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Concord Grape Juice Supplementation and Neurocognitive Function in Human Aging

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ABSTRACT: Polyphenol compounds found in berry fruits, in particular flavonoids, have been associated with health benefits including improvement in cognition and neuronal function with aging. Concord grape juice contains polyphenols, including anthocyanins and flavanols, and previous research has shown improvement in a number of human health conditions with grape juice supplementation. In the current study, older adult subjects with mild cognitive impairment consumed Concord grape juice or placebo for 16 weeks and were administered assessments of memory function and brain activation pre- and postintervention. Participants who consumed grape juice showed reduced semantic interference on memory tasks. Relatively greater activation in anterior and posterior regions of the right hemisphere was also observed with functional magnetic resonance imaging in the grape juice treated subjects. These findings provide further evidence that Concord grape juice can enhance neurocognitive function in older adults with mild memory decline.

KEYWORDS: *Concord grape juice, memory, mild cognitive impairment, brain activation, aging*

INTRODUCTION

Polyphenols found in berry fruits such as blueberries and grapes have been associated with several health benefits. Preclinical studies have implicated berry-derived polyphenols in moderation of oxidative stress and inflammation, increased neuronal signaling, and improved metabolic function among other effects.[1−3]

Purple grape juice, in particular, is rich in polyphenolic compounds including a variety of flavonoids. Recent data indicate that anthocyanins comprise 46% of detected polyphenols in samples of Concord grape juice.[4] In animal studies, anthocyanins have been shown to cross the blood−brain barrier,[5] to accumulate in a number of brain regions including those essential to cognitive function, and to enhance memory performance.[6] In a key animal experiment examining Concord grape juice supplementation and cognitive performance in aging, different concentrations of grape juice produced differential effects, with lower dosing associated with improvement in learning and higher dosing with improvements in motor performance.[7] That study indicated, for the first time, neural benefits with grape juice supplementation in the context of aging and suggested the possibility that dosing may be an important factor.

Brief to moderate-term supplementation with grape juice also has been studied in a number of human disease conditions, and benefit has been shown with respect to blood pressure control, improved endothelial function, and reduced low-density lipoprotein (LDL) cholesterol oxidation.[8−10] Such effects are associated with increased nitric oxide synthesis attributed to the actions of flavanols,[11] which are well represented in Concord grape juice[4] and would be expected to enhance overall vascular function, including circulation in the brain.

In a previous, controlled human trial assessing effects of grape juice supplementation on cognition, we found improvement in memory function in older adults with mild cognitive impairment (MCI), a risk condition for dementia.[12] This effect was observed after daily supplementation for 12 weeks with 100% Concord grape juice. In light of the positive results of that initial study, we designed a second trial in the same population. We extended the duration of the treatment to determine whether additional benefit might accrue with a longer treatment period, and we performed functional magnetic resonance imaging (fMRI) studies to assess potential changes in brain activation during a working memory task.

MATERIALS AND METHODS

Participants. The research protocol was approved by the University of Cincinnati Medical Institutional Review Board, and all participants reviewed and signed an informed consent document. We recruited older adults from the region in and around Cincinnati, OH, by means of print advertising in the major daily newspaper. As in the prior trial, we enrolled a sample of individuals 68 years old and older with mild, age-related memory decline who met criteria for MCI. MCI is considered to be a risk condition for advancing cognitive impairment[13] with 5−10% annual progression to dementia, in particular Alzheimer's disease.[14] Individuals with MCI have greater than expected memory deficit for age but continue to function independently albeit with less efficiency. Prospective participants with diagnosed or suspected dementia, diabetes, kidney disease, liver

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disease, serious psychiatric conditions, and substance abuse were excluded.

