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Effect of slow release-Fampridine on muscle strength, rate of force development, functional capacity and cognitive function in an enriched population of MS patients. A randomized, double blind, placebo controlled study

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ABSTRACT

Design: This study was conducted as a randomized, double blind, placebo-controlled parallel group trial preceded by open label enrichment phase.
Objectives: The objectives of this study were 1) to examine the effect of SR-Fampridine treatment on muscle strength in terms of maximal voluntary contraction (MVC) and rate of force development (RFD) of the lower extremities and 2) to replicate previously published data on the effect of slow release-Fampridine (SR-Fampridine) on the functional capacity of the lower limbs, the upper limb and cognitive function, in persons with multiple sclerosis (pwMS).
Methods: Previously identified responders to SR-Fampridine were randomized to SR- Fampridine or placebo treatment for four weeks. On days 0 and 26–28 participants underwent testing by isokinetic dynamometry, Nine Hole Peg Test (9-HPT), Symbol Digit Modalities Test (SDMT), Six Spot Step Test (SSST), Timed 25 Foot Walk Test (T25FW) and 5-Times Sit-to-Stand (5-STS).
Results: A statistical significant effect of SR-Fampridine on MVC was demonstrated during knee extension, knee flexion and hip flexion of the weakest leg, as well as on RFD during knee extension and knee flexion of the weakest leg. Furthermore, a significant effect of SR-Fampridine on T25FW, SSST and 5-STS was demonstrated.
Conclusion: Gold standard dynamometry assessment of muscle strength showed improved MVC and RFD in persons with MS treated with SR-Fampridine compared to placebo. Furthermore, previous findings on the effects of SR-Fampridine on functional capacity of the lower limbs were replicated.
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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by recurrent demyelination, which can lead to axonal conduction block (Judge and Bever, 2006). Symptoms include paresthesia, palsy, fatigue, optic neuritis, diplopia, vertigo, and bladder disturbances (Compston and Coles, 2008). Mobility problems are reported by more than 67% (Gottberg et al., 2006), and persons with MS (pwMS) report walking to be the most important bodily function (Heesen et al., 2008). Thus, measures to alleviate mobility problems are highly warranted.

Slow release-Fampridine (SR-Fampridine) can improve conduction in demyelinated nerves through inhibition of voltage gated potassium channels (Bostock et al., 1981; Hayes, 2004). 35–43% of pwMS, in the Extended Disability Status Scale (EDSS) (Kurtzke, 1983) range of 4–7, can achieve improvements in maximal walking speed by ≈25%, when assessed by the Timed 25 Foot Walk (T25FW) (Goodman et al, 2009, 2010). Furthermore, SR-Fampridine can improve muscle strength in the lower extremities when assessed manually (Lower Extremity Manual Muscle Test, LEMMT) (Goodman et al., 2009, 2010). Our group has previously shown that SR-Fampridine treatment not only improves gait speed, but also results in improved performance during

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more complex walking tasks such as the Six Spot Step Test (SSST), which also challenges lower limb coordination and balance (Nieuwenhuis et al., 2006). Of note, improved cognitive function assessed by the Symbol Digit Modalities Test (SDMT) was also observed in a subset of patients (Jensen et al., 2014a).

In pwMS maximal isokinetic and isometric muscle strength (maximal voluntary contraction, MVC) (Armstrong et al., 1983; Ng et al., 2004), as well as rate of force development (RFD, ΔForce/Δtime) are often reduced (Armstrong et al., 1983; Ng et al., 2004). Strength impairment seems more pronounced in the lower extremities than in the upper extremities (Schwid et al., 1999), probably caused by loss in muscle mass, changes in fiber-type composition and/or reduced neural activation (Kent-Braun et al., 1997; de Haan et al., 2000). In pwMS muscle strength and RFD are related to a number of daily functional tasks, such as walking speed (Thoumie et al., 2005; Kjolhede et al., 2015a), walking distance (Kjolhede et al., 2015a; Dalgas et al., 2009), and rising from a chair (Kjolhede et al., 2015a; Moller et al., 2012). It seems relevant to investigate to what extent SR-Fampridine treatment can improve MVC and RFD in pwMS. Despite previous reports of improved muscle strength following SR- Fampridine treatment interpretations should be cautious since 1) LEMMT has not been validated in pwMS, 2) data provided no information on individual muscle groups, 3) no information on RFD has been provided, and 4) LEMMT is regarded as a poor measure of maximal muscle strength as compared to dynamometry (Cuthbert and Goodheart, 2007).

Consequently, the objectives of this study were to 1) to examine the effect of SR- Fampridine treatment on muscle strength in terms of MVC and RFD of the lower extremities and 2) to replicate previously published data on the effect of slow SR- Fampridine on the functional capacity of the lower limbs, the upper limb and cognitive function, in persons with pwMS (Jensen et al., 2014a).

Specifically, we compared 1) concentric and isometric MVC of thigh and hip muscles assessed by dynamometry, 2) RFD assessed during isometric contractions by dynamometry, 3) upper limb function assessed by the 9-Hole Peg Test (9-HPT), 4) cognitive function assessed by SDMT, and 5) functional capacity of the lower extremities assessed by SSST, T25FW and the 5 Times Sit to Stand (5-STS), in PwMS receiving SR- Fampridine versus placebo.

2. Materials and methods

2.1. Study design

This study was a randomized, double blind, placebo-controlled parallel group trial preceded by open label enrichment.

In the open label enrichment phase participants were tested at baseline by T25FW, SSST, 5-STS, 9-HPT, and SDMT. Afterwards they were treated with SR-Fampridine 10 mg BID and then retested by the same tests after 26–28 days of treatment. Participants in the enrichment phase were naïve to SR-Fampridine. Further details on the enrichment phase have been published elsewhere (Jensen et al., 2014a).

2.1.1. Study overview

Study design and flow chart is depicted in Fig. 1.

