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A Randomised Placebo-Controlled Study of Purified Anthocyanins on Cognition in Individuals at Increased Risk for Dementia

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ABSTRACT

Importance: *Identifying nutritional compounds which can reduce cognitive decline in older people is a hugely important topic.* ***Objective:*** *To study the safety and effect of anthocyanins in maintaining cognitive functioning in people at increased risk for dementia.* ***Design, setting, and participants:*** *Participants (206 individuals, aged 60–80 years) diagnosed with either mild cognitive impairment (MCI) or two or more cardiometabolic disorders (i.e., diabetes, hypertension, obesity) were enrolled at three different centres in Norway.* ***Intervention:*** *Participants were randomly assigned to four capsules with a total of 320 mg/d of naturally purified anthocyanins or placebo 1:1 for 24 weeks.* ***Main outcomes and measures:*** *The primary outcome was the Quality of Episodic Memory composite measure (0–100) from an online cognitive test battery CogTrack, which was administered at baseline and monthly for the next 24 weeks. Secondary outcomes included other cognitive*

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scores from the CogTrack battery. We applied mixed effects models with a baseline test score, group, time and their interaction as fixed effects, as well as other predefined baseline covariates. The primary comparison was the group difference at week 24 based on a modified intention-to-treat principle. ***Results:*** *: The primary analysis did not show a significant group difference at 24 weeks (78.2 versus 76.8; adjusted mean difference 1.4 (95% confidence interval -0.9−3.7); effect size 0.15; p = 0.23). However, there was a significant difference in slopes during weeks 8−24 (p = 0.007); the anthocyanin group improved while the placebo group worsened. No differences were found for the secondary cognitive outcomes. Anthocyanin capsules were well-tolerated and safe to use.* ***Conclusion:*** *Anthocyanin supplementation for 24 weeks was safe and well tolerated in people with MCI or cardiometabolic disorders. We found no significant group difference in episodic memory at the end of the study but statistically significant differences in slopes. Further studies are warranted to explore whether anthocyanins supplementation can reduce cognitive decline in people at increased risk of dementia.* ***Trial registration:*** *ClinicalTrials.gov, (Identifier NCT03419039). http://www.clinicaltrials.gov/, NCT03419039.* (Am J Geriatr Psychiatry 2022; ■■:■■−■■)

INTRODUCTION

A total of 50 million people are living with dementia worldwide, and these numbers are to nearly triple by 2050.[1] Currently, there are no disease-modifying treatments available outside the US, and the marketing authorization for Aduhelm in the US has been withdrawn. A growing body of evidence suggests that some modifiable factors, including cardiometabolic disorders such as hypertension, diabetes and hypercholesterolemia, as well as lifestyle factors such as physical exercise and diet, are associated with an increased risk of developing dementia.[2] Notably, age-dependent dysregulation of inflammation, oxidative stress, dysregulation of cerebral capillaries, atherosclerosis, gut microbiome,[3] and endothelial changes are postulated to have a role in age-related cognitive diseases, including Alzheimer's disease (AD) and cerebrovascular and metabolic disorders.[4,5]

Anthocyanins, a flavonoid subclass found in dark berries and fruits, are among the dietary factors that may have positive effects on the pathogenesis of AD.[6] Findings from both animal and human cell studies suggest that they have antioxidant and anti-inflammatory effects and improve the blood lipid profile.[7-9] In addition, anthocyanins have been shown to increase flow-mediated dilatation [10] and to cross the blood−brain barrier,[11] making them promising candidates for dementia prevention studies. Indeed, findings from observational studies suggested that food with high concentrations of anthocyanins could improve cognition and reduce the risk of developing dementia.[12] In addition, intervention studies performed in different populations, including older people and people with mild cognitive impairment (MCI), have suggested positive cognitive effects of blueberries,[13,14] but trials have usually been based on small sample sizes, short duration, and used food-based anthocyanins with less accurate amounts. We have shown a well-defined anthocyanin capsule to be well tolerated.[15] To increase the understanding of whether anthocyanins can improve cognition and reduce the risk of dementia, we have performed a randomized study with >200 participants, with monthly computerized cognitive testing and with imaging, genetic, and biofluid marker description.