We administered structured interview instruments and objective cognitive assessments to determine eligibility for study inclusion. The Academic and Medical History Questionnaire[15] was administered to obtain demographic information and information concerning medical conditions and medication and supplement usage. The Clinical Dementia Rating (CDR[16]) was used to assess level of cognitive decline as evident in change in everyday functional capability. Information was gathered from the prospective subject and an informant to establish the CDR classification, which includes normal, early decline, and mild, moderate, and severe dementia. In addition to the classification score representing stage of memory impairment, the sum of boxes score was computed to quantify severity of impairment.[17] We also administered the Montreal Cognitive Assessment (MOCA[18]) and the Rey Auditory Verbal Learning Test (RAVLT[19]) as objective measures of overall cognitive ability and memory function, respectively. We enrolled individuals classified with early decline on the CDR corresponding to MCI and excluded those classified as normal and those with mild, moderate, or severe dementia. Also, those who performed above threshold scores established for MCI on the MOCA[18] and the RAVLT[19] were excluded. We also administered the Geriatric Depression Scale (GDS[20]) to assess the level of depressive symptoms. Although depression is not uncommon in early neurodegeneration, higher levels of depressive symptoms can influence performance on memory measures. We excluded prospective participants with GDS scores >15 so that depression would not confound performance on the outcomes measures.

We enrolled 21 participants, 11 men and 10 women. The mean (SD) and median ages of the sample were 76.9 (6.1) and 76 years, respectively. The minimum and maximum ages were 68 and 90 years, and the interquartile range was 11. The mean (SD) and median educational levels were 13.7 (2.1) and 13 years, respectively. The minimum and maximum educational levels were 12 and 18 years, and the interquartile range was 4. Nine of the 21 participants, or 43% of the subject sample, had been diagnosed with hypertension. There were four hypertensive individuals in the Concord grape juice group and five hypertensive individuals in the placebo group. All of these participants were being treated with antihypertensive medications prior to and during the intervention. The medications included calcium channel blockers, β-adrenergic blockers, angiotensin-converting enzyme inhibitors, and diuretics. There was no difference between the hypertensive and nonhypertensive subjects on any demographic, cognitive, metabolic, or anthropometric factor and no difference between those with and without hypertension for any of these factors within the grape juice group or the placebo group.

Procedure. All subjects were administered memory and mood assessments at the preintervention enrollment visit and again at the final visit after 16 weeks of supplementation. The California Verbal Learning Test-II (CVLT[21]) was administered to assess memory function. This task involves learning a list of 16 common words. Recall and recognition memory scores were derived as well as indices of interference of nontarget material on memory performance. Nontarget material includes error responses that represent intrusion of items that are semantically related to target items. The design of the CVLT is such that interference errors are facilitated in susceptible individuals. As noted, the GDS[20] was used to assess the level of depressive symptoms. We also measured anthropometric parameters and obtained fasting blood samples. Glucose and insulin assays were performed by the Biochemistry Laboratory of the University of Cincinnati Clinical Translational Research Center.

We solicited participation in the brain imaging protocol from subjects who had no counterindication to exposure to the procedure such as implantation of a metal-containing medical device or claustrophobia. Eight subjects, four per group, were included in the fMRI studies, which were performed at the University of Cincinnati Center for Imaging Research. Subjects performed a sequential letter, *n*-back working memory task that was programmed and administered using EPrime[22] during fMRI data acquisition. The task involved presentation of a series of letters of the alphabet for 500 ms with an interstimulus interval of 2500 ms. The subjects were instructed to respond to each letter by pressing one of two response buttons indicating either "yes", that the letter had appeared *n* items previously, or "no", that it had not. In the 0-back condition subjects were instructed to respond "yes" when a particular stimulus (the letter "x") was displayed. Accordingly, the 0-back condition represented a sustained attention task with minimal working memory load as there was no information retention requirement. In the 1-back condition, the subject was required to recognize when the currently displayed letter was the same as the letter displayed 1 letter previously. In the 2-back condition, the subject was required to recognize when the current letter matched the letter presented 2 letters back. Consequently, working memory load increased across the conditions with the greatest load elicited during the 2-back condition. Figure 1 illustrates stimulus presentation sequences and examples of targets for each of the *n*-back conditions.

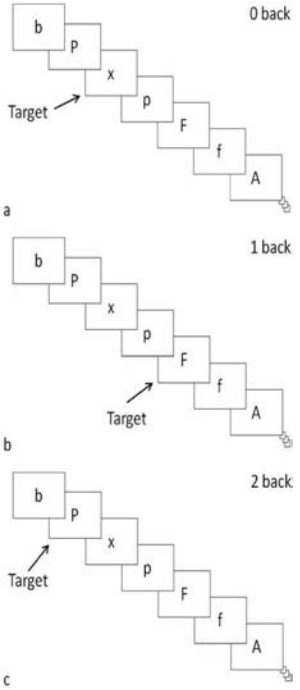

Figure 1. Depiction of *n*-back stimuli presentation for each of the working memory conditions. The stimulus duration was 500 ms, and the interstimulus interval was 2500 ms. In the 0-back condition (a), the instruction was to respond by pressing the "yes" button each time the "x" appears. In the 1-back (b) and 2-back (c) conditions, instructions indicate that the "yes" button should be pressed each time the current letter is the same as the letter presented 1 or 2 items earlier, respectively. Examples of the target responses are shown for each condition sequence. The order of stimulus item presentation is shown from right to left.

