Abstract

Context: Current research suggests that dark cocoa may reduce fatigue; however, the effect on fatigue in people with MS (pwMS) has never been established. The objective of this feasibility study was to explore the acute effect of high flavonoid cocoa on measures of fatigue and glycaemic response. **Methods:** This was a randomised crossover participant blind exploratory study in 12 participants (2 male and 10 female) with MS-related fatigue (>4 on the Fatigue Severity Scale; FSS). After fasting overnight, participants consumed the high flavonoid cocoa drink (350 mg gallic acid equivalents {GAE}/g) or a low flavonoid cocoa control (120 mg GAE/g), consuming the alternative drink on the next visit. Fatigue was self-reported on a 100mm visual analogue scale at 30-minute time intervals for 2 hours post cocoa consumption and every 2 hours for the rest of the day. Fatigability was monitored using a 6 minute walk test (6MWT) at the end of the visit (2 hrs), and activity monitors worn for 24 hours commencing at 12noon on the day of testing. The feasibility of performing the trial including outcome measures was documented. **Results:** A moderate effect was found in self-reported fatigue throughout the day in favour of the high flavonoid group (Cohen's d 0.32, 95% non-central t CI -0.57-1.20). Fatigability measures did not change. Participants consumed and enjoyed the cocoa, all participants completed the study and outcome measures were accepted. **Conclusion:** The results of this study support further trials to investigate the feasibility and efficacy of pure cocoa as a dietary supplement for fatigue in pwMS.

Abbreviations

BG, blood glucose; GR, glycaemic response; VAS, visual analogue scale; GAE, gallic acid equivalents; pwMS, people with Multiple Sclerosis; FFQ, food frequency questionnaire; BI, Barthels Index; FSS, fatigue severity scale

Introduction

Fatigue is one of the most debilitating symptoms in people with Multiple Sclerosis (pwMS), greatly affecting quality of life (Tabrizi and Radfar, 2015). The exact cause of fatigue in MS is unknown, however various mechanisms may influence fatigue severity.

Foods rich in flavonoids may show potential for reducing fatigue, through several proposed mechanisms. There is currently available evidence that suggests oxidative stress may contribute to the pathology in MS, which in turn may be improved or inhibited by the antioxidant properties in flavonoids (van Horssen et al., 2008). In addition, it has been suggested that the functional properties of flavonoids allow for penetration through the blood-brain barrier, potentially leading to improved neurosignaling, as well as rehabilitation of neuronal function (Solanki et al., 2015). A pathological inflammatory response may be responsible for the fatigue experienced in MS, for example TNF-alpha levels have been found to be elevated in fatigued pwMS compared to those who were non fatigued (Braley and Chervin, 2010). Luteolin, a naturally occurring flavonoid, has been found to benefit the disease course of pwMS, for example by inhibiting activated peripheral blood leukocytes and mast cells and mast cell dependent T cell activation (Theoharides, 2009). Katz, Doughty and Ali (2011) suggest that cocoa may be beneficial towards MS remission as its flavonoid content may promote blood flow to the brain, and may therefore lead to additional nerve repair, better metabolic clearance from the brain and greater oxygen availability. Therefore foods containing flavonoids may be used in conjunction with other disease modifying treatments (DMTs) in pwMS to reduce relapses and improve the severity of the symptoms experienced.

Cocoa is rich in flavonoids and is a popular and easily accessible product. A recent systematic review and meta-analysis investigated 42 randomized control trials, and found cocoa to be significantly beneficial for vascular endothelial function and inflammation

(Hooper et al., 2012). Cocoa has been shown to improve fatigue in people with Chronic Fatigue Syndrome (CFS). Sathyapalan et al. (2010) conducted a double blinded, randomised, pilot crossover study, daily providing participants with 45g of high flavonoid chocolate. After eight weeks participants reported significant reductions in fatigue and disability. Poor sleep quality has previously been shown to be significantly correlated with fatigue in MS (Attarian et al., 2004). Flavonoids have also been shown to improve sleep quality and therefore may reduce daytime fatigue in those with MS (Ngan & Cunduit, 2011).

