping up stairs, brush hairs *etc.* After evaluation, the
174 total score was presented as a percentage, with healthy participants reaching 100%.²³ The
175 physiotherapist performing the clinical examination was experienced to assess patients
176 suffering from neuromuscular disorders and was not blinded to the evaluation.

177

178 *Neuromuscular tests*

179 Maximal voluntary contractions (MVC) of the dorsiflexor muscles were carried out
180 unilaterally in a custom made device ²⁴ consisting of a fixed footplate, where the foot was
181 firmly strapped to avoid any movement and ensure the quality of the isometric force
182 measurement. A strain-gauged transducer (model OMF06M, linear range 0-15 kN, precision
183 $\pm 0.5 \%$, sensitivity 10 mV/kN; OMICRON, Gambais, France) was placed on the footplate to
184 measure force production. During all contractions, the participants were seated on a chair,
185 with their knee slightly flexed according to the comfort of the participant. The foot was firmly
186 tightened with belts over the footplate with an ankle angle of 90°. The participants were not
187 constrained and were allowed to seat as comfortably as they could, however, during MVCs,
188 they were not allowed to hold the seat and were asked to remain as steady as they could.

189 Bipolar surface electromyography (sEMG) electrodes (10 mm diameter, 20 mm inter-
190 electrode distance) recorded the electrical activity of the tibialis anterior (TA) and the soleus
191 (SOL) muscles. The reference electrode was placed on the bony part of the contralateral
192 patella. Skin was cleaned and abraded prior to the placement of electrodes, and low resistance
193 impedance between electrodes ($<5 \text{ k}\Omega$) was obtained. A Biopac MP 150 system (Biopac
194 systems, Inc., Holliston, MA, USA) was used to record sEMG data at a sampling rate of 2000
195 Hz. Electromyographic signals were amplified with a bandwidth frequency ranging from 1 Hz
196 to 500 Hz (common mode rejection ratio = 11 dB; impedance input = 1000 MV; gain = 1000).

197

198 The tests were performed on both legs, one at a time, in a random order with at least 10
199 minutes of rest period between each leg. Two MVCs of dorsiflexion were performed on each
200 leg to determine the maximal strength production and the concomitant sEMG signals of both
201 the TA and the SOL muscles. A 60-second rest was allowed between each contraction. Then,
202 a fatiguing task consisting of an isometric 2-minute MVC ²⁵ of dorsiflexion was performed
203 with recordings of the sEMG of TA and SOL muscles. No visual feedback was provided to

204 the participants and they were asked to perform an all-out effort while they received strong
205 verbal encouragement.

206

207 *Questionnaire and 6-min walk test*

208 Quality of life of the participants was evaluated with the Medical Outcomes Study Short-
209 Form 36 (SF-36) questionnaire.²⁶ A 6-min walk test (6MWT) was used to assess the greatest
210 distance participants could walk in 6 minutes on a 20-meter shuttle.

211

212

213 **Data analysis**

214 The MVC was considered as the mean value over a 500-ms period around the peak force. The
215 best of the two trials was analyzed. During the fatiguing task, the percentage of MVC loss
216 was calculated as the difference of a 1-second window width at the start and a 1-second
217 window width at the end of the 2-minute MVCs. All sEMG data were analyzed over the same
218 window width as the force data where the root mean square (RMS) of the TA and the SOL
219 (TA RMS and SOL RMS) was quantified and the loss in TA RMS was computed. RMS was
220 calculated with commercially available software (AcqKnowledge 4.1, Biopac Systems, Inc.,
221 Holliston, MA, USA), while the rest of the outcome measures were analyzed with Matlab
222 R2010b (The MathWorks, Inc., Natick, MA, United-States).

223

224 **Statistical analysis**

225 Statistical processing was performed using Statistica[®] software for Microsoft Windows
226 (StatSoft, version 8.0, Tulsa, OK, USA). The Shapiro-Wilk test was used to test whether
227 outcome measures were normally distributed, and depending on the results the appropriate
228 statistical test was performed. When data were normally distributed, an unpaired Student t-test
229 was performed to compare FSHD1 and HP groups and a paired Student t-test was used to
230 disclose training-induced changes within group (FSHD1 and HP). When data did not follow a
231 normal distribution, equivalent non-parametric tests, the Mann-Whitney *U* test and the
232 Wilcoxon signed ranks test were performed. A two-way ANOVA (leg × time) with repeated
233 measures on time was performed on the intensity values recorded during each training
234 session, while the Friedman ANOVA was applied for the discomfort and fatigue VAS values
235 as they did not follow a normal distribution. In all statistical analysis the significance level
236 was set at $p < 0.05$. Unless specified, normal distributed data are expressed as means ± SD
237 (standard deviation of the mean), in the entire manuscript and in the tables and figures, while

238 non-normally distributed data are expressed as median \pm IQR (inter quartile range) in tables
239 and box-plots are used in figures.

240

241 **RESULTS**

242 **FSHD1 patients and healthy participants before the training period**

243 The plasma CK concentration was higher in FSHD1 patients before the training period
244 ($t=4.38$; $p<0.001$; Table 1). The MMT (Right: $U=5.0$; $p<0.001$; Left: $U=0.0$; $p<0.001$;) and
245 MFM scores of the FSHD1 patients were significantly impaired compared to the HP before
246 the training period ($U=0.0$; $p<0.001$; Table 1). Similarly, the distance covered during the
247 6MWT by the FSHD1 patients was shorter compared to the HP ($t=-2.63$; $p=0.02$; Table 1).
248 Lastly, quality of life assessed by means of SF-36 questionnaire (Table 2) revealed lower
249 values of FSHD1 patients compared to the HP for the following subscores: physical
250 functioning ($U=6.5$; $p<0.001$), social functioning ($U=18.0$; $p<0.05$), vitality ($U=14.5$; $p<0.05$),
251 general health ($U=5.0$; $p<0.001$) and the standardized physical component ($U=7.0$; $p<0.01$).

252

253 *Neuromuscular tests and fatiguing task*

254 As illustrated in the Figure 1A, the peak force during ankle dorsiflexion MVC was
255 significantly lower in FSHD patients than in HP prior to the training period for both legs
256 (Right, Pre: $U=88.0$; $p<0.001$; Left, Pre: $U=102.0$; $p<0.001$). The associated amplitude of the
257 TA RMS during dorsiflexion MVC (Figure 1B) was lower in the FSHD1 patients compared
258 to the HP before (Right: $U=95.0$; $p<0.001$; Left: $U=90.0$; $p=0.013$) the training period. In
259 addition, the amplitude of the SOL RMS during dorsiflexion MVC was found lower in the
260 FSHD1 patients before on the right leg ($U=85.0$; $p=0.007$), but not on the left leg ($p=0.282$).
261 Although not significantly different for the left leg ($p=0.095$), the group of patients with
262 FSHD1 exhibited a lower force reduction during the 2-minute MVC than the HP group before

263 the training period on the right leg (Right: $U=10.0$; $p=0.003$; Figure 2A). No difference in
264 percentage of TA RMS and SOL RMS reduction between groups was found before the
265 training period for either leg ($p>0.05$).

266

267 **Effects of NMES training in FSHD1 patients and healthy participants**

268 Individual patient/healthy participant's compliance to the training program was maximal (i.e.,
269 100% of the scheduled training sessions attended). Whatever the group considered, plasma
270 CK did not change significantly during the NMES training period ($p>0.05$). The NMES
271 training did not modify the SF-36 questionnaire subscores and the values of the FSHD1
272 patients remained lower than those of the HP (Table 2). Also, no significant changes of the
273 MFM and 6MWT assessments were observed after the training period ($p>0.05$) and the values
274 of the FSHD1 patients remained lower than those of the HP (Table 1). Although no
275 significant differences were found, the MMT values of the FSHD1 patients tended to slightly
276 increase after the training period ($p=0.067$; Figure 3) for both the right and left legs. Also,
277 when considering exclusively legs (i.e., fourteen legs) matching the inclusion criterion of
278 having a MMT score of ankle dorsiflexion comprised between 2 to 4, a significant training
279 effect was observed ($p=0.027$; data not illustrated).

280

281 *Neuromuscular tests and fatiguing task*

282 No significant training effect was found in dorsiflexion strength ($p>0.05$; Figure 1A) and in
283 the associated RMS amplitude of the TA ($p>0.05$; Figure 1B) and the SOL muscles in either
284 group for both sides. All these variables of the FSHD1 patients remained lower than those of
285 the HP (Figures 1 and 2).

286 The force reduction during the 2-minute MVC did not changed after the training period for
287 both legs in the FSHD1 patients and HP ($p>0.05$; Figure 2A). Although no significant

288 difference was found for the left leg, the TA RMS reduction of the right leg during the 2-
289 minute MVC was significantly greater after the training period for the FSHD1 patients
290 ($t=3.33$; $p>0.05$; Figure 2B). No significant change was noted for the HP. Also, no change of
291 the SOL RMS reduction was found after the training period for either leg or group.

292

293 *Characteristics of the NMES training program*

294 As illustrated in the Figure 4, the stimulation intensity of the NMES training was significantly
295 increased for the FSHD1 groups on both legs ($F=1.89$; $p<0.05$) as well as for the HP ($F=3.26$;
296 $p<0.001$). The self-reported evaluation of the discomfort level throughout the training reduced
297 on the right leg only in the group of patients with FSHD1 ($\chi^2=36.1$; $p=0.041$; Left leg:
298 $\chi^2=28.2$; $p=0.208$), whereas no change was observed for the group of healthy participants
299 ($p>0.05$). Likewise, the self-reported fatigue level reduced significantly in the group of
300 patients with FSHD1 along the training only in the left leg (Left leg: $\chi^2=40.3$; $p=0.014$; Right
301 leg: $\chi^2=34.3$; $p=0.061$). No change was observed for either leg in the HP group ($p>0.05$).

302

303 **DISCUSSION**

304 The purpose of this study was to investigate whether a short term bilateral NMES training on
305 the *tibialis anterior* muscles in adults with FSHD1 would be well tolerated and would
306 improve muscle strength, endurance and motor function. All participants completed the 8-
307 week NMES training program and no side effects were reported during or after the training
308 period. Unfortunately, this program did not improve ankle dorsiflexion maximal muscle
309 strength, nor muscle endurance or motor function in patients with FSHD1, although a
310 tendency towards an increase was observed for the MMT scores. Also, no significant
311 improvements were noted for the healthy participants.

312 All the participants (*i.e.*, FSHD1 patients and HP) completed the NMES training program and
313 according to the CK measurements, no rhabdomyolysis was induced by the NMES protocol.
314 Also, discomfort and fatigue VAS values reported throughout the protocol remained clinically
315 low (mean VAS<2) and did not significantly increase during the 8-week period. These results
316 agree with previous studies investigating the effects of NMES training programs in
317 neuromuscular diseases¹⁷ and confirm that NMES exercise is well tolerated by FSHD1
318 patients and HP.