There were 34 stimulus items in each condition block. Four runs, each consisting of the three *n*-back conditions, were administered, and the order in which the condition blocks were presented within each run was varied. To assess activation during the condition with greatest working memory load, we analyzed brain activity during correct target identification events (hits) for the 2-back condition.

Brain imaging data were acquired using a 4.0 T Varian INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA, USA). The visual stimuli for the *n*-back task were projected through nonferromagnetic high-resolution video goggles (Resonance Technologies, Inc., Northridge, CA, USA). We acquired four runs of T2*-weighted gradient-echo echo planar images (EPI) consisting of 35 contiguous 4 mm axial slices covering the entire brain (TR/TE 3000/25 ms, FOV 256 × 256 mm, flip angle 85°). A multiecho reference

scan was obtained between each of the four runs to correct for ghost and geometric distortions. After the fMRI data acquisition, a T1-weighted 3-D anatomical image was acquired using a modified driven equilibrium Fourier transform (MDEFT) sequence[23] (TMD = 1.1 s, TR = 13 ms, TE = 6 ms, FOV = 25.6 × 19.2 × 19.2 cm, matrix 256 × 192 × 96 flip angle 20°) to provide anatomical coregistration of fMRI data.

Raw (binary) MRI data were reconstructed using in-house software developed with the Interactive Data Language program (IDL; www.ittvis.com) with hamming filtering in the *X*, *Y*, and *Z* planes. Images were subsequently processed, analyzed, and visualized with the Analysis of Functional Neuroimages (AFNI) software.[24,25] Images were visually inspected for gross movement and were motion corrected using a six-parameter rigid-body transformation with Fourier interpolation.[26] All data sets were normalized to standard space using tools in AFNI to match each subject's image to the International Consortium for Brain Mapping's ICBM452 template from UCLA's Laboratory of Neuroimaging (www.loni.ucla.edu) and masked to exclude nonbrain voxels. fMRI data were converted to percent signal change.

Treatment. The grape juice and placebo beverage were provided by Welch Foods, Inc., Concord, MA, USA. The juice was 100% Concord grape juice derived by hot press and pasteurized with no added ingredient. This juice was analyzed by reverse-phase HPLC-MS^n, and information concerning the specific polyphenol constituents has been documented elsewhere.[4] The proportions of the chief polyphenolic constituents of the juice, on a molar basis, were anthocyanins (46%), tartaric acid esters of hydroxycinnamates (29%), and procyanidins (10%).[4] The total polyphenol concentration was determined by using the Folin−Ciocalteu procedure.[27] Anthocyanins were determined according to a spectrophotometric procedure,[28] and proanthocyanidins were determined with normal-phase high-performance liquid chromatography after solid-phase extraction of the juice with a Sephadex LH-20.[29,30] Oxygen radical absorbance capacity (ORAC) was determined using fluorescein as the assay probe.[31]

The placebo beverage was designed to match the grape juice with respect to color, taste, total calories, and sugar profile (ratio of glucose to fructose). The placebo contained no juice or polyphenolic compounds. Table 1 contains additional information on the quantification of the composition of the juice and placebo from lots used in this study.

Table 1. Quantification of Concord Grape Juice and Placebo Beverage Composition[a]

	100% Concord grape juice	placebo (0% juice)
°Brix	16.2	16.5
total polyphenolics, mg/L as gallic acid equiv	2091	ND[b]
anthocyanin, mg/L as malvidin equiv	425	ND
proanthocyanidins, mg/L as catechin equiv	888	ND
vitamin C (ascorbic acid)	ND	ND
ORAC, μmol TE/L	35765	NA[c]

[a]Values were derived by averaging determinations from three lots of the Concord grape juice and placebo beverage used in this study that were refrigerated at −1 °C and sampled across a 12 month storage period. Total polyphenol concentration was determined according to the Folin−Ciocalteu procedure.[27] Anthocyanins were determined by a spectrophotometric procedure.[28] Proanthocyanidins were determined by normal-phase high-performance liquid chromatography after solid-phase extraction of the juice with a Sephadex LH-20.[29,30] Oxygen radical absorbance capacity (ORAC) was determined using fluorescein as the assay probe.[31] Data were provided by Welch Foods, Inc. A complete determination of polyphenolic content by reverse phase HPLC-MS^n of one lot of the Concord grape juice has been documented elsewhere.[4] [b]ND = none detected. [c]NA = not analyzed.

