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ORIGINAL COMMUNICATION

# Targeting phosphocreatine metabolism in relapsing–remitting multiple sclerosis: evaluation with brain MRI, $^{1}$H and $^{31}$P MRS, and clinical and cognitive testing

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## Abstract

**Background/objectives** Fluoxetine and prucalopride might change phosphocreatine (PCr) levels via the cAMP–PKA pathway, an interesting target in the neurodegenerative mechanisms of MS.

**Methods** We conducted a two-center double-blind, placebo-controlled, randomized trial including 48 relapsing–remitting MS patients. Patients were randomized to receive placebo ($n=13$), fluoxetine ($n=15$), or prucalopride ($n=14$) for 6 weeks. Proton ($^{1}$H) and phosphorus ($^{31}$P) magnetic resonance spectroscopy (MRS) as well as volumetric and perfusion MR imaging were performed at weeks 0, 2, and 6. Clinical and cognitive testing were evaluated at weeks 0 and 6.

**Results** No significant changes were observed for both $^{31}$P and $^{1}$H MRS indices. We found a significant effect on white matter volume and a trend towards an increase in grey matter and whole brain volume in the fluoxetine group at week 2; however, these effects were not sustained at week 6 for white matter and whole brain volume. Fluoxetine and prucalopride showed a positive effect on 9-HPT, depression, and fatigue scores.

**Conclusion** Both fluoxetine and prucalopride had a symptomatic effect on upper limb function, fatigue, and depression, but this should be interpreted with caution. No effect of treatment was found on $^{31}$P and $^{1}$H MRS parameters, suggesting that these molecules do not influence the PCr metabolism.

**Keywords** Multiple sclerosis · Fluoxetine · Prucalopride · Magnetic resonance spectroscopy · Phosphocreatine

## Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) involving two main pathophysiological processes: inflammatory mediated demyelination correlating with focal CNS lesions and clinical relapses and a more diffuse mixed neurodegenerative and inflammatory process correlating with brain atrophy and slow accrual of clinical disability [1–3]. Whereas multiple treatments exist for the inflammatory part of the disease, MS patients have been left without therapeutic options in the more neurodegenerative stages of the disease [4].

Astrocytes have been put forward as an interesting cellular target for treating neurodegeneration and neuroinflammation [5]. It has been shown that the expression of the Beta-2 (B2) adrenergic receptor is decreased in astrocytes in MS patients [6, 7]. Astrocytic B2 adrenergic stimulation has been shown to modulate neuroinflammation and axonal survival during metabolic stress [8, 9]. Moreover,

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it has been reported that this receptor can positively stimulate the transcription of creatine kinase B (CK-B) through the cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) metabolism [10, 11]. CK-B is a key enzyme in the phosphocreatine (PCr) metabolism as it transfers the phosphate group ($P_i$) from PCr to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP). ATP, as a crucial energy source, maintains the ion balance between the intra- and extracellular space. A disturbance in the PCr metabolism may lead to accumulation of potassium in the extracellular space, preventing proper intracellular transport of glutamate resulting in glutamatergic excitotoxicity and subsequent axonal degeneration [12].

Proton magnetic resonance spectroscopy ($^1$H MRS) studies have taught us that the *N*-acetylaspartate (NAA) levels are decreased in white matter lesions and normal appearing white matter (NAWM) of patients with MS, implying a diffuse disruption of the neuroaxonal integrity [13, 14]. The role of a disturbance in the PCr metabolism in this neurodegenerative process was confirmed in a recent phosphor MRS ($^{31}$P MRS) study as Kauv and coworkers found that both in relapsing–remitting MS (RRMS) and secondary progressive MS (SPMS) patients had higher PCr levels compared to healthy controls [15]. The ATP levels were negatively correlated with expanded disability status scales (EDSS), suggesting that more progressed patients had less ATP production [15]. This confirmed the findings of Steen and coworkers who found an increase in PCr/ATP and PCr/$phosphate_{total}$ in patients with progressive MS. Results from samples of NAWM of progressive MS patients showed that PCr metabolism is impaired due to decreased CK-B levels, raising the possibility that a defective PCr metabolism in astrocytes might contribute to the degeneration of oligodendrocytes and axons in MS [14]. Two therapeutic compounds, fluoxetine and prucalopride, are known to respectively stimulate PKA and cAMP production, potentially correcting the aforementioned imbalance in PCr metabolism [16, 17]. Since both products also have CNS anti-inflammatory effects, they are good candidates for treating both disease components in MS [18–23]. The aim of this study, therefore, was to investigate the potential effect of fluoxetine and prucalopride in treating this dysmetabolism. Using MRS, we focused on the early metabolic changes that could indicate an effect on the subclinical neurodegeneration that is already present in the early stages of the disease.