319 Although well tolerated, this study failed to show a positive significant effect of NMES on
320 muscle strength, muscle endurance and motor function of the ankle dorsiflexor muscles in
321 FSHD1 patients, as well as in the HP. These observations are somehow surprising considering
322 that benefits of NMES training programs on muscle strength are widely reported in literature
323 for similar, or even shorter training periods in healthy participants^{18,19}. In addition, the use of
324 NMES was shown to be effective in patients with muscular dystrophies¹²⁻¹⁵ and in patients
325 with FSHD1.^{16,17}

326 Although, the characteristics of stimulation parameters used here (35 Hz of frequency and
327 200- μ s pulse duration) may be questioned, these NMES parameters were chosen since they
328 were successfully used in previous rehabilitation settings in patients with muscular
329 dystrophies.^{13,17} However, FSHD1 patients can have fat infiltration in TA muscles^{6,27} and
330 strong alteration of the sarcomeric contractile properties, preferentially of type II fibers,^{28,1}
331 reducing the overall capacity of the muscle to contract. Nevertheless, this reason cannot
332 account for the absence of improvements in the HP. Considering that frequencies above 50 Hz
333 are suggested to maximize the training effect of NMES on muscle strength in healthy
334 participants,²² it may be suggested that these frequencies should be considered in future
335 NMES studies in FSHD1 patients.

336 The lack of significant improvements could also be attributable to the frequency of the
337 sessions and/or the duration of the training. Only three sessions per week for eight weeks
338 were performed, whereas Colson et al.¹⁷ trained their FSHD1 patients five days per week over
339 a 5-month training period. Similarly, Milner-Brown and Miller¹⁶ obtained significant
340 improvement in strength after a 2-hour session performed 5 days per week during 14 months.
341 Therefore, a higher volume training period (greater number of sessions or duration) might be
342 required to obtain significant strength improvements in FSHD1 patients. Finally, as
343 previously suggested, the stimulation intensity was constantly increased throughout the
344 training period to ensure strength adaptations.²² However, this stimulation intensity increment
345 might have been too moderate to improve muscle strength. Therefore, it seems that the main
346 reason for the absence of strength increase in FSHD1 patients has to be related to the
347 frequency and/volume of the NMES sessions. Moreover, in the FSHD1 patients, the impaired
348 muscle function of the TA at the beginning of the study can be suggested as a possible reason
349 candidate for the training to be ineffective. Since no training effect was seen in the HP group
350 either, this cannot be stated with certitude. Finally, the *soleus* muscle activity of the right leg
351 during the dorsiflexion was found to be of lower magnitude compared to the left leg for the
352 FSHD1 patients before the training. This observation confirms that important imbalance exists
353 between limbs (i.e., asymmetric muscle weakness) and that particular neuromuscular
354 adjustments/compensations could occur with the disease in order to maintain functional
355 movements. These neuromuscular imbalances and their influence on functional daily tasks
356 such as balance/walking should be further investigated in FSHD patients.

357 Interestingly and although it did not change with the training period, the MVC loss was much
358 lower in the FSHD1 patients than in the HP during the 2-minute fatiguing task. This may
359 indicate that patients with FSHD1 experienced a lower amount of muscle fatigue compared to
360 the HP,²⁵ before and after the training, likely for several reasons. First, as the amplitude of the

361 TA EMG RMS reduced similarly, this suggests that the neural drive to the muscles would
362 have become suboptimal with fatigue for both groups in the same fashion during the fatiguing
363 exercise.²⁹ Second, patients with FSHD1 have strong alteration of the sarcomeric contractile
364 properties of type II fibers,^{1,28} which could lead to muscles more resistant to fatigue. Third,
365 weaker participants are shown to be less fatigable than stronger ones,³⁰ as the intramuscular
366 pressure is lower the negative feedback from afferent groups III and IV is therefore
367 diminished.³⁰ Even though, the group of patients with FSHD1 showed a greater TA EMG
368 RMS loss after the training in comparison to the loss before the training, this decrease was
369 similar in both groups. Since the patients group showed lower force losses, it can be suggested
370 that at the task truncation, the group of patients with FSHD were experiencing a lower amount
371 of muscle fatigue compared to the HP group.

372

373 Study limitations

374 A limitation of this study is related to the low number of FSHD1 patients and HP included.
375 The reasons may be as follows: i) the pool of patients with FSHD1 is rather low and/or
376 geographically spread, ii) it is unlikely to include enough patients with FSHD1 that have the
377 identical muscle weakness as well as possible matching response to the training program.
378 Nevertheless, all the participants completed the NMES training sessions scheduled. Second,
379 the heterogeneity of the adaptations to the NMES training program may have been too
380 important to highlight specific adaptations within each group. Also, no FSHD1 control group
381 (i.e., FSHD 1 patients not receiving NMES) was included in order to assess the possible
382 changes of measured variables due to the progression of the disease during the 8-week period.
383 Then, although the reliability of strength measurements is often questionable in fragile
384 populations, the measurements seemed to be sufficiently accurate since interclass correlation
385 coefficient for the ankle dorsiflexion MVC ranged from 0.96 (right leg) to 0.98 (left leg) and

386 from 0.87 (right leg) to 0.93 (left leg) for the associated TA EMG RMS values.
387 Notwithstanding these limitations, the present results may be useful to assist clinicians to plan
388 in the design of rehabilitation programs with the use of NMES in FSHD1 patients. For
389 example, in light of the present results and based on the literature, we proposed that future
390 clinical studies should design NMES training programs including between three to five
391 training sessions per week (for a muscle group) with frequencies ranging above 50 Hz with a
392 pulse duration ranging from 100 μ s to 500 μ s for a minimum duration of 20 to 30 minutes
393 (duty cycle ~50%). Although the exact training duration period has yet to be determined, a
394 minimum of three months seemed required to expect positive adaptations.

395

396 **CONCLUSION**

397 In summary, the present results show that an 8-week bilateral NMES training (20 minutes per
398 session, 3 sessions per week) of the tibialis anterior muscle did not improve muscle strength,
399 endurance and motor function in patients with FSHD1. Whether this non-responsiveness is
400 due to the impaired neuromuscular function of the ankle dorsiflexor muscles and/or to the
401 duration of the NMES protocol or to the stimulation intensity level reached during the NMES
402 sessions still remain to be shown. As suggested by Colson et al.¹⁷, it is likely that the efficacy
403 of the NMES training would depend on rapidity of starting NMES training as soon as the
404 FSHD1 diagnosis is made to maximize the training effects.

405

406

407 **REFERENCES**

408

- 409 1. Lassche S, Stienen GJM, Irving TC, et al. Sarcomeric dysfunction contributes to
410 muscle weakness in facioscapulohumeral muscular dystrophy. *Neurology*.
411 2013;80(8):733-737.
- 412 2. Tawil R, Van Der Maarel SM. Facioscapulohumeral muscular dystrophy. *Muscle*
413 *Nerve*. 2006;34(1):1-15.
- 414 3. Kalkman JS, Schillings ML, van der Werf SP, et al. Experienced fatigue in
415 facioscapulohumeral dystrophy, myotonic dystrophy, and HMSN-I. *J Neurol*
416 *Neurosurg Psychiatry*. 2005;76(10):1406-1409.
- 417 4. McDonald CM. Physical activity, health impairments, and disability in neuromuscular
418 disease. *Am J Phys Med Rehabil*. 2002;81(11 Suppl):S108-S120.
- 419 5. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BGM, Bleijenberg G. The
420 development of a model of fatigue in neuromuscular disorders: A longitudinal study. *J*
421 *Psychosom Res*. 2007;62(5):571-579.
- 422 6. Olsen DB, Gideon P, Jeppesen TD, Vissing J. Leg muscle involvement in
423 facioscapulohumeral muscular dystrophy assessed by MRI. *J Neurol*.
424 2006;253(11):1437-1441.
- 425 7. Dorobek M, Szmidt-Sałkowska E, Rowińska-Marcińska K, Gawel M, Hausmanowa-
426 Petruszewicz I. Relationships between clinical data and quantitative EMG findings in
427 facioscapulohumeral muscular dystrophy. *Neurol Neurochir Pol*. 2013;47(1):8-17.
- 428 8. Pastorello E, Cao M, Trevisan CP. Atypical onset in a series of 122 cases with
429 FacioScapuloHumeral Muscular Dystrophy. *Clin Neurol Neurosurg*. 2012;114(3):230-
430 234.
- 431 9. van der Kooi EL, Vogels OJ, van Asseldonk RJ, et al. Strength training and albuterol in

- 432 facioscapulohumeral muscular dystrophy. *Neurology*. 2004;63(4):702-708.
- 433 10. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC.
434 Strength training and aerobic exercise training for muscle disease. *Cochrane database*
435 *Syst Rev*. 2010;(1):CD003907.
- 436 11. Maddocks M, Gao W, Higginson IJ, Wilcock A. Neuromuscular electrical stimulation
437 for muscle weakness in adults with advanced disease. *Cochrane database Syst Rev*.
438 2013;1(1):CD009419.
- 439 12. Scott OM, Vrbová G, Hyde S a, Dubowitz V. Responses of muscles of patients with
440 Duchenne muscular dystrophy to chronic electrical stimulation. *J Neurol Neurosurg*
441 *Psychiatry*. 1986;49(12):1427-1434.
- 442 13. Scott OM, Hyde SA, Vrbová G, Dubowitz V. Therapeutic possibilities of chronic low
443 frequency electrical stimulation in children with Duchenne muscular dystrophy. *J*
444 *Neurol Sci*. 1990;95(2):171-182.
- 445 14. Zupan A. Long-term electrical stimulation of muscles in children with Duchenne and
446 Becker muscular dystrophy. *Muscle Nerve*. 1992;15(3):362-367.
- 447 15. Zupan A, Gregoric M, Valencic V, Vandot S. Effects of electrical stimulation on
448 muscles of children with Duchenne and Becker muscular dystrophy. *Neuropediatrics*.
449 1993;24(4):189-192.
- 450 16. Milner-Brown HS, Miller RG. Muscle strengthening through electric stimulation
451 combined with low-resistance weights in patients with neuromuscular disorders. *Arch*
452 *Phys Med Rehabil*. 1988;69(1):20-24.
- 453 17. Colson SS, Benchortane M, Tanant V, et al. Neuromuscular electrical stimulation
454 training: a safe and effective treatment for facioscapulohumeral muscular dystrophy
455 patients. *Arch Phys Med Rehabil*. 2010;91(5):697-702.
- 456 18. Thériault R, Boulay MR, Thériault G, Simoneau JA. Electrical stimulation-induced