Since approximately 40% of pwMS with mobility problems have been shown to respond to SR-Fampridine treatment, participants (the top 40%) showing the most marked improvements on SSST were categorized as responders. Responders were randomized in a 1:1 ratio to SR-Fampridine 10 mg BID or placebo BID using a computer based randomization, which was performed in blocks of four. SR-Fampridine tablets and placebo tablets were similar in appearance. Tablets and sealed envelopes were packaged at the Hospital Pharmacy at Odense University Hospital to ensure the blinding of the investigator. Bottles of 4×14 tablets were handed out to the participants by the investigator. Participants returned the bottles at end of trial, and a pill count was performed. Intervention commenced after a one-week washout period which has been suggested to be sufficient (Bever et al., 1994).

2.2. Subjects

2.2.1. Recruitment

Eligible patients were identified through the four MS-clinics in the region of southern Denmark (Odense, Vejle, Esbjerg and Sønderborg), by going through patient files or by personal consultation. The corresponding author recruited all participants.

2.2.2. Inclusion and exclusion criteria

Subjects were required to meet the McDonald criteria for MS (Polman et al., 2011), be 18–60 years, have an EDSS between 4 and 7 with a pyramidal functional subscore of ≥2, and fulfill the responder criterion. Subjects were excluded if having: a history of epileptic seizures, MS relapse or change in immunomodulatory treatment within 60 days, cancer within five years, clinically important systemic disease or concomitant treatment with carvedilol, propranolol, or metformin.

Information on disease duration was retrieved from The Danish Multiple Sclerosis Registry (Bronnum-Hansen et al., 2011).

2.3. Standard protocol approvals, registrations, and patient consents

This study was performed in accordance with the Declaration of Helsinki and monitored by the GCP-unit at Odense University Hospital, Denmark. The study was approved by The Regional Scientific Ethical Committees for Southern Denmark (journal number, S-20120023), and by the Danish Medical Agency (journal number, 2012012850).

2.4. Experimental assessments

2.4.1. Muscle strength

Maximal concentric and isometric muscle strength for the knee extension (KE), knee flexion (KF) and hip flexion (HF) were determined using isokinetic dynamometry (Kinetic Communicator 500H, Chattecx Corp., TN, USA). Isokinetic dynamometry is known to be safe and reliable in pwMS (Armstrong et al., 1983) and the reliability and validity of the applied dynamometer has been described elsewhere (Farrell and Richards, 1986). Subjects were placed sitting during KE and KF, and standing during HF. Both legs were tested separately. Concentric KE and KF strength was evaluated at slow (30°/s) and fast (180°/s) angular velocities at a range of motion (ROM) of 90–20° (full extension=0°). Concentric HF strength was tested at slow (30°/s) and moderate (120°/s) angular velocities, using a ROM of 0–45° (neutral standing=0°). Isometric KE/KF strength tests were performed at a fixed knee joint angle of 70°, while isometric HF strength was tested at 20° fixed hip flexion angle. After a 5-min standardized warm-up on a stationary ergometer bike, subjects were tested at each test mode separately with instructions to contract as hard and fast as possible. Typically ≥5 contractions were performed in each test mode, until no further increase in peak force could be observed. Force signals were digitally sampled at 1000 Hz and converted into joint moments and corrected for gravity (Aagaard et al., 2002, 1995). Concentric tests were analyzed for peak moment, while isometric tests were analyzed for peak moment and contractile RFD, with RFD calculated in the 0–100 ms time interval relative to onset, and also determined as the peak tangential slope of the moment-time curve (i.e. peak RFD) (Aagaard et al., 2002).

2.4.2. Functional capacity and cognitive functions

To evaluate functional capacity SSST (Nieuwenhuis et al., 2006), T25FW (Rudick et al., 2002) and 5-STS (Moller et al., 2012) were performed, while SDMT (Parmenter et al., 2007) was performed to evaluate cognitive functions, and 9-HPT (Goodkin et al., 1988) was

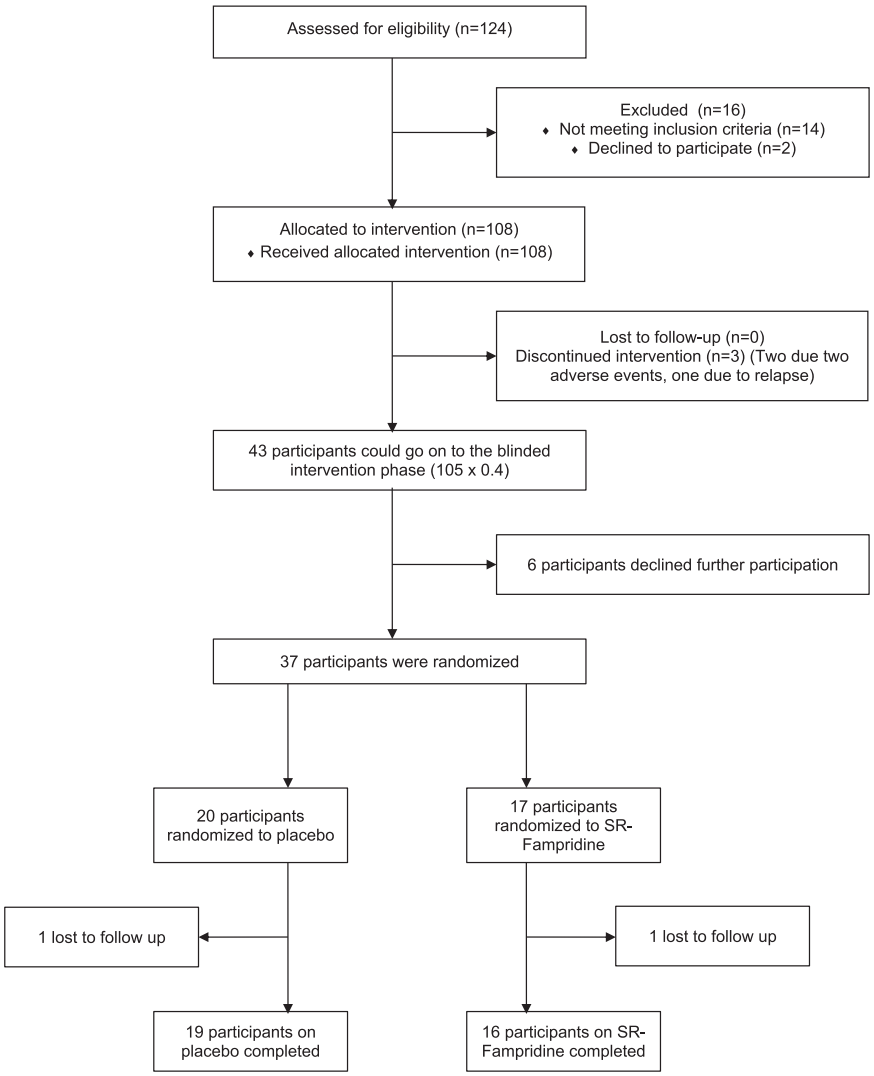

Fig. 1. Flow chart of participants.

performed to evaluate hand dexterity.