Flavonoid rich foods have also been shown to influence postprandial blood glucose levels (Coe et al. 2013). Glucose tolerance may be altered in pwMS (Mahler et al., 2012; Wens et al. 2013; White et al., 2006) and an association has been found between the availability of glucose to the brain and perceived fatigue (Roelcke et al., 1997). This has therefore raised the question as to whether improved glucose tolerance may reduce the fatigue experienced in those with the disease. However to date there has been no exploration of the response in this group.

The current randomised crossover exposure response participant blind exploratory study will assess the effect of high flavonoid cocoa versus low flavonoid cocoa on fatigue and fatigability as measured by mobility in pwMS. Glycaemic response (GR) after the consumption of the drink, was also measured.

Method

This was a randomised crossover participant blind exploratory study in 12 participants (aged 54 ± 10.56 years, 2 male and 10 female) with MS-related fatigue. Participants were expected to attend two test visits at Oxford Brookes University and in a randomised order (determined electronically by a random number table) consumed either the low flavonoid control or the high flavonoid cocoa on different days, with at least three days between test visits (Figure 1). The present study was approved by the Oxford Brookes University Research Ethics Committee: UREC Registration No: 150938. All procedures were carried out accordingly to Declaration of Helsinki guidelines and policies, and retained data was managed accordingly to the Oxford Brookes University's policy on Academic Integrity.

Procedure

PwMS were recruited from local support groups throughout the Thames Valley and via advertisements posted at Oxford Brookes University. After expressing interest in the study, participants where provided with the study information sheet and were given a minimum of 24 hours to review the information and ask the researchers any questions regarding the trial. Once potential participants agreed to take part in the trial and after initial eligibility was checked over the phone, a combined screening and first test visit was arranged where signed consent was taken.

Participants were asked to keep a 24 hour food diary the day before each visit, and to repeat this diet before the next test day. In addition to reduce variability in testing, participants were also asked to avoid vigorous exercise, and to limit their alcohol and caffeine intake on the day prior to testing (≤ 2 units and ≤ 3 cups respectively). Additionally, participants were asked to fast overnight for 10 – 12 hours prior to visits, which began between 7-10 am, and on the first assessment visit a health questionnaire was administered

(asking about smoking habits, current or previous diseases, current medication or supplement intake, dietary habits).

Screening and descriptive data

At the screening/ first test visit demographics were recorded including MS subtype, compliance with the fasting protocol, blood pressure (mmHg) was recorded and mean fasting blood glucose (BG) was measured. Participant independence in daily living was assessed using the Barthels Index (BI; Nicholl et al., 2004). Each test occasion lasted no longer than three hours, and participants were required to leave a minimum of 24 hours between test days.

Participants

Participants were excluded if they reported any sudden changes in MS symptoms within the last three months, had a change in their DMTs and/ or medications that could influence fatigue in the past three weeks, had a metabolic disease or were presently on medication interfering with insulin or glucose metabolism, had been diagnosed with a condition other than MS affecting the CNS, had an allergy or intolerance to ingredients used during testing, experienced fatigue from any condition other than MS, were pregnant or lactating, were clinically depressed, had a BMI outside 18.5 - 30 kg/m² (body composition was confirmed using Tanita BC-418MA), had impaired glucose tolerance (7.8-11.1 mmol/L), or had a fatigue severity score less than 4 on the Fatigue Severity Scale (FSS; Krupp et al. 1989). This scale asks nine questions about various aspects of perceived fatigue, 1 = not fatigued at all and 7 = very fatigued.

Acute response to flavonoid drink/ intervention

Participants were randomly administrated a high polyphenol test or low polyphenol control cocoa drink (Table 1), to consume within a maximum time of 15 minutes. Drinks were matched as closely as possible for available carbohydrate (avCHO) and energy content. Due to the idea that pwMS are following the Overcoming Multiple Sclerosis (OMS) diet, which excludes diary from the diet, the drink was made with Alpro rice milk (Tesco, UK). The total polyphenol content of the drinks had previously been established (Santos and Coe, 2016), with the high flavonoid cocoa powder containing 350mg gallic acid equivalents (GAE)/g, whilst the low flavonoid control powder had instead been established to contain 120mg GAE/g.