- 457 changes in performance and fiber type proportion of human knee extensor muscles. *Eur*
458 *J Appl Physiol.* 1996;74(4):311-317.
- 459 19. Colson SS, Martin A, Van Hoecke J. Effects of electromyostimulation versus voluntary
460 isometric training on elbow flexor muscle strength. *J Electromyogr Kinesiol.*
461 2009;19(5): e311-319.
- 462 20. Lacôte M, Chevalier A-M, Miranda A, Bleton JP. *Évaluation Clinique de La Fonction*
463 *Musculaire.* Maloine; 2008.
- 464 21. Maffiuletti NA. Physiological and methodological considerations for the use of
465 neuromuscular electrical stimulation. *Eur J Appl Physiol.* 2010;110(2):223-234.
- 466 22. Filipovic A, Kleinöder H, Dörmann U, Mester J. Electromyostimulation--a systematic
467 review of the influence of training regimens and stimulation parameters on
468 effectiveness in electromyostimulation training of selected strength parameters. *J*
469 *Strength Cond Res.* 2011;25(11):3218-3238.
- 470 23. Bérard C, Payan C, Hodgkinson I, Fermanian J, MFM Collaborative Study Group. A
471 motor function measure for neuromuscular diseases. Construction and validation study.
472 *Neuromuscul Disord NMD.* 2005;15(7):463-470.
- 473 24. Simoneau EM, Billot M, Martin A, Van Hoecke J. Antagonist mechanical contribution
474 to resultant maximal torque at the ankle joint in young and older men. *J Electromyogr*
475 *Kinesiol.* 2009;19(2):e123-e131.
- 476 25. Schillings ML, Kalkman JS, Janssen HMHA, van Engelen BGM, Bleijenberg G,
477 Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. *Clin*
478 *Neurophysiol.* 2007;118(2):292-300.
- 479 26. Brazier JE, Harper R, Jones NM, et al. Validating the SF-36 health survey
480 questionnaire: new outcome measure for primary care. *BMJ.* 1992;305(6846):160-164.
- 481 27. Kan HE, Klomp DWJ, Wohlgemuth M, et al. Only fat infiltrated muscles in resting

- 482 lower leg of FSHD patients show disturbed energy metabolism. *NMR Biomed.*
483 2010;23(6):563-568.
- 484 28. D'Antona G, Brocca L, Pansarasa O, Rinaldi C, Tupler R, Bottinelli R. Structural and
485 functional alterations of muscle fibres in the novel mouse model of
486 facioscapulohumeral muscular dystrophy. *J Physiol.* 2007;584(3):997-1009.
- 487 29. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev.*
488 2001;81(4):1725-1789.
- 489 30. Hunter SK, Enoka RM. Sex differences in the fatigability of arm muscles depends on
490 absolute force during isometric contractions. *J Appl Physiol.* 2001;91(6):2686-2694.
491
492

493 **FIGURES LEGENDS**

494 **Figure 1A** Box-plots of the dorsiflexion Maximal Voluntary Contraction (N) for the FSHD1
495 patients and the HP groups for the right and left legs, before (dark fill-in) and after (white fill-
496 in) the NMES training. Boxes represent group median and interquartile range values and
497 whiskers are the highest and lowest values. Significant group differences $p<0.001$ (***)).

498 **Figure 1B** Box-plots of the RMS amplitude of the Tibialis Anterior during the dorsiflexion
499 Maximal Voluntary Contraction for the FSHD1 patients and the HP groups for the right and
500 left legs, before (dark fill-in) and after (white fill-in) the NMES training. Boxes represent
501 group median and interquartile range values and whiskers are the highest and lowest values.
502 Significant group differences $p<0.05$ (*) and $p<0.001$ (***)).

503 **Figure 2.** Box-plot of the percentage of force production loss (A, left panel) and of the RMS
504 of the tibialis anterior (TA) (B, right panel) during the 2-minute sustained ankle dorsiflexion
505 endurance exercise, for the right and left legs, before (dark fill-in) and after (white fill-in) the
506 8-week training for patients with facioscapulohumeral muscular dystrophy (FSHD1) and
507 healthy participants (HP). Boxes represent group median and interquartile range values and
508 whiskers are the highest and lowest values. Columns represent group mean values and error
509 bars the standard error of the group mean. Significant group differences: $p<0.05$ (*), $p<0.01$
510 (**).

511 **Figure 3** Box-plot of the manual muscle testing (MMT) of the dorsiflexion for both for legs
512 obtained before (dark fill-in) and after (white fill-in) the 8-week training period for
513 facioscapulohumeral muscular dystrophy (FSHD1). Boxes represent group median and
514 interquartile range values and whiskers are the highest and lowest values. Dashed lines
515 display individual data.

516 **Figure 4** Mean and standard error (mean \pm SE) of the stimulation intensity (mA) for the
517 FSHD1 (grey line) and the HP (black line) for the right (plain lines) and left (dashed lines)
518 throughout the 24 sessions of the 8-week NMES training.

1 **Title:** Short-term neuromuscular electrical stimulation training of the tibialis anterior did not
2 improve strength and motor function in facioscapulohumeral muscular dystrophy patients
3

4 **ABSTRACT**

5 **Objective:** To investigate the effects on motor function, muscle strength and endurance of
6 short term neuromuscular electrical stimulation (NMES) training of the *tibialis anterior* (TA)
7 muscles in patients with facioscapulohumeral muscular dystrophy type 1 (FSHD1) in
8 comparison with healthy controls.

9 **Design:** This prospective study included ten patients with FSHD1 and ten healthy participants
10 (HP). Maximal voluntary isometric contraction (MVC) of ankle dorsiflexion (DF) and a 2-
11 minute sustained DF MVC with surface electromyography recordings (sEMG) of the TA and
12 the soleus muscles were measured and motor function clinical tests were performed before
13 and after the training period.

14 **Results:** No significant short term training effect was found in any of the investigated
15 variables for either group, although a tendency towards an increase was noted for the manual
16 muscle testing of the FSHD1. Patients with FSHD1 showed lower MVC force and lower
17 maximal TA sEMG amplitude than HP. During the 2-minute sustained MVC, the percentage
18 of force loss was lower for the FSHD1 patients, suggesting that they were experiencing a
19 lower amount of muscle fatigue compared to the HP group.

20 **Conclusion:** The present NMES protocol was not strenuous enough and/or the parameters of
21 stimulation were not adequate to improve dorsiflexion strength, muscle endurance and motor
22 function in FSHD1 patients and HP.
23
24

25 **KEY WORDS:** Isometric strength; Muscle endurance; Electromyography; Neuromuscular
26 disorder
27

28 INTRODUCTION

29 With a European prevalence of 4/100,000, the facioscapulohumeral muscular dystrophy
30 (FSHD) is the most common inherited muscular dystrophy disease. The FSHD is genetically
31 heterogeneous and two types of FSHD (*i.e.*, FSHD1, 95% of patients and FSHD2, 5% of
32 patients) have been identified.¹ Independently of the type of FSHD (*i.e.*, 1 or 2), the disease is
33 characterized by a progressive asymmetric muscle weakness and atrophy usually spreading
34 from facial to shoulder girdle, arms, abdominal and lower limb muscles.² In addition to
35 muscle weakness, fatigue and pain are the two other most frequently reported symptoms. In
36 particular, severe fatigue, a major burden in daily life activities, is reported by 61% of patients
37 with FSHD³ conducting to a sedentary lifestyle through a reduced level of physical activity.⁴
38 The reduced level of muscle strength has been identified as a key factor in explaining low
39 level of physical activity and high experienced fatigue.⁵ In patients with FSHD1, *tibialis*
40 *anterior* muscles can be affected in earlier stages of the disease than other lower limb muscles
41 ^{6,7} and this decline in *tibialis anterior* function is frequently considered as the first disabling
42 symptom.⁸ Since the *tibialis anterior* has a strong functional role in gait and balance, both its
43 weakness and fatigue may lead to a loss of mobility and increase the risk of falling.⁴ Since no
44 therapeutic treatments are yet available for FSHD,² it is of interest to propose alternative
45 procedures to moderate the progressive loss of strength, endurance and muscle function.
46 Aerobic exercises have been proposed to improve muscle function in patients with FSHD, but
47 some studies failed to show improvements on strength of such training, even though no
48 deleterious effects were reported.^{9,10} Neuromuscular electrical stimulation (NMES) is another
49 type of exercise broadly used in rehabilitation settings.¹¹ When NMES training was performed
50 on patients suffering from disabling forms of muscular dystrophy, such as Duchenne and
51 Becker dystrophies, tolerance and efficacy were shown to maintain or even improve muscle
52 strength.¹²⁻¹⁵ Comparable results in the *tibialis anterior* and the quadriceps muscles were

53 reported in a group of mixed patients with neuromuscular disorders including patients with
54 FSHD.¹⁶ More recently, NMES training, performed on shoulder girdle and knee extensor
55 muscles, was found to be safe and effective in improving strength and muscle function in
56 patients with FSHD1.¹⁷ The two studies that have investigated the NMES training programs
57 in FSHD^{16,17} involved long training periods of 14 and 5 months respectively. Although
58 beneficial effects of short term (less than 8 weeks) NMES training programs on muscle
59 strength and/or endurance in healthy participants^{18,19} or patients with muscular dystrophy¹²
60 were found, such programs have not been implemented in patients with FSHD. Therefore, the
61 objective of this study was to investigate the effect of a bilateral 8-week NMES training on
62 the *tibialis anterior* muscle in adults with FSHD1. It was hypothesized to observe muscle
63 strength and endurance gains in dorsiflexion as well as improved motor function in patients
64 with FSHD1.

65

66 **METHODS**

67

68 **Participants**

69 Ten adults with FSHD1 (mean \pm standard deviation (SD): 5 females and 5 males; age $62.3 \pm$
70 10.2 year; height: 168.5 ± 12.8 cm; body mass: 73.7 ± 15.2 kg) and 10 healthy participants
71 (HP) age matched (7 females and 3 males; age 56 ± 4.8 year; height: 171.5 ± 9.01 cm; body
72 mass: 74.8 ± 12.4 kg) volunteered to take part in the study and written informed consent was
73 obtained from all participants. The study was carried out according to the Declaration of
74 Helsinki and approved by the local Institutional Human Ethics Committee. The trial was
75 declared.