2.5. Statistical method

The STATA 12 software package was used for statistical analysis.

A Mixed Effects Model was applied to evaluate between group results. The Model considered the observed value of a given response variable as the sum of fixed parts, β, consisting of a constant and the contributions of the co-variates; treatment group, EDSS, age, sex and disease duration, and random parts, ζ and ε, consisting of contributions from the measurements and the individuals; $y_{ij}=\beta_0+\beta_1\times$treatment $group_j+\beta_2\times EDSS_j+\beta_3\times age_j+\beta4\times sexj+\beta5\times$disease durationj$+\zeta i+\varepsilon ij$ where i denotes the i'th measurement in the j'th individual. Distribution of the residuals was checked using q–q-plots, histograms and Shapiro-Wilks test. Normality was met for KE MVC 30°/s in the strongest leg, KE RFD 0–100 ms in the strongest leg, HF MVC 30°/s in both legs, HF RFD 0–100 ms in the weakest leg, and KF RFD 0–100 ms in the strongest leg. On the remaining outcomes a boxcox transformation was performed. In order to check for overall effects on mechanical muscle function composite scores for MVC and RFD obtained for KE, KF and HF were generated for each subject by summing all individual strength data (i.e. concentric MVC at 30°/s and 180°/s (120°/s for HF) plus isometric MVC and RFD 0–100 ms plus peak RFD, respectively). All strength variables were normalized to body mass. A correlation analysis applying Spearman's rank correlations was performed in order to control for the influence of the co-variates age, sex, EDSS, and disease duration. For dynamometry measures sex was the only significant co-variate with males being stronger than females. EDSS was significant for T25FW, SSST and 5-STS. Sex was significant for 9-HPT with females performing better than males.

All dynamometry measures and T25FW, SSST and 5-STS were considered dependent. Due to the risk of detecting a false positive by chance a correction procedure was executed. With 33 measurements the Bonferroni correction was considered to be to conservative. Consequently, a Benjamini-Hochberg correction (Benjamini et al., 2001) was performed with a False Discovery Rate of 0.1.

A priori power calculation based on the expected difference off vs. on treatment in time to complete SSST was performed (expected time off SR-Fampridine treatment=10.7 s and on SR-Fampridine treatment=7.5 s; standard deviation (SD) ± 5.2 s (based on Nieuwenhuis et al. (2006); p=0.05; power 0.9), yielding n=25 in each study arm.

3. Results

One hundred and eight participants entered the open label enrichment phase. Two participants dropped out due to adverse events and one due to a relapse. One hundred and five completed the enrichment phase. Subsequently, 43 participants were invited to the intervention phase. Six participants declined further participation and subsequently, 20 subjects were randomized to placebo, while 17 were randomized to

Table 1
Baseline demographics.

	Placebo ± SD	SR-Fampridine ± SD	p-value
EDSS	5.5 ± 0.7	5.8 ± 0.8	0.09
Age	48.4 ± 6.4	50.8 ± 6.5	0.1
Gender	65% women	47% women	0.1
Disease duration	9.8 ± 5.9	9.5 ± 5.4	0.8

SR-Fampridine. Two were lost to follow-up (Fig. 1).

There were no significant differences in baseline demographics (Table 1).

3.1. Muscle strength and rate of force development

3.1.1. Concentric muscle strength - weakest leg

KE MVC at 30°/s improved 17% ± 30.7 in the SR-Fampridine as compared to −0.9% ± 16.2 in the placebo group (p=0.03). At 180°/s the improvement in the SR-Fampridine group was 19.4% ± 42.7 vs. −3.6% ± 11.9 in the placebo group (p=0.02).

3.1.2. Isometric muscle strength and RFD - weakest leg

KE RFD peak improved 35.4% ± 63.2 in the SR-Fampridine group compared to −4.5% ± 31.9 in the placebo group (p=0.006), RFD 0–100 ms for KE improved by 31.4% ± 47.3 in the SR-Fampridine group vs. −2.8% ± 28.2 in the placebo group (p=0.005). KF MVC was improved by 39.2% ± 113.3 in the SR- Fampridine group as compared to −11.2% ± 25.6 in the placebo group (p=0.01), while KF peak RFD improved 57.8% ± 69.4 in the SR-Fampridine group vs. −5.6% ± 44.8 in the placebo group (p < 0.001). Likewise RFD 0–100 ms improved 73.2% ± 131.5 in the SR- Fampridine group as compared to −2.3 ± 33.8 in the placebo group (p=0.006).

3.1.3. Concentric muscle strength - strongest leg

KF MVC at 30°/s improved by 14.4% ± 21.9 in the SR-Fampridine group vs. −2.3% ± 10.0 in the placebo group (p=0.001).

3.1.4. Isometric muscle strength and RFD - strongest leg

In KE RFD 0–100 ms there was an improvement of 63.5% ± 82.9 in the SR-Fampridine group compared to 20.4% ± 53.7 in the placebo group (p=0.02). KF RFD peak improved by 72.6% ± 107.2 in the SR-Fampridine group and 18.1% ± 49.7 in the placebo group (p=0.04). KF RFD 0–100 ms improved by 81.2% ± 112.8 in the SR-Fampridine group compared to 24.5% ± 87.4 in the placebo group (p=0.02) (details are given in Tables 2, 3 and in Fig. 2).

