

# A randomised double-blind placebo-controlled feasibility trial of flavonoid-rich cocoa for fatigue in people with relapsing and remitting multiple sclerosis

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**Title:** A randomised double-blind placebo-controlled feasibility trial of flavonoid-rich cocoa for fatigue in people with Relapsing and Remitting Multiple Sclerosis.

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Key words

Fatigue, Cocoa, Diet, Multiple Sclerosis, Flavonoids

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1 **Abstract**

2

3 The impact of flavonoids on fatigue has not been investigated in Relapsing and Remitting Multiple  
4 Sclerosis (RRMS).

5

6 **Objective:** To determine the feasibility and estimate the potential effect of flavonoid-rich cocoa on  
7 fatigue and fatigability in RRMS.

8

9 **Methods:** A randomised double-blind placebo-controlled feasibility study in people recently  
10 diagnosed with RRMS and fatigue, throughout the Thames Valley (ISRCTN: 69897291). During a six  
11 week intervention participants consumed a high or low flavonoid cocoa beverage daily. Fatigue and  
12 fatigability were measured at three visits (weeks 0, 3 and 6). Feasibility and fidelity were assessed  
13 through recruitment and retention, adherence and a process evaluation.

14

15 **Results:** 40 pwMS (10 men, 30 women, age  $44 \pm 10$  yrs) were randomised and allocated to high  
16 (n=19) or low (n=21) flavonoid groups and included in analysis. Missing data was <20% and  
17 adherence to intervention of allocated individuals was >75%. There was a small effect on fatigue  
18 (Neuro-QoL: effect size {ES} 0.04; confidence interval {CI} -0.40-0.48) and a moderate effect on  
19 fatigability (six-minute walk test: ES 0.45; CI -0.18 - 1.07). There were seven adverse events (four  
20 control, three intervention), only one of which was possibly related and it was resolved.

21

22 **Conclusion:** A flavonoid beverage demonstrates the potential to improve fatigue and fatigability in  
23 RRMS.

24 **Introduction**

25 Ninety percent of people with Multiple Sclerosis (pwMS) experience fatigue [1]. Fatigue and  
26 fatigability are difficult to treat and greatly affect health related quality of life in pwMS [2].  
27 Fatigability is a term derived from the broader definition of fatigue which refers to an inability to  
28 maintain both physical and cognitive performance [3]. The aetiology of fatigue in MS is complex  
29 including possible neural, inflammatory, metabolic and psychological mechanisms [2] [4]. Whilst a  
30 number of behavioural and drug approaches for fatigue management have been explored, to date the  
31 strongest evidence of success in reducing MS related fatigue is from exercise interventions [5] [6].  
32 However success is limited and other approaches or combination therapies need to be investigated [7].

33 Dark chocolate containing 70-85% cocoa solids is well known for its high antioxidant and  
34 flavonoid content. Over a four week period, dark chocolate consumption has been shown to improve  
35 subjective fatigue in those with Chronic Fatigue Syndrome (CFS) [8] [9], and results from a small  
36 randomised controlled pilot study using a short-term cocoa intervention suggested an increase in sleep  
37 quality and reduction in fatigue [10]. A simple dietary supplement could be implemented alongside  
38 other behavioural interventions early after diagnosis as an adjunctive therapeutic approach to support  
39 pwMS to manage fatigue. There is currently limited evidence-based guidance to inform tailored  
40 dietary advice for symptom management in pwMS. Most diet based studies to date have looked at the  
41 risk of development or relapses in MS [11] [12]. However, modifiable lifestyle factors strongly  
42 correlate to clinically significant fatigue and remain a target for therapeutic trials [13]. To date there  
43 has been limited research assessing the effect of dietary interventions in pwMS [14], but they have  
44 identified good adherence to dietary interventions.

45 We propose that a flavonoid approach for managing MS related fatigue may be moderately  
46 effective, inexpensive, and safe [15] and that it may be exerting its effects by reducing inflammation  
47 and oxidative stress. The aim of the current trial was to evaluate the feasibility and estimate potential  
48 effect to inform a follow-on substantive trial. The following key objectives were assessed: 1) The  
49 acceptance of the study design and diet intervention by participants; 2) Monitoring recruitment rate  
50 and the process of randomisation, adherence to the protocol and loss to follow up; 3) Efficiency of  
51 data collection methods; 4) The estimate of effect size for fatigue, fatigability and other measures.

52  
53

54 **Methods**

55 This was a parallel, randomised, double-blind placebo-controlled trial to assess feasibility and  
56 efficacy (Trial registration ISRCTN: 69897291; Ethical approval National Research Ethics Service  
57 {Solihull West Midlands} reference: 199515). Oxford Brookes University acted as sponsor and the  
58 study was conducted in accordance with the Declaration of Helsinki.