METHODS

Study Design and Participants

We performed a 24-week randomised, double-blind, placebo-controlled Phase II study, performed at three centres in Norway during 2018−020.

The study was reviewed and approved by The Norwegian Regional Ethics Committee (2017/374) and registered with ClinicalTrials.gov (identifier NCT03419039). All participants reviewed and signed written consent to participate in this study before

enrollment into the trial according to Good Clinical Practice principles.

Participants were recruited from referrals to geriatric, psychiatric, neurology, cardiology, or memory outpatient clinics at two university hospitals and one geriatric practice in southern Norway from an ongoing cohort study and via community and social media advertising. Participants were prescreened for eligibility by a telephone interview, and potential candidates were then assessed by a face-to-face interview with a research nurse and a study physician to determine eligibility. Participants were contacted by telephone after 4 weeks and seen in the clinic at weeks 12 and 24 (final visit) to ensure adherence to the study protocol and assessment of any adverse events during the trial. They were asked to maintain their usual dietary and lifestyle habits during the study period.

Inclusion and Exclusion Criteria

Inclusion criteria were age 60−80 years, and having either A) MCI determined by Winblad criteria [16] with or without cardiometabolic disorders (CMD) or B) cognitively healthy with ≥2 of the below stated CMD known to be associated with increased risk of cognitive decline and dementia [2]: Cerebrovascular disease, stable cardiovascular disease as visualised by angiography, and metabolic disorders such as diabetes mellitus, hypercholesterolemia, overweight defined by body mass index ≥25, or hypertension.

The exclusion criteria included dementia, Parkinson's disease, stroke within 5 years and other somatic disease which - according to the study physician − might adversely affect cognitive functioning, clinically significant depression, use of anticoagulants, and any use of the investigational product during the 12 months prior to inclusion. Patients were excluded if they had difficulties using the computerised tests. The complete list of inclusion and exclusion criteria is displayed elsewhere.[17]

Procedures and Clinical Data

Diagnostic procedures

A study physician, in most cases a licensed specialist in psychiatry, geriatrics or neurology, obtained clinical data on medical and psychiatric history and sociodemographic data and performed a physical examination.

A short neuropsychological test battery was administered, consisting of CERAD memory,[18] Trail making A and B,[19] Mini-Mental State Exam (MMSE),[20] and the Informant Questionnaire on Cognitive Decline in Elderly,[21] and the Geriatric Depression Scale (15 items).[22] In addition, the Clinical Dementia Rating scale,[23] which elicits relevant information from an informant (typically a partner or adult child), was administered. These measures were used to determine a diagnosis of MCI according to Winblad criteria.[16] These criteria are not specific to any disease and were chosen since the anthocyanins are likely to work on various mechanisms relevant to different cognitive disorders and not only AD. The diagnostic assessment also included routine blood analysis and structural brain MRI scans.[17] For apolipoprotein (APOE) genotyping, the genomic DNA was extracted from peripheral blood using standard methods. Allelic discrimination analysis was performed using predesigned TaqMan SNP genotyping assays for rs7412 and rs429358 (C____904973_10 and C___3084793_20, Thermo Scientific) and TaqPath ProAmp Master Mix (ThermoFisher), as described by the manufacturer. The amplification reactions were performed using the ABI PRISM 7300 Real-Time PCR System with SDS v1.4 software. Also, in a subset, cerebrospinal fluid (CSF) abeta42-amyloid measurement was performed as a marker of AD pathology.[24] A subgroup of patients (139, 87 in CMD and 52 in MCI group) consented to lumbar puncture enabling cerebrospinal fluid analysis of AD markers was quantified using the commercially kit from Meso Scale Discovery. Abnormal values of Ab42 were used to detect amyloid positivity indicating Alzheimer's disease pathology, with values <495.9 pg/ml as a threshold.[24]

The interventional product

The intervention consisted of two Medox capsules (a standardised nutraceutical product that contained 80 mg of naturally purified anthocyanins from bilberry (Vaccinium myrtillus), and black currant (Ribes nigrum)). Each capsule contains 50% Maltodextrin Glucidex IT 19, 50% bilberry (V. myrtillus) and black currant (R. nigrum) extract powder with 80-mg anthocyanin citrates as the 3-O-rutinosides of cyanidin and delphinidin and the 3-O-b-galactopyranosides, 3-O-b-glucopyranosides, and 3-O-a-arabinopyranosides of

cyanidin, peonidin, delphinidin, petunidin, and malvidin.. Identically appearing placebo capsules (91% maltodextrin and 9% citric acid) were given twice daily, i.e., 320 mg anthocyanins per day.