## Materials and methods

### Subjects

The study protocol (concordant with the revised Declaration of Helsinki) was approved by the Ethics Committee of the University Hospitals of Brussels and Ghent [24]. All participants provided written informed consent, following a complete description of the study. Subjects with RRMS, diagnosed according to the revised McDonald 2010 criteria, were allowed to participate when they fulfilled the following inclusion criteria: an EDSS score of $<7$, age between 18 and 60 years, no relapse or corticosteroid treatment within the 3 months prior to scanning, on a stable immunomodulatory treatment for at least 3 months if being treated with such a drug (only interferons and copaxone) [25]. In women of childbearing age, adequate contraception had to be guaranteed. Exclusion criteria were the use of certain medications (antidepressants, MAO inhibitors, serotonin agonists, and medication that lengthens the QT interval), pregnancy or breastfeeding, kidney failure, severe gastrointestinal problems, diabetes mellitus, moderate-to-severe depression (DSM-IV criteria for depression), contra-indications for MRI, and (serious) adverse event on fluoxetine or prucalopride in personal history.

### Study design

In this two-center, double-blind, placebo-controlled study, RRMS patients were randomized and divided into three treatment groups. The fluoxetine group received 20 mg/day of fluoxetine for 2 weeks, followed by 40 mg/day for 4 weeks. The prucalopride group started with 1 mg/day for 2 weeks, followed by 2 mg/day for 4 weeks. The third group was a control group, with patients receiving a placebo during 6 weeks. After screening, the baseline visit with baseline MRI/MRS took place at week 0 and two follow-up MRI/MRS visits at weeks 2 and 6. Clinical and cognitive testing was performed at weeks 0 and 6. Concomitant drugs and adverse events were registered, as was the compliance rate. The study was registered at the European Union Drug Regulating Authorities (Eudra-CT: 2010-023996-25).

### MRI and MRS

All measurements were performed on one of the two whole-body 3T MRI systems (Siemens—University Hospital Ghent and Philips—University Hospital Brussels). All data were analyzed by icometrix, a company specialized in neuroimaging in MS. For more detailed information about the acquisition parameters of the brain imaging, we refer to the appendix for MRI methodology.

The anatomical MRI consisted of 3D FLAIR and 3D T1-weighted images. The anatomical data were processed by icometrix using the CE-labeled and FDA-cleared software called MSmetrix, to extract volumetric measurements for whole brain (WB), grey matter (GM), white matter (WM), as well as for MS lesion volume [26, 27]. $^1$H MRS measurements were preceded by the 3D T1-weighted scan, which

was reformatted to obtain coronal and sagittal series. This allowed accurate positioning of the 2D spectroscopic imaging slice in the centrum semiovale above the corpus callosum. Signal amplitudes for metabolites NAA, total creatine (tCr, i.e., creatine and phosphocreatine), and total choline (tCho, i.e., glycerophosphocholine, phosphorylcholine and choline) were determined. Ratios of the metabolites over both tCr and water were used for evaluation. Total creatine is usually assumed to be stable, although in MS subjects, this can be debated, and therefore, ratio over water was added, especially as it allows comparison between the two scanners [14, 28, 29]. For $^{31}$P MRS, signal amplitudes Pi, PCr, α-, β-, and γ-ATP were quantified. Only those metabolite concentrations with a percentage-estimated standard error (Cramer–Rao lower bound) of less than 50% were considered as reliable in the fitted spectrum. $^{31}$P ratios were determined using the α-ATP resonance as a reference. Although β-ATP is often used as reference for ATP (since it does not contain contributions of other compounds known to be present in brain tissue), we resorted to use α-ATP for normalization, because β-ATP was not always reliably quantified due to low signal-to-noise ratio [14]. The α-ATP, as shown in Fig. 1, was clearly visible and could be determined accurately.

In a subgroup of 33 patients, brain tissue perfusion measurements were performed using arterial spin labeling (ASL), enabling the calculation of cerebral blood flow (CBF) and bolus arrival time (BAT).

## Clinical and cognitive testing

The following tests were performed: timed-25-foot-walk test (T25-FW), nine-hole-peg test (9-HPT), Hauser ambulation index (HAI), California verbal learning test II (CVLT-II versions 1 and 2, respectively), symbol digit modalities test (SDMT), controlled oral word association test (COWAT)—semantic and phonetic, revised Beck depression inventory (BDI-II), and the modified fatigue impact scale (MFIS).

## Outcome measures

Primary outcome measures included the change in PCr/α-ATP and NAA/tCr levels, following 2 and 6 weeks of treatment. We expected that changes in metabolites occur quickly after starting the study medication. Secondary outcome measures were changes in brain volume, ASL-based perfusion, as well as clinical and cognitive changes between the first and last study visits.

## Statistical analysis

The level of PCr/ATP was used to determine the sample size, based on the study of Steen and coworkers [14]. A sample