76 Adults patients diagnosed with FSHD1 were recruited from the outpatient record of the
77 physical medicine and rehabilitation department at the hospital and were included into the

78 study according to the following criteria: number of 4q35 D4Z4 <11 repeats (mean of the
79 group 6.89 ± 1.37 units), no mutation on *SMCHD1* gene ; muscle weakness of ankle
80 dorsiflexion from 2 to 4 at least on one leg, assessed by manual muscle testing (MMT).²⁰
81 Exclusion criteria comprised previous NMES training of the lower-limb; history of cancer,
82 joints pathologies, or collagenopathies, parturient, or breast-feeding woman or simultaneous
83 participation to another research study.

84

85 **Neuromuscular electrical stimulation training**

86 Bilateral neuromuscular electrical stimulation (NMES) training sessions of the *tibialis*
87 *anterior* muscles were performed with a Compex (Rehab 400, Cefar-Compex, DJO France
88 SAS, Mouguerre, France) portable battery-powered stimulator. Participants either exercised at
89 home or were trained by one of the experimenters or a physiotherapist. All healthy
90 participants as well as four patients with FSHD1 carried out their training sessions at home. In
91 the case participants trained at home, a weekly appointment was set-up with one of the
92 experimenters to provide feedback and to control the quality of the training. During these
93 training sessions participants were seated (hips, knees and ankles angles at 90°) with their feet
94 fixed. During the training sessions, the participants were instructed to place comfortably their
95 feet under a heavy-weighted object so that the feet would be firmly stuck and would not move
96 during the contractions. The participants were simultaneously stimulated bilaterally with self-
97 adhesive electrodes (2 mm thick) made of elastomer (5 cm x 5 cm) that were positioned as
98 follows: the positive electrode was placed on the superior part of the muscle, whereas the
99 negative electrode was placed on the medial part of the muscle, over the muscle bulk.

100 The NMES training program lasted for 8 weeks, with 3 sessions a week. Each session was
101 composed of a 2-minute warm-up, followed by the 20-minute working out session, and
102 finishing with 3 minutes of relaxation. The NMES program consisted in isometric

103 contractions of 9s (rise time: 1.5s; steady tetanic stimulation time: 6s; fall time: 1.5s) followed
104 by a pause lasting 7 seconds (duty cycle: 56.25%) at 35 Hz and with a 200 μ s pulse-width.
105 These stimulation parameters were chosen accordingly to previous successful NMES using
106 low-frequency protocols in patients with neuromuscular disorders to increase muscle strength.
107 ¹²⁻¹⁷ Participants were encouraged to increase stimulation intensity progressively every 5
108 minutes throughout each session up to individual tolerance threshold (i.e., discomfort/pain)
109 since strength gains would be dependent on the stimulation intensity.^{21,22} As individual
110 tolerance threshold varied among participants, they were instructed to increase progressively
111 stimulation intensity during the warm-up period to ensure a visible muscle contraction.
112 However, since the feet were secured, no joint movement was induced. Moreover, during
113 each of the training sessions, the participants or the physiotherapist, according to the training
114 modality (i.e., supervised or at home) had to fill-out a questionnaire consisting in reporting the
115 following: date and time of the training session; the mean intensity of the neuromuscular
116 electrical stimulation delivered; self-evaluation of their perceived fatigue after the training
117 session; the discomfort related to the NMES delivered during training sessions. Visual
118 analogue scales (VAS) were used to score perceived fatigue and discomfort. A score of 0 mm
119 indicated no fatigue or no discomfort and 100 mm indicated unbearable fatigue or maximum
120 discomfort.

121

122 **Study design**

123 To disclose the effect of the 8-week NMES training, at pre and post training, the participants
124 had first a blood sample collection, followed by a clinical examination and neuromuscular
125 tests. Following this, they answered a questionnaire during a 30 min period of rest and then
126 performed a 6-min walk test (6MWT).

127

128 *Blood samples collection*

129 To establish tolerance to NMES training, Plasma Creatine Kinase (CK) was measured after
130 the fourth week (W4), and once randomly during the training in addition to before and after
131 the 8-week training period (W8). Blood samples were collected and analyzed at the hospital.
132 The first and last CK measurements were determined at rest, whereas the 4-week and the
133 random test were performed within two hours following the NMES training. Plasma CK
134 activity was determined spectrophotometrically by an automatic analyzer using a test kit
135 (Roche/Hitachi Automated Clinical Chemistry Analyzer, Modular P-800, Roche Diagnostics,
136 Meylan, France). The CK activity was considered as a biological marker of training-induced
137 damage for each participant.

138

139 *Clinical examination*

140 Muscle function and strength of the TA were assessed manually by a physiotherapist, by
141 manual muscle testing - MMT.²⁰ Depending on the amplitude of the ankle dorsiflexion,
142 without extension of the hallux, scores were ranked from 0 where the muscle is no longer
143 capable of force production to 5 representing the absence of muscle impairment.

144 The Motor Function Measurement (MFM) assessed the functional capacity of daily life
145 activities: standing still, weight transfers, sitting, proximal and distal motor ability of muscles,
146 walking, standing up, raising up arms, stepping up stairs, brush hairs *etc.* After evaluation, the
147 total score was presented as a percentage, with healthy participants reaching 100%.²³ The
148 physiotherapist performing the clinical examination was experienced to assess patients
149 suffering from neuromuscular disorders and was not blinded to the evaluation.

150

151 *Neuromuscular tests*

152 Maximal voluntary contractions (MVC) of the dorsiflexor muscles were carried out
153 unilaterally in a custom made device ²⁴ consisting of a fixed footplate, where the foot was
154 firmly strapped to avoid any movement and ensure the quality of the isometric force
155 measurement. A strain-gauged transducer (model OMF06M, linear range 0-15 kN, precision
156 $\pm 0.5 \%$, sensitivity 10 mV/kN; OMICRON, Gambais, France) was placed on the footplate to
157 measure force production. During all contractions, the participants were seated on a chair,
158 with their knee slightly flexed according to the comfort of the participant. The foot was firmly
159 tightened with belts over the footplate with an ankle angle of 90°. The participants were not
160 constrained and were allowed to seat as comfortably as they could, however, during MVCs,
161 they were not allowed to hold the seat and were asked to remain as steady as they could.

162 Bipolar surface electromyography (sEMG) electrodes (10 mm diameter, 20 mm inter-
163 electrode distance) recorded the electrical activity of the tibialis anterior (TA) and the soleus
164 (SOL) muscles. The reference electrode was placed on the bony part of the contralateral
165 patella. Skin was cleaned and abraded prior to the placement of electrodes, and low resistance
166 impedance between electrodes ($<5 \text{ k}\Omega$) was obtained. A Biopac MP 150 system (Biopac
167 systems, Inc., Holliston, MA, USA) was used to record sEMG data at a sampling rate of 2000
168 Hz. Electromyographic signals were amplified with a bandwidth frequency ranging from 1 Hz
169 to 500 Hz (common mode rejection ratio = 11 dB; impedance input = 1000 MV; gain = 1000).

170

171 The tests were performed on both legs, one at a time, in a random order with at least 10
172 minutes of rest period between each leg. Two MVCs of dorsiflexion were performed on each
173 leg to determine the maximal strength production and the concomitant sEMG signals of both
174 the TA and the SOL muscles. A 60-second rest was allowed between each contraction. Then,
175 a fatiguing task consisting of an isometric 2-minute MVC ²⁵ of dorsiflexion was performed
176 with recordings of the sEMG of TA and SOL muscles. No visual feedback was provided to

177 the participants and they were asked to perform an all-out effort while they received strong
178 verbal encouragement.

179

180 *Questionnaire and 6-min walk test*

181 Quality of life of the participants was evaluated with the Medical Outcomes Study Short-
182 Form 36 (SF-36) questionnaire.²⁶ A 6-min walk test (6MWT) was used to assess the greatest
183 distance participants could walk in 6 minutes on a 20-meter shuttle.

184

185

186 **Data analysis**

187 The MVC was considered as the mean value over a 500-ms period around the peak force. The
188 best of the two trials was analyzed. During the fatiguing task, the percentage of MVC loss
189 was calculated as the difference of a 1-second window width at the start and a 1-second
190 window width at the end of the 2-minute MVCs. All sEMG data were analyzed over the same
191 window width as the force data where the root mean square (RMS) of the TA and the SOL
192 (TA RMS and SOL RMS) was quantified and the loss in TA RMS was computed. RMS was
193 calculated with commercially available software (AcqKnowledge 4.1, Biopac Systems, Inc.,
194 Holliston, MA, USA), while the rest of the outcome measures were analyzed with Matlab
195 R2010b (The MathWorks, Inc., Natick, MA, United-States).

196

197 **Statistical analysis**

198 Statistical processing was performed using Statistica[®] software for Microsoft Windows
199 (StatSoft, version 8.0, Tulsa, OK, USA). The Shapiro-Wilk test was used to test whether
200 outcome measures were normally distributed, and depending on the results the appropriate
201 statistical test was performed. When data were normally distributed, an unpaired Student t-test
202 was performed to compare FSHD1 and HP groups and a paired Student t-test was used to
203 disclose training-induced changes within group (FSHD1 and HP). When data did not follow a
204 normal distribution, equivalent non-parametric tests, the Mann-Whitney *U* test and the
205 Wilcoxon signed ranks test were performed. A two-way ANOVA (leg × time) with repeated
206 measures on time was performed on the intensity values recorded during each training
207 session, while the Friedman ANOVA was applied for the discomfort and fatigue VAS values
208 as they did not follow a normal distribution. In all statistical analysis the significance level
209 was set at $p < 0.05$. Unless specified, normal distributed data are expressed as means ± SD
210 (standard deviation of the mean), in the entire manuscript and in the tables and figures, while

211 non-normally distributed data are expressed as median \pm IQR (inter quartile range) in tables
212 and box-plots are used in figures.

213

214 **RESULTS**

215 **FSHD1 patients and healthy participants before the training period**

216 The plasma CK concentration was higher in FSHD1 patients before the training period
217 ($t=4.38$; $p<0.001$; Table 1). The MMT (Right: $U=5.0$; $p<0.001$; Left: $U=0.0$; $p<0.001$;) and
218 MFEM scores of the FSHD1 patients were significantly impaired compared to the HP before
219 the training period ($U=0.0$; $p<0.001$; Table 1). Similarly, the distance covered during the
220 6MWT by the FSHD1 patients was shorter compared to the HP ($t=-2.63$; $p=0.02$; Table 1).
221 Lastly, quality of life assessed by means of SF-36 questionnaire (Table 2) revealed lower
222 values of FSHD1 patients compared to the HP for the following subscores: physical
223 functioning ($U=6.5$; $p<0.001$), social functioning ($U=18.0$; $p<0.05$), vitality ($U=14.5$; $p<0.05$),
224 general health ($U=5.0$; $p<0.001$) and the standardized physical component ($U=7.0$; $p<0.01$).