59

60 *Recruitment*

61 Recruitment was through neurology clinics in the Thames Valley, UK. In addition, local MS Society  
62 branches were made aware of the trial and given contact details, and an advertisement for the study  
63 along with the participant information sheet (PIS) was made available on the MS Society website.  
64 Individuals were able to self-refer to the study by contacting the researchers.

65

66 *Setting*

67 The intervention took place in the home of each participant. All testing took place at Oxford Brookes  
68 University (OBU), Oxford, UK except for optional home visits at week 3. Assessments took place  
69 between 7.30 and 10 am and the intervention lasted a total of 6 weeks with three testing visits  
70 (baseline, week 3 and 6).

71

72 *Randomisation and allocation*

73 After recruitment, participants were allocated the next available study number by the blinded assessor.  
74 The study number related to a computer-generated randomisation list held by the principle  
75 investigator and randomised individuals (1:1) into the intervention or the control group. The  
76 randomisation list used minimisation to balance groups for gender and if individuals were on disease  
77 modifying treatments (DMTs) at baseline. The list provided a three digit code that related to a code on  
78 identical pre-package sachets (made up by a co-investigator) containing either intervention or control  
79 cocoa. The sachets were then dispensed to the participant. The intervention began three days after  
80 baseline. Group allocation was concealed throughout the study and analysis.

81

82 *Eligibility*

83 Eligibility criteria were: adults aged  $\geq 18$  years with a  $< 10$  year clinical diagnosis of RRMS, either  
84 treatment naïve or taking first line DMTs (supplementary file 1), no relapse or sudden change in MS  
85 symptoms within the previous three months, no contraindications to providing a blood sample or  
86 tolerating the cocoa drink, fatigue greater than 4 out of 7 on the Fatigue Severity Scale (FSS) [16],  
87 had no other conditions that may be associated with fatigue (e.g. anaemia), not on medication for the  
88 treatment of depression, an Expanded Disability Status Scale (EDSS) score of  $< 4.5$  [17], sufficient  
89 mental capacity to consent, able to walk with or without a walker for at least 16 meters, no condition  
90 affecting the central nervous system other than MS (migraine and headache were allowed), not

91 pregnant or lactating and no objection to the researchers contacting their general practitioner (family  
92 doctor) and neurologist.

93

#### 94 *Intervention*

95 Participants were provided with cocoa by the lead researcher at baseline and at week three, and they  
96 were asked to consume the drink in their homes daily. Cocoa was consumed after an overnight fast at  
97 the same time each morning. After the consumption of the drink, they were instructed to wait 30  
98 minutes before consuming any other food or beverage and/ or take their medication. Participants were  
99 instructed to take their medication and to follow their usual diet for the rest of the day. The cocoa  
100 drinks (intervention and control) were designed to differ only in flavonoid content (low versus high  
101 flavonoid; supplementary file 2). Cocoa powder was provided to participants in air tight individual  
102 sachets. They were asked to add the sachet content to a mug and to add heated rice milk prior to  
103 consuming the drink. Instructions on preparation were provided to ensure all participants followed the  
104 same protocol. Unused cocoa powder was collected by the researcher at the next assessment.

105

#### 106 *Outcomes*

107 The primary aim was to assess feasibility of the dietary intervention in terms of recruitment rate and  
108 the process of randomisation, adherence to the protocol and loss to follow up, safety and process. We  
109 documented adverse events (AEs). Duration of participation and dropout from the intervention were  
110 also recorded. Appropriateness of data collection methods was determined through completion of  
111 questionnaires and missing data, and through the process evaluation, and estimates of effect {effect  
112 sizes (ES) and confidence intervals (CI)} were calculated for the measures and demographics were  
113 collected at baseline.

114

#### 115 *Fatigue and fatigability*

116 Throughout the six-week intervention participants were asked to rate their level of fatigue on a  
117 numerical rating scale (NRS) through daily 'fatigue texts' sent at 10:00, 15:00 and 20:00 every day,  
118 rating their fatigue between 1-10 (10 worst). They replied to the text message of '*on a scale from 1-  
119 10, with 1 being no fatigue and 10 being the worst fatigue you have experienced, how fatigued are  
120 you at the current time?*'. They did this at 10 am, 3 pm and 8 pm every day for six weeks. Fatigability  
121 was measured at baseline and week 6 using the six minute walk test (6MWT) [18]. Participants were  
122 asked to walk at their normal, comfortable walking pace back and forth on a measured 16-metre track  
123 in a University corridor. Distance walked was measured. At baseline, week 3 and 6 the levels of  
124 subjective fatigue experienced over the past seven days were measured using the Neuro-QOL short  
125 form questionnaire [19]. The Adult Memory and Information Processing Battery (AMIPB) [20] was  
126 used to measure cognitive fatigue. The AMIPB required the second highest number in each row to be

127 circled, with 15 rows of five double digit numbers. Participants had two minutes and their attempts  
128 were timed, with incorrect answers being noted.

