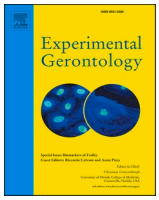
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A randomized, placebo-controlled trial of purified anthocyanins on cognitive function in individuals at elevated risk for dementia: Analysis of inflammatory biomarkers toward personalized interventions

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ABSTRACT

Background: Dementia poses a significant global health challenge. Anthocyanins neutralize free radicals, modulate signaling pathways, inhibit pro-inflammatory genes, and suppress cytokine production and may thus have positive cognitive effects in people at increased risk of dementia. We aim to investigate the effects of purified anthocyanins on cognitive function in people at increased risk of dementia according to their inflammation status based on blood-based inflammatory biomarkers.

Methods: This is a secondary analysis of a 24-week randomized, double-blind, placebo-controlled trial. Cluster analysis was performed to categorize two groups based on their individual inflammatory biomarker profile using multiplex sandwich ELISA for the quantitative measurement of cytokines. Descriptive statistics and longitudinal models assessed cognitive outcomes. The primary comparison was the group difference at week 24 based on a modified intention-to-treat analysis.

Results: Cluster analysis revealed two distinct inflammatory biomarker profiles. In Cluster 1 (high levels of inflammation biomarkers), anthocyanin treatment showed a statistically significant improvement on cognitive function compared to placebo at 24 weeks. No significant differences were observed in Cluster 2 (low levels of inflammation biomarkers). The demographic characteristics, cognitive scores, and biomarker distributions were

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similar between treatment groups at baseline. However, cluster 1 exhibited higher BMI, diabetes prevalence, medication usage, and lower HDL cholesterol levels.
Conclusion: Individuals with elevated levels of inflammation markers benefited from anthocyanin treatment to enhance cognitive performance, whereas those with lower levels did not. The anti-inflammatory and antioxidant properties of anthocyanins make them a promising intervention, and future prospective trials in people with increased inflammation are warranted.

1. Introduction

Dementia poses a significant global health challenge. Recent scientific endeavors have explored potential interventions to mitigate cognitive decline in individuals at an elevated risk for dementia. Anthocyanins have antioxidant and anti-inflammatory properties and represent a promising prevention avenue in cognitive and cardiovascular health (Reis et al., 2016; Kent et al., 2022). Through the modulation of pro-inflammatory and antioxidant transcription factor signaling pathways, particularly the inhibition of nuclear factor-kappa B (NF-κB) activation and enhancement of nuclear factor erythroid 2-related factor 2 (Nrf2) activity, anthocyanins curtail the expression of pro-inflammatory genes, reducing the production of inflammatory mediators and increasing activity of endogenous antioxidant defense pathways (Karlsen et al., 2007). Furthermore, these compounds inhibit enzymes like cyclooxygenase and lipoxygenase, pivotal in pro-inflammatory mediator synthesis. Anthocyanins also suppress the production of inflammatory cytokines, enhance the activity of endogenous antioxidant enzymes, safeguard mitochondria from oxidative damage, and regulate inflammasome activation, thereby ameliorating the impact of inflammaging (Kozłowska and Dzierżanowski, 2021).

Neuroinflammation plays a crucial role in the progression of dementia. Individuals who are more susceptible to a persistent inflammatory state may experience accelerated neurodegeneration and cognitive decline, driven by interactions between central and peripheral immune compartments (Langworth-Green et al., 2023). Understanding this mechanism is critical for devising effective interventions. The bidirectional relationship between beta-amyloid and inflammation significantly contributes to cognitive deterioration (Karran and De Strooper, 2022), underscoring the importance of elucidating this link for the development of therapeutic strategies aimed at reducing or preventing inflammation. Given the importance of inflammation for age-related cognitive decline and dementia (Langworth-Green et al., 2023), anthocyanins represent a strong candidate as a dementia prevention intervention (Suresh et al., 2022).

We recently reported a 24-week placebo-controlled randomized study of the impact of anthocyanins on cognitive function in older individuals at increased risk of dementia. No statistically significant differences were found, but there were some signals indicating potential pro-cognitive benefits, indicating that some people may benefit (Aarsland et al., 2023).

Precision medicine is an approach that takes into account individual differences in people's genes, environments, and lifestyles (Akhoon, 2021). Therefore, given the importance of inflammation for cognitive decline and anthocyanins' anti-inflammatory effects, this study aims to test the impact of purified anthocyanins on cognitive function in individuals with different inflammation statuses.

2. Methods

This is a secondary analysis of the "AnthoCyanins In people at risk for Dementia study" (ACID). Here we conducted a Phase II study over 24 weeks, employing a randomized, double-blind, and placebo-controlled design, across three centers in Norway from 2018 to 2020. The detailed methods are described elsewhere (Aarsland et al., 2023; Khalifa et al., 2020). A number of sub-group analyses were planned a priori, but this unbiased analysis was not initially planned.