Anthocyanin and placebo capsules were provided by the manufacturer Medpalett AS, Sandnes, Norway.[17] The dosage was determined based on previous clinical trials that reported relevant biological changes,[15,25] good tolerability and posed no risk of adverse effects.[15] Participants were asked to return empty blister packages and unused capsules.

Randomization, blinding, and packaging

Capsules were identically packaged by the manufacturer, shipped to the three centres, and dispensed after allocation in the study.

Participants were allocated in a 1:1 ratio to anthocyanins or placebo based on block randomisation (varying block sizes of 4 or 6) within six strata based on two recruitment groups (i.e., MCI with or without CMD versus CMD only) and three centers. Anonymous randomisation lists were created by the trial statistician, whereas the final allocation of treatment groups was performed at random at the medication production site. The randomisation lists with ID numbers and anonymous treatment arms were sent to the production site. Participants and all study staff, data analyst and laboratory technicians were kept blind throughout the study period. Unblinding was performed after publication of the final version of the signed statistical analysis plan (ClinicalTrials.gov, NCT03419039), locking of the database, and completion of statistical analyses.

Outcome Measures

The online digital cognitive test battery, CogTrack®, is an online set of cognitive tests with proven utility, reliability, sensitivity, and validity,[26] as well as reliable sensitivity to change over time.[27] It consists of 10 tests; pattern separation presentation, word presentation, immediate word recall, simple reaction time, choice reaction time, digit vigilance, spatial working memory, numeric working memory, delayed word recall, word recognition and picture recognition.[28] Based on factor analysis,[29] these subtests were combined into the following domains: attention, memory, and cognitive speed. Importantly, the tests employ multiple parallel forms in order to reduce learning effects.

A training session was performed, and the baseline session was performed before taking the first study dose. Cognitive testing was performed monthly, and collection and registration of the cognitive data were performed securely online.[26] Participants were instructed to perform the test at the same time of day on all occasions, preferably in the morning, to ensure testing procedures were standardised as much as possible (i.e., temperature and coffee intake).

Primary outcome measure

The primary outcome measures the Quality of Episodic Memory (QEM) composite score,[30] which combines the word and picture recognition outcome data from the CogTrack® at week 24. A modified version of this outcome measure was used due to the non-completeness of the word recall data. Thus, the word recognition and picture recognition accuracy outcomes were used. The range of values is 0−100.

Secondary outcome measures

Secondary outcomes from the CogTrack® battery include attentional Intensity Index, sustained Attention Index, cognitive Reaction Time, attentional Fluctuation Index, speed of Memory Retrieval, and Quality of Working Memory.

Power and sample size calculations

Sample size calculations were based on a comparison of the mean of QEM, the primary outcome, between the anthocyanins arm and the placebo arm at the end of study. We assumed an effect size of 0.40, which is considered to be of mild to moderate size and clinically relevant. A sample size of 99 patients per arm is required for a two-sided test of this difference between the intervention and placebo arm with a statistical power of 80% and with an alpha level of 0.05 (using the statistical calculator at https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html.) Allowing for approximately 10% drop-out, we aimed to recruit a total of 110 participants in each arm. Adjustments for baseline scores will likely increase the statistical power.

Statistical Analysis

Descriptive statistics (mean, standard deviation, minimum and maximum scores) of the baseline data are presented by treatment arm. Follow-up measures of QEM were compared between treatment arms in a mixed effects model with baseline QEM, treatment arm, time of test and the interaction between treatment arm and time as fixed effects, and including random intercepts and random time slopes, i.e., in a mixed modelling approach to ANCOVA. The primary comparison was the marginal mean difference between active and placebo at the end of the study (week 24). The model was fitted using restricted maximum likelihood (REML) estimation. Due to the apparent continued learning effect in the primary outcome, it was decided, by an external statistician, to exclude the first follow-up (at 4 weeks) from the model. Further adjustments were made for the stratifying variables centre and recruitment group (MCI versus CMD) and for age, gender and education, which are known predictors of the outcome.[31] The chosen analysis is unbiased for missing at random intermittent missingness in the outcome as well as dropouts.[32] Missing values in baseline covariates were imputed by stratified mean imputation.[33]