129

130 Questionnaires administered at baseline, week 3 and 6 were: the Physical Activity Scale for the  
131 Elderly (PASE) [21], EQ5D-5L [22], Preference-Based Multiple Sclerosis Index (PBMSI) [23] and  
132 the Hospital Anxiety and Depression Scale (HADS) [24]. Demographic information was collected. A  
133 previously published protocol paper gives detail about each measure [25]. Activity was monitored  
134 with seven-day wrist worn accelerometers (Axivities ®) prior to, and over two separate weeks of the  
135 trial (week 2-3 and 5-6).

136

137 Blood markers of inflammation were measured including: TNF-alpha, reduced glutathione, a marker  
138 of antioxidant status and lipid peroxidation.

139

140 Detailed description of measures can be found in supplementary file 1.

141

#### 142 *Process evaluation*

143 Upon exiting the study at week 6, each participant was interviewed about the intervention process,  
144 ease of adherence, tolerance and acceptability of the flavonoid drink and the collection of outcome  
145 measures. Participants were asked their opinion on the importance of the research question proposed  
146 to inform future trials. The thirteen topics used included difficulties with intervention delivery,  
147 scheduling of assessments and outcome measure acceptability and suggestions on how to improve the  
148 intervention process. A proportion of the data (20%) was coded by two different team members to  
149 check on reliability of the coding scheme. Transcripts of interviews were examined to identify themes  
150 and categories. Codes were applied to these broad themes, which were then broken down further into  
151 sub-codes. Agreement on concepts and coding by blinded assessors were sought between two  
152 members of the research team to ensure reliability.

153

#### 154 *Analysis*

155 Feasibility was analysed through evaluation of eligibility, recruitment and retention [26].

156 Completeness of outcome measures was reported and 80% was set as a criterion for success.

157 Retention was measured by the proportion of participants who were lost to follow-up. Successful  
158 adherence to the intervention was defined as at least 75% of the participants having completed cocoa  
159 consumption. Further aspects of adherence were measured by the percentage of fatigue texts  
160 completed by participants.

161 Primary analysis followed the intention to treat principal utilised the complete case data set.

162 Results were presented using point estimates and 95% confidence intervals. For the fatigue texts the  
163 fatigue NRS was calculated as the area under the curve (AUC) for each group, ignoring area beneath

164 the baseline, and was calculated geometrically [27]. Data was transformed to improve model fit, or  
165 different regression approaches were used (e.g. Negative Binomial, or Poisson regression). The results  
166 for the 6MWT are reported as mean  $\pm$  SD and for the comparison of baseline and post-intervention as  
167 mean difference  $\pm$  standard error [(SE); 95% (CI)]. A Linear Mixed Model (LMM) was used to  
168 differences between groups throughout all time points. Alternatively and wherever the variables were  
169 categorical, such as the EQ5D-5L sub categories, a generalized estimating equation (GEE) with  
170 appropriate distributions (e.g. Negative Binomial) method was implemented. Both methods used  
171 SAS/STAT 14.3. Fatigue and fatigability measures including the Neuro-QoL short form fatigue, the  
172 6MWT and the AMIPB were further analysed to determine difference between responders and non-  
173 responders and relative risk scores then calculated. Responders on the NeuroQoL, for both the control  
174 and intervention groups, were classified as those who had a clinically meaningful change of 10 points  
175 out of 100 (with the questionnaire total score converted from an original total of 40 between baseline  
176 and week 6. Responders on the 6MWT were classified as those who had a minimally important  
177 change (MIC) in covered distance of 21.6m [27] between baseline and week 6.

178 Data over three time points for activity data was analysed using a Freidman's test and  
179 between group effects were calculated using a Mann-Witney test. Process evaluation included  
180 frequencies for adhering to the intervention, session content and progression which was analysed  
181 descriptively with confidence intervals and regression where possible. A standard content analysis  
182 techniques were employed.

183



184 **Results**

185

186 Between May 2016 and August 2017, 40 pwMS were recruited from four neurology clinics including:  
187 Oxford University Hospitals NHS Trust (John Radcliffe Hospital site), Milton Keynes hospital, Royal  
188 Berkshire hospital or Buckingham hospital or through advertisements and online media (MS Society  
189 webpage and MS Trust Facebook page).

190

191 Figure 1 shows participant flow. It was not possible to determine the total number of people screened.

192

193

194

195 *Feasibility*

196 Fifty-three people showed an initial interest in the trial, but decided not to take part due to: not having  
197 enough time to take part in the trial, did not like chocolate, or were unable to take part in nutrition  
198 trials due to being on weight loss programs or having gastric surgery. All but one person consumed  
199 the cocoa with rice milk (one consumed almond milk). One person discontinued the control  
200 intervention but was not lost to follow up and one person consented to be on the trial but decided not  
201 to consume the cocoa from the start of the intervention. Adherence to intervention overall was 100%  
202 (19/19) for the intervention group and 90% (19/21) for the control group. Missing data from NRS was  
203 less than 20% of total responses, but reporting did drop in week 6. Blood measures were only  
204 achieved in 20 participants (after a maximum of three attempts). Overall missing data for secondary  
205 measures was less than 20%. There were seven AEs during the trial, caused by worsening fatigue,  
206 feelings of nausea, or a general feeling of being unwell. AEs were considered unlikely to be related  
207 (five), possibly related but expected/ resolved (one) and not related to the intervention (one). Four  
208 AEs were associated with the control cocoa and three with the high flavonoid cocoa. There were no  
209 incidences of un-blinding of the researcher nor participant. Five out of 40 people were seen at home at  
210 week 3.