Recruitment took place through referrals to various clinics—geriatric, psychiatric, neurology, cardiology, and memory outpatient clinics—at two university hospitals and one geriatric practice in southern Norway. Additional participants were drawn from an ongoing cohort study and through community and social media advertisements. Preliminary eligibility screening occurred via a telephone interview, followed by a face-to-face assessment by a research nurse and a study physician to confirm eligibility. Regular follow-ups were conducted at weeks 4, 12, and 24 (final visit) through telephone calls and clinic visits to ensure protocol adherence and assess any adverse events during the trial. Throughout the study period, participants were instructed to maintain their regular dietary and lifestyle habits.

2.1. Participants

Among the 263 individuals initially screened, 201 eligible participants (100 men, 101 women) were ultimately enrolled, consisting of 65 with Mild Cognitive Impairment (MCI) and 136 with Cardiometabolic disorders (CMD). 103 participants were randomized to receiving anthocyanins and 98 receiving a placebo. A high completion rate of 98.5% was achieved, with only one participant discontinuing due to diarrhea and four others due to protocol deviation (Supplemental Fig. 1). Challenges arose due to the pandemic and the untimely passing of Professor Keith Wesnes, responsible for managing CogTrack battery data. Consequently, difficulties in accessing CogTrack data and supervising data flow occurred, leading to a notable number of missing cognitive test sessions. Additionally, the last assessment for 15 participants was delayed by up to 12 weeks, with a median delay of 7 weeks.

2.2. Inclusion and exclusion criteria

Inclusion criteria comprised individuals aged 60–80 years falling into either category A) A concise neuropsychological assessment battery was administered, including the CERAD memory test, Trail Making Tests A and B, the Mini-Mental State Examination (MMSE), and the Informant Questionnaire on Cognitive Decline in the Elderly. Additionally, the 15-item Geriatric Depression Scale and the Clinical Dementia Rating scale were utilized, with the latter gathering pertinent information from an informant, usually a partner or an adult child. These assessments were employed to diagnose Mild Cognitive Impairment (MCI) following the Winblad criteria. (Winblad et al., 2004) with or without cardiometabolic disorders (CMDs), or category B) cognitively healthy individuals with at least two of the specified CMDs known to be associated with an increased risk of cognitive decline and dementia (Livingston et al., 2020). These CMDs include cerebrovascular disease, stable cardiovascular disease visualized by angiography, and metabolic disorders such as diabetes mellitus, hypercholesterolemia, overweight defined by a body mass index (BMI) of $\geq$25, or hypertension.

Exclusion criteria encompassed dementia, Parkinson's disease, a stroke within the past 5 years, or any other somatic disease that, according to the study physician, might adversely affect cognitive functioning. Other exclusion criteria included clinically significant depression, the use of anticoagulants, and any use of the investigational product in the 12 months preceding inclusion. Patients were also excluded if they faced difficulties in using computerized tests. The complete list of inclusion and exclusion criteria and the flowchart of the sample selection can be found in the previous publication (Aarsland

et al., 2023; Khalifa et al., 2020).

2.3. Procedures and clinical information

2.3.1. The interventional product

Capsules including a standardized nutraceutical product containing 80 mg of naturally purified anthocyanins from bilberry (*Vaccinium myrtillus*) and black currant (*Ribes nigrum*). The capsules, identical in appearance, contained a mixture of 50 % Maltodextrin Glucidex IT 19 and 50 % extract powder from bilberry (*V. myrtillus*) and black currant (*R. nigrum*), with each capsule providing 80 mg of anthocyanin citrates. The placebo capsules, indistinguishable in appearance, contained 91 % maltodextrin and 9 % citric acid. Participants were required to take four capsules daily, divided into two doses (two capsules per dose), equating to a total of 320 mg of anthocyanins per day. The capsules were supplied by the manufacturer Medpalett AS, Sandnes, Norway (Khalifa et al., 2020), and the dosage, four capsules daily divided in two doses, was determined based on previous trials indicating relevant biological changes, good tolerability, and no risk of adverse effects (Zhang et al., 2020).

2.3.2. Randomization, blinding, and packaging

Participants were allocated in a 1:1 ratio to anthocyanins or placebo using block randomization within six strata based on two recruitment groups (MCI with or without CMD versus CMD only) and three centers. The randomization lists, with ID numbers and anonymous treatment arms, were created by the trial statistician and sent to the production site. The final allocation of treatment groups was done randomly at the medication production site. Throughout the study period, participants, study staff, data analysts, and laboratory technicians remained blinded. Unblinding occurred post the publication of the final version of the signed statistical analysis plan, database locking, and completion of statistical analyses (ClinicalTrials.gov, NCT03419039).