Fatigue VAS

Fatigue was recorded on a horizontal 100mm VAS every 30 minutes following drink consumption and throughout testing, categorizing 0mm as 'not at all fatigued' and 100mm as 'extremely fatigued' (Kos et al., 2006). Participants continued to record fatigue every two hours after testing was completed, until six hours after leaving the lab.

Fatigability

Fatigability was monitored using a 6 minute walk test (6MWT) performed at the end of the visit (2 hrs), and through activity monitoring.

Activity monitoring

A GENEActiv (Geneactive, UK) was used to record physical activity for a 24 hour period. Data was sampled at 100Hz at a +/-8g range at 3.9mg resolution and recorded from the non-dominant wrist. Post measurement, data was epoched to 1seconds samples and analysed in a bespoke spreadsheet (Excell, Microsoft Office 2011, US) and expressed as percentages of

physical activity level (sedentary, light, moderate, vigorous) per hour, according to sample frequency adjusted Single Vector Magnitude cut-offs described by Esliger et al. (2011).

6 Minute Walk Test

The 6MWT has previously been proved to be an accurate tool to establish physical fatigue in MS (Goldman, Marrie and Cohen, 2008). On each test occasion, the 6MWT was performed approximately 120 minutes after the test meal was consumed. Participants were instructed to walk back and forth along a 14m long corridor at a pace they deemed comfortable, rounding a cone at the end of each lap. During the walk, participants were at all times accompanied by appropriately trained personnel, and were informed they may stop and rest if needed. The walk was timed using a hand held stopwatch and distance walked was measured in metres.

GR

GR was measured using the previously validated method of Wolever (2004) and redesigned by Coe et al. (2013), and is in line with procedures recommended by the Food and Agriculture Organization/World Health Organization. A total of eight blood measures were taken using an automatic blood glucose analyser (Glucose 201+, Hemocue AB, Sweden). Mean fasting BG was calculated using two 5µl finger-prick samples at -5 and 0 min, and measurements were collected at 15, 30, 45, 60, 90 and 120 minutes after the cocoa consumption. BG levels were compared to WHO guidelines to determine if any of the participants had fasting BG or postprandial BG values outside of the healthy range.

Statistical Analysis

This trial was not designed to determine efficacy and therefore no formal sample size calculation was under taken. Descriptive statistics were expressed, including demographic characteristics.

Fatigue raw data, and GR-AUC data was calculated using a fixed effects model with two treatments (control versus test) adjusted using the Tukey-Kramer and comparing time and treatment interaction. GR VAS AUC was calculated geometrically using the trapezoidal rule at each time point relative to baseline values (Wolever, 2004). For outcome data the Linear Mixed Models (LMM) procedure of SAS 9.4 was used to determine the mean changes in measures, as response variables, according to exposures (test and control) and three repeated measurements, using baseline as a covariate. Further and based on the differences of LS (Marginal) means between two groups (test versus control; pairwise comparisons), provided by LMM analysis, powers, effect sizes (Cohen's d) and their 95% non-central confidence limits were calculated.

For physical activity, a model with time as a repeated factor, a treatment factor with two levels (placebo and test) and 3rd factor with 3 levels (sedentary, light, moderate and vigorous) was considered. Day and night required introduction of one more factor with two levels (day and night). Activity data was aggregated over 6 hours (4 levels) for sedentary, light and moderate activity. Activity awake versus sleep data was aggregated for day and night (2 levels). A random effect model aggregated for total activities with sequence incorporated.

Results

A summary of descriptive data may be viewed in Table 2. Participants who had had MS for a number of years presented with relapsing remitting, primary and secondary progressive subtypes. All were high functioning and independent in daily activities. As can be seen in Table 2 four out of the 12 participants were on special diets, three of who were on the OMS diet, and one on a gluten free diet. Seven people were taking Vitamin D supplements. Two participants reported daily taking the antidepressant medication Amitriptyline (10mg and 50mg) and two additional participants reported currently taking drugs for fatigue including Modafinil and Amandatine (200mg and 100mg).