211 Demographic and clinical data are shown in Table 1. There were no significant differences  
212 for demographics between the groups at baseline ( $p>0.05$ ).

213

214 [Insert Table 1]

215

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217

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219

220 Table 1. Demographic information at baseline for both groups

221

222

	Intervention (n = 19)	Control (n = 21)
223 Demographic data		
224 Age (years)	41 ± 11	46 ± 8
225 Women	14 (74%)	16 (76%)
226		
227 BMI (kg/m <sup>2</sup> )	26 ± 7	25 ± 6
228 Treatment naïve	7/19(37%)	6/21(29%)
229		
230 Medications		
231		
232 Anti-depressants/anxiolytic	6	6
233 Anti-convalescents/anti-	3	6
234 spastics		
235 Sedating analgesic	0	1
236 Other sedating	2	7
237		
238 Other (excluding DMTs)	25	24
239 Smokers	0(0%)	2(10%)
240 Uses assistive device	2(11%)	6(29%)
241 Report food allergy	2(11%)	2(10%)
242 Reported food intolerance	6(32%)	5(24%)
243		
244 Reported taking special diet	4(21%)	2(10%)
245		
246 Fatigue Severity Scale (FSS)	5 ± 2	5 ± 3
247 Total		
248 Barthel Index (BI) Total	20 ± 2	19 ± 8
249 Physical Activity for the	90 ± 32	102 ± 31
250 Elderly (PASE) Total		

251 Values are means ± standard deviations, or total number of people in ( ) considering the percentage of

252 the total sample population. FSS and Barthel Index totals are reported as medians ± ranges. An

253 independent t-test was used to compare means for age, BMI, PASE. A Mann-Witney U test was used

254 to compare medians for FSS and BI for non-parametric measures to determine differences between

255 the intervention groups. A chi-squared test was used to compare means for nominal data. There were

256 no significant differences between groups for any baseline measures (p>0.05).

257 *Outcome measures*

258

259 Between group effect sizes were considered from all three assessments points. Efficacy potential of  
260 fatigue and feasibility was determined. A breakdown of the outcome measures is shown in Table 2.

261

262 [Insert Table 2]

263

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276 Table 2. Results for outcome measures at baseline, week 3 and week 6 of the intervention <sup>a</sup>.

277

	Control Baseline (n=21)	3 weeks	6 weeks	Intervention Baseline (n=20)	3 weeks	6 weeks	Relative risk c	Effect sizes with 95% CI b
<b>Fatigue</b>								
Neuro-QoL	25.86 ± 1.27	22.55 ± 1.18	22.95 ± 1.51	27.63 ± 0.88	24.21 ± 1.21	22.95 ± 1.17	1.45	0.04 (- 0.40-0.48)
<b>Fatigability</b>								
AMIPB								
Incorrect	1 ± 2	1 ± 1	1 ± 2	0 ± 1	0 ± 1	0 ± 1		-0.13 (- 0.34-0.09)
Time	53.83 ± 3.83	55.80 ± 5.00	52.43 ± 3.44	58.85 ± 4.01	57.66 ± 4.07	58.67 ± 4.74		0.11 (- 0.34-0.55)
6 minute walk	344 ± 17.67	n/a	354.5 ± 19.29	360.9 ± 13.15	n/a	394.6 ± 18.11	1.80	0.45 (0.18 - 1.07)
Numerical rating scale fatigue	n/a	35 + 12	34 + 16	n/a	33 + 18	31 + 18		
<b>QoL</b>								
EQ5D-5L								
Mobility	2 ± 3	2 ± 3	1.5 ± 3	1 ± 2	1 ± 2	1 ± 2		-0.02 (- 0.26-0.22)
Self-care	1 ± 2	1 ± 2	1 ± 2	1 ± 1	1 ± 1	1 ± 1		-0.30 (- 0.43— 0.16)
Usual activities	2 ± 3	2 ± 2	2 ± 3	2 ± 2	2 ± 2	2 ± 2		-0.12 (- 0.33-0.11)
Pain/ discomfort	2 ± 3	2 ± 3	2 ± 3	2 ± 3	2 ± 2	2 ± 3		-0.25 (- 0.45— 0.02)
Anxiety/ depression	1 ± 2	1 ± 2	1 ± 2	2 ± 2	2 ± 2	2 ± 2		0.15 (- 0.07-0.36)
Health today VAS	67.71 ± 3.30	68.50 ± 3.98	74.15 ± 3.56	71.58 ± 3.48	71.95 ± 3.25	72.79 ± 3.30		0.24 (- 0.21-0.69)
HADS								
Depression	5 ± 8	4 ± 7	4.5 ± 9	4 ± 5	5 ± 7	5 ± 7		0.09 (- 0.15-0.33)
Anxiety	7.62± 0.74	7.25± 0.70	6.65± 0.80	7.32± 0.83	7.21± 0.83	6.63± 0.82		0.14 (- 0.31-0.58)
PBMSI								
Walking	2 ± 2	2 ± 2	1.5 ± 2	1 ± 2	1 ± 2	1.5 ± 2		-0.15 (- 0.35-0.07)
Fatigue	2 ± 2	2 ± 2	2 ± 2	2 ± 2	2 ± 2	2 ± 2		-0.12 (- 0.29-0.06)
Mood	1 ± 2	1 ± 2	1 ± 2	1 ± 2	1 ± 2	1 ± 2		-0.10 (- 0.30-0.11)
Concentration	2 ± 3	2 ± 2	2 ± 2	2 ± 2	2 ± 2	2 ± 2		0.05 (- 0.15-0.26)
Roles/ responsibility	1 ± 2	1 ± 2	1 ± 2	1 ± 2	1 ± 1	1 ± 2		-0.06 (- 0.25-0.14)
<b>Mechanism</b>								
Plasma								
Lipid peroxidation	0.66 ± 0.01	0.68 ± 0.01	0.67 ± 0.01	0.69 ± 0.01	0.69 ± 0.01	0.70 ± 0.02		0.50 (0- 0.99)
TNF-alpha	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01		0.01 (- 0.22-0.23)
Glutathione	0.0043 ± 0.0047	0.0045 ± 0.0031	0.0035 ± 0.0030	0.0034 ± 0.0051	0.0035 ± 0.0041	0.0060 ± 0.0040		0.07 (- 0.55-0.69)