2.3.3. Outcome

CogTrack® is an online digital cognitive test battery known for its established utility, reliability, sensitivity, and validity (Brooker et al., 2019), as well as its proven responsiveness to changes over time (Wesnes et al., 2017; Creese et al., 2019). Comprising 10 tests, including 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, CogTrack® utilizes factor analysis to group these subtests into domains such as attention, memory, and cognitive speed (Wesnes et al., 2017). Notably, the tests incorporate multiple parallel forms to minimize potential learning effects.

Prior to the first study dose, participants underwent a training session, and the baseline session was conducted. Cognitive testing occurred monthly, with the collection and registration of cognitive data conducted securely online (Wesnes et al., 2017). Participants were instructed to consistently perform the test at the same time of day, preferably in the morning, to standardize testing conditions as much as possible (considering factors such as temperature and coffee intake).

The primary outcome measures is the composite score for Quality Episodic Memory (QEM) (Downey et al., 2018), amalgamating the outcome data for word and picture recognition from CogTrack® at the 24-week mark. The potential score range spans from 0 to 100.

In this post-hoc study only, the primary outcome was considered.

2.4. Inflammation biomarkers

2.4.1. Methods Quanterix SP-X®

Cytokines play crucial roles in the immune response, with some being pro-inflammatory and others anti-inflammatory. Pro-inflammatory cytokines include IL12p70, IL1β, IFN-γ, IL6, IL8, IL22, and TNF-α, all of which promote inflammation and immune responses against pathogens. In contrast, IL4, IL5, and IL10 are generally anti-inflammatory, helping to regulate and reduce inflammation. These cytokines work together to balance the body's immune response, ensuring effective defense against infections while preventing excessive inflammation that could lead to tissue damage. These specific cytokines selection are based on their established presence in the literature related to AD. Additionally, the selection was influenced by the assay performance. Cytokines are notoriously challenging to detect in biofluids, but this assay has been tested to ensure reliable detection. The SP-X Extended immunoassay kit (Quanterix Product Number 85–0329) comprised a multiplex sandwich ELISA for the quantitative measurement of cytokines IL12p70, IL1b, IL4, IL5, IFN-γ, IL6, IL8, IL22, TNF-α, and IL10. Briefly, calibrator antigen stocks for all analytes were reconstituted and serially diluted using the Sample Diluent. Human plasma samples were vortexed, centrifuged (10,000 $g \times 5$ min) and diluted 1:4 with Sample Diluent. The plate arrays were washed with 25× Wash Buffer on a Simoa Microplate Washer (Quanterix). Following a 120-min incubation (23 °C, 525 rpm), plates were washed and incubated with Biotinylated Detecting Antibodies for 30 min (23 °C, 525 rpm). Subsequently, streptavidin-HRP conjugate was added and further incubated for 30 min, and plates were washed before adding Luminol and Hydrogen peroxide. Plates were imaged on the Simoa SP-X using a CCD camera (577 nm excitation, 620 nm emission). Subsequently, the %CV was recalculated based on these adjusted concentration values, assessing new averages and standard deviations. Inflammation biomarkers in their original scale can be found in supplemental Table 1.

2.5. Ethical considerations

Ethical approval for the study was obtained from The Norwegian Regional Ethics Committee (2017/374), and the trial was registered with ClinicalTrials.gov under the identifier NCT03419039. Before enrollment, all participants underwent a comprehensive review of the study, and their informed written consent was obtained, adhering to the principles of Good Clinical Practice.

2.6. Cluster analysis

A non-hierarchical analysis was conducted using the K-means method, utilizing information from inflammatory biomarkers available at the baseline of the study to identify their specific profiles and assess susceptibility to treatment. To perform the cluster analysis, we initially conducted exploratory data analysis to understand the data distributions. Data transformation using the bestNormalize package in R Studio (Peterson, 2021; Peterson and Cavanaugh, 2020), was necessary due to observed bias in the variables (see supplementary material 1).

Subsequently, Euclidean distances of the transformed data were computed, determining the optimal number of clusters according to the majority rule. Following this rule, two clusters were constructed and described. Finally, a descriptive analysis of clinical and biological characteristics was conducted to understand the features of each cluster. Percentages were estimated for categorical variables and means and standard deviations were calculated for quantitative variables. Differences between cluster groups at baseline were assessed using Pearson's chi-squared test for categorical variables and independent samples *t*-test for quantitative variables to determine significant distinctions in baseline characteristics between the groups.

2.7. Sample size calculation

Sample size calculations were based on the full original study, using a comparison of the mean of QEM, between the anthocyanins arm and the placebo arm at the end of study. We assumed an effect size of 0.40, requiring a sample size of 99 patients per arm for a two-sided test of this difference between the groups with a statistical power of 80 % and with an alpha level of 0.05. Allowing for approximately 10 % dropout, we

aimed to recruit a total of 110 participants in each arm. Thus, the study was not powered for sub-group analyses.