No adverse events or side-effects were reported or observed during this study and all participants finished the drink on all occasions. Participants were overall content with the taste and sensory properties of the drink, none reported a dislike of the drink. Participants seemed to find it feasible to comply to consuming a relatively similar diet the day before each test day.

Fatigue Visual Analogue Scales

A moderate effect was found in self-reported fatigue throughout the day in favour of the high flavonoid group post consumption (Cohen's d 0.32, 95% non-central t CI -0.57-1.20). Figure 2 indicates a trend for fatigue to be reduced in the hours after leaving the lab through until evening.

Activity monitoring

There was more activity during the sleeping hours (10pm-9am) after the consumption of the low flavonoid compared to the high flavonoid drink (Figure 3). Moderate physical activity

tended to be higher five hours post high flavonoid cocoa consumption compared to moderate physical activity after the control (Cohen's d 0.47, 95% CI: -0.36-1.27).

6 Minute Walk Test

All participants completed the 6MWT on both visits. There was a wide range in distance walked with an average of 273±115m, ranging from 120 – 475m (Figure 4). This was expected due to the varied levels of disability among the participants, of which three needed the assistance of an aid (cane or frame) to complete the walk.

Glycaemic Response

All participants had normal fasting BG levels and 2 hour postprandial BG levels as defined by the WHO (diabetes if fasting BG of >7.0 mmol/l or 2 hour postprandial BG >11.1 mmol/l and impaired glucose tolerance if fasting BG >7.0 mmol/l and 2 hour postprandial BG >7.8 and <11.1 mmol/l); however two participants were exclude from the analysis due to abnormally high BG levels at time points between 0-120 mins. There was a trend for the high flavonoid cocoa to slightly decrease GR in the early stages postprandial (Cohen's d - 0.07, 95% t CI -94-0.81; Figure 5).

Discussion

The results of this trial ultimately show that a single drink of flavonoid rich cocoa produced a modest effect size reduction in perceived fatigue ratings compared to a low flavonoid control drink. This is the first study in pwMS to explore flavonoid use as a treatment for fatigue. The positive effect on fatigue alongside good tolerance and no adverse effects support the need for a powered trial of the longer-term effects. The results of the study may help direct future studies to identify specific questions and employ more efficient designs.

A single dose of flavonoids produced a modest effect on fatigability as measured the 24 hour activity levels yet showed no impact on the 6MWT. Specifically, later in the day five hours after cocoa consumption an effect was observed in activity levels. However, performance on these mobility measures will have been affected by a number of other biopsychosocial factors and it may have been that the 6MWT was administered too early to see an effect on activity levels. In the current study there was a trend for activity during the day to be greater after the high flavonoid cocoa consumption, and activity during the sleeping hours was less. This observation is a positive observation as both poor sleep quality and reduced physical activity are common in MS (Attarian et al., 2004) and a number of participant's commented on poor sleep quality the night before testing.

Moreover, the fatigue VAS does not differentiate between primary fatigue which is a direct result of the disease, and secondary fatigue which is a result of other symptoms. High flavonoid cocoa has previously been shown to improve factors which may lead to secondary fatigue such as sleep quality, and it may be speculated a further frequent intake of high flavonoid cocoa may allow for the beneficial mechanisms to take effect, possibly reducing secondary fatigue while allowing for improved fatigue VAS accuracy (Bisson et al., 2008). Also from assessing the activity data, participants overall seemed to perform low levels of

257 physical activity and spent a majority of their time in sedentary/ light activity. Therefore
258 although further studies would need to be performed, it appears that high flavonoid cocoa
259 may contribute to improving physical activity during the day and better sleep patterns at night
260 in those with MS.