280 a. Values are means ± SE for normally distributed values and medians ± ranges (1st to 3rd quartile) for categorical data.  
281 b. Effect sizes and CI's are Cohen's d, based on non-central t distributions of least squares means differences for continuous  
282 variables, using SAS 9.4 and Cliff's delta, for categorical variables, using R 3.5.  
283 c. Relative risks were calculated for measure of fatigue and fatigability for responders versus non responders.

284 [Insert Table 3]

285  
286

287 Table 3. Physical activity levels (reported in minutes per day on average) using an accelerometer watch prior to  
288 the intervention, between 9 to 16 days (3 weeks) into the intervention and between 35 to 42 days(6 weeks) at the  
289 end of the intervention, broken down into sedentary light moderate and vigorous classification.  
290

	Baseline control	3 weeks	6 weeks	Baseline intervention	3 weeks	6 weeks
<b>Activity</b>	1052.97	1031.17	1049.98±1	1049.20	1090.68±	1061.76
Sedentary	± 23.78	±28.07	9.12	± 29.97	32.06	±22.14
Light	302.48 ± 15.15	304.68 ±17.78	305.16 ±14.22	317.59 ±24.28	293.98 ± 23.53	311.70 ± 18.44
Moderate	83.44 ±13.67	101.76 ± 14.29	82.59 ±10.12	71.78±9.63	76.04±9.64	82.04±9.91
Vigorous	1.67 ±0.69	1.65 ±0.82	1.99 ±1.19	1.98 ±0.93	2.74 ±1.05	1.55±0.49

303 Vales are means ± SE. Total activity for each participant was broken down into sedentary, light, moderate and  
304 vigorous and average for all participants in each group, over each 7-day time period.  
305  
306  
307

### 308 *Fatigue and fatigability*

309 Fatigue was further analysed to determine difference between responders and non-responders. There  
310 was no difference in means between groups ( $p>0.05$ ) yet the Relative Risk (RR) for those who  
311 responded and therefore had improved fatigue in the intervention group (11 out of 19 responders) to  
312 the control group (8 out of 20 responders) was 1.45. The AMIPB was assessed in a similar way with a  
313 change in 1 SD of an improvement in 11.5 seconds considered clinically meaningful. Based on these  
314 criteria only one person improved in the intervention group and no one in the control group and  
315 therefore a RR was not calculated.