2.8. Statistical analysis

A longitudinal data analysis was conducted to assess whether the anthocyanins treatment group followed the same trend over time as the placebo treatment group in each of the two inflammation groups. To achieve this, adjusted and unadjusted mixed-effect models with random intercepts and slopes were fitted. These models were performed for each cluster as well as for the general sample, incorporating the variables of treatment, time (measurement), and the interaction between time and treatment. Age and gender were included as covariates in the adjusted models. Additionally, a visual analysis was conducted by plotting individual and overall trajectories of subjects in each treatment arm for each cluster and for the general sample. A significance level of 0.05 was considered for hypothesis testing. Statistical analyses were performed using R software version 4.2.1.

3. Results

The study included 201 participants, with 103 receiving anthocyanins and 98 receiving a placebo, with no significant baseline differences (Table 1). Dropouts and missing data can be found in the flowchart (Supplementary Fig. 1).

3.1. Clustering

Analysis of inflammatory biomarkers revealed variations among participants, leading to the identification of two distinct patterns: cluster 1, characterized by higher levels of inflammation markers, and cluster 2, characterized by lower levels of inflammation markers (Fig. 1, Tables 1 and 2). Several significant differences were noted, notably higher levels of IL12p70, IL1b, IL4, IL5, IL6, IL10, INFγ, and TNFα in cluster 1 (Table 3, Fig. 1).

Significant clinical differences were also observed between the clusters, with cluster 1 exhibiting higher body mass index, prevalence of diabetes, medication usage, and leukocyte counts, whereas cluster 2 showed higher levels of HDL cholesterol (Table 2).

After creating the clusters is possible to observe important differences between the different interleukins between cluster 1 and cluster 2. (Table 3).

3.2. Longitudinal models

At the 24-week follow-up, the primary fully adjusted analysis, accounting for age and sex, revealed a statistically significant improvement in cognitive performance between treatment arms, Cluster 1 (Estimate 0.769 SE 0.300, *p*-value of 0.012). In contrast, in Cluster 2, there was no significant difference between the treatment groups (Table 4, Fig. 2).

4. Discussion

Here we present a secondary analysis of the ACID study (Aarsland et al., 2023), investigating the effects of purified anthocyanins on cognitive function in older individuals at risk of dementia. We divided the cohort into two subgroups based on cluster analysis of their plasma levels of several cytokines. We found a significant statistical difference in the primary outcome between anthocyanin treatment and placebo in individuals with high levels of inflammatory markers, but not in those with lower levels of inflammation cytokine. These promising findings need to be supported by a prospectively designed study including people with increased inflammation markers.

There is growing interest in supplements for dementia prevention (Basri et al., n.d.; McGrattan et al., 2022). However, most studies have failed to find positive effects, including omega 3 supplements, the Mediterranean diets, vitamin E or anthocyanins (Castellanos-Perilla

Table 1
Baseline characteristics of the sample and markers distribution according to clusters and treatment type.

Characteristics	Cluster 1		*P* value	Cluster 2		P value	Overall		P value
	Intervention	Placebo		Intervention	Placebo		Intervention	Placebo	
	(n = 45)	(n = 44)		(n = 58)	(n = 54)		(n = 103)	(n = 98)	
Age, mean (SD)	69.3 (4.67)	69.3 (5.56)	0.995	68.1 (5.29)	69.1 (5.27)	0.298	68.6 (5.04)	69.2 (5.38)	0.422
Sex, (%)									
Female	20 (44.4 %)	21 (47.7 %)	0.922	32 (55.2 %)	28 (51.9 %)	0.871	52 (50.5 %)	49 (50.0 %)	1.000
Male	25 (55.6 %)	23 (52.3 %)		26 (44.8 %)	26 (48.1 %)		51 (49.5 %)	49 (50.0 %)	
Modified Quality of Episodic Memory Baseline, mean (SD	73.2 (8.22)	74.9 (9.76)	0.364	74.9 (9.22)	72.5 (9.42)	0.167	74.2 (8.79)	73.6 (9.60)	0.652
Modified Quality of Episodic Memory Final, mean (SD)	78.5 (10.9)	77.1 (10.7)	0.592	77.6 (10.9)	77.3 (11.3)	0.889	78.0 (10.8)	77.2 (11.0)	0.647
IL12p70, mean (SD)	1.32 (4.39)	0.506 (0.845)	0.225	0.109 (0.111)	0.136 (0.376)	0.619	0.640 (2.94)	0.302 (0.654)	0.258
IL1b, mean (SD)	0.300 (0.640)	0.380 (0.727)	0.581	0.085 (0.277)	0.170 (0.442)	0.228	0.179 (0.480)	0.264 (0.594)	0.263
IL4, mean (SD)	1.70 (5.76)	1.19 (2.77)	0.591	0.246 (0.613)	0.235 (0.690)	0.930	0.883 (3.88)	0.663 (1.97)	0.610
IL5, mean (SD)	0.466 (0.861)	0.380 (0.358)	0.539	0.121 (0.168)	0.110 (0.131)	0.688	0.272 (0.604)	0.231 (0.290)	0.540
INFg, mean (SD)	0.396 (1.09)	0.283 (0.433)	0.525	0.074 (0.048)	0.085 (0.062)	0.303	0.215 (0.737)	0.174 (0.308)	0.609
IL6, mean (SD)	5.91 (14.5)	3.21 (4.29)	0.239	1.050 (0.716)	1.380 (1.070)	0.066	3.170 (9.850)	2.20 (3.11)	0.342
IL8, mean (SD)	10.7 (18.2)	7.06 (3.57)	0.196	7.04 (4.01)	6.260 (3.32)	0.265	8.630 (12.4)	6.62 (3.44)	0.117
IL22, mean (SD)	0.951 (0.828)	0.724 (0.522)	0.126	0.645 (0.602)	0.696 (0.580)	0.649	0.779 (0.723)	0.709 (0.552)	0.440
TNFa, mean (SD)	1.94 (2.27)	1.83 (1.78)	0.798	0.808 (0.373)	0.824 (0.383)	0.825	1.30 (1.62)	1.27 (1.32)	0.895
IL10, mean (SD)	0.855 (1.04)	0.770 (0.570)	0.633	0.466 (0.280)	0.590 (0.532)	0.130	0.626 (0.738)	0.671 (0.554)	0.702