The treatment was administered in a double-blind manner. Subjects were assigned randomly to the active supplement (n = 10) or placebo (n = 11) group and consumed either grape juice or placebo beverage each day for 16 weeks. Daily dosage for each subject was determined according to body weight and maintained in the range of 6.3−7.8 mL/kg, consistent with our prior study and with other human trials. Subjects weighing between 45 and 57 kg were prescribed 355 mL/day. Those weighing between 58 and 68 kg consumed 444 mL/day. Those between 69 and 82 kg consumed 532 mL/day, and those weighing between 83 and 95 kg consumed 621 mL/day. Daily consumption was divided into three administrations with the morning, midday, and evening meals to reduce burden and the risk of gastrointestinal distress associated with consuming a large fluid volume.

Grape juice and placebo were bottled in glass containers and stored in a cold room at 4 °C until distributed to the subjects, who were instructed to keep the bottles refrigerated at home. Bottle caps from emptied containers were collected from the subjects during and at the end of the intervention as a means of checking adherence to the consumption protocol.

Statistical Analyses. The primary statistical analyses for the memory and mood data included analyses of covariance (ANCOVA) comparing outcome measures obtained at the final visit between groups and using the corresponding measure from the preintervention visit as covariate control to isolate the effect of the treatment.[32] Treatment outcome values were reported as adjusted means, which represent measures obtained at the final visit adjusted for preintervention values of the corresponding measure. Separate ANCOVA analyses were performed for each outcome domain. For statistically significant effects we computed Cohen's *f*, the effect size statistic for the ANCOVA analyses, which can be classified as small (0.1), medium (0.25), and large (0.40).[33] In addition, *t* tests were performed on the preintervention demographic, anthropometric, and metabolic variables to assess differences between the groups on these factors.

For the brain imaging data, a region-of-interest (ROI) approach was used. Six homologous areas in each hemisphere of the brain were identified for a total of 12 brain regions involved in working memory function. These working memory ROIs had been established empirically[34] and included (1) bilateral inferior frontal cortex, (2) bilateral middle frontal cortex, (3) bilateral superior frontal cortex, (4) bilateral anterior cingulate gyrus, (5) bilateral hippocampus, and (6) bilateral superior parietal cortex. We used the automatic anatomical labeling atlas in AFNI to create these ROIs.[35] The mask was applied to each individual's fMRI activation map. Activation within each ROI for the hit events was extracted and used for the ROI image analyses. Analysis of variance (ANOVA) was performed to determine meaningful ROI activation and ANCOVA were used to examine between-group differences in activation. Partial eta-squared (η^2) values were derived to assess effect sizes for significant effects, characterized as minimal (0.04), medium (0.25), and strong (0.64).[36]

RESULTS

Table 2 contains data on the subject sample characteristics at enrollment and after the 16 week intervention. There was no preintervention difference between the groups with respect to any factor. Age and education levels were consistent with our prior intervention studies in this population.[12,37] The CDR sum of boxes[17] and MOCA[18] scores were in the expected range for a sample of MCI subjects. Mean weight and waist circumference values are representative of the high population base rates for overweight and obesity.[38] The level of depressive symptoms as measured by the GDS was low in both groups.[20]

At 16 weeks, there was no between-group difference for performance on the CVLT learning task (38.8 vs 37.3, adjusted means, $F(1,18) = 0.45$, $p = 0.50$). There was a trend favoring the placebo group for recognition memory performance (13.8 vs 12.4, adjusted means, $F(1,81) = 3.31$, $p = 0.09$). However, as shown in Figure 2, the subjects consuming the placebo