316 There was a medium effect size for distance walked in six minutes (0.45 {CI 0.18-1.07})  
317 between the groups with the intervention showing a larger increase in the metres walked in 6 minutes  
318 after the intervention (Table 2). The RR for responders in the intervention group compared to control  
319 was 1.80 in favour of the intervention group.  
320

### 321 *Process evaluation*

322 Both groups had similar positive experiences about the scheduling of the assessments. 13/19 in the  
323 intervention vs 18/20 people in the control indicated no impact of the measurement instruments used  
324 on weekly routine over the six weeks. Similar numbers in both groups identified that the outcome  
325 measures were acceptable. *Randomisation*: Most comments from both groups indicated acceptance  
326 towards the process of randomisation. *Taste*: Similar numbers (8/19 intervention vs 9/20 control)  
327 reported positive comments about the taste of the cocoa. *Procedures and routine of preparing the*  
328 *drink*: A few individuals in both groups identified that it was inconvenient to wait for food after drink  
329 consumption (4/19 intervention vs 3/20 control), and that the process did not always fit in with their

330 lifestyle e.g., traveling. Other individuals (15/19 intervention vs 17/20 control) identified that they  
331 worked it into a routine. *Continuation of consuming the drink*: half of both groups (9/19 intervention  
332 vs 11/20 control) said they would continue the drink if offered. A further four people in both groups  
333 said they would continue if it was beneficial or if they could implement their own routine.

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344 **Discussion**

345 PwMS engaged with the dietary intervention, with fatigue and fatigability measures responding more  
346 in the intervention group with effect sizes calculated. The current study was shown to be feasible and  
347 well received by pwMS, with high adherence to the intervention and excellent data completion. Our  
348 study establishes that the use of dietary interventions is feasible and may offer possible long-term  
349 benefits to support fatigue management, by improving fatigue and walking endurance. We further  
350 propose that considering the possible anti-inflammatory mechanism, flavonoids may be used as an  
351 adjunctive approach alongside other therapeutic interventions and suggest the possible benefit of such  
352 combined approaches for fatigue management. However further full powered trials would need to be  
353 performed.

354         The time post diagnosis was extended from five to ten years in order to recruit to target (n =  
355 40). Adherence (>75%), retention and amount of missing data (<80%) were within the acceptable  
356 ranges. Most missing data was from the NRS texts over the six weeks which is not surprising as this  
357 was the most time-consuming part of the assessment. Completion was still above 80% of total with  
358 the first five weeks, with week six showing the most missing data. Mild AEs have previously been  
359 reported when consuming high flavonoid cocoa including nausea and vomiting, gastrointestinal  
360 disturbances and headaches [28]. The AEs reported in the current trial did not cause any safety  
361 concerns and were similar between groups.

362         This is the first study to suggest the potential for fatigability being improved through a  
363 6MWT after six weeks of a flavonoid intervention, with a moderate effect size. In the current study  
364 the intervention showed a MIC over time with 33.7m (SE: 8.4) in contrast to the control group 10.2m  
365 (SE: 9.6). Fatigue correlates with a decrease of physical endurance [29] and walking speed [30] and  
366 therefore a treatment targeting fatigue could also improve walking performance. Flavonoid have been  
367 found to increase cerebral blood flow by inducing widespread stimulation of brain perfusion, and this  
368 could also influence mood, cognitive performance, fatigue perception and ability to perform specific  
369 movement tasks [31]. When considering other symptoms, pain was shown to improve in the flavonoid  
370 group over the six weeks as measured by the EQ5D-5L with a moderate effect size. The antioxidant  
371 properties of flavonoids are thought to lesson neuropathic pain by alleviating oxidative stress and thus  
372 reducing neuron damage caused by lipid peroxidation. [32]. We did not measure objective measures  
373 of pain in this trial and therefore further research is warranted to explore the pain improvement.  
374 Indeed previous research has pointed towards higher motivation in physical activity in pwMS when  
375 symptoms such as pain were improved [33]. This may also allow pwMS to become more active and  
376 mobile, as noted by the improvements on the 6MWT.

377         The process evaluation revealed that overall the trial was well accepted, with the timing of  
378 assessments and the outcome measures being convenient and low burden, respectively and the  
379 participants in both groups found the taste of the cocoa enjoyable, or noted it as neither tasty nor  
380 unacceptable. In both groups the blinding and randomisation process were accepted, and a majority of

381 pwMS in both groups declared their willingness to continue consuming the cocoa long term,  
382 especially if benefits to fatigue were found. However findings around the preparation of the drink and/  
383 or scheduling the timing of drink consumption into ones routine are factors to consider for future  
384 trials.

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### 386 **Limitations**

387 As a feasibility study a powered investigation using a sample size of 80 is now needed. For blood  
388 measures, the lack of ability of the phlebotomist to collect blood from several of the participants led to  
389 missing data. From the data collected, there were small effect sizes in blood indicators, apart from  
390 lipid peroxidation which showed a moderate effect size and therefore this area needs further  
391 investigation. The NRS fatigue data was analysed as a total whole change over the six weeks, and  
392 therefore more sensitive analysis may have discovered changes within the six weeks. Fatigue may be  
393 both physical and cognitive and coexists with and is impacted by a number of factors including  
394 anxiety and depression. This study measured a number of these factors including: motor (6MWT) and  
395 cognitive (AMIPB) fatigue, and anxiety and depression (HADS) which could be further investigated  
396 in a study powered for this. In this pilot we set out to reduce variability from these factors where  
397 possible, for example we excluded individuals with a clinical diagnosis or receiving treatment for  
398 depression (such as individuals on antidepressants)

399

400 It should be considered that, while we used a wide range of recruitment methods reducing the risk of  
401 recruitment bias, participants were recruited from affluent areas of the UK. Acknowledging this  
402 limitation, we nevertheless propose the responses in outcome measures are largely generalizable to  
403 relatively healthy pwMS.