The primary effect estimate was the average marginal difference between treatment arms evaluated at 24 weeks in the fully adjusted model, presented with a 95% confidence interval and with the p-value from the two-sided contrast test. Superiority of anthocyanins was claimed if the hypothesis of no difference at 24 weeks was rejected on an alpha-level of 0.05 and if also the adjusted mean QEM in the anthocyanins arm was higher than in the placebo arm at 24 weeks. Stata functions mixed, and margins were used for the analysis.

Standardised effect size was calculated by dividing the model-based difference at 24 weeks by the pooled standard deviation of QEM at baseline. Secondary outcomes (all continuous) were subject to similar analyses as the main outcome; however, the results of these analyses were considered exploratory.

Further, exploratory analyses included comparing slopes, and sensitivity analyses included restricting the primary analysis to the per-protocol sample, exploring non-linear effects of time, and dealing with drop-outs using joint modelling.

Pre-defined subgroup analyses of the primary outcome included MCI versus normal cognition, old versus middle-aged (defined as above or below median age), with versus without CMD, with or without APOE e4 genotype, and normal versus abnormal CSF AD markers.

As there was only one primary null hypothesis to be tested in this trial, there was no adjustment for multiplicity.

RESULTS

From 263 people screened, 206 eligible participants (103 men, 103 women) (69 MCI, 137 CMD) were enrolled, 106 receiving anthocyanins and 100 placebo (Fig. 1). The treatment groups were well-balanced for demographic, clinical, genetic and biomarker features (Table 1). Nearly all (98.5%) completed the final study assessment. One participant discontinued due to diarrhoea, and four due to protocol deviation (Fig. 1). Due to the pandemic and the unexpected passing of Professor Keith Wesnes, responsible for handling the CogTrack battery data, difficulties occurred with access to CogTrack data and supervision of data flow and a considerable number of cognitive test sessions were missing, and for 15 participants, the last assessment was delayed by up to 12 weeks (median 7, IQR 6−11).

Cognitive Results of the Whole Cohort

The baseline cognitive scores were comparable between the two treatment groups (Table 2). At week 24, in the primary fully adjusted analysis, the primary outcome measure, QEM, did not differ significantly between the two groups: 78.2 versus 76.8 points intervention versus control; adjusted mean difference 1.4 (95% CI -0.9−3.7); p = 0.23, effect size = 0.15 (95% CI -0.10−0.41),. However, there was a significant (p = 0.007) difference in slope between the two treatment groups over the included follow-up time (8−24 weeks), favouring the anthocyanin group (Fig. 2). This was further explored in a non-linear modelling of the evolvements in the two groups (Fig. 3), which confirmed the findings from the linear modelling on the restricted follow-up time (mean difference at 24 weeks 1.3; 95% CI -1.3−3.9, p = 0.33). A Chi-square (df = 2) test of interaction terms in this model gave p = 0.055 for the difference in evolvement over time between the treatment groups. Restricting the analysis to the per-

FIGURE 1. CONSORT flow diagram of the study.