225

226 *Neuromuscular tests and fatiguing task*

227 As illustrated in the Figure 1A, the peak force during ankle dorsiflexion MVC was
228 significantly lower in FSHD patients than in HP prior to the training period for both legs
229 (Right, Pre: $U=88.0$; $p<0.001$; Left, Pre: $U=102.0$; $p<0.001$). The associated amplitude of the
230 TA RMS during dorsiflexion MVC (Figure 1B) was lower in the FSHD1 patients compared
231 to the HP before (Right: $U=95.0$; $p<0.001$; Left: $U=90.0$; $p=0.013$) the training period. In
232 addition, the amplitude of the SOL RMS during dorsiflexion MVC was found lower in the
233 FSHD1 patients before on the right leg ($U=85.0$; $p=0.007$), but not on the left leg ($p=0.282$).
234 Although not significantly different for the left leg ($p=0.095$), the group of patients with
235 FSHD1 exhibited a lower force reduction during the 2-minute MVC than the HP group before

236 the training period on the right leg (Right: $U=10.0$; $p=0.003$; Figure 2A). No difference in
237 percentage of TA RMS and SOL RMS reduction between groups was found before the
238 training period for either leg ($p>0.05$).

239

240 **Effects of NMES training in FSHD1 patients and healthy participants**

241 Individual patient/healthy participant's compliance to the training program was maximal (i.e.,
242 100% of the scheduled training sessions attended). Whatever the group considered, plasma
243 CK did not change significantly during the NMES training period ($p>0.05$). The NMES
244 training did not modify the SF-36 questionnaire subscores and the values of the FSHD1
245 patients remained lower than those of the HP (Table 2). Also, no significant changes of the
246 MFM and 6MWT assessments were observed after the training period ($p>0.05$) and the values
247 of the FSHD1 patients remained lower than those of the HP (Table 1). Although no
248 significant differences were found, the MMT values of the FSHD1 patients tended to slightly
249 increase after the training period ($p=0.067$; Figure 3) for both the right and left legs. Also,
250 when considering exclusively legs (i.e., fourteen legs) matching the inclusion criterion of
251 having a MMT score of ankle dorsiflexion comprised between 2 to 4, a significant training
252 effect was observed ($p=0.027$; data not illustrated).

253

254 *Neuromuscular tests and fatiguing task*

255 No significant training effect was found in dorsiflexion strength ($p>0.05$; Figure 1A) and in
256 the associated RMS amplitude of the TA ($p>0.05$; Figure 1B) and the SOL muscles in either
257 group for both sides. All these variables of the FSHD1 patients remained lower than those of
258 the HP (Figures 1 and 2).

259 The force reduction during the 2-minute MVC did not changed after the training period for
260 both legs in the FSHD1 patients and HP ($p>0.05$; Figure 2A). Although no significant

261 difference was found for the left leg, the TA RMS reduction of the right leg during the 2-
262 minute MVC was significantly greater after the training period for the FSHD1 patients
263 ($t=3.33$; $p>0.05$; Figure 2B). No significant change was noted for the HP. Also, no change of
264 the SOL RMS reduction was found after the training period for either leg or group.

265

266 *Characteristics of the NMES training program*

267 As illustrated in the Figure 4, the stimulation intensity of the NMES training was significantly
268 increased for the FSHD1 groups on both legs ($F=1.89$; $p<0.05$) as well as for the HP ($F=3.26$;
269 $p<0.001$). The self-reported evaluation of the discomfort level throughout the training reduced
270 on the right leg only in the group of patients with FSHD1 ($\chi^2=36.1$; $p=0.041$; Left leg:
271 $\chi^2=28.2$; $p=0.208$), whereas no change was observed for the group of healthy participants
272 ($p>0.05$). Likewise, the self-reported fatigue level reduced significantly in the group of
273 patients with FSHD1 along the training only in the left leg (Left leg: $\chi^2=40.3$; $p=0.014$; Right
274 leg: $\chi^2=34.3$; $p=0.061$). No change was observed for either leg in the HP group ($p>0.05$).

275

276 **DISCUSSION**

277 The purpose of this study was to investigate whether a short term bilateral NMES training on
278 the *tibialis anterior* muscles in adults with FSHD1 would be well tolerated and would
279 improve muscle strength, endurance and motor function. All participants completed the 8-
280 week NMES training program and no side effects were reported during or after the training
281 period. Unfortunately, this program did not improve ankle dorsiflexion maximal muscle
282 strength, nor muscle endurance or motor function in patients with FSHD1, although a
283 tendency towards an increase was observed for the MMT scores. Also, no significant
284 improvements were noted for the healthy participants.

285 All the participants (*i.e.*, FSHD1 patients and HP) completed the NMES training program and
286 according to the CK measurements, no rhabdomyolysis was induced by the NMES protocol.
287 Also, discomfort and fatigue VAS values reported throughout the protocol remained clinically
288 low (mean VAS<2) and did not significantly increase during the 8-week period. These results
289 agree with previous studies investigating the effects of NMES training programs in
290 neuromuscular diseases¹⁷ and confirm that NMES exercise is well tolerated by FSHD1
291 patients and HP.

292 Although well tolerated, this study failed to show a positive significant effect of NMES on
293 muscle strength, muscle endurance and motor function of the ankle dorsiflexor muscles in
294 FSHD1 patients, as well as in the HP. These observations are somehow surprising considering
295 that benefits of NMES training programs on muscle strength are widely reported in literature
296 for similar, or even shorter training periods in healthy participants^{18,19}. In addition, the use of
297 NMES was shown to be effective in patients with muscular dystrophies¹²⁻¹⁵ and in patients
298 with FSHD1.^{16,17}

299 Although, the characteristics of stimulation parameters used here (35 Hz of frequency and
300 200- μ s pulse duration) may be questioned, these NMES parameters were chosen since they
301 were successfully used in previous rehabilitation settings in patients with muscular
302 dystrophies.^{13,17} However, FSHD1 patients can have fat infiltration in TA muscles^{6,27} and
303 strong alteration of the sarcomeric contractile properties, preferentially of type II fibers,^{28,1}
304 reducing the overall capacity of the muscle to contract. Nevertheless, this reason cannot
305 account for the absence of improvements in the HP. Considering that frequencies above 50 Hz
306 are suggested to maximize the training effect of NMES on muscle strength in healthy
307 participants,²² it may be suggested that these frequencies should be considered in future
308 NMES studies in FSHD1 patients.

309 The lack of significant improvements could also be attributable to the frequency of the
310 sessions and/or the duration of the training. Only three sessions per week for eight weeks
311 were performed, whereas Colson et al.¹⁷ trained their FSHD1 patients five days per week over
312 a 5-month training period. Similarly, Milner-Brown and Miller¹⁶ obtained significant
313 improvement in strength after a 2-hour session performed 5 days per week during 14 months.
314 Therefore, a higher volume training period (greater number of sessions or duration) might be
315 required to obtain significant strength improvements in FSHD1 patients. Finally, as
316 previously suggested, the stimulation intensity was constantly increased throughout the
317 training period to ensure strength adaptations.²² However, this stimulation intensity increment
318 might have been too moderate to improve muscle strength. Therefore, it seems that the main
319 reason for the absence of strength increase in FSHD1 patients has to be related to the
320 frequency and/volume of the NMES sessions. Moreover, in the FSHD1 patients, the impaired
321 muscle function of the TA at the beginning of the study can be suggested as a possible reason
322 candidate for the training to be ineffective. Since no training effect was seen in the HP group
323 either, this cannot be stated with certitude. Finally, the *soleus* muscle activity of the right leg
324 during the dorsiflexion was found to be of lower magnitude compared to the left leg for the
325 FSHD1 patients before the training. This observation confirms that important imbalance exists
326 between limbs (i.e., asymmetric muscle weakness) and that particular neuromuscular
327 adjustments/compensations could occur with the disease in order to maintain functional
328 movements. These neuromuscular imbalances and their influence on functional daily tasks
329 such as balance/walking should be further investigated in FSHD patients.

330 Interestingly and although it did not change with the training period, the MVC loss was much
331 lower in the FSHD1 patients than in the HP during the 2-minute fatiguing task. This may
332 indicate that patients with FSHD1 experienced a lower amount of muscle fatigue compared to
333 the HP,²⁵ before and after the training, likely for several reasons. First, as the amplitude of the

334 TA EMG RMS reduced similarly, this suggests that the neural drive to the muscles would
335 have become suboptimal with fatigue for both groups in the same fashion during the fatiguing
336 exercise.²⁹ Second, patients with FSHD1 have strong alteration of the sarcomeric contractile
337 properties of type II fibers,^{1,28} which could lead to muscles more resistant to fatigue. Third,
338 weaker participants are shown to be less fatigable than stronger ones,³⁰ as the intramuscular
339 pressure is lower the negative feedback from afferent groups III and IV is therefore
340 diminished.³⁰ Even though, the group of patients with FSHD1 showed a greater TA EMG
341 RMS loss after the training in comparison to the loss before the training, this decrease was
342 similar in both groups. Since the patients group showed lower force losses, it can be suggested
343 that at the task truncation, the group of patients with FSHD were experiencing a lower amount
344 of muscle fatigue compared to the HP group.

345

346 Study limitations

347 A limitation of this study is related to the low number of FSHD1 patients and HP included.
348 The reasons may be as follows: i) the pool of patients with FSHD1 is rather low and/or
349 geographically spread, ii) it is unlikely to include enough patients with FSHD1 that have the
350 identical muscle weakness as well as possible matching response to the training program.
351 Nevertheless, all the participants completed the NMES training sessions scheduled. Second,
352 the heterogeneity of the adaptations to the NMES training program may have been too
353 important to highlight specific adaptations within each group. Also, no FSHD1 control group
354 (i.e., FSHD 1 patients not receiving NMES) was included in order to assess the possible
355 changes of measured variables due to the progression of the disease during the 8-week period.
356 Then, although the reliability of strength measurements is often questionable in fragile
357 populations, the measurements seemed to be sufficiently accurate since interclass correlation
358 coefficient for the ankle dorsiflexion MVC ranged from 0.96 (right leg) to 0.98 (left leg) and

359 from 0.87 (right leg) to 0.93 (left leg) for the associated TA EMG RMS values.
360 Notwithstanding these limitations, the present results may be useful to assist clinicians to plan
361 in the design of rehabilitation programs with the use of NMES in FSHD1 patients. For
362 example, in light of the present results and based on the literature, we proposed that future
363 clinical studies should design NMES training programs including between three to five
364 training sessions per week (for a muscle group) with frequencies ranging from above 50 Hz
365 with a pulse duration ranging from 100 μ s to 500 μ s for a minimum duration of 20 to 30
366 minutes (duty cycle ~50%). Although the exact training duration period has yet to be
367 determined, a minimum of three months seemed required to expect positive adaptations.