3.1.5. Composite scores – weakest leg

KE MVC changed 11.6% ± 24.1 in the SR-Fampridine group vs. −0.8% ± 11.3 in the placebo group (p=0.03). For KF MVC a change of 11.2% ± 29.5 was seen in the SR-Fampridine vs. −4.8% ± 12.9 in the placebo group (p=0.03). HF MVC changed by 14.3% ± 14.9 in the SR-Fampridine group vs. −1.3% ± 20.5 in the placebo group (p=0.05), while KE RFD changed by 23.3% ± 32.2 in the SR-Fampridine group vs. −2.2% ± 30.3 in the placebo group (p=0.002). Finally, KF RFD changed by 34.7% ± 43.0 in the SR-Fampridine group vs. −6.7% ± 32.2 in the placebo group (p < 0.001).

3.1.6. Composite scores - strongest leg

There were no significant differences in composite scores in the strongest leg. Details for composite scores are given in Table 4 and Fig. 3.

3.2. Functional capacity

T25FW improved by −13.6% ± 18.3 in the SR-Fampridine group compared to 4.7% ± 24.1 in the placebo group (p=0.02). SSST improved −11.4% ± 17.7 in the SR-Fampridine group and 3.8% ± 19.6 in the placebo group (p=0.005). 5-STS improved by −7.6% ± 37.1 in the SR- Fampridine group vs. 1.6% ± 13.7 in the placebo group (p=0.006). There were no significant differences between groups on 9-HPT and SDMT (details are given in Table 5).

3.3. Correlations between changes in functional capacity of the lower limbs and muscle strength

T25FW was significantly correlated with KE MVC at 30°/s (ρ=−0.3, p=0.05), composite KE MVC (ρ=−0.5, p=0.05) and KF RFD peak (ρ=−0.5, p=0.001) in the weakest leg, and KE RFD 0–100 ms in the strongest leg (ρ=−0.5, p=0.001). SSST was significantly correlated with KE RFD 0–100 ms in the strongest leg (ρ=−0.4, p=0.01), and composite KE MVC (ρ=−0.6, p=0.02) in the weakest leg. 5-STS was significantly correlated with KF RFD peak of the weakest leg (ρ=−0.5, p=0.002), and KE RFD 0–100 ms in the strongest leg (ρ=−0.5, p=0.005).

4. Discussion

In this enriched MS population we demonstrated significant effects of SR-Fampridine in the lower extremities on 1) concentric and isometric MVC as well as RFD of the lower extremities, and 2) on functional capacity. We did not demonstrate significant effects on 9-HPT or SDMT.

Table 2
Concentric MVC in the lower extremities (mean values ± SD). KE=Knee extension. KF=Knee flexion. HF=Hip flexion. Nm=Newton meter. MVC=Maximal voluntary contraction.

Concentric		Placebo Pre	Post	Mean change (%)	SR-Fampridine Pre	Post	Mean change (%)	p-value/FDR
Weakest leg								
KE (Nm)	MVC 30°/s	88.5 ± 52.9	85.9 ± 44.6	−2.5 ± 15.8 (−0.9 ± 16.2)	89.7 ± 30.7	102.6 ± 36.7	13.5 ± 24.6 (17.0 ± 30.7)	**0.03/0.04**
	MVC 180°/s	53.9 ± 29.8	51.7 ± 27.7	−2.3 ± 5.9 (−3.6 ± 11.9)	54.7 ± 19.6	60.3 ± 24.7	8.0 ± 18.9 (19.4 ± 42.7)	**0.02/0.03**
KF (Nm)	MVC 30°/s	46.9 ± 23.4	48.7 ± 25.7	1.8 ± 7.2 (3.8 ± 16.1)	51.3 ± 17.7	58.2 ± 25.4	7.1 ± 19.6 (14.7 ± 46.1)	0.4/0.08
	MVC 180°/s	32.4 ± 21.0	31.1 ± 21.8	−1.3 ± 5.6 (−6.6 ± 34.8)	39.2 ± 17.8	41.2 ± 20.0	2.7 ± 14.3 (8.3 ± 34.5)	0.16/0.06
HF (Nm)	MVC 30°/s	62.7 ± 35.4	64.1 ± 23.8	1.4 ± 17.3 (10.8 ± 39.5)	63.2 ± 26.6	75.6 ± 33.8	12.4 ± 15.6 (19.8 ± 17.3)	0.14/0.06
	MVC 120°/s	46.9 ± 28.3	50.8 ± 32.9	3.9 ± 21.6 (10.8 ± 40.0)	50.4 ± 31.0	56.9 ± 31.5	6.5 ± 8.7 (27.4 ± 53.3)	0.19/0.07
Strongest leg								
KE (Nm)	MVC 30°/s	113.7 ± 54.8	118.3 ± 54.0	4.6 ± 12.0 (5.5 ± 12.5)	103.3 ± 35.4	111.8 ± 33.3	6.5 ± 15.3 (8.9 ± 16.7)	0.47/0.09
	MVC 180°/s	69.7 ± 34.4	68.8 ± 32.0	−1.0 ± 8.6 (−0.4 ± 9.7)	62.6 ± 24.1	68.5 ± 23.6	3.6 ± 11.9 (7.2 ± 19.2)	0.14/0.06
KF (Nm)	MVC 30°/s	68.7 ± 30.8	68.6 ± 33.5	−0.1 ± 5.1 (−2.3 ± 10.0)	62.3 ± 22.6	73.1 ± 26.4	9.0 ± 13.0 (14.4 ± 21.9)	**0.001/0.006**
	MVC 180°/s	41.5 ± 29.5	47.8 ± 27.1	6.3 ± 21.9 (15.2 ± 42.3)	44.6 ± 20.8	53.8 ± 20.7	7.4 ± 12.8 (20.8 ± 29.3)	0.06/0.05
HF (Nm)	MVC 30°/s	88.8 ± 45.3	87.0 ± 37.5	−1.8 ± 25.1 (−1.5 ± 21.3)	68.8 ± 27.5	77.5 ± 34.4	8.8 ± 31.8 (4.1 ± 33.3)	0.9/0.1
	MVC 120°/s	69.6 ± 43.0	70.2 ± 40.0	0.6 ± 16.7 (5.7 ± 23.1)	61.5 ± 28.6	71.4 ± 34.7	9.9 ± 20.7 (22.1 ± 46.8)	0.3/0.08

Table 3
Isometric MVC and RFD in the lower extremities (mean values ± SD). KE=Knee extension. KF=Knee flexion. HF=Hip flexion. Nm=Newton meter. Nm/s=Newton meter per second. MVC=Maximal voluntary contraction. RFD=Rate of force development.