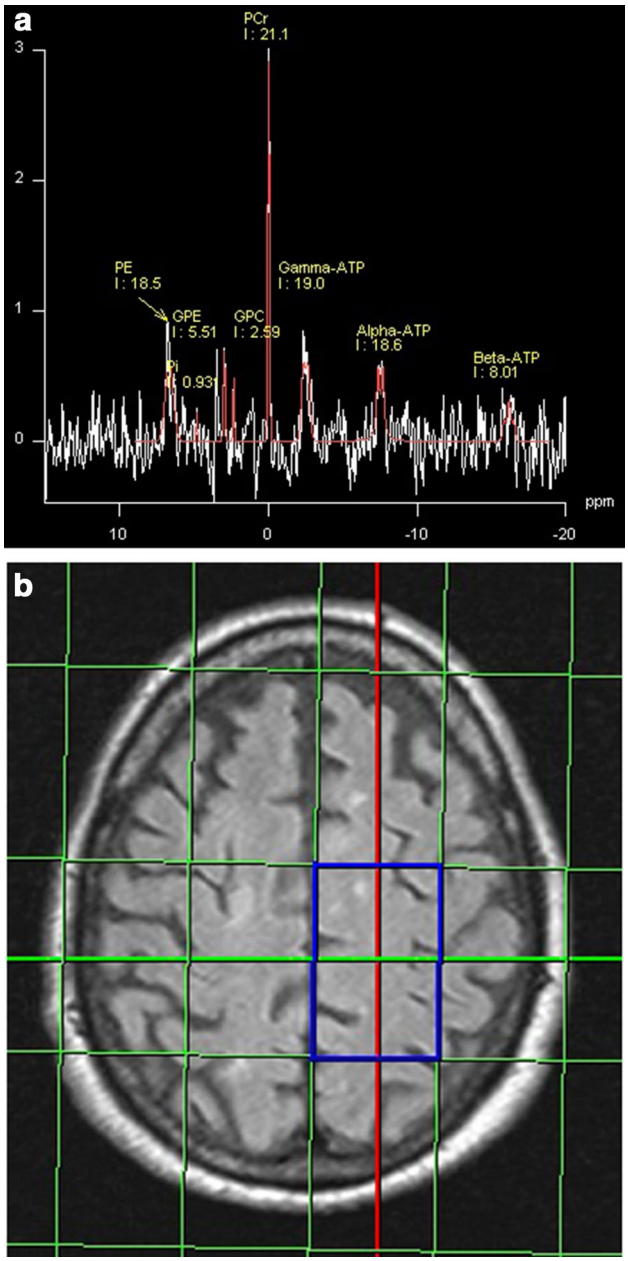


**Fig. 1** Spectrum above shows a typical 31P spectrum from this trial

of 16 in each group has 80% power to detect a decrease of 20% with a significance level of 0.05 (two-tailed) [14]. The duration of the study was chosen based on both preclinical and clinical research, showing that fluoxetine has a fairly fast effect on cerebral metabolites [18, 30–34].

We used mixed linear models to evaluate the difference between the three different treatment groups over the three different timepoints. First, normality of the residuals was assessed. If a *P* value smaller than 0.05 was found in the mixed linear models, the longitudinal changes were analyzed for each treatment group separately between the three

different timepoints using a paired *t* test. IBM SPSS statistics (version 22) was used to perform the analysis. Due to missing values in the subgroup of patients that underwent ASL scans, a basic paired *t* test between the different timepoints was performed. A voxel threshold was set to $P<0.05$ after familywise error correction. Given the exploratory nature of the ASL analysis, also less stringent thresholds (uncorrected $P<0.001$ and $P<0.01$) were used. In case clear trends were found, we further extended the analysis using a within-subject ANOVA between the timepoints to evaluate the global effect.

## Results

### Demographics

Forty-eight patients were included in the study. Figure 2 shows the CONSORT diagram of the study. The mean compliance of the study medication was 96.6% (SD ± 18.76). The mean age at baseline was 42.8 years (SD ± 10.07) for the placebo group, 45.4 years (SD ± 9.44) for the fluoxetine group, and 42.2 years (SD ± 12.46) for the prucalopride group. There was no significant difference in age at baseline ($P=0.661$). The female/male ratio was 10/4 for the placebo group, 10/6 for the fluoxetine group, and 8/7 for the prucalopride group ($P=0.610$). The mean EDSS was 1.4 (SD ± 0.93): 1.2 (SD ± 0.84) in the placebo group, 1.4 (SD ± 1.10) in the fluoxetine group, and 1.6 (SD ± 0.81) in the prucalopride group. The median EDSS was 1.0 (range 3.00) in the placebo group, 1.5 (range 4.00) in the fluoxetine group, and 1.5 (range 3.50) in the prucalopride group. The mean disease duration was 10.4 years (SD ± 7.98). The mean time since the last relapse was 4.4 years (SD ± 3.48). One patient did not have a DMT; the other patients were either on Avonex ($n=23$), Betaferon ($n=9$), Rebif ($n=11$), or Copaxone ($n=1$).