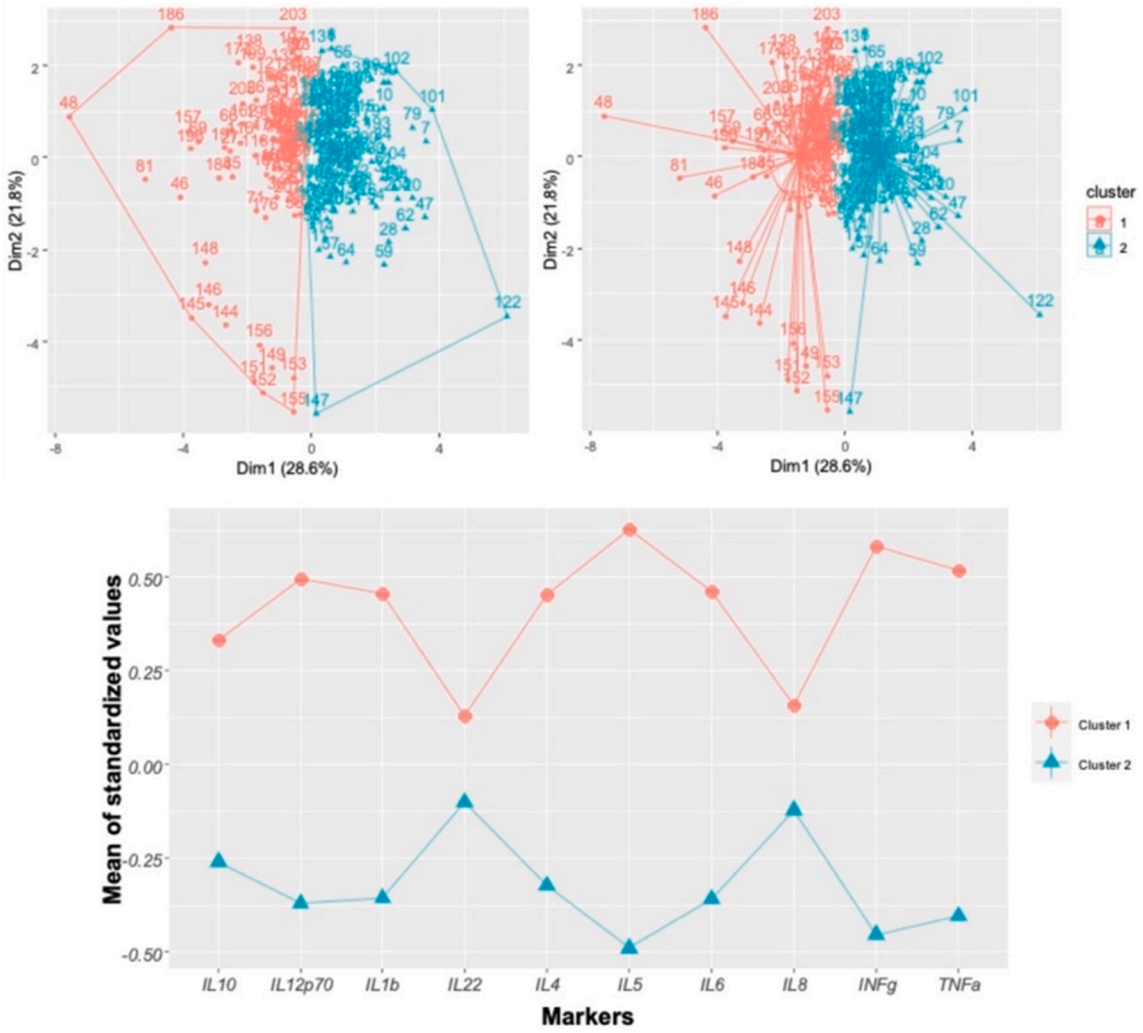


Fig. 1. Cluster analysis according to inflammatory biomarkers levels. Dim: Dimention; IL: Interleukine; INFg:Interferon-gamma; TNFa: Tumor Necrosis Factor-alpha. The figure shows the distribution of inflammatory biomarkers among participants, grouped into two clusters according to their inflammation profiles. Cluster 1 is characterized by elevated levels of inflammatory biomarkers, while Cluster 2 has lower levels.