261 This was a one-day study which may not have been long enough to produce a larger
262 effect. Sathyapalan et al. (2010) administered high flavonoid cocoa daily over a period of
263 eight weeks to find significant differences. A meta-analysis of Hooper et al. (2012) similarly
264 suggests positive changes to health may depend on the quantity of cocoa consumed. The dose
265 of polyphenols present in the high flavonoid drink in the current study may have been a
266 limitation as the drinks contained 350 mg of gallic acid equivalents (GAE)/g and 120 mg
267 GAE/g for the high flavonoid and low flavonoid beverages, respectively. In Field et al.
268 (2011), on results obtained for spatial memory and performance on aspects of choice reaction
269 time task, high flavonoid cocoa containing 773 mg flavonoids was used, with significant
270 results found compared to the control (low flavonoid cocoa).

271 The underlying mechanisms were not fully explored in this acute response to a single
272 dose trial; however, foods rich in flavonoids have been shown to stabilise postprandial BG
273 levels (Davies et al., 2012) and consequently reduce fatigue in healthy individuals (Micha et
274 al., 2011). In this study there was no effect of dark cocoa on BG, however there was a trend
275 for the high flavonoid cocoa to reduce the BG in the early stages after consumption and
276 stabilise levels later after consumption. Although all participants showed normal BG levels as
277 defined by WHO, there were some abnormally high patterns in GR during the two hours after
278 the cocoa drink consumption. Therefore the GR in people with MS should be considered in
279 future trials when assessing nutrition and fatigue, and the effect of pure cocoa on fasting BG
280 would need further exploration.

Diet has recently been shown to be an important factor in influencing clinical outcomes in MS (Grossman & Wahls, 2016). Research has found that pwMS are not only willing to consider dietary approaches to manage their symptoms (Brenton & Goldman, 2016) yet many are already on various diets in order to do so (Schwarz et al., 2008). It was therefore hypothesised that this population would be more open to a dietary intervention. Indeed results show that four out of 12 participants were on special diets for their condition, and seven were taking some form of Vitamin D supplements.

Although not for managing depression, two participants reported daily intake of the antidepressant drug Amitriptyline (50 and 10mg), while two additional participants reported currently taking drugs for fatigue including Modafinil and Amandatine (200mg and 100mg). Amitriptyline has previously been shown to cause sleepiness, as well as drowsiness in patients (Frost et al., 2011). Contrastively, Modafinil and Amandatine have previously been reported to decrease fatigue (Ashtari et al., 2009, Brown et al., 2010). Therefore these medications may have increased variability and decreased ability to see a difference in results. However, pwMS are on these medications and therefore the study is more reflective of real world practice.

No participants in the trial vocalised issues with the outcome measures or with the fasting protocol. From the accelerometer results, it seemed that participants wore the watch for the entire testing period. Overall we propose that the flavonoid cocoa drink was well tolerated and enjoyed by all participants and is both suitable for a trial of longer term use and feasible that this drink would be consumed as part of a daily diet. There were no adverse events, and all participants completed the study. Participants were compliant with the study protocol and approved of outcome measures. This, in combination with the moderate effect size on reducing fatigue, shows promise for further studies into the role of flavonoid rich cocoa on fatigue in MS.

Limitations

Due to the novel and exploratory nature of the study a pragmatic approach was used determine sample size and no formal sample size calculation was under taken. However, post hoc analysis found moderate effect sizes at measurement points where differences in fatigue were greatest in the high flavonoid group.

In the current study, whilst the participants were, assessors were not blinded to the intervention. However, the protocol was delivered in randomised format and according to fixed standardised operating instruction sets and procedures. Also a number of measures including the accelerometer data and the fatigue ratings were recorded at home following the test day. Furthermore all data processing was performed blinded and a statistician blinded to the groupings performed analysis. The inclusion/ exclusion criteria in this study was very broad including those with all types of MS and on any medication for their condition and/ or other health conditions (excluding glycaemic control medication). Therefore the heterogeneity of the participant characteristics in addition to the small sample size may have reduced the observed effect. However the study enabled the procedures to be tested across subtypes and establish a follow on study as feasible.

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