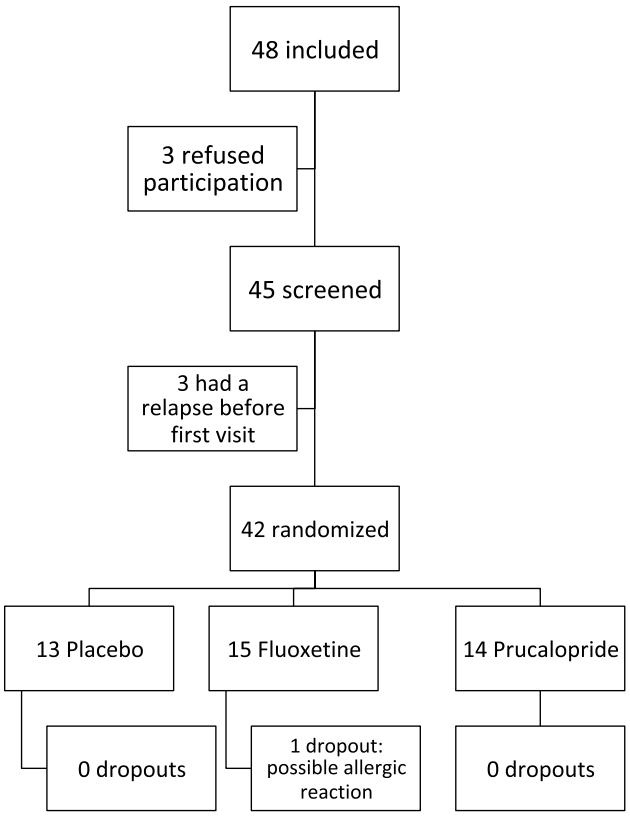


**Fig. 2** CONSORT diagram of the study

### $^{1}$H and $^{31}$P MRS

We did not find any significant differences between the three treatment groups for the different timepoints for both the $^{1}$H MRS and $^{31}$P MRS indices. Table 1 shows the results of spectroscopy for each parameter.

### Volumetric MRI

When evaluating WB, WM, GM, and lesion volumes over time and study groups, we found a significant difference ($P=0.042$) for the WM volume. Table 2 shows the mean values and the standard deviations of the different volumetric parameters. We then looked into the different timepoints in the separate study groups. For the placebo group, we found a decrease in white matter volume between week 0 and week 2 ($P=0.017$). For the fluoxetine and prucalopride groups, we did not find any difference for WM between the different timepoints. Figure 3 shows the percentage change from baseline of the different volumetric parameters over time for the fluoxetine, prucalopride, and placebo groups. Using a *t* test, we compared the percentage change of volume from baseline for both week 2 and week 6. Between placebo and fluoxetine, we found a significant difference ($P=0.008$) at week 2, showing a higher WM volume in the fluoxetine group. We observed a similar effect for the WB volume at week 2 with a near significant difference ($P=0.058$). These differences were not observed at week 6. Furthermore, we found a significant difference between prucalopride and placebo at week 2 ($P=0.018$) showing a higher WM volume in the prucalopride group.

### ASL

A subgroup of 33 patients underwent ASL, but none of the treatment groups showed significant changes with respect to BAT. The intake of prucalopride did not lead to significant changes in CBF over time either. For fluoxetine, we found a few voxels in a cluster that showed a significant increase in CBF over time between the baseline scan and

**Table 1** Results of MRS data for the placebo, fluoxetine, and prucalopride groups

| | Week 0 | *N* | Week 2 | *N* | Week 6 | *N* | *P* value |
|---|---|---|---|---|---|---|---|
| NAA/tCr | | | | | | | 0.797 |
| Placebo | 1.19 (0.171) | 13 | 1.19 (0.198) | 11 | 1.23 (0.275) | 12 | |
| Fluoxetine | 1.28 (0.148) | 15 | 1.33 (0.178) | 14 | 1.32 (0.136) | 13 | |
| Prucalopride | 1.19 (0.136) | 13 | 1.15 (0.194) | 13 | 1.20 (0.184) | 13 | |
| $NAA/H_2O$ | | | | | | | 0.139 |
| Placebo | 0.12 (0.035) | 13 | 0.12 (0.034) | 11 | 0.12 (0.036) | 11 | |
| Fluoxetine | 0.13 (0.026) | 15 | 0.14 (0.034) | 14 | 0.13 (0.022) | 14 | |
| Prucalopride | 0.12 (0.014) | 13 | 0.12 (0.019) | 13 | 0.15 (0.110) | 13 | |
| $tCr/H_2O$ | | | | | | | 0.059 |
| Placebo | 0.10 (0.023) | 13 | 0.10 (0.018) | 11 | 0.12 (0.055) | 12 | |
| Fluoxetine | 0.10 (0.015) | 15 | 0.10 (0.020) | 14 | 0.10 (0.013) | 13 | |
| Prucalopride | 0.10 (0.007) | 13 | 0.11 (0.012) | 13 | 0.11 (0.008) | 13 | |
| $tCho/H_2O$ | | | | | | | 0.603 |
| Placebo | 0.04 (0.006) | 13 | 0.04 (0.005) | 11 | 0.04 (0.012) | 12 | |
| Fluoxetine | 0.04 (0.007) | 15 | 0.04 (0.008) | 14 | 0.04 (0.008) | 13 | |
| Prucalopride | 0.04 (0.004) | 13 | 0.04 (0.005) | 13 | 0.04 (0.007) | 13 | |
| tCho/tCr | | | | | | | 0.459 |
| Placebo | 0.37 (0.065) | 13 | 0.38 (0.044) | 11 | 0.35 (0.056) | 12 | |
| Fluoxetine | 0.39 (0.043) | 15 | 0.39 (0.036) | 14 | 0.38 (0.065) | 13 | |
| Prucalopride | 0.37 (0.037) | 13 | 0.36 (0.033) | 13 | 0.37 (0.057) | 13 | |
| PCr/α-ATP | | | | | | | 0.961 |
| Placebo | 1.77 (0.484) | 11 | 1.68 (0.217) | 8 | 1.76 (0.610) | 8 | |
| Fluoxetine | 1.62 (0.303) | 10 | 1.62 (0.301) | 12 | 1.64 (0.220) | 8 | |
| Prucalopride | 1.66 (0.383) | 9 | 1.56 (0.239) | 7 | 1.57 (0.262) | 9 | |
| $P_i$/α-ATP | | | | | | | 0.337 |
| Placebo | 0.72 (0.315) | 10 | 0.50 (0.057) | 6 | 0.61 (0.378) | 7 | |
| Fluoxetine | 0.50 (0.096) | 8 | 0.59 (0.216) | 11 | 0.56 (0.133) | 7 | |
| Prucalopride | 0.59 (0.240) | 6 | 0.53 (0.153) | 6 | 0.52 (0.135) | 7 | |