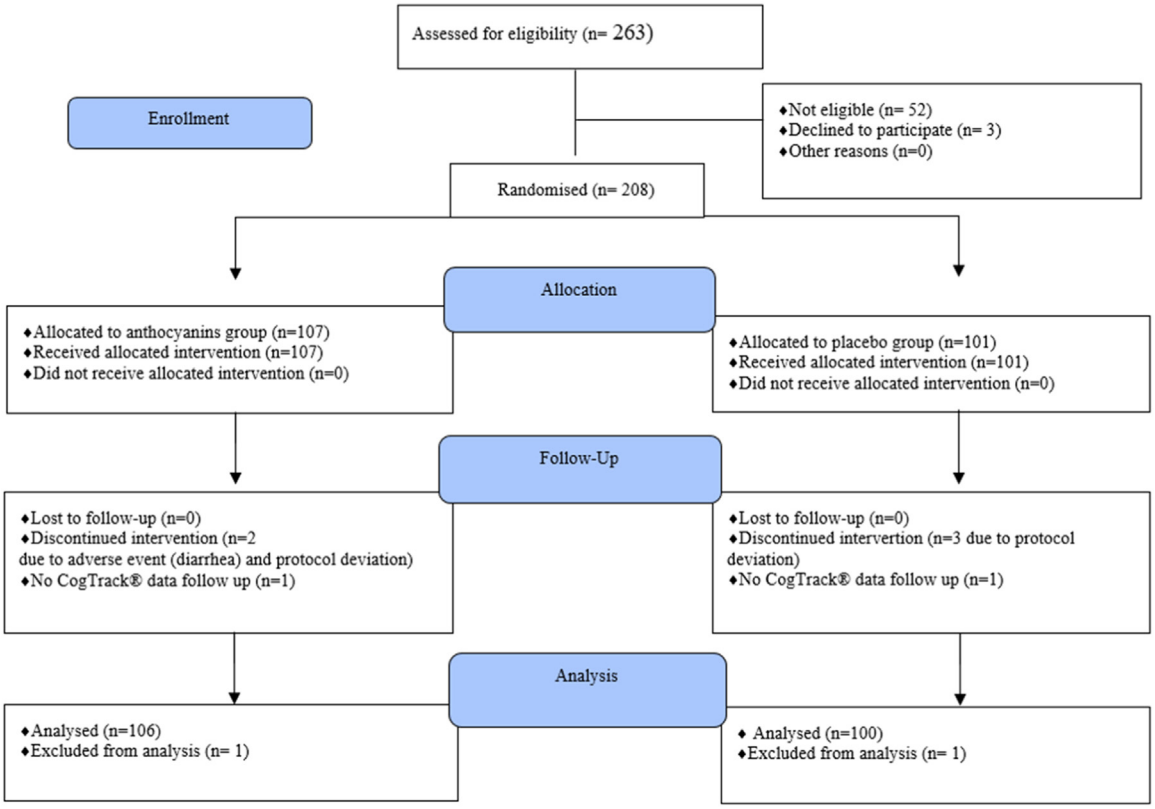


TABLE 1. Baseline Characteristics of Participants in the Anthocyanins Group and Placebo Group

Characteristic	Anthocyanins Treatment Group (n = 106)	Placebo Treatment Group (n = 100)
Age in years	68.9 (5.1); 60−79	69.7 (5.5); 60−80
Female	53 (50%)	50 (50%)
Education in years	14.3 (3.4); 7−22	13.9 (2.9); 8−20
BMI	27.2 (4.0); 18.6−38.4	28.2 (4.6); 19.6−41.4
Smoker	10 (9%)	5 (5%)
MMSE score	28.99 (1.41)	28.77 (1.56)
Diagnostic group		
MCI	35 (33%)	34 (34%)
CMD	71 (67%)	66 (66%)
CSF Amyloid beta 42/40 positive	23 (41.07%)	18 (38.30%)
APOE ε4 carriers	45 (42%)	43 (43%)

Data presented as n (%) or as mean (SD).

Abbreviations: MMSE: Mini-Mental State Examination; BMI: Body mass index; MCI: Mild cognitive impairment; CMD: cardiometabolic disorders; CSF: Cerebrospinal fluid; APOE ε4: Apolipoprotein E ε4 allele

protocol sample or dealing with dropouts using joint modelling did not change our findings. There were no significant between-group differences for the secondary cognitive outcome measures (Table 3).

Subgroup Analyses

The Subgroup analyses indicated that those with CMD, age ≤69 years, and APOE e4 allele present, showed more pronounced positive cognitive effects of anthocyanins than the other groups (Table 3). Tests of interactions between subgroups and treatment were however, not statistically significant.