Isometric		Placebo Pre	Post	Mean change (%) (%)	SR-Fampridine Pre	Post	Mean change (%)	p-value/ FRD
Weakest leg								
KE	MVC (Nm)	111.9 ± 61.5	109.4 ± 56.2	−2.5 ± 11.8 (−2.3 ± 14.6)	113.4 ± 35.8	120.7 ± 45.6	8.0 ± 24.7 (7.1 ± 20.0)	0.5/0.09
	RFD peak (Nm/s)	612.7 ± 420.9	571.0 ± 433.2	−41.7 ± 234.5 (−4.5 ± 31.9)	636.6 ± 404.7	737.3 ± 434.9	124.1 ± 281.8 (35.4 ± 63.2)	**0.006/**
	RFD 0–100 ms (Nm/s)	339.2 ± 240.0	329.6 ± 245.5	−9.6 ± 98.6 (−2.8 ± 28.2)	311.0 ± 256.0	395.0 ± 255.8	97.7 ± 141.9 (31.4 ± 47.3)	**0.02** **0.005/ 0.009**
KF	MVC (Nm)	35.0 ± 21.1	32.4 ± 22.6	−2.6 ± 7.1 (−11.2 ± 25.6)	37.5 ± 20.6	43.6 ± 26.9	5.8 ± 13.0 (39.2 ± 113.3)	**0.01/0.03**
	RFD peak (Nm/s)	173.3 ± 127.1	147.8 ± 117.5	−25.5 ± 59.9 (−5.6 ± 44.8)	190.0 ± 165.9	251.1 ± 195.8	69.6 ± 102.9 (57.8 ± 69.4)	**< 0.001/**
	RFD 0–100 ms (Nm/s)	75.4 ± 53.9	70.0 ± 58.4	−5.3 ± 33.3 (−2.3 ± 33.8)	78.2 ± 79.5	98.4 ± 76.2	23.5 ± 39.1 (73.2 ± 131.5)	**0.003** **0.006/ 0.02**
HF	MVC (Nm)	91.8 ± 40.0	84.9 ± 35.6	−3.9 ± 16.0 (−0.5 ± 16.9)	87.2 ± 31.6	95.2 ± 40.3	8.1 ± 27.1 (9.9 ± 26.7)	0.18/0.07
	RFD peak (Nm/s)	502.7 ± 408.6	402.8 ± 316.7	−99.9 ± 191.7 (−9.2 ± 65.1)	551.9 ± 384.7	586.9 ± 459.2	35.0 ± 434.2 (23.2 ± 85.1)	0.16/0.06
	RFD 0–100 ms (Nm/s)	243.4 ± 209.6	210.1 ± 171.3	−27.1 ± 105.4 (−11.1 ± 84.4)	225.7 ± 191.7	287.6 ± 226.9	61.9 ± 177.7 (18.1 ± 125.7)	0.25/0.07
Strongest leg								
KE	MVC (Nm)	145.9 ± 61.8	147.6 ± 64.9	1.7 ± 12.5 (0.3 ± 9.7)	126.6 ± 40.8	138.4 ± 35.2	8.9 ± 25.6 (11.7 ± 30.6)	0.12/0.05
	RFD peak (Nm/s)	887.8 ± 584.5	952.8 ± 604.0	64.9 ± 215.6 (13.1 ± 31.6)	793.7 ± 583.2	860.4 ± 365.9	41.2 ± 371.4 (29.2 ± 51.3)	0.44/0.09
	RFD 0–100 ms (Nm/s)	546.6 ± 383.5	29.2 ± 167.2	29.2 ± 167.2 (20.4 ± 53.7)	405.4 ± 296.8	542,2 ± 250.0	117.8 ± 151.4 (63.5 ± 82.9)	**0.02/0.03**
KF	MVC (Nm)	50.7 ± 28.4	53.6 ± 30.0	2.9 ± 9.8 (4.5 ± 19.1)	47.1 ± 21.8	50.7 ± 26.2	4.1 ± 8.7 (9.6 ± 22.9)	0.6/0.05
	RFD peak (Nm/s)	362.9 ± 290.7	390.3 ± 287.4	27.4 ± 133.3 (18.1 ± 49.7)	313.4 ± 352.4	432.2 ± 311.9	116.8 ± 241.4 (72.6 ± 107.2)	**0.04/0.1**
	RFD 0–100 ms (Nm/s)	164.6 ± 119.8	169.3 ± 112.4	4.6 ± 73.4 (24.5 ± 87.4)	112.4 ± 110.1	177.0 ± 126.7	58.6 ± 72.0 (81.2 ± 112.8)	**0.02/0.03**
HF	MVC (Nm)	105.3 ± 46.7	118.5 ± 52.7	13.2 ± 22.3 (14.9 ± 22.6)	111.1 ± 77.8	108.9 ± 37.4	−2.2 ± 62.9 (−2.1 ± 28.3)	0.09/0.05
	RFD peak (Nm/s)	611.5 ± 455.6	623.7 ± 446.5	12.1 ± 198.5 (10.1 ± 49.1)	621.6 ± 417.4	828.1 ± 766.1	206.5 ± 641.3 (37.6 ± 85.3)	0.4/0.08
	RFD 0–100 ms (Nm/s)	382.4 ± 297.1	386.6 ± 258.7	4.2 ± 135.4 (36.5 ± 105.5)	378.6 ± 296.7	413.5 ± 382.5	34.9 ± 336.5 (49.5 ± 168.7)	0.6/0.09

4.1. Effects of SR-Fampridine on mechanical muscle function in pwMS

This is the first study to employ dynamometry assessment of MVC and RFD in order to evaluate the effect of SR-Fampridine treatment on mechanical muscle function in pwMS. Previously an effect of 3.8–4.4% on muscle strength when assessed by LEMMT has been reported (Goodman et al., 2009, 2010). In comparison we demonstrated averaged improvements in isokinetic MVC of KE, KF and HF of ~13%.