et al., 2024; Guerchet et al., 2014). One possible explanation for the failure of these clinical trials may be the lack of a personalized approach. Not all patients with dementia have an elevated inflammatory profile, and thus anti-inflammatory intervention may not be effective for everyone (Shen et al., 2019), explaining the non-difference found in cluster 2.

We analyzed a panel of inflammation biomarkers through clustered analysis. Cluster 1 was found to be significantly associated with well-known proinflammatory conditions, such as diabetes, higher comorbidity (more medications needed), increased leucocyte count, higher body mass index (BMI), and lower HDL. The latter may indicate an excess of adipose tissue and increased secretion of established pro-inflammatory molecules such as IL5, IL12, TNF-α and IL1β (Hotamisligil and Spiegelman, 1994; Hotamisligil et al., 1993). When examining the variations among the inflammatory markers used to form the clusters, cluster 1 had notably higher levels of proinflammatory cytokines, such as IL12p70, IL1β, IL5, TNF-γ, IL6, and TNF-α (Table 2). It is worth noting that two anti-inflammatory cytokines, IL4 and IL10, were also elevated in cluster 1, likely as a compensatory response to the overall pro-inflammatory state (Schnyder and Handschin, 2015). Possibly due to its anti-inflammatory properties, the participants in cluster 1 receiving anthocyanins had improved cognition compared to the participants in cluster 1 receiving placebo. No treatment effect was observed in cluster 2, despite having higher levels of HDL, a measure of good cholesterol, and low scores in most of the proinflammatory interleukins.

Although the mechanisms by which anthocyanins improve cognitive function in cluster 1 were not investigated, their anti-inflammatory and antioxidative properties are well-known. There is increasing evidence from both clinical and pre-clinical research of the widespread involvement of inflammatory processes in dementia (Kinney et al., 2018) and pre-dementia states (Tao et al., 2018), increasing the odds for neuroprogression (Chitnis and Weiner, 2017) Overall, these findings support our initial hypothesis that anthocyanins can be a promising intervention for individuals at risk of dementia and with elevated inflammation (Kent et al., 2022).

Study strengths include the sub-group analysis was based on an unbiased purely statistical analysis of a considerable number of cytokines measured using state-of-the-art technology. The intervention used meticulously standardized naturally purified anthocyanins sourced from blueberries, which enhances the precision of evaluating the biological effects of the examined phenolic compounds, eliminating the potential interference from other bioactive food components and food matrices. Additionally, a homogenous population from Norway with similar habits and diets was used. The findings highlight the need to shift toward precision and personalized nutrition approaches and tailoring medical interventions to the individual's phenotypic and nutrigenomic profiles.

Table 2
Cluster differences according to the baseline sample.

Characteristics	Overall (n = 201)	Cluster 1 (n = 89)	Cluster 2 (n = 112)	P value
Age	68.9 (5.20)	69.3 (5.10)	68.6 (5.28)	0.326
Sex				
Female	101 (50.2%)	41 (46.1 %)	60 (53.6 %)	0.360
Male	100 (49.8 %)	48 (53.9 %)	52 (46.4 %)	
Education in years	14.1 (3.20)	13.6 (2.82)	14.4 (3.45)	0.068
Modified Quality of Episodic Memory Baseline, mean (SD)	73.9 (9.18)	74.0 (9.00)	73.7 (9.35)	0.816
Modified Quality of Episodic Memory Final, mean (SD)	77.6 (10.9)	77.8 (10.7)	77.5 (11.0)	0.834
Diagnostic group				
MCI	136 (67.7 %)	67 (75.3 %)	69 (61.6 %)	0.138
CMD	65 (32.3 %)	22 (24.7 %)	43 (38.4 %)	
BMI	27.7 (4.30)	28.7 (4.63)	26.9 (3.85)	0.003
Smoker				
No	186 (92.5%)	80 (89.9 %)	106 (94.6 %)	0.315
Yes	15 (7.5 %)	9 (10.1 %)	6 (5.4 %)	
Alcohol consumption				
No	22 (10.9 %)	10 (11.2 %)	12 (10.7 %)	0.999
Yes	178 (88.6 %)	79 (88.8 %)	99 (88.4 %)	
Missing	1 (0.5%)	0 (0%)	1 (0.9%)	
Geriatric Depression scale	1.13 (1.33)	1.04 (1.29)	1.21 (1.37)	0.395
Mini Mental Status Examination	28.9 (1.49)	28.9 (1.48)	28.9 (1.50)	0.913
Medication	3.59 (2.48)	4.02 (2.45)	3.24 (2.46)	0.026
Diabetes				
No	165 (82.1 %)	66 (74.2 %)	99 (88.4 %)	0.015
Yes	36 (17.9 %)	23 (25.8 %)	13 (11.6 %)	
Hypertension				
No	66 (32.8 %)	24 (27.0 %)	42 (37.5 %)	0.141
Yes	134 (66.7%)	65 (73.0%)	69 (61.6%)	
Missing	1 (0.5%)	0 (0%)	1 (0.9%)	
CRP *	2.04 (2.93)	2.23 (2.41)	1.85 (3.37)	0.531
Albumin *	39.2 (2.63)	38.8 (2.96)	39.6 (2.22)	0.145
Leukocytes *	5.88 (1.37)	6.19 (1.41)	5.57 (1.28)	0.024
Thrombocytes*	239 (61.5)	237 (59.5)	241 (63.8)	0.739
Cholesterol*	4.80 (1.08)	4.55 (1.09)	5.04 (1.04)	0.025
LDL	3.06 (1.06)	2.86 (1.08)	3.25 (1.01)	0.069
HDL	1.35 (0.353)	1.26 (0.272)	1.44 (0.399)	0.009
Triglycerides *	1.59 (0.886)	1.71 (0.790)	1.48 (0.967)	0.217