Table 2. Subject Sample Characteristics by Group[a]

	preintervention			postintervention		
	placebo	grape	*p*	placebo	grape	*p*
age, years	75 (6)	78 (5)	0.19			
education, years	13.8 (2)	13.7 (2)	0.90			
CDR[b] sum boxes	1.0 (0.3)	1.1 (0.3)	0.35			
MOCA[c]	22.8 (2)	23.4 (2)	0.59			
GDS[d]	6.0 (4)	5.7 (3)	0.81	5.9 (5)	6.8 (4)	0.66
BP,[e] systolic	143 (19)	138 (25)	0.59	139 (20)	137 (14)	0.79
BP, diastolic	82 (15)	76 (10)	0.32	81 (13)	78 (10)	0.58
glucose, mg/dL	100 (7)	104 (12)	0.42	100 (7)	100 (11)	0.99
insulin, μU/mL	22 (9)	19 (4)	0.25	25 (7)	20 (4)	0.12
weight, kg	74.9 (8)	76.7 (9)	0.67	74.1 (8)	76.0 (9)	0.61
waist, cm	95.1 (11)	94.3 (9)	0.85	95.2 (12)	93.8 (9)	0.77

[a]Mean (SD) scores and *p* values associated with independent sample *t* tests, df = 19. [b]CDR = Clinical Dementia Rating. [c]MOCA = Montreal Cognitive Assessment. [d]GDS = Geriatric Depression Scale. [e]BP = blood pressure.

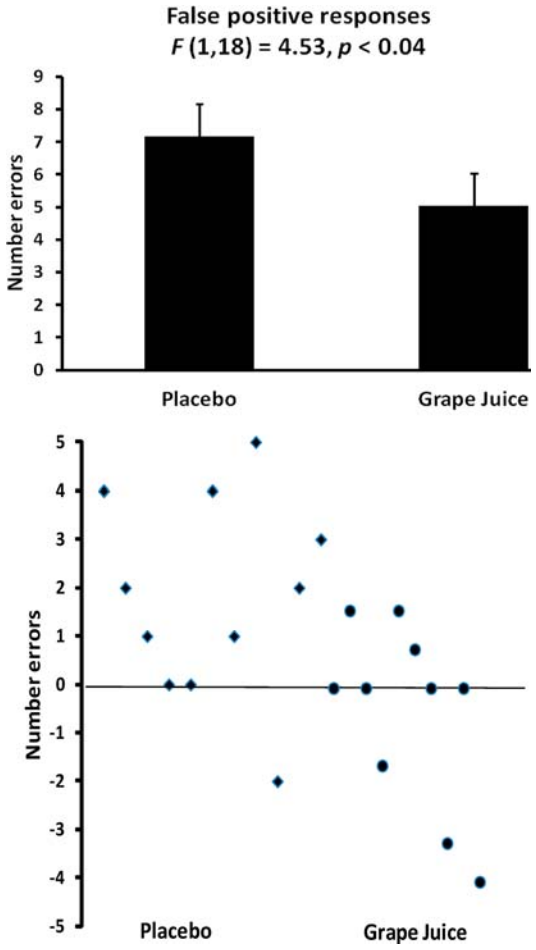

Figure 2. Subjects who received Concord grape juice exhibited fewer intrusion errors during recognition memory testing, indicating reduced interference of nontarget material in memory. The upper panel shows mean error performance by group at 16 weeks adjusted for preintervention performance, 7.2 vs 5.0, $p = 0.04$. Error bars represent standard error. The lower panel shows scatter plots of intrusion error change scores (final score at 16 weeks less preintervention score) for each group: ◆, placebo subject; ●, Concord grape juice subject.

beverage committed significantly more interference errors on this recognition memory task (7.16 vs 5.03, adjusted means, $F(1,18) = 4.53$, $p = 0.04$). The effect size for this result was large (Cohen's $f = 0.50$) and reflected better ability to discriminate learned, target items from nontarget foils for the subjects who consumed grape juice. There was no effect of the intervention on mood, (5.7 vs 6.9, adjusted mean GDS scores, $F(1,18) = 0.86$, $p = 0.36$).