KE MVC in the weakest leg seems more affected by the effects of SR- Fampridine than KF MVC and HF MVC.

Moreover, no change in HF MVC or HF RFD was observed. This is surprising as pwMS present with a supranuclear distribution of palsy, being more affected in KF and HF. One possible explanation is that HF dynamometry was technical difficult to perform. Participants were standing up, sometimes with difficulties standing on the supporting leg. Hence, the variance of measurements was higher than that of KE and KF.

Changes in three out of 12 concentric measures were significant compared to eight out of 18 isometric measurements. This could be due to the fixation of the extremity during isometric measurements, making them technically easier to perform.

In the strongest leg KF MVC appeared to be more positively affected by SR- Fampridine treatment than KE MVC and HF MVC, respectively. Interestingly, Dalgas et al. similarly found a better effect in KF MVC than in KE MVC in the strongest legs following 12 weeks of resistance training in pwMS, suggesting a marked potential for strength improvements in the hamstring muscles (Dalgas et al., 2009). However, the present composite strength scores did not support this, mainly because the isometric KF MVC remained un-affected following SR-Fampridine treatment.

Kjølhede et al. (2015b) demonstrated ≈15–20% increase in KE RFD after 24 weeks of resistance training in pwMS. We demonstrated similar improvements in composite KE RFD ranging from 13.3% ± 32.3 to 23.3% ± 32.2.

The effect of SR-Fampridine was more pronounced in the weakest leg compared to the strongest leg, probably due to the larger margin for improvement.

Also, the effect of SR-Fampridine administration was more pronounced for RFD compared to MVC, probably reflecting an enhanced ability to generate rapid rise in muscle force. This accentuated boosting of contractile RFD may reflect an effect of SR-Fampridine on neural activation, known to be of major importance for RFD in vivo (Aagaard et al., 2002; Grimby et al., 1981) and which may show the highest adaptive potential in the weakest leg (due to the potential presence of more marked neural deficits). RFD has been suggested to be a major determinant of the maximal force and velocity (Aagaard et al., 2002), and positive associations between lower limb RFD and postural balance have been demonstrated (Izquierdo et al., 1999; Jakobsen et al., 2011). Consequently, gains in RFD may reduce the incidence of falls due to an increased ability to exert a rapid rise in muscle force (Aagaard et al., 2002), underlining the potential important clinical implication of the increase in RFD observed with SR-Fampridine treatment in the present study.

The Benjamini-Hochberg correction (with FDR=0.1) shows that isometric KF RFD peak is the cut-off (p-value=FDR). For the composite scores HF MVC on the weakest leg is the cut-off (p=0.05, FDR=0.04). This indicates that the risk of false positives is minimal.

4.2. Upper limb function and cognitive function

Contrasting our previous findings (Jensen et al., 2014a), no significant change was observed in 9-HPT. This may be due to the lower power as the observed values corresponded to those seen in our

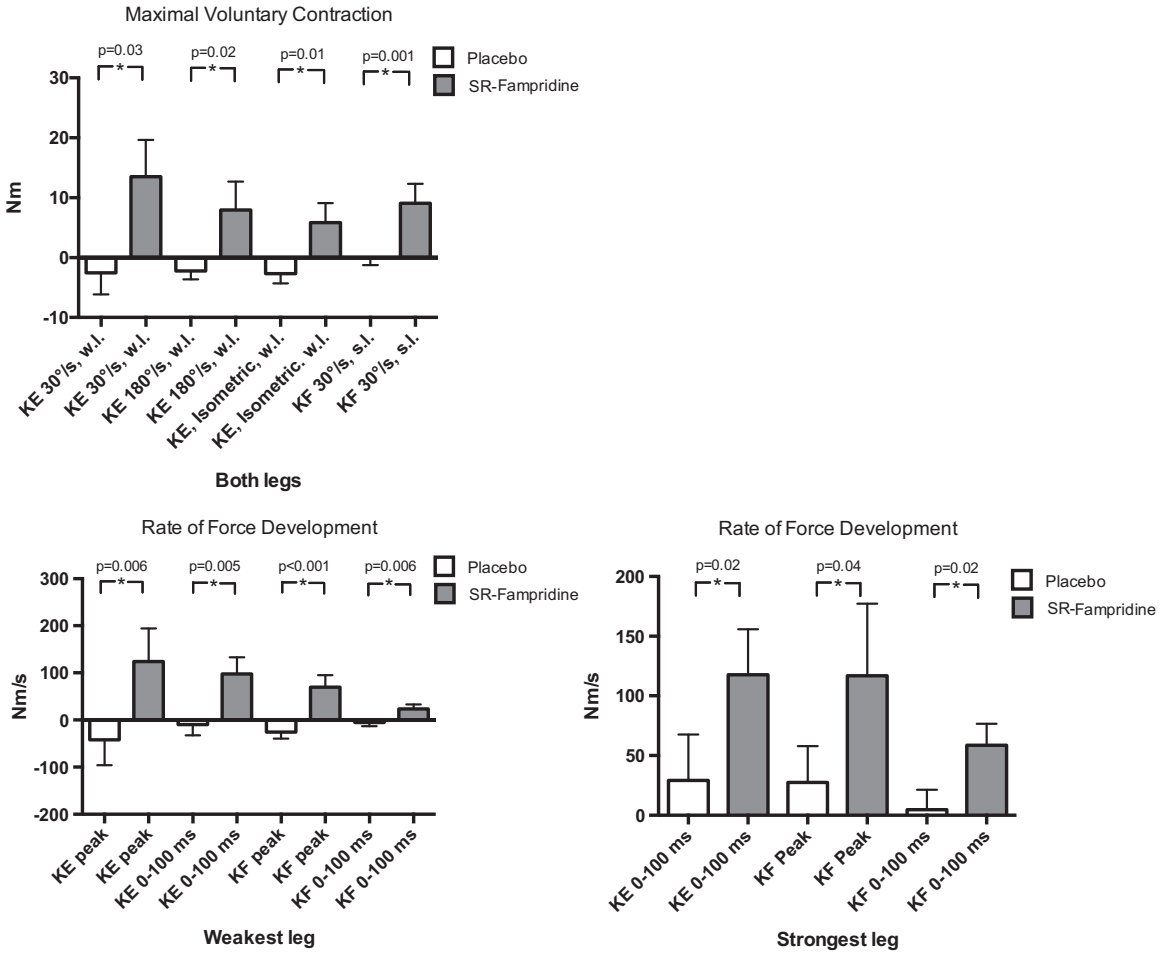

Fig. 2. Statistical significant differences in muscle strength and rate of force development in the lower extremities. KE=knee extension. KF=Knee flexion. w.l=weakest leg. s.l.=strongest leg.