368

369 **CONCLUSION**

370 In summary, the present results show that an 8-week bilateral NMES training (20 minutes per
371 session, 3 sessions per week) of the tibialis anterior muscle did not improve muscle strength,
372 endurance and motor function in patients with FSHD1. Whether this non-responsiveness is
373 due to the impaired neuromuscular function of the ankle dorsiflexor muscles and/or to the
374 duration of the NMES protocol or to the stimulation intensity level reached during the NMES
375 sessions still remain to be shown. As suggested by Colson et al.¹⁷, it is likely that the efficacy
376 of the NMES training would depend on rapidity of starting NMES training as soon as the
377 FSHD1 diagnosis is made to maximize the training effects.

378

379

380 **REFERENCES**

381

- 382 1. Lassche S, Stienen GJM, Irving TC, et al. Sarcomeric dysfunction contributes to
383 muscle weakness in facioscapulohumeral muscular dystrophy. *Neurology*.
384 2013;80(8):733-737.
- 385 2. Tawil R, Van Der Maarel SM. Facioscapulohumeral muscular dystrophy. *Muscle*
386 *Nerve*. 2006;34(1):1-15.
- 387 3. Kalkman JS, Schillings ML, van der Werf SP, et al. Experienced fatigue in
388 facioscapulohumeral dystrophy, myotonic dystrophy, and HMSN-I. *J Neurol*
389 *Neurosurg Psychiatry*. 2005;76(10):1406-1409.
- 390 4. McDonald CM. Physical activity, health impairments, and disability in neuromuscular
391 disease. *Am J Phys Med Rehabil*. 2002;81(11 Suppl):S108-S120.
- 392 5. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BGM, Bleijenberg G. The
393 development of a model of fatigue in neuromuscular disorders: A longitudinal study. *J*
394 *Psychosom Res*. 2007;62(5):571-579.
- 395 6. Olsen DB, Gideon P, Jeppesen TD, Vissing J. Leg muscle involvement in
396 facioscapulohumeral muscular dystrophy assessed by MRI. *J Neurol*.
397 2006;253(11):1437-1441.
- 398 7. Dorobek M, Szmidt-Sałkowska E, Rowińska-Marcińska K, Gaweł M, Hausmanowa-
399 Petruszewicz I. Relationships between clinical data and quantitative EMG findings in
400 facioscapulohumeral muscular dystrophy. *Neurol Neurochir Pol*. 2013;47(1):8-17.
- 401 8. Pastorello E, Cao M, Trevisan CP. Atypical onset in a series of 122 cases with
402 FacioScapuloHumeral Muscular Dystrophy. *Clin Neurol Neurosurg*. 2012;114(3):230-
403 234.
- 404 9. van der Kooi EL, Vogels OJ, van Asseldonk RJ, et al. Strength training and albuterol in

- 405 facioscapulohumeral muscular dystrophy. *Neurology*. 2004;63(4):702-708.
- 406 10. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC.
407 Strength training and aerobic exercise training for muscle disease. *Cochrane database*
408 *Syst Rev*. 2010;(1):CD003907.
- 409 11. Maddocks M, Gao W, Higginson IJ, Wilcock A. Neuromuscular electrical stimulation
410 for muscle weakness in adults with advanced disease. *Cochrane database Syst Rev*.
411 2013;1(1):CD009419.
- 412 12. Scott OM, Vrbová G, Hyde S a, Dubowitz V. Responses of muscles of patients with
413 Duchenne muscular dystrophy to chronic electrical stimulation. *J Neurol Neurosurg*
414 *Psychiatry*. 1986;49(12):1427-1434.
- 415 13. Scott OM, Hyde SA, Vrbová G, Dubowitz V. Therapeutic possibilities of chronic low
416 frequency electrical stimulation in children with Duchenne muscular dystrophy. *J*
417 *Neurol Sci*. 1990;95(2):171-182.
- 418 14. Zupan A. Long-term electrical stimulation of muscles in children with Duchenne and
419 Becker muscular dystrophy. *Muscle Nerve*. 1992;15(3):362-367.
- 420 15. Zupan A, Gregoric M, Valencic V, Vandot S. Effects of electrical stimulation on
421 muscles of children with Duchenne and Becker muscular dystrophy. *Neuropediatrics*.
422 1993;24(4):189-192.
- 423 16. Milner-Brown HS, Miller RG. Muscle strengthening through electric stimulation
424 combined with low-resistance weights in patients with neuromuscular disorders. *Arch*
425 *Phys Med Rehabil*. 1988;69(1):20-24.
- 426 17. Colson SS, Benchortane M, Tanant V, et al. Neuromuscular electrical stimulation
427 training: a safe and effective treatment for facioscapulohumeral muscular dystrophy
428 patients. *Arch Phys Med Rehabil*. 2010;91(5):697-702.
- 429 18. Thériault R, Boulay MR, Thériault G, Simoneau JA. Electrical stimulation-induced

- 430 changes in performance and fiber type proportion of human knee extensor muscles. *Eur*
431 *J Appl Physiol.* 1996;74(4):311-317.
- 432 19. Colson SS, Martin A, Van Hoecke J. Effects of electromyostimulation versus voluntary
433 isometric training on elbow flexor muscle strength. *J Electromyogr Kinesiol.*
434 2009;19(5): e311-319.
- 435 20. Lacôte M, Chevalier A-M, Miranda A, Bleton JP. *Évaluation Clinique de La Fonction*
436 *Musculaire.* Maloine; 2008.
- 437 21. Maffiuletti NA. Physiological and methodological considerations for the use of
438 neuromuscular electrical stimulation. *Eur J Appl Physiol.* 2010;110(2):223-234.
- 439 22. Filipovic A, Kleinöder H, Dörmann U, Mester J. Electromyostimulation--a systematic
440 review of the influence of training regimens and stimulation parameters on
441 effectiveness in electromyostimulation training of selected strength parameters. *J*
442 *Strength Cond Res.* 2011;25(11):3218-3238.
- 443 23. Bérard C, Payan C, Hodgkinson I, Fermanian J, MFM Collaborative Study Group. A
444 motor function measure for neuromuscular diseases. Construction and validation study.
445 *Neuromuscul Disord NMD.* 2005;15(7):463-470.
- 446 24. Simoneau EM, Billot M, Martin A, Van Hoecke J. Antagonist mechanical contribution
447 to resultant maximal torque at the ankle joint in young and older men. *J Electromyogr*
448 *Kinesiol.* 2009;19(2):e123-e131.
- 449 25. Schillings ML, Kalkman JS, Janssen HMHA, van Engelen BGM, Bleijenberg G,
450 Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. *Clin*
451 *Neurophysiol.* 2007;118(2):292-300.
- 452 26. Brazier JE, Harper R, Jones NM, et al. Validating the SF-36 health survey
453 questionnaire: new outcome measure for primary care. *BMJ.* 1992;305(6846):160-164.
- 454 27. Kan HE, Klomp DWJ, Wohlgemuth M, et al. Only fat infiltrated muscles in resting

- 455 lower leg of FSHD patients show disturbed energy metabolism. *NMR Biomed.*
456 2010;23(6):563-568.
- 457 28. D'Antona G, Brocca L, Pansarasa O, Rinaldi C, Tupler R, Bottinelli R. Structural and
458 functional alterations of muscle fibres in the novel mouse model of
459 facioscapulohumeral muscular dystrophy. *J Physiol.* 2007;584(3):997-1009.
- 460 29. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev.*
461 2001;81(4):1725-1789.
- 462 30. Hunter SK, Enoka RM. Sex differences in the fatigability of arm muscles depends on
463 absolute force during isometric contractions. *J Appl Physiol.* 2001;91(6):2686-2694.
464
465

466 **FIGURES LEGENDS**

467 **Figure 1A** Box-plots of the dorsiflexion Maximal Voluntary Contraction (N) for the FSHD1
468 patients and the HP groups for the right and left legs, before (dark fill-in) and after (white fill-
469 in) the NMES training. Boxes represent group median and interquartile range values and
470 whiskers are the highest and lowest values. Significant group differences $p<0.001$ (***)).

471 **Figure 1B** Box-plots of the RMS amplitude of the Tibialis Anterior during the dorsiflexion
472 Maximal Voluntary Contraction for the FSHD1 patients and the HP groups for the right and
473 left legs, before (dark fill-in) and after (white fill-in) the NMES training. Boxes represent
474 group median and interquartile range values and whiskers are the highest and lowest values.
475 Significant group differences $p<0.05$ (*) and $p<0.001$ (***)).

476 **Figure 2.** Box-plot of the percentage of force production loss (A, left panel) and of the RMS
477 of the tibialis anterior (TA) (B, right panel) during the 2-minute sustained ankle dorsiflexion
478 endurance exercise, for the right and left legs, before (dark fill-in) and after (white fill-in) the
479 8-week training for patients with facioscapulohumeral muscular dystrophy (FSHD1) and
480 healthy participants (HP). Boxes represent group median and interquartile range values and
481 whiskers are the highest and lowest values. Columns represent group mean values and error
482 bars the standard error of the group mean. Significant group differences: $p<0.05$ (*), $p<0.01$
483 (**).

484 **Figure 3** Box-plot of the manual muscle testing (MMT) of the dorsiflexion for both for legs
485 obtained before (dark fill-in) and after (white fill-in) the 8-week training period for
486 facioscapulohumeral muscular dystrophy (FSHD1). Boxes represent group median and
487 interquartile range values and whiskers are the highest and lowest values. Dashed lines
488 display individual data.

489 **Figure 4** Mean and standard error (mean \pm SE) of the stimulation intensity (mA) for the
490 FSHD1 (grey line) and the HP (black line) for the right (plain lines) and left (dashed lines)
491 throughout the 24 sessions of the 8-week NMES training.

Table 1. Mean and standard deviation (mean \pm SD) of the plasma Creatine Kinase (CK) values obtained before training (Pre), after the fourth week (Mid), after the 8-week training period (Post) and during the random measurement made during the training period after the training session (Random), as well as the motor function measurement (MFM) and the 6-min walk test performance obtained before (Pre) and after (Post) the 8-week training period for facioscapulohumeral muscular dystrophy (FSHD1) and healthy participants (HP).