The numbers shown are mean values (standard deviation). The *P* values are those of the linear mixed models analysis

**Table 2** Mean (standard deviation) of the different volumetric parameters (in ml) for the different timepoints and treatment groups. The columns at the right of the mean (standard deviation) represent the number of patients taken into account in the analysis

| | Week 0 | *N* | Week 2 | *N* | Week 6 | *N* | *P* value |
|---|---|---|---|---|---|---|---|
| WB | | | | | | | 0.147 |
| Placebo | 1446.03 (60.239) | 13 | 1445.79 (64.327) | 12 | 1448.47 (63.128) | 12 | |
| Fluoxetine | 1453.38 (61.605) | 15 | 1455.82 (66.828) | 14 | 1465.95 (58.180) | 13 | |
| Prucalopride | 1454.88 (46.076) | 14 | 1453.32 (47.743) | 13 | 1451.31 (47.811) | 14 | |
| WM | | | | | | | 0.042 |
| Placebo | 549.40 (29.675) | 13 | 547.42 (28.556) | 12 | 553.36 (26.707) | 12 | |
| Fluoxetine | 534.63 (30.354) | 15 | 537.87 (30.390) | 14 | 538.65 (32.047) | 13 | |
| Prucalopride | 554.40 (27.848) | 14 | 558.83 (27.211) | 13 | 554.41 (28.841) | 14 | |
| GM | | | | | | | 0.393 |
| Placebo | 896.64 (52.715) | 13 | 898.38 (55.818) | 12 | 895.14 (56.436) | 12 | |
| Fluoxetine | 912.76 (52.994) | 15 | 917.93 (53.769) | 14 | 927.33 (52.535) | 13 | |
| Prucalopride | 900.48 (38.603) | 14 | 894.64 (37.145) | 13 | 896.98 (43.826) | 14 | |
| Lesion volume | | | | | | | 0.499 |
| Placebo | 10.64 (10.064) | 13 | 9.86 (10.390) | 12 | 9.84 (9.648) | 12 | |
| Fluoxetine | 5.36 (6.376) | 15 | 5.26 (4.936) | 14 | 4.50 (4.071) | 13 | |
| Prucalopride | 8.46 (11.925) | 14 | 9.08 (12.534) | 13 | 9.18 (12.660) | 14 | |