Table 4
Composite scores of MVC and RFD for KE, KF and HF in the weakest and the strongest leg (mean values ± SD). KE=Knee extension. KF=Knee flexion. HF=Hip flexion. Nm=Newton meter. Nm/s=Newton meter per second. MVC=Maximal voluntary contraction. RFD = Rate of force development.

		Placebo Pre	Post	Mean change (%)	SR-Fampridine Pre	Post	Mean change (%)	p-value/FDR
Weakest leg								
MVC (Nm)	KE	254.3 ± 141.9	247.0 ± 126.6	−7.3 ± 28.1 (−0.8 ± 11.3)	258.0 ± 80.1	283.6 ± 103.4	29.5 ± 63.6 (11.6 ± 24.1)	**0.03/0.03**
	KF	114.4 ± 63.4	112.2 ± 68.3	−2.2 ± 12.4 (−4.8 ± 12.9)	128.1 ± 51.0	143.1 ± 69.8	15.6 ± 43.1 (11.2 ± 29.5)	**0.03/0.03**
	HF	212.0 ± 95.3	201.6 ± 89.1	−2.8 ± 41.3 (−1.3 ± 20.5)	195.1 ± 77.1	277.7 ± 100.7	29.9 ± 42.6 (14.3 ± 14.9)	**0.05/0.04**
RFD (Nm/s)	KE	951.9 ± 654.4	900.6 ± 670.4	−51.3 ± 311.2 (−2.2 ± 30.3)	947.7 ± 651.3	1132.3 ± 687.4	221.9 ± 408.5 (23.3 ± 32.2)	**0.002/0.02**
	KF	248.7 ± 179.2	217.8 ± 170.9	−30.8 ± 85.7 (−6.7 ± 32.2)	268.2 ± 231.9	349.6 ± 263.1	93.1 ± 119.4 (34.7 ± 43.0)	**< 0.001/ 0.008**
	HF	774.0 ± 597.0	612.9 ± 482.3	−138.6 ± 259.0 (−8.4 ± 54.4)	777.6 ± 552.5	874.4 ± 651.7	96.8 ± 557.8 (12.4 ± 51.2)	0.09/0.07
Strongest leg								
MVC (Nm)	KE	329.4 ± 146.9	334.7 ± 147.3	5.3 ± 20.1 (1.5 ± 6.3)	292.5 ± 95.2	318.7 ± 87.8	19.0 ± 36.7 (8.3 ± 13.4)	0.06/0.05
	KF	160.9 ± 81.6	170.0 ± 89.0	9.2 ± 27.3 (4.1 ± 19.6)	154.0 ± 60.5	177.6 ± 67.9	20.5 ± 26.5 (13.7 ± 16.5)	0.08/0.06
	HF	268.8 ± 132.2	281.9 ± 122.8	13.2 ± 45.7 (8.3 ± 16.4)	241.4 ± 107.0	257.8 ± 97.7	16.5 ± 72.0 (10.9 ± 28.2)	0.9/0.1
RFD (Nm/s)	KE	1434.4 ± 964.2	1528.6 ± 961.9	94.2 ± 371.9 (14.6 ± 36.4)	1199.0 ± 860.6	1402.6 ± 610.6	159.1 ± 498.0 (13.3 ± 32.3)	0.2/0.08
	KF	527.6 ± 299.7	559.6 ± 381.8	32.0 ± 190.2 (18.6 ± 56.1)	425.8 ± 92.6	609.2 ± 413.3	175.4 ± 270.7 (41.2 ± 63.5)	0.1/0.08
	HF	993.9 ± 747.3	1010.3 ± 697.1	16.3 ± 303.2 (1.6 ± 8.8)	1000.2 ± 703.8	1241.5 ± 1140.9	241.4 ± 947.8 (33.4 ± 93.9)	0.3/0.09

previous study. Our findings were substantially lower than reported by Goodman et al. (2007). No obvious explanation for this is offered, but it is noticed that participants had slightly longer disease duration than in the current study. Participants performed substantially worse compared to normative data from healthy adults between 46 and 50 years (18.3 s) (Oxford Grice et al., 2003) and none of our participants were able to reach this normative level after SR-Fampridine treatment. Theoretically SR-Fampridine would have the same effect on the upper limbs as on the lower limbs, which was not the case in the present study. This may be due to the more complex coordination of hand and finger movements than seen during more gross motor activities. Also, the tracts to the lower limbs may be more prone to demyelinating damage. Participants were selected based on their walking capacity. Thus, upper limb impairment was not part of the eligibility criteria. Studying short-term and long-term effects of SR-Fampridine in 52 patients, Ruck et al. were also not able to detect a significant effect on

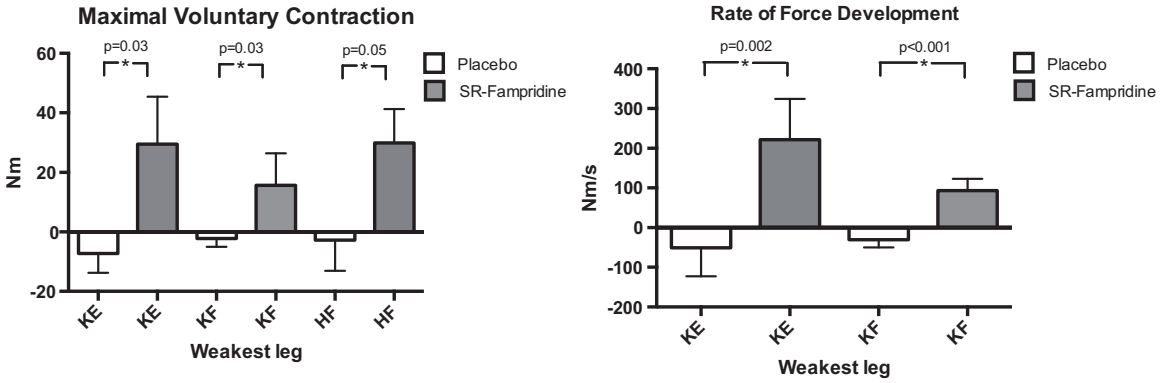

Fig. 3. Statistical significant differences in composite scores of muscle strength in the weakest leg. No statistical significant differences were found in the strongest leg. KE=Knee