CK (U/L)	FSHD1	HP
CK (U/L)		
<i>Pre</i>	213.1 \pm 46.7	118.6 \pm 38.3***
<i>Mid</i>	185.5 \pm 52.7	131.4 \pm 49.7
<i>Post</i>	208.3 \pm 48.5	124.8 \pm 40.6***
<i>Random</i>	205.8 \pm 32.4	119.9 \pm 37.4***
MFM		
<i>Pre</i>	68.86 \pm 19.35	100.0 \pm 0.0***
<i>Post</i>	66.97 \pm 17.42	100.0 \pm 0.0***
6-min walk test (m)		
<i>Pre</i>	309.67 \pm 132.14	462.22 \pm 113.66*
<i>Post</i>	311.11 \pm 126.88	475.25 \pm 131.07*

Significantly different from FSHD1: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 2. Medical Outcomes Study Short-Form 36 (SF-36) scores obtained before (Pre) and after (Post) the 8-week training period for patients with facioscapulohumeral muscular dystrophy (FSHD1) and healthy participants (HP).

	FSHD1		HP	
	Pre	Post	Pre	Post
<i>SF-36 subscores</i>				
Physical Functioning	43.0 ± 30.1	35.0 ± 22.2	92.5 ± 12.8***	96.5 ± 6.7
Physical role	70.0 ± 32.9	71.9 ± 41.1	92.5 ± 23.8	90.0 ± 21.1
Bodily pain	57.5 ± 23.6	52.6 ± 24.9	80.0 ± 23.6	80.9 ± 23.1
Mental Health	75.6 ± 14.9	73.0 ± 16.1	84.0 ± 13.7	81.6 ± 20.0
Emotional role	96.3 ± 11.1	70.8 ± 45.2	100.0 ± 0.0	100.0 ± 0.0
Social Functioning	75.0 ± 21.5	75.0 ± 22.2	97.5 ± 7.9*	93.8 ± 15.9
Vitality (Energy/Fatigue)	48.9 ± 20.4	47.3 ± 17.8	74.0 ± 16.6*	72.5 ± 22.9
General Health	59.9 ± 14.9	50.4 ± 17.6	87.4 ± 11.9***	87.4 ± 15.6
Health Change	50.0 ± 28.9	43.8 ± 17.7	57.5 ± 23.7	57.5 ± 16.9
Standardized physical component	34.9 ± 11.5	34.8 ± 7.9	53.1 ± 6.2**	53.4 ± 5.3
Standardized mental component	55.8 ± 3.7	52.0 ± 10.4	56.6 ± 5.3	54.9 ± 9.3

Significantly different from FSHD1: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Figure 1A