Given the small sample size and the preliminary nature of the brain imaging study, we chose to perform between-group comparisons of the final visit activation in each of the 12 identified ROIs associated with working memory. We observed group differences for two regions: right superior parietal cortex ($F(1,7) = 7.46$, $p < 0.05$, partial $\eta^2 = 0.55$) and a marginally significant effect for right middle frontal cortex ($F(1,7) = 5.26$, $p = 0.06$, partial $\eta^2 = 0.47$). In each case, activation was greater in the grape juice treated group relative to the placebo-treated group: right superior parietal cortex (0.07 ± 0.06 vs -0.01 ± 0.02) and right middle frontal cortex (0.10 ± 0.04 vs -0.01 ± 0.08). An ANCOVA was then performed to examine activation in these regions using the corresponding preintervention measures of activation as covariate controls to assess group differences while isolating the effect of the intervention. The ANCOVA demonstrated a significant effect for right middle frontal cortex ($F(1,7) = 8.56$, $p = 0.05$, partial $\eta^2 = 0.68$) and a marginally significant effect of right superior parietal cortex ($F(1,7) = 6.05$, $p = 0.07$, partial $\eta^2 = 0.60$), indicating greater activation in the grape juice treated group relative to the placebo-treated group in both of these regions. Figure 3 shows these regions of greater activation observed for the grape juice subjects.

DISCUSSION

Although learning and retention scores were not improved with supplementation, we observed reduced interference during recognition memory for subjects receiving 100% Concord grape juice. The latter effect indicated that the grape juice supplemented subjects were better able to discriminate previously learned material from foils on the recognition memory task. Paradoxically, the trend toward better performance among the placebo-treated subjects on the recognition task is attributable to their poorer ability to distinguish learned items from foils. That is, placebo-treated subjects produced more correct responses because they discriminated relatively poorly and endorsed memory for items to which they had not been previously exposed. Such indiscriminant endorsement of items on the recognition memory task would, de facto, increase the number of correct responses but also increase endorsement of incorrect foils. Intrusion errors of this sort are characteristic

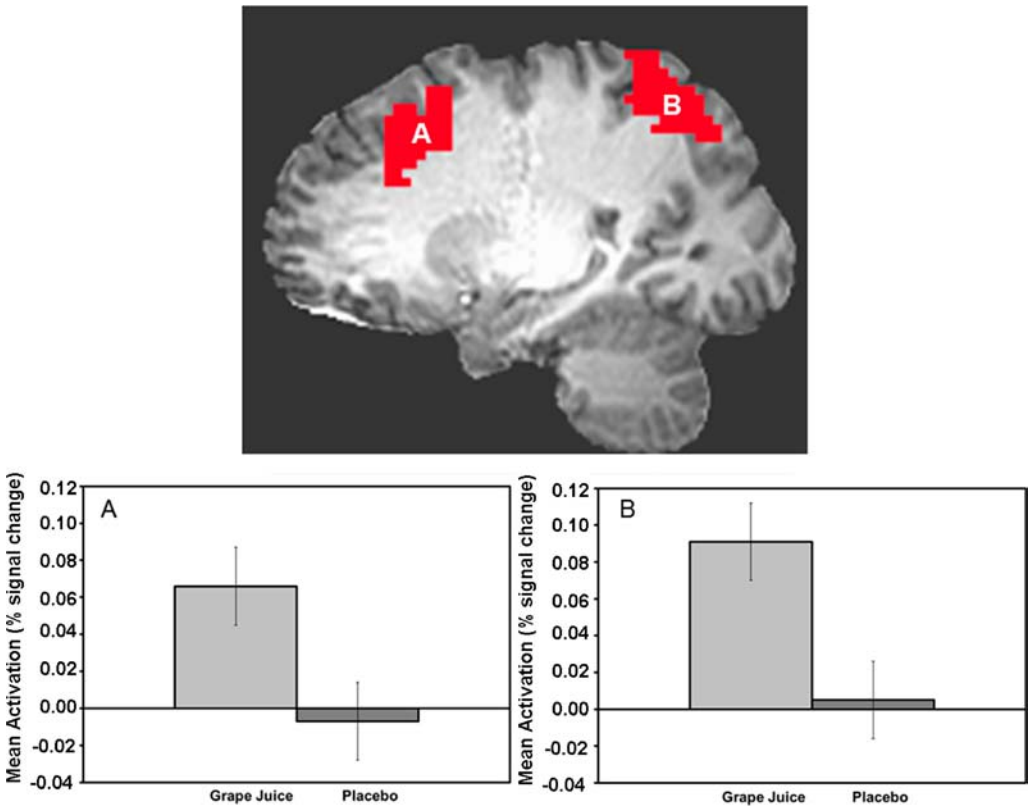

Figure 3. (Top) Right middle prefrontal (A) and right superior parietal (B) cortical regions of interest (ROI) overlaid on a T1-weighted anatomic image. These regions were relatively more activated in subjects who consumed 100% Concord grape juice as compared with those who consumed the placebo beverage. The red color defines the anatomical location of each ROI. (Bottom) Mean MRI signal intensity as a function of treatment for the right middle prefrontal cortex ROI (A) and the right superior parietal cortex ROI (B) during the sequential letter working memory task. Error bars represent standard error.