Table 5
Functional capacity in the lower extremities, cognition and hand dexterity (mean values ± SD). T25FW=Timed 25 Foot Walk. SSST=Six Spot Step Test. 5-STS=5 Times Sit to Stand. 9-HPT=9-Hole Peg Test. SDMT=Symbol Digit Modalities Test.

	Placebo Pre	Post	Mean change (%)	SR-Fampridine Pre	Post	Mean change (%)	p-value/FDR
T25FW	8.3 ± 3.8	8.6 ± 4.2	0.3 ± 1.8 (4.7 ± 24.1)	14.1 ± 17.0	11.3 ± 9.2	−3.3 ± 9.5 (−13.6 ± 18.3)	**0.02/0.03**
SSST	13.9 ± 6.5	14.5 ± 7.4	0.6 ± 3.2 (3.8 ± 19.6)	20.2 ± 14.8	17.5 ± 9.6	−3.25 ± 8.0 (−11.4 ± 17.7)	**0.005/0.009**
5-STS	13.6 ± 4.9	13.8 ± 5.0	0.2 ± 2.3 (1.6 ± 13.7)	16.4 ± 6.2	13.9 ± 5.2	−2.5 ± 4.0 (−7.6 ± 37.1)	**0.006/0.02**
9-HPT	27.9 ± 11.4	28.5 ± 9.3	0.6 ± 4.4 (4.1 ± 12.0)	29.2 ± 10.7	29.1 ± 11.2	−0.3 ± 2.6 (−1.0 ± 8.4)	0.1
SDMT	45.8 ± 17.2	46.7 ± 17.8	0.9 ± 3.5 (2.5 ± 9.3)	39.0 ± 10.0	41.3 ± 13.1	1.7 ± 5.9 (3.4 ± 15.7)	0.9

9-HPT, neither after two weeks nor 9–12 months of treatment (Ruck et al., 2014).

No effect of SR-Fampridine treatment was seen on SDMT. However, in a subset of patients change above the clinical relevant threshold for SDMT was observed (SR- Fampridine: 26.7% vs. Placebo: 11.1%). This is in line with Smits et al. who investigated the effect of 4-aminopyridine on cognition by a comprehensive neuropsychological battery including SDMT (Smits et al., 1994). They also did not find effect on SDMT, but they did find prominent changes in individual participants and a trend towards significant improvements in the Delayed Recall condition and Paced Auditory Serial Addition Test. Rossini et al. (2001) also investigated the effect of 4-aminopyridine on cognition but were neither able to demonstrate a significant improvement.

Ruck et al. (2014) found a significant effect on Paced Auditory Serial Addition Test after 9–12 months, but not after two weeks of treatment. Thus, it seems possible that SR- Fampridine may be able to improve cognition in a subset of patients. The reasons for not.

reaching a significant level could be 1) short treatment period, 2) responsiveness of the applied instrument and/or 3) cognitive deficits not being part of the eligibility criteria.

Hence, the suggested effect on cognition in a subset of participants must be interpreted with caution as neither sample size, neither study time nor SDMT are sufficient to draw definite conclusions.

4.3. Lower limb functional capacity

In the SR-Fampridine group percentage improvements on T25FW and SSST were 13.6% ± 18.3% and 11.4% ± 17.7, respectively. For the T25FW this comparable to our previous findings, whereas it is lower for SSST (11.2% and 17.0%, respectively) (Jensen et al., 2014a). In our previous study SSST was more sensitive to change than the T25FW. We were not able to demonstrate the same difference in the present study, which may be due to insufficient power. In the SR-Fampridine group 33.3% improved ≥20% on the SSST, thus demonstrating a clinically relevant improvement (Hobart et al., 2013), while 25% improved ≥20% on the T25FW (Jensen et al., 2014a).

The sit-to-stand performance (5-STS) demonstrated significant improvement in the SR-Fampridine group of 7.6% ± 37.1 compared to the placebo group (2% worsened). The percentage improvement was lower than previously reported (16.6%) (Jensen et al., 2014b), but still confirms the positive effect of SR-Fampridine on the functional capacity to erect from a sitting position. In the SR-Fampridine group 25% improved ≥25.5%, which has previously been reported as a reliable change (Moller et al., 2012).

5. Study limitations

A total of 16 and 19 participants completed the trial in the SR-Fampridine and placebo groups, respectively, which was lower than the planned number of participants (n=25 in each group). This may have contributed to a reduced power in the statistical comparisons between groups. Six participants withdrew consent between the enrichment phase and the intervention phase, due to the risk of receiving placebo.

Also the responder criterion applied may be a limitation, as it has not been validated.

6. Conclusion

SR-Fampridine treatment improved overall MVC strength of KE, KF and HF, while also inducing overall improvements in RFD of KE and KF of particular the weakest leg. Furthermore, we replicated previously published open label data on functional capacity in an RCT setting. The previously reported better responsiveness of SSST compared to T25FW could not be replicated.

ClinicalTrials.gov identifier

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Declaration of conflicting interests

HBJ has received research support from Biogen Idec and travel grants and/or teaching honoraries from Biogen Idec, Novartis, Almirall and Merck Serono and serves as an advisory board member for Novartis.

JLN has no conflicting interests.

MR has received travel grants and consultancy honoraries from Biogen Idec, Genzyme, TEVA and Novartis and serves as an advisory board member for Biogen Idec.

UD has received research support, travel grants and/or teaching honoraries from Biogen Idec, Merck Serono and Sanofi Aventis. UD served as PI for the Biogen Idec sponsored ACTIMS study.

PA has no conflicting interests.

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