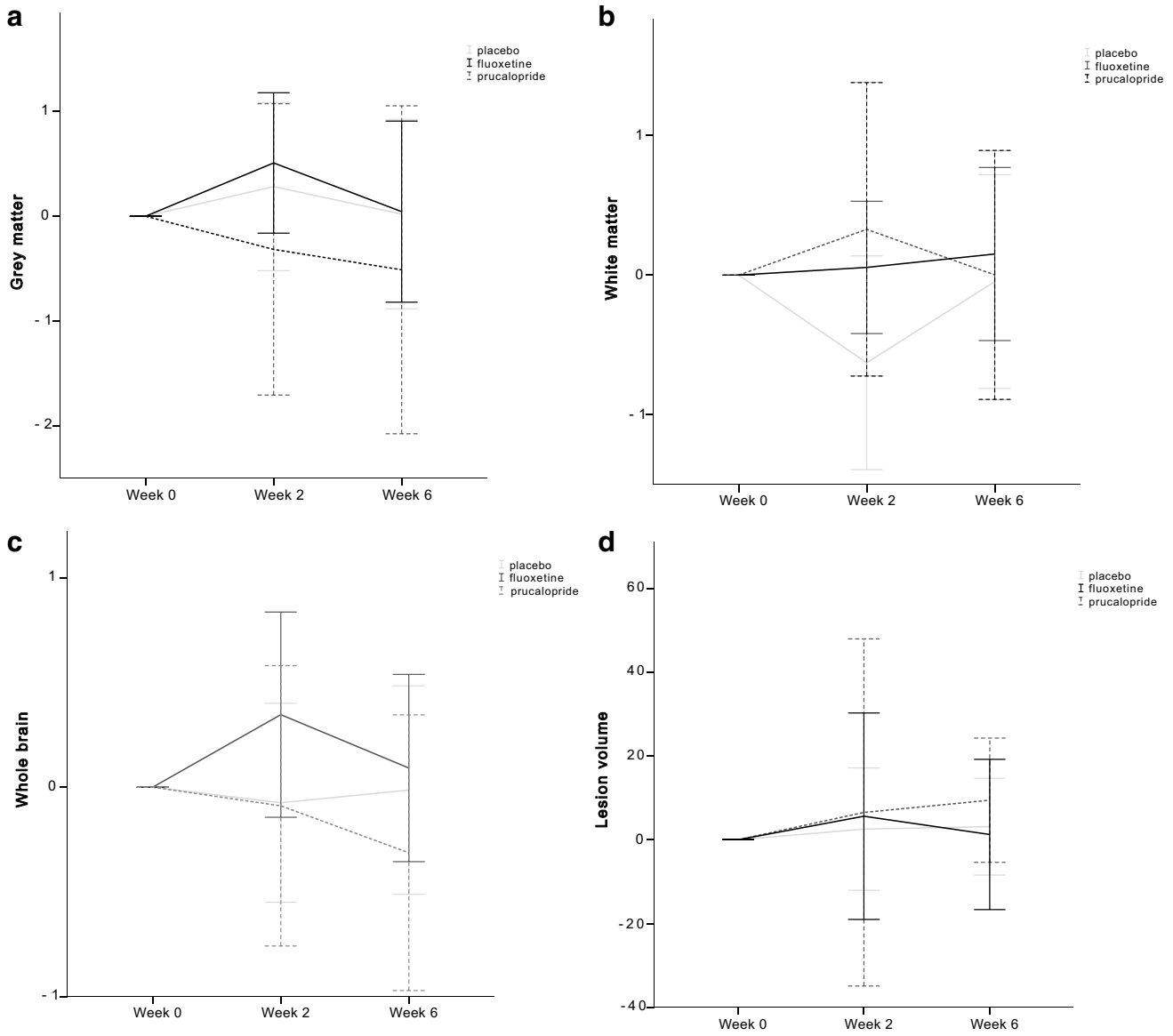


**Fig. 3** Evolution of the volumetric measures (in ml) (WB, WM, GM, and lesion volume) over the different timepoints for the three treatment groups. The values represent the percentage difference from baseline (week 0). The error bars represent ± 1 SD

the 2-week follow-up scan, and between the baseline and the 6-week follow-up scan ($P < 0.001$). When using a less stringent $P$ value (i.e., $< 0.01$), we observed changes both from baseline to 2-week follow-up and from baseline to 6-week follow-up in the left frontal region. However, after correction for multiple comparisons (FWE, $P < 0.05$), none of the observed effects remained significant. For the placebo group, we found some indication ($P < 0.001$) for reduced CBF at 6-week follow-up compared to both baseline and 2-week follow-up. The anatomical location of the changes was, however, not consistent over time, and after correction, none of the effects remained significant (FWE, $P < 0.05$). The peak significant voxels indicated a trend representing a positive effect of fluoxetine in terms of CBF; i.e., CBF decreased over time in the placebo group and CBF increase in the fluoxetine group.

## Clinical and cognitive parameters

We did not find a significant effect of the study medications on neither the T25-FW test ($P = 0.613$), nor the HAI ($P = 0.515$). On the total 9-HPT, no significant difference was found between week 0 and week 6 ($P = 0.536$); there was a trend towards a decrease in the dominant hand ($P = 0.078$), but not in the non-dominant hand ($P = 0.908$), as illustrated in Fig. 4. We also looked at the difference

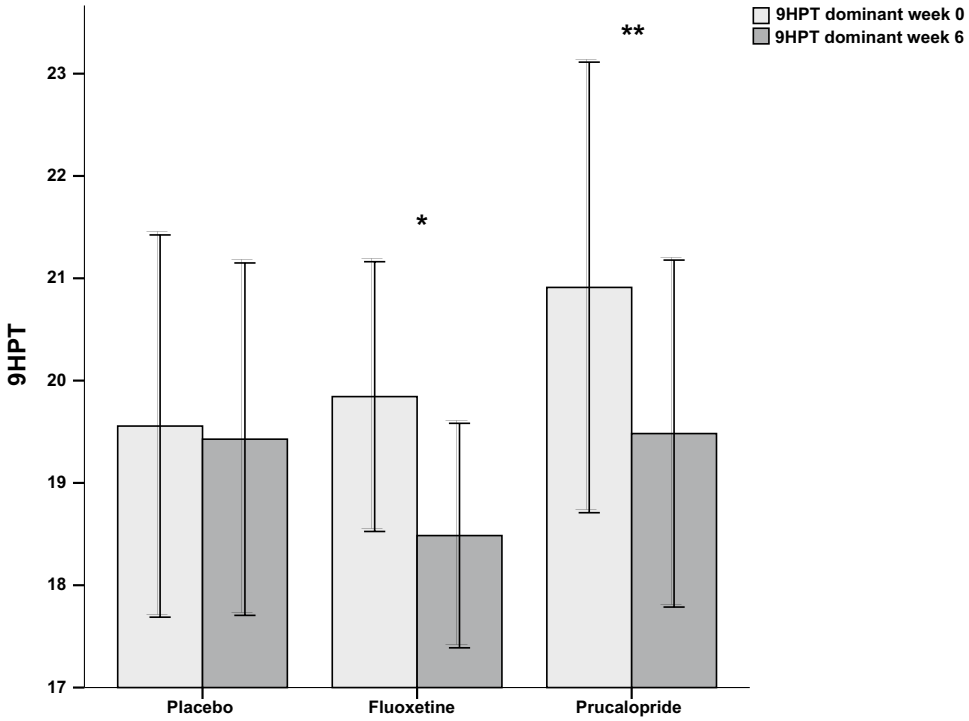


**Fig. 4** Bar chart of the evolution of the 9-HPT in the dominant hand between week 0 and week 6 in the different treatment groups. The error bars represent the 95% confidence interval. $^{*}P<0.05$, $^{**}P<0.01$