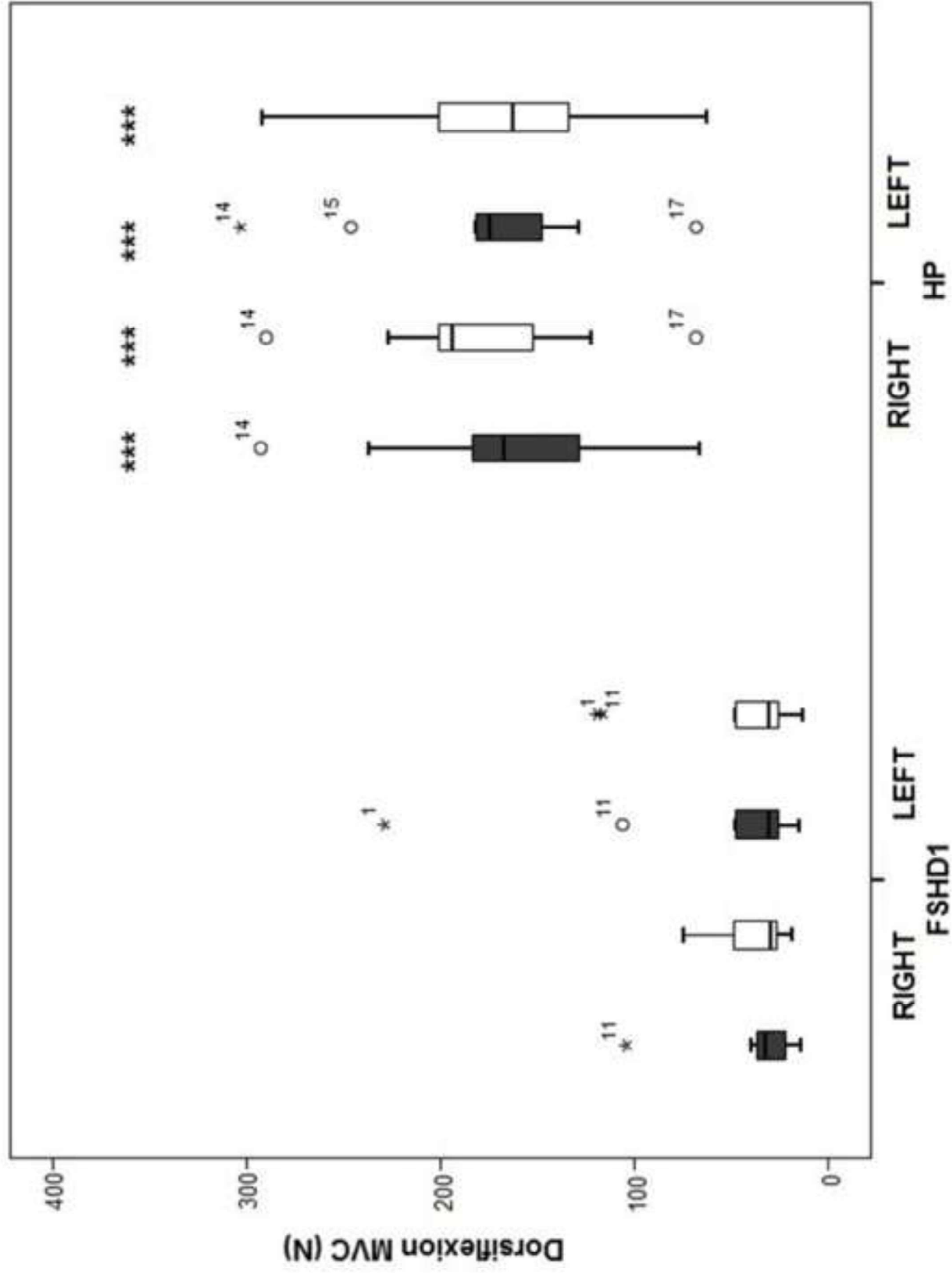


Figure 1B

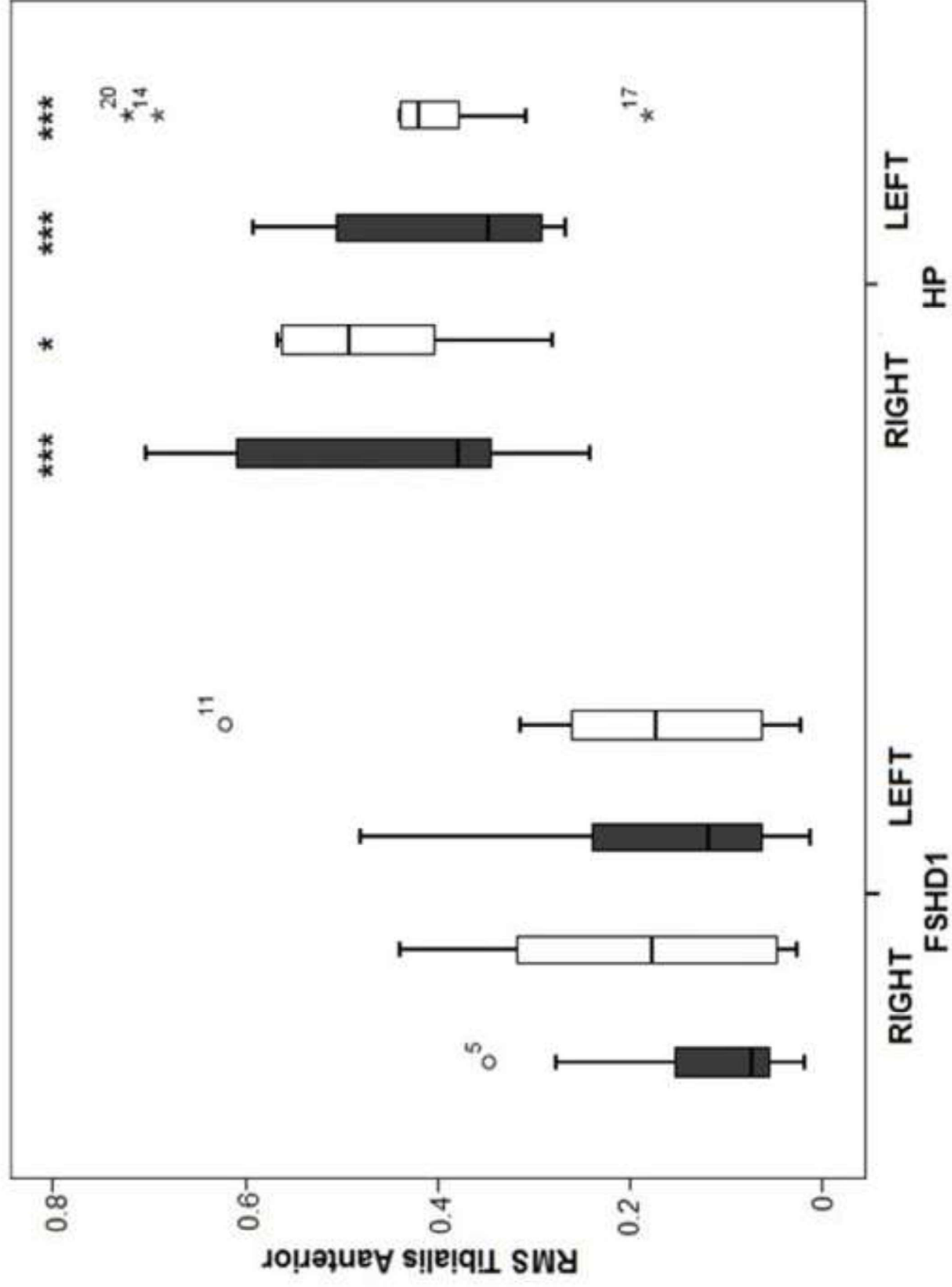


Figure 2A

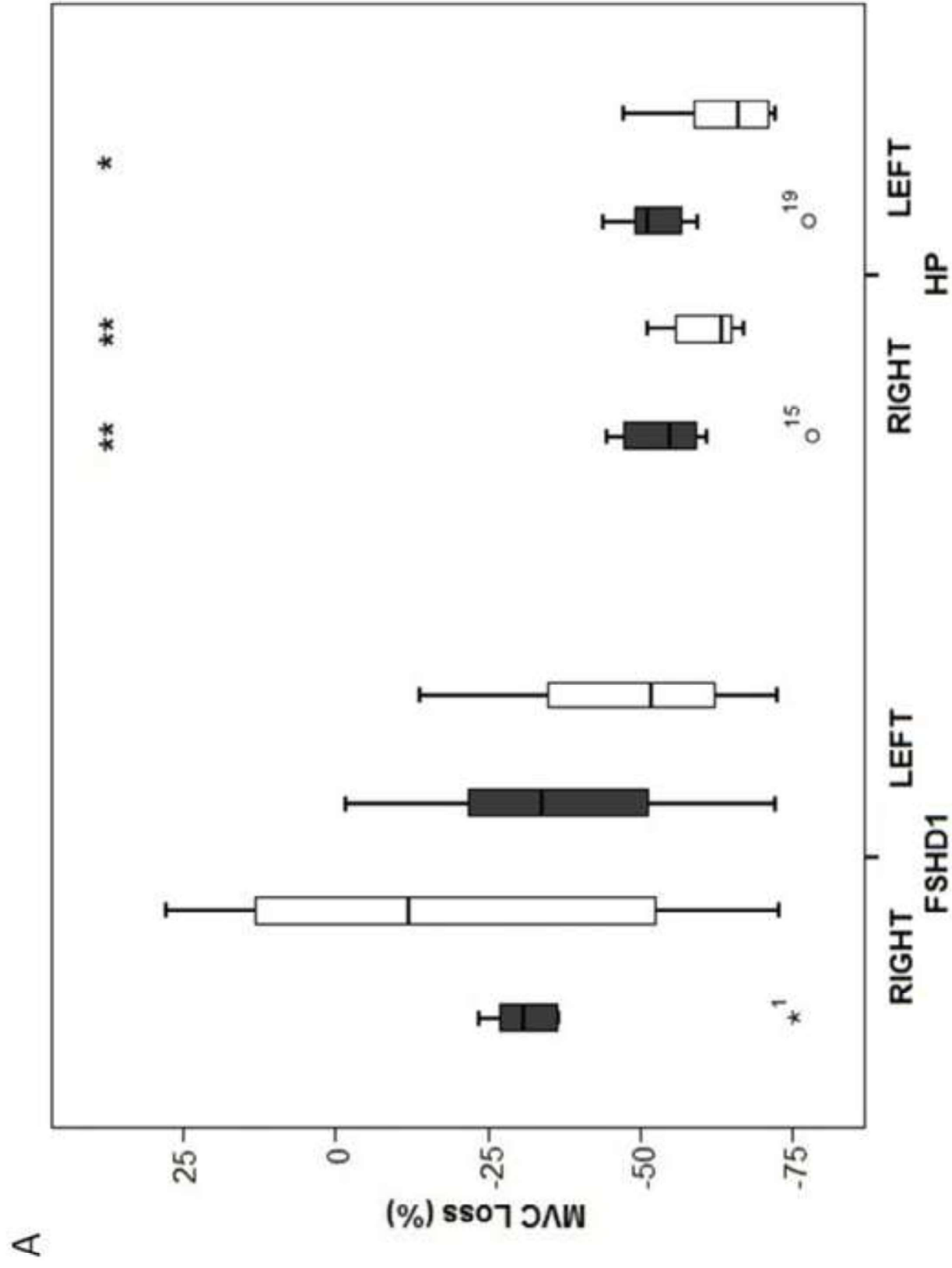
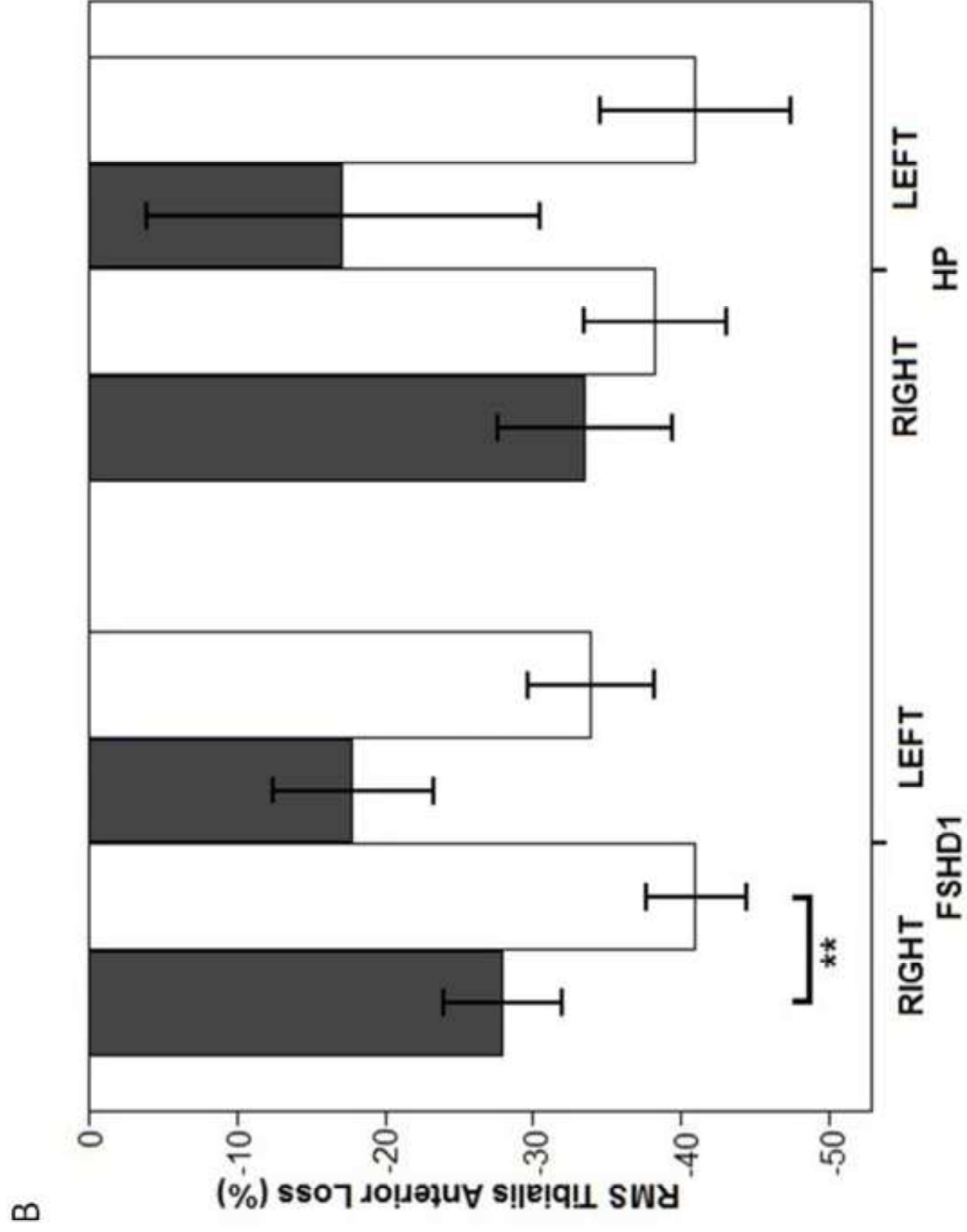


Figure 2B



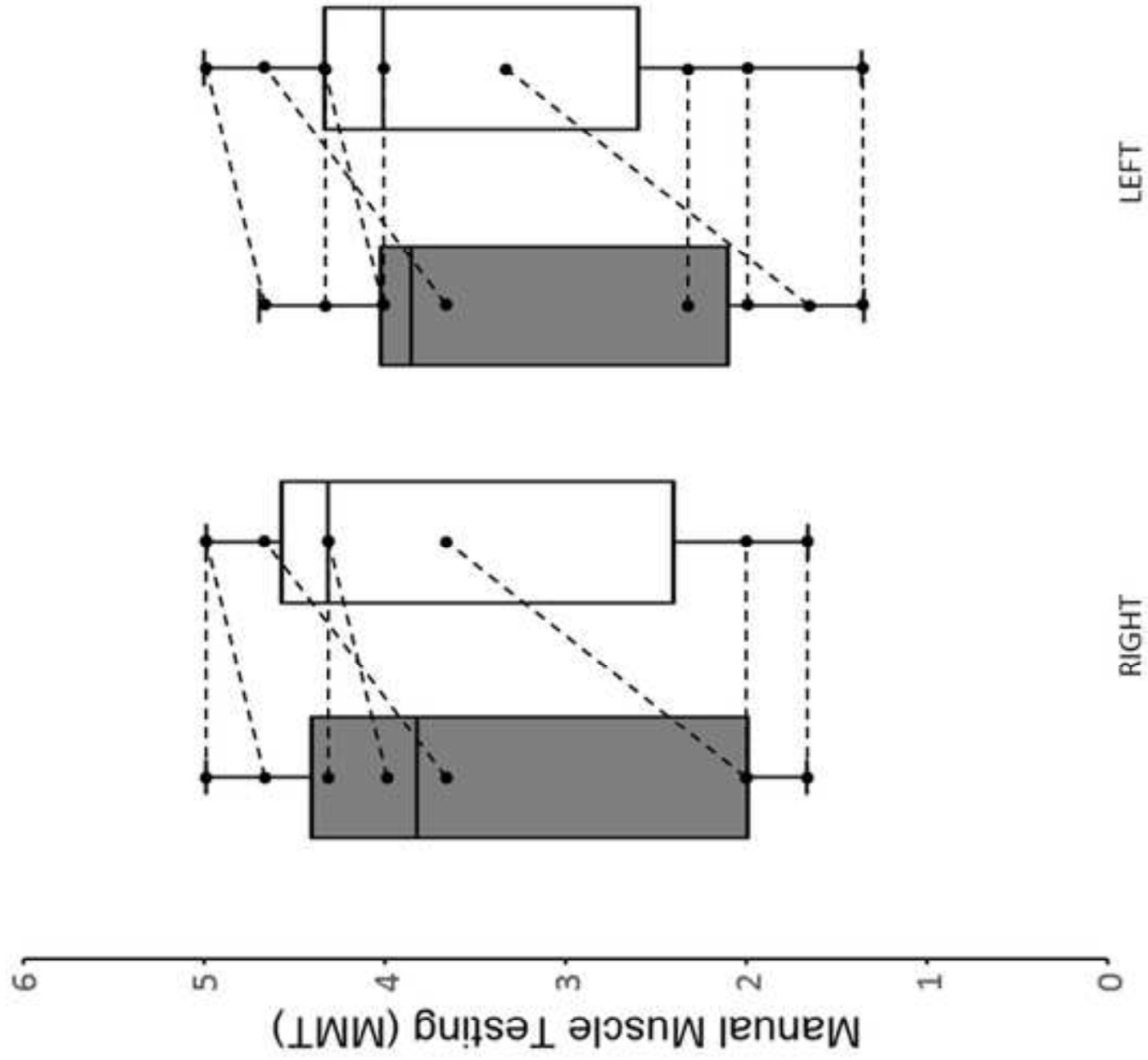


Figure 3

Figure 4