404

### 405 **Conclusion**

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407 The use of dietary approaches to reduce fatigue and associated factors in pwMS may be an easy, safe  
408 and cost effect way to impact on quality of life and independence, allowing people to feel more in  
409 control of their condition. A full evaluation including wider geography, longer follow up and cost  
410 effectiveness is now indicated. This technology has the potential to be implemented in the UK and  
411 worldwide, and alongside other rehabilitation including exercise, DMTs and physiotherapy.

412

413 Figure 1 Flow of recruitment legend: The screening and enrolment process for a 6 week randomised  
414 double-blind placebo-controlled feasibility trial in people with Multiple Sclerosis. A total of 40 people  
415 were included in the final analysis, and reasons for not receiving the allocated intervention (cocoa  
416 drink) and discontinuation are presented.

417



418 *Contributorship*

419 Coe, Collett, Soundy, Clegg, Cavey, Wade, Palace, De Luca, Harrison, Buckingham and Dawes were  
420 involved in the design and ongoing conduct of the project.

421 Coes and Dawes were responsible for the overall conduct of the project.

422 Coe, Cossington, Soundy, Durkin, Kirsten, and Dawes were responsible for the data collection and  
423 day to day running of the trial.

424 Izadi was involved in the statistical analysis of the project.

425 All authors were involved in the writing and proof reading of the project.

426 Wade was responsible for AEs.

427

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429 Oxford Brookes University

430

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440

441 *Conflict of interest*

442 There was no conflict of interest

443

444 *Ethical approval*

445 Ethical approval was granted from the National Research Ethics Service {Solihull West Midlands}  
446 reference: 199515)

447 **References**

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- 450 1. Strober, L.B., *Fatigue in multiple sclerosis: a look at the role of poor sleep*. Front Neurol,  
451 2015. **6**: p. 21.
- 452 2. Wolkorte, R., D.J. Heersema, and I. Zijdewind, *Muscle Fatigability During a Sustained Index*  
453 *Finger Abduction and Depression Scores Are Associated With Perceived Fatigue in Patients*  
454 *With Relapsing-Remitting Multiple Sclerosis*. Neurorehabil Neural Repair, 2015. **29**(8): p.  
455 796-802.
- 456 3. Kluger, B.M., L.B. Krupp, and R.M. Enoka, *Fatigue and fatigability in neurologic illnesses:*  
457 *proposal for a unified taxonomy*. Neurology, 2013. **80**(4): p. 409-16.
- 458 4. Srinivasan, R., et al., *Evidence of elevated glutamate in multiple sclerosis using magnetic*  
459 *resonance spectroscopy at 3 T*. Brain, 2005. **128**(Pt 5): p. 1016-25.
- 460 5. Asano, M. and M.L. Finlayson, *Meta-analysis of three different types of fatigue management*  
461 *interventions for people with multiple sclerosis: exercise, education, and medication*. Mult  
462 Scler Int, 2014. **2014**: p. 798285.
- 463 6. Langeskov-Christensen, M., et al., *Potential pathophysiological pathways that can explain*  
464 *the positive effects of exercise on fatigue in multiple sclerosis: A scoping review*. J Neurol Sci,  
465 2017. **373**: p. 307-320.
- 466 7. Schwarz, S., et al., *Complementary and alternative medicine for multiple sclerosis*. Mult Scler,  
467 2008. **14**(8): p. 1113-9.
- 468 8. Sathyapalan, T., et al., *High cocoa polyphenol rich chocolate may reduce the burden of the*  
469 *symptoms in chronic fatigue syndrome*. Nutr J, 2010. **9**: p. 55.
- 470 9. Sathyapalan T, C.P., Beckett S, Rigby AS & Atkin SL, *High cocoa polyphenol rich chocolate*  
471 *improves the symptoms of chronic fatigue*. Endocrine Abs, 2006. **6**.
- 472 10. Coe, S., et al., *Flavonoid rich dark cocoa may improve fatigue in people with multiple*  
473 *sclerosis, yet has no effect on glycaemic response: An exploratory trial*. Clin Nutr ESPEN,  
474 2017. **21**: p. 20-25.
- 475 11. Hedstrom, A.K., et al., *High consumption of coffee is associated with decreased multiple*  
476 *sclerosis risk; results from two independent studies*. J Neurol Neurosurg Psychiatry, 2016.  
477 **87**(5): p. 454-60.
- 478 12. Azary, S., et al., *Contribution of dietary intake to relapse rate in early paediatric multiple*  
479 *sclerosis*. J Neurol Neurosurg Psychiatry, 2018. **89**(1): p. 28-33.
- 480 13. Weiland, T.J., et al., *Clinically significant fatigue: prevalence and associated factors in an*  
481 *international sample of adults with multiple sclerosis recruited via the internet*. PLoS One,  
482 2015. **10**(2): p. e0115541.
- 483 14. Bisht, B., et al., *A multimodal intervention for patients with secondary progressive multiple*  
484 *sclerosis: feasibility and effect on fatigue*. J Altern Complement Med, 2014. **20**(5): p. 347-55.
- 485 15. Yadav, V., L. Shinto, and D. Bourdette, *Complementary and alternative medicine for the*  
486 *treatment of multiple sclerosis*. Expert Rev Clin Immunol, 2010. **6**(3): p. 381-95.
- 487 16. Herlofson, K., et al., *Fatigue in early Parkinson's disease. Minor inconvenience or major*  
488 *distress?* Eur J Neurol, 2012. **19**(7): p. 963-8.
- 489 17. Kurtzke, J.F., *Rating neurologic impairment in multiple sclerosis: an expanded disability*  
490 *status scale (EDSS)*. Neurology, 1983. **33**(11): p. 1444-52.
- 491 18. Goldman, M.D., R.A. Marrie, and J.A. Cohen, *Evaluation of the six-minute walk in multiple*  
492 *sclerosis subjects and healthy controls*. Mult Scler, 2008. **14**(3): p. 383-90.
- 493 19. Cella, D., et al., *Neuro-QOL: brief measures of health-related quality of life for clinical*  
494 *research in neurology*. Neurology, 2012. **78**(23): p. 1860-7.