Adverse Events

The capsules were well tolerated, and compliance was very high (median 98% of planned doses, IQR

TABLE 2. Performance on the Computerized Cognitive Subtests at Baseline and Study End

Cogtrack Variables	Anthocyanins Treatment		Placebo Treatment	
	Baseline (n = 104)	W24 (n = 79)	Baseline (n = 98)	W24 (n = 73)
Modified Quality of Episodic Memory (primary)	74.1 (8.8)	78.2 (10.8)	73.6 (9.6)	77.2 (11.0)
Attentional Intensity Index	1451 (169)	1425 (203)	1452 (147)	1416 (151)
Sustained Attention Index	91.9 (6.4)	93.4 (6.3)	91.0 (7.3)	93.9 (5.5)
Cognitive Reaction Time	191.2 (65)	171 (59)	210.1 (62.3)	183 (63)
Attentional Fluctuation Index	24.2 (16.0)	20.4 (10.0)	23.0 (11.1)	20.5 (8.1)
Speed of Memory Retrieval	3708 (1089)	3139 (794)	3633 (739)	3242 (853)
Quality of Working Memory	77.7 (19.9)	84.6 (17.6)	79.3 (19.9)	86.6 (13.7)

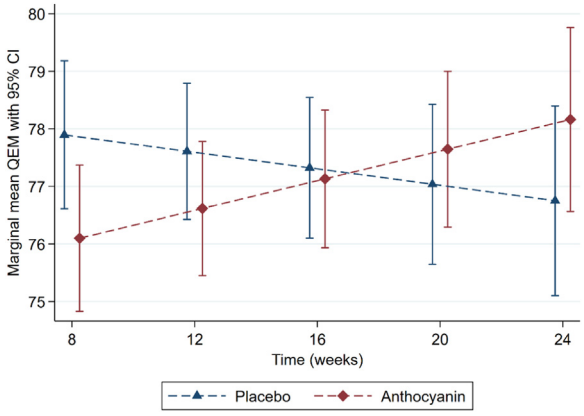
Data presented as mean (SD).

93–99). Sixteen adverse events were reported (Table 4), nine in the anthocyanins group and seven in the placebo group, with no significant difference (p = 0.66; Chi-square test). Clinically significant laboratory changes were not detected.

DISCUSSION

We report findings from a Phase II study of anthocyanins focusing on cognition in older people at risk of developing dementia. There was no significant difference in the primary cognitive outcome between groups at the 24-week time point, with an effect size of 0.15, but there was a significant difference in cognition favouring the anthocyanin group in the slope analysis. In addition, the longitudinal pattern of change in cognition indicated an initial improvement followed by a decline in the placebo group [34] while indicating an ongoing improvement in the group receiving anthocyanin supplementation. There were no differences between groups in the secondary cognitive outcomes. There were indications of different response in the a priori subgroup analyses, i.e., in people with CMD, younger age, and APOE e4 allele seemed to have a better cognitive response to anthocyanins than the other groups, although interaction analyses were not significant. The study was not powered to detect subgroup differences, but these findings warrant further exploration.The anthocyanin supplementation; capsules were found to be safe and well tolerated.

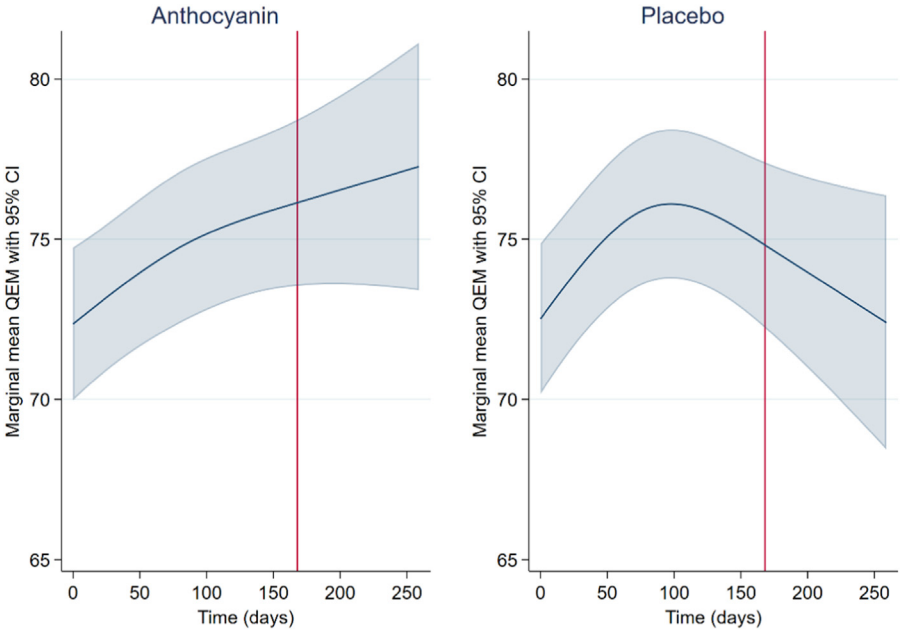
FIGURE 2. Adjusted marginal means of Quality of Episodic Memory at the different follow-up visits for the anthocyanin and the placebo group. Results from the main analysis using linear mixed effects regression.