* Representation of a subsample (51.7 % missing information). BMI: Body Mass Index, CRP: C-Reactive Protein, LDL: Low-Density Lipoprotein and HDL: High-Density Lipoprotein.

Table 3
Levels of the different included interleukins.

Characteristics	Overall (n = 201)	Cluster 1 (n = 89)	Cluster 2 (n = 112)	P value
IL12p70, mean (SD)	0.478 (2.16)	0.920 (3.18)	0.122 (0.272)	0.021
IL1b, mean (SD)	0.220 (0.539)	0.340 (0.682)	0.126 (0.366)	0.009
IL4, mean (SD)	0.776 (3.09)	1.45 (4.51)	0.240 (0.648)	0.014
IL5, mean (SD)	0.252 (0.477)	0.423 (0.660)	0.116 (0.151)	< 0.001
INFg, mean (SD)	0.195 (0.569)	0.340 (0.832)	0.079(0.055)	0.004
IL6, mean (SD)	2.70 (7.38)	4.57 (10.8)	1.210 (0.916)	0.004
IL8, mean (SD)	7.65 (9.26)	8.90 (13.2)	6.660 (3.700)	0.125
IL22, mean (SD)	0.745 (0.644)	0.839 (0.699)	0.670 (0.590)	0.071
TNFa, mean (SD)	1.29 (1.48)	1.88 (2.03)	0.816 (0.376)	< 0.001
IL10, mean (SD)	0.653 (0.653)	0.813 (0.835)	0.526 (0.423)	0.004

The limitations of this study should also be noted. Firstly, this was a post-hoc sub-group analyses and not powered for sub-group analyses. Then, we did not calculate the effect size as it would be unreliable given the small sample size. Furthermore, there was no comprehensive dietary assessment during the study duration, which raises the possibility of undocumented dietary changes that might impact anthocyanin absorption. Despite instructing participants against significant lifestyle or dietary alterations, it is still possible that such changes occurred. Also, it is important to consider the observed learning effect for the primary outcome. Overall improved memory scores observed in both the intervention and control groups could be attributed to a combined placebo effect and a learning effect (Aarsland et al., 2023). The repeated exposure to cognitive assessments may have contributed to enhanced performance over time, regardless of the actual impact of the intervention itself. This potential confounding factor should be considered when interpreting the results. As a result, W4 observation was excluded from the analyses (Aarsland et al., 2023). It is also important to consider the heterogeneity of the cohort, which included two distinct groups, MCI and CMD, that may involve different pathways. This heterogeneity could have limited the ability to detect differences, although it might also enhance the clinical applicability of such interventions across a broader population. Additionally, the population was geographically restricted, potentially limiting the generalizability of the results, as specific lifestyle habits and genetic predispositions could influence the effects of anthocyanins. Therefore, the findings should be interpreted with caution. Future anthocyanin trials should focus on participants with higher levels of inflammation, include larger sample sizes, and incorporate correlations with oxidative stress biomarkers.

5. Conclusion

People with higher levels of inflammation demonstrated significantly better cognitive function after anthocyanin treatment compared to placebo at 24 weeks. Additionally, these individuals had higher BMI, a greater prevalence of diabetes, increased medication usage, and lower HDL cholesterol levels compared to those with lower inflammatory profiles. The anti-inflammatory and antioxidant properties of anthocyanins suggest they could be a promising intervention, warranting future prospective trials in individuals with elevated inflammation levels.