- 495 20. Vlaar, A.M. and D.T. Wade, *The Adult Memory and Information Processing Battery (AMIPB)*  
496 *test of information-processing speed: a study of its reliability and feasibility in patients with*  
497 *multiple sclerosis*. Clin Rehabil, 2003. **17**(4): p. 386-93.
- 498 21. Washburn, R.A., et al., *The Physical Activity Scale for the Elderly (PASE): development and*  
499 *evaluation*. J Clin Epidemiol, 1993. **46**(2): p. 153-62.
- 500 22. Herdman, M., et al., *Development and preliminary testing of the new five-level version of EQ-*  
501 *5D (EQ-5D-5L)*. Qual Life Res, 2011. **20**(10): p. 1727-36.
- 502 23. Kuspinar, A., et al., *Using existing data to identify candidate items for a health state*  
503 *classification system in multiple sclerosis*. Qual Life Res, 2014. **23**(5): p. 1445-57.
- 504 24. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale*. Acta Psychiatr  
505 Scand, 1983. **67**(6): p. 361-70.
- 506 25. Coe, S., et al., *A protocol for a randomised double-blind placebo-controlled feasibility study*  
507 *to determine whether the daily consumption of flavonoid-rich pure cocoa has the potential to*  
508 *reduce fatigue in people with relapsing and remitting multiple sclerosis (RRMS)*. Pilot  
509 Feasibility Stud, 2018. **4**: p. 35.
- 510 26. Craig, P., *A new CONSORT extension should improve the reporting of randomized pilot and*  
511 *feasibility trials*. J Clin Epidemiol, 2017. **84**: p. 30-32.
- 512 27. Cella, M. and T. Chalder, *Measuring fatigue in clinical and community settings*. J Psychosom  
513 Res, 2010. **69**(1): p. 17-22.
- 514 28. Crews, W.D., Jr., D.W. Harrison, and J.W. Wright, *A double-blind, placebo-controlled,*  
515 *randomized trial of the effects of dark chocolate and cocoa on variables associated with*  
516 *neuropsychological functioning and cardiovascular health: clinical findings from a sample of*  
517 *healthy, cognitively intact older adults*. Am J Clin Nutr, 2008. **87**(4): p. 872-80.
- 518 29. CRE-2008-0419, *Test-retest Reliability of the StepWatch Activity Monitor Outputs in*  
519 *Individuals with Chronic Stroke measure*. Clinical Rehabilitation, 2008.
- 520 30. Huisinga, J.M., et al., *Is there a relationship between fatigue questionnaires and gait*  
521 *mechanics in persons with multiple sclerosis?* Arch Phys Med Rehabil, 2011. **92**(10): p. 1594-  
522 601.
- 523 31. Nehlig, A., *The neuroprotective effects of cocoa flavanol and its influence on cognitive*  
524 *performance*. Br J Clin Pharmacol, 2013. **75**(3): p. 716-27.
- 525 32. Azevedo, M.I., et al., *The antioxidant effects of the flavonoids rutin and quercetin inhibit*  
526 *oxaliplatin-induced chronic painful peripheral neuropathy*. Mol Pain, 2013. **9**: p. 53.
- 527 33. Fragoso, Y., Santana, DLB, Pinto, RC, *The positive effects of a physical activity program for*  
528 *multiple sclerosis patients with fatigue* Neurorehabilitation, 2008. **23**(2): p. 153-57.

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