The results of the study have to be interpreted in the context of power. The Alzheimer's Disease Neuroimaging Initiative (ADNI) collaboration has, e.g., suggested that sample sizes of more than 400 per group are needed over a minimum of 12 months to have the power to detect a 25% treatment effect on cognition and even larger sample sizes are needed for global and functional measures.[35] The current study was, therefore, very much a preliminary evaluation. Previous research indicates that polyphenols need to be supplemented for 12–24 months before significant cognitive health benefits emerge.[36] In this context, the highly significant advantage in our primary cognitive measure on the slope analysis is encouraging and supports the need for a larger and longer trial to evaluate what is a potentially exciting intervention.

Our findings are in line with previous studies. A recent systematic review including 49 randomised trials reported improvements in memory and other cognitive measures as well as endothelial function,

FIGURE 3. Results from non-linear modelling of the evolvements in the two groups. The effect of time (continuous) was modelled using restricted cubic splines with three knots. The vertical lines indicate 168 days i.e., 24 week.



although most studies were performed in healthy adults.[37] In two small studies of people with subjective cognitive impairment,[38] improvements effect on one cognitive test, whereas most tests did not show statistically significant differences. Two studies, including people with MCI, reported enhanced neuronal activation during functional MRI after 16 weeks [39] and decreased concentration of one of several inflammatory serum markers [40] in the anthocyanin group.

Strengths and Limitations

Polyphenol-rich matrix may contain other bioactive compounds; thus, the addition of other bioactives may alter both the bioavailability and the biological effect of the studied phenolic compounds.[41] In the present study, the interventional product was well-standardised naturally purified anthocyanins from bilberry; this allows a more accurate evaluation of the biological effects of the studied phenolic compounds without the confounding presence of other bioactive food components and food matrices.

Limitations include the lack of a detailed dietary assessment during the study period, i.e., we cannot exclude that dietary changes during the study period may have occurred, which could influence the absorption of anthocyanins. We did, however, instruct participants not to make major lifestyle or dietary changes during the study. Furthermore, an apparent learning effect for the primary outcome led to a change in the statistical analysis plan, i.e., excluding W4 from the primary analysis and the slope analysis. The apparent difference in slopes W8-W24 must be seen in the light of the improvement from baseline to W8 in the placebo group. The inclusion of two different groups, i.e., MCI and CMD, led to a heterogeneous cohort which may have reduced the opportunity to find differences. The sample size in the two subgroups was too small to achieve sufficient statistical power to detect small treatment effects in the subgroups. The design, with only one dose of anthocyanins, did not allow us to explore a possible dose-effect response. Anthocyanins may have limited bioavailability. In our recent pilot study [15] measuring plasma concentrations, only two of 29 metabolites

TABLE 3. Summary of Regression Results for Primary and Secondary Cognitive Outcomes, Anthocyanins versus Placebo at 24 Weeks