of age-related memory decline and have been related to diminished inhibitory control in working memory.[39] Intrusion errors reflecting poorer ability to discriminate tend to be more prominent in early neurodegeneration than complete memory failure,[40] the latter being observed in more advanced cognitive decline and dementia. These results indicate that subjects who consumed Concord grape juice acquired new information at the same level as those who consumed placebo but were better able to suppress interference of extraneous material, thus making better discriminations when retrieving previously presented material and making memory judgments.

We also observed increased activation on fMRI in the right anterior and posterior cortical regions during performance of the *n*-back working memory task. Increased regional fMRI activation represents greater hemodynamic response, which would be consistent with observations of vascular benefit associated with grape juice supplementation,[8−10] and greater hemodynamic response is strongly associated with increased neuronal activity. A similar increase in hemodynamic response with fMRI has been demonstrated in young healthy individuals in the context of controlled, short-term supplementation with flavanol-rich cocoa.[41] In that study increased activation was observed in right hemisphere brain regions similar to those showing increased activation in this study. This similar blood oxygen level dependent (BOLD) response suggests similar benefit with respect to cerebral blood flow following supplementation with another flavanol-containing product, suggesting that this effect may be attributable specifically to the flavanol content of Concord grape juice.[4] In addition, it is possible that the enhanced activation observed in the characteristic working memory ROIs[34,42] reflected increased neural recruitment associated with improved cognitive control as demonstrated in reduced interference on the recognition memory task. A similar, beneficial effect of reduced memory interference in association with increased right hemisphere activation also was found in a nonpolyphenol supplementation study in this population,[43] suggesting that better suppression of cognitive interference may be an important effect of enhanced neuronal activation in older adults with very early neurodegeneration.

The current findings are consistent with the prior human trial with Concord grape juice supplementation.[12] In addition, the effect size estimates for both the memory and imaging findings were large, despite the modest sample size. However, as noted, these findings represent preliminary observations in a relatively small sample of older adults with early memory decline, and replication will be important. In particular, imaging studies with larger samples that would allow utilization of voxel-wise rather than ROI analyses will be important to confirm the observed activation effects.

In future studies, the inclusion of different dosing conditions and intervention periods to assess the possibility of over- and underdosing will be essential. Notably, we did not observe a more robust memory effect in this longer trial relative to the previous 12 week intervention with Concord grape juice. Many of the prior animal and human trials have been conducted with intervention periods of 6−8 weeks, and one prior grape juice study in animals showed differential neurocognitive responses

at different doses within the same time frame.[7] In addition, another study from that laboratory[44] demonstrated motor and cognitive benefits with low and moderate concentrations of walnut supplementation but impairment of both motor function and cognition with higher dosing. It will be of interest to compare lower doses and briefer interventions with moderate to high dosing for longer duration such as that employed in our trial. Whereas our daily dosage was quite consistent with other human trials showing vascular benefit,[8−10] the intervention period was longer, and it is not known whether certain benefits may have been moderated because of the more extended treatment. Furthermore, there may be concern regarding applicability if experimental dosing is greater than typical consumption in everyday life. A recent study detected polyphenols and metabolites in urine and plasma of healthy individuals following supplementation with Concord grape juice for a very brief period,[4] and associating different dosing regimens with bioavailability will be useful in clinical trials. Ultimately, obtaining such measures and correlating them with neurocognitive outcomes would provide essential information concerning the effects of polyphenol supplementation and effective dosing.

On balance, the findings provide further indications that polyphenol-rich Concord grape juice supplementation has benefit for neurocognitive function in older adults with mild memory decline. For the first time, we show preliminary data indicating increased neural activation in cortical regions along with improved memory function in this population.

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Notes

The authors declare no competing financial interest.

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