CRediT authorship contribution statement

Miguel German Borda: Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization. **George E. Barreto:** Writing – review & editing, Formal analysis, Data curation. **Jonathan Patricio Baldera:** Writing – review & editing, Software, Methodology. **Chiara de Lucia:** Writing – review & editing,

Table 4
Unadjusted and adjusted mixed-effect models for cognitive performance among clusters.

Variables	Cluster 1		P value	Cluster 2		P value	Overall		P value
	Estimate	Std. Error		Estimate	Std. Error		Estimate	Std. Error	
Unadjusted models									
Treatment	−3.564	1.871	0.060	2.004	1.724	0.247	−0.443	1.276	0.729
Time	0.072	0.211	0.733	0.694	0.196	0.001	0.412	0.145	0.005
Treatment * Time	0.771	0.300	0.012	−0.272	0.270	0.315	0.193	0.203	0.344
Adjusted models°									
Age	−0.383	0.165	0.023	−0.718	0.132	< 0.001	−0.589	0.102	< 0.001
Sex (reference: Female)	−2.720	1.681	0.109	−1.183	1.395	0.398	−1.614	1.057	0.129
Treatment	−3.518	1.794	0.053	1.124	1.588	0.436	−0.823	1.192	0.491
Time	0.072	0.211	0.734	0.689	0.194	0.001	0.410	0.145	0.005
Treatment * Time	0.769	0.300	0.012	−0.281	0.267	0.229	0.184	0.202	0.364

° Adjusted by age and sex.

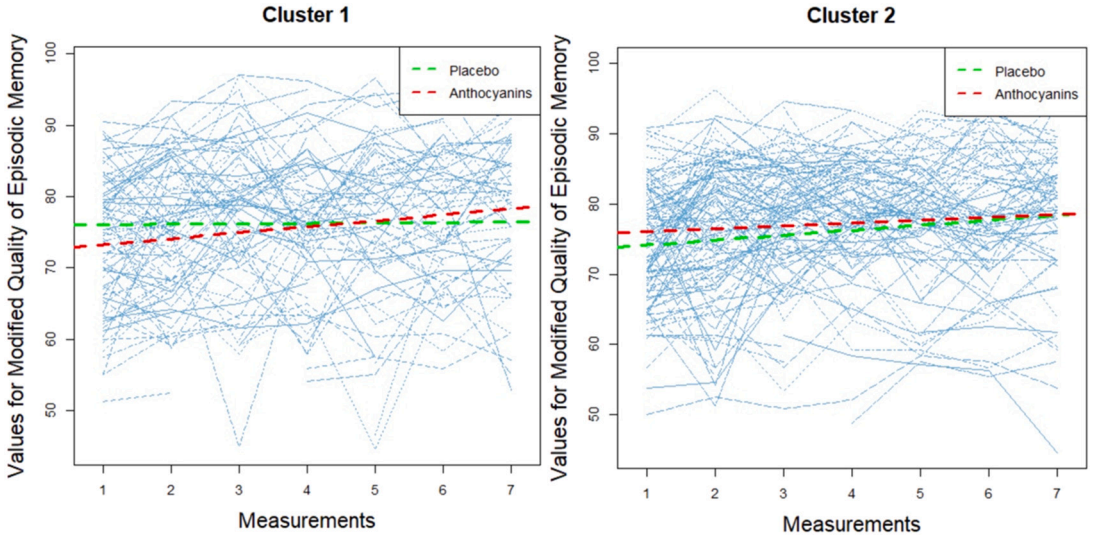


Fig. 2. Comparison of Quality of Episodic Memory between clusters among 24-weeks follow-up.
This figure compares the evolution of episodic memory quality in participants belonging to clusters 1 and 2 over 24 weeks of treatment with anthocyanins or placebo. A significant improvement in memory is observed for participants in Cluster 1 who received anthocyanins.

Formal analysis, Data curation. **Khadija Khalifa:** Writing – original draft, Methodology, Investigation, Conceptualization. **Anne Katrine Bergland:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis. **Ilaria Pola:** Writing – review & editing, Validation, Supervision. **Felipe Botero-Rodríguez:** Writing – review & editing, Writing – original draft, Methodology. **Richard Siow:** Writing – review & editing, Validation. **Miia Kivipelto:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Henrik Zetterberg:** Writing – review & editing, Visualization, Funding acquisition, Data curation. **Nicholas J. Ashton:** Writing – review & editing, Supervision, Resources, Methodology. **Clive Ballard:** Writing – review & editing, Project administration, Investigation, Data curation. **Dag Aarsland:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following potential conflicts of interest:

- Henrik Zetterberg (HZ) has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave. He has also given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche. He is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is part of the GU Ventures Incubator Program.
- Anne Katrine Bergland (AKB) has received support for conference participation from Evonik.

All other authors declare that they have no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2024.112569.

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