	Linear Model (W8-W24)				Non-linear model (BL-W24)			
	n/obs	Estimated Diff (95% CI)	p-value	p (Difference iSlopes)	n/obs	Estimated Diff (95% CI)	p-value	p (Difference in Evolvements)
Primary outcome								
Quality of Episodic Memory modified	204/792	1.4 (−0.9; 3.7)	0.23	0.007	206/1186	1.3 (−1.3; 3.9)	0.33	0.06
Within subgroups:								
MCI	68/262	−0.3 (−4.5; 3.8)	0.88	0.77	69/393	1.5 (−3.5; 6.5)	0.56	0.53
No MCI	136/530	2.1 (−0.7; 4.8)	0.15	0.002	137/793	1.2 (−1.9; 4.3)	0.45	0.09
> 69 years	87/329	−0.9 (−4.2; 2.5)	0.62	0.18	88/495	0.1 (−3.9; 4.2)	0.95	0.10
≤ 69 years	117/463	2.9 (−0.3; 6.0)	0.07	0.017	118/691	2.1 (−1.5; 5.7)	0.26	0.27
APOE ε4 carriers	87/347	3.3 (−0.7; 7.3)	0.11	0.022	88/516	4.5 (0.0; 8.9)	0.048	0.041
APOE ε4 non-carriers	117/445	0.0 (−2.7; 2.7)	0.99	0.15	118/670	-0.9 (−4.0; 2.3)	0.59	0.62
Low CSF abeta42	40/162	−0.8 (−5.7; 4.1)	0.75	0.47	41/239	-6.9 (−13.3; −0.5)	0.033	0.86
Normal CSF abeta42	62/258	2.0 (−1.3; 5.4)	0.23	0.42	62/380	1.8 (−2.5; 6.1)	0.41	0.57
Secondary outcomes								
Power of Attention	204/792	-8 (−49; 33)	0.70	0.72	206/1185	2 (−49; 53)	0.95	0.91
Continuity of Attention	204/792	-0.2 (−1.5; 1.1)	0.78	0.68	206/1185	0.5 (−1.2; 2.3)	0.55	0.36
Cognitive Reaction time	204/791	6 (−10; 22)	0.49	0.97	206/1185	-6 (−26; 15)	0.59	0.13
Variability of Attention	204/792	0.2 (−3.1; 3.4)	0.92	0.91	206/1185	0.5 (−3.1; 4.1)	0.78	0.95
Speed of Memory	204/792	−46 (−206; 114)	0.57	0.41	206/1186	34 (−213; 281)	0.79	0.06
Quality of working memory	204/792	−0.9 (−4.7; 2.9)	0.63	0.87	206/1186	-1.2 (−5.5; 3.1)	0.58	0.90

p values <0.05 are written in bold.

Abbreviations: n/Obs; number of subjects/number of observations, Mild cognitive impairment; APOE e4, Apolipoprotein E ε4 allele; CSF: Cerebrospinal fluid.

For COGRTM, an influential outlier was excluded.

For CONT_ATT, Variability of attention and Speed of memory and Quality of working memory, analyses were also performed on transformed variables (to improve on the distribution of residuals) without any changes in the conclusions.

TABLE 4. Reported Adverse Events

Type of Symptoms	Anthocyanins Treatment Group (n = 106)	Placebo Treatment Group (n = 100)
Gastrointestinal disorders (n=8)		
Mild constipation	1	2
Stomach discomfort	1	1
Diarrhea	1	1
Heart burn	1	0
Skin (n = 1)		
Skin rash	1	0
Bleeding diathesis (n = 4)		
Bleeding gum	1	1
Rectal bleeding[b]	0	1
Bruising after a blood draw	1	0
Cardiac disorders (total,n = 3)		
Angina	1[a]	1[a]
Hypertension	1[a]	0

[a] Serious adverse events: Hospital admission

[b] Worsening of chronic hemorrhoids

differed significantly between the treated and non-treated participants. Thus we cannot exclude the possibility that the concentrations of relevant metabolites in the brain are too low to have a neuroprotective effect. However, we have previously found that a dose-dependent effect of the same compound has beneficial vascular effects, indicating that sufficient concentrations of biologically relevant metabolites can be achieved.[10] Another possibility is that anthocyanins can produce biologically relevant changes that can be detected in the gut microbiome, imaging, blood or other biofluids. This needs to be explored in future studies.

Finally, the impact of the pandemic and the passing away of professor Wesnes led to missing or delayed assessments.

CONCLUSIONS

In summary, although the primary analysis did not demonstrate positive cognitive effects after six months of treatment with anthocyanins, positive signals were identified, supporting further work to clarify the role of anthocyanins as dementia prevention. Future studies need to explore the potential mechanisms leading to cognitive improvement, how they relate to

bioavailability of anthocyanins and metabolites, the optimal dosage, and the duration of treatment.

DATA STATEMENT

The data has not been previously presented orally or by poster at scientific meetings

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Conflict of interest Disclosures

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The remaining authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.jagp.2022.10.002.

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