over time in the different treatment groups and found that the 9-HPT of the dominant hand was significantly faster in the fluoxetine group ($P=0.017$) and in the prucalopride group ($P=0.007$). Although Fig. 4 shows a trend towards a possibly difference of the baseline 9-HPT, we did not find a significant difference over the different study groups ($P=0.663$). The BDI showed a tendency to decrease in time (week 0 versus week 6) in both the fluoxetine and the prucalopride group, although the overall effect on BDI was not significant ($P=0.152$), as illustrated in Fig. 5. When using paired *t* tests to investigate the difference between week 0 and week 6 for the different treatment groups separately, we found a significant decrease for both the fluoxetine ($P=0.041$) and the prucalopride group ($P=0.041$). For the MFIS, there was no difference between the different treatment groups over time ($P=0.228$). In the prucalopride group, we found a significant decrease between week 0 and week 6 on the MFIS ($P=0.014$), which was not the case for the fluoxetine group ($P=0.133$) (Fig. 5). These results need to be interpreted with caution as the overall effect measured by the mixed models, showed no significant effect.

We observed no significant difference over time between the different treatment groups for the SDMT ($P=0.899$), CVLT-II ($P=0.636$), COWAT semantic ($P=0.126$), and phonetic ($P=0.258$).

## Adverse events

We did not observe any alarming or new adverse events in the three treatment groups (Table 3). There were no serious adverse events (SAEs) reported during the study.

## Discussion

Our study aimed to investigate the potential neuroprotective effects of fluoxetine and prucalopride in MS. Both drugs are inexpensive and well tolerated as confirmed in our study population. Short-term use of prucalopride and fluoxetine seemed safe in patients with MS.

Our study failed in its primary outcome measure, as fluoxetine and prucalopride did not significantly change the levels of PCr, as measured by $^{31}$P MRS. An important remark is that MRS examines the whole brain metabolism and that a compartmental effect might be concealed, especially if it is small. On the contrary, the volumetric measurements on the anatomical MRI did show an effect of the treatment on the WM volume, with fluoxetine and prucalopride seemingly having a more favorable effect on this parameter. Although not significant, a trend was seen in the fluoxetine group for increase in WB volume. The positive effect on the WM and the WB volume was, however, not observed at week 6.

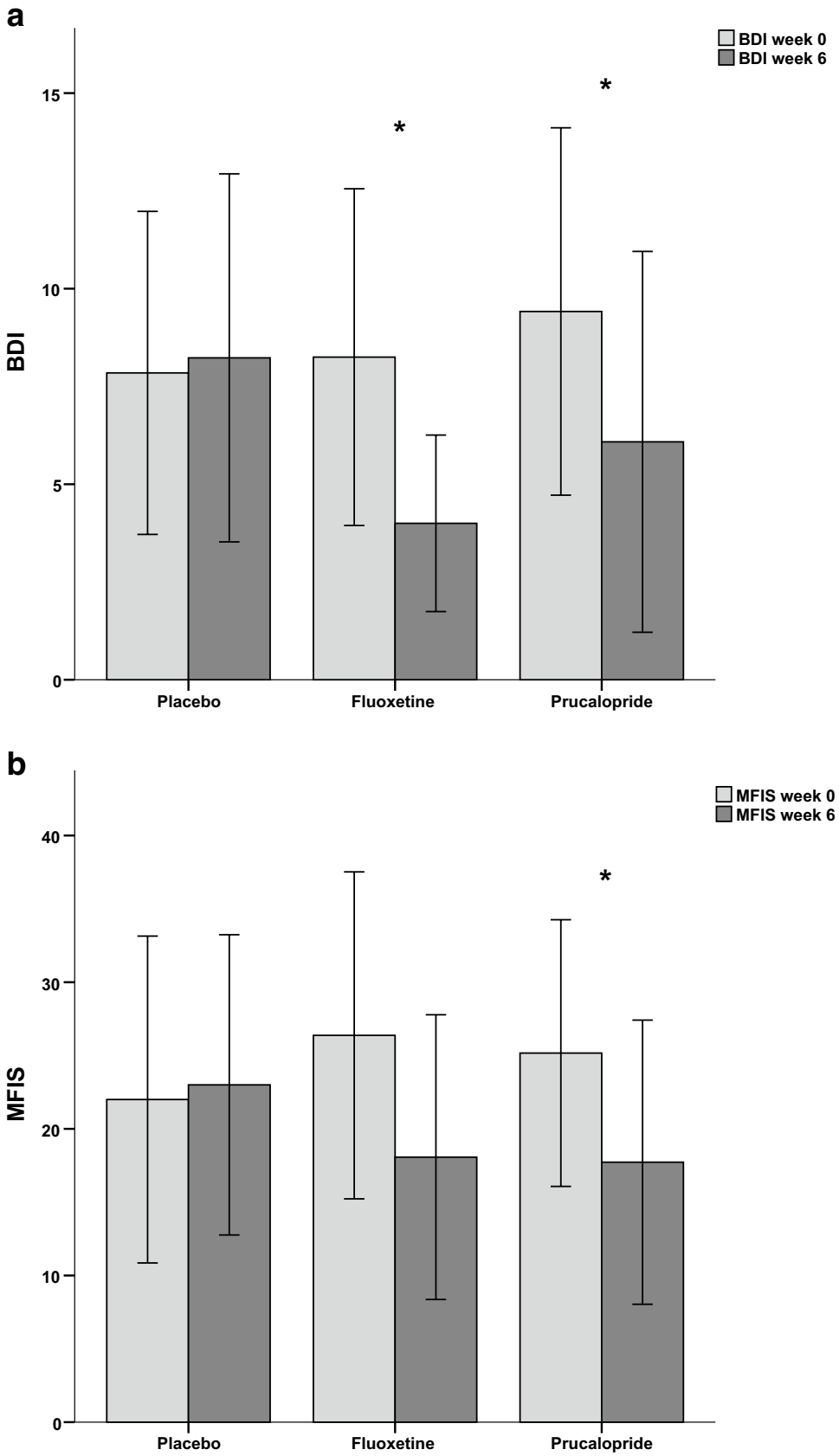


**Fig. 5** Bar chart of the evolution of the BDI and MFIS score in the different treatment groups. The error bars represent the 95% confidence interval. $^{*}P < 0.05$

**Table 3** Recorded adverse events of the trial

| Adverse events | Placebo | Fluoxetine | Prucalopride |
|---|---|---|---|
| Diarrhea | 3/13 | 2/16 | 2/13 |
| Gastric problems | | 2/16 | 1/13 |
| Weight gain | 1/13 | | |
| Muscle cramps | 1/13 | | |
| Fatigue | 1/13 | 1/16 | 1/13 |
| Swelling | 1/13 | | |
| Flu-like symptoms | | 1/16 | |
| URTI | 1/13 | | 1/13 |
| Urticaria | | 1/16 | |
| Itch | 1/13 | 1/16 | |
| Abnormal menstrual bleeding | | 1/16 | |
| Loss of appetite | | 1/16 | |
| Polyuria | | 1/16 | |
| Paresthesia | | 1/16 | |
| Palpitations | | | 1/13 |
| Sleeping problems | | | 1/13 |
| Headache | | | 1/13 |

Our volumetric analysis was only intended to pick up early signals of metabolic changes impacting brain volume; of course, neuroprotective effects reducing brain volume loss can only be evaluated if patients are treated and observed for a longer time considering the variation in volumetric measures and percentage of expected brain atrophy [29, 35, 36]. In the fluoxetine group, a few voxels showed that a significant increase in CBF, which after correcting for multiple testing, was not retained.

We observed a significant increase in fine motor skills of the dominant hand in both the fluoxetine group and the prucalopride group. It should be noted that the included patients show a mild handicap (mean EDSS of 1.4); therefore, the 9-HPT probably is a more sensitive marker for motor skills than the T25-FW in this patient group. Fluoxetine and prucalopride have a quite early influence on the upper limb functioning as measured by the 9-HPT. This result should be interpreted with caution, as the study was not powered to evaluate these clinical changes. In recent literature, a 20% change from baseline in the 9-HPT (or T25-FW) is considered as a threshold for significant change. However, we did not observe a difference between the treatment arms in the proportion of patients showing a 20% change in the 9-HPT (dominant hand) [37]. Of course, the duration of the treatment was to short to be able to attain this kind of change. Furthermore, our study revealed a decrease in depressive symptoms in both the fluoxetine and prucalopride groups. The MFIS score was significantly decreased in the prucalopride group and showed a trend towards a decrease in the fluoxetine group. Taking into account that fluoxetine is an antidepressant and prucalopride has shown to exert an antidepressant effect in animal models, the co-administration of both compounds might have a synergistic and especially a more rapid effect on depression [38]. It might thus be interesting to combine both drugs in MS patients to see if the observed effects could be potentiated. The antidepressant effect of both drugs might play a role in the clinical effect we see in this study. To our knowledge, this is the first time that prucalopride has shown a potential influence on depressive symptoms in humans.

Considering the small sample size, our study was not intended to observe effects on clinical progression. A study investigating the effects on clinical parameters during long-term administration of the drugs should be conducted.

In conclusion, fluoxetine showed to have transitory effects on white matter volume, with a trend towards an increase in whole brain volume. Clinically, we see a positive effect on fine motor skills, depression, and a trend towards a decrease in fatigue. Prucalopride showed similar effects on these clinical data, but no effect on volumetrics. The clinical data should be interpreted with caution, as the study was not powered to evaluate this. Furthermore, there was no change observed in any of the spectroscopic parameters following treatment, which suggests that nor fluoxetine, nor prucalopride have an influence on the PCr metabolism.

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## Compliance with ethical standards

**Conflicts of interest** The authors declare that there is no conflict of interest.

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