

Improvements in quantitative EEG following consumption of a natural citicoline-enhanced beverage

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Abstract

The present study examined the impact of a taurine-free drink enhanced with citicoline and other natural ingredients on electrophysiological markers of mental alertness. Ten healthy adult participants enrolled in a double-blind, placebo-controlled crossover study and were randomized to receive either placebo or the citicoline supplement on the first visit. Measures of electrical brain activity using electroencephalogram (EEG) were collected 30 min after consuming the beverage. Seven days after the initial assessment participants completed the alternative condition (placebo or citicoline beverage). Compared to placebo, significant improvements were found in frontal alpha EEG and N100 event related potentials (ERP) associated with the citicoline-enhanced supplement. These preliminary findings suggest that a novel brain drink containing compounds known to increase choline in the brain significantly improved attention as measured by ERP and EEG. These findings suggest that a viable and alternative brain supplement without potential compounds such as taurine may augment attentional mechanisms in healthy individuals.

Keywords: *citicoline, EEG, nutrition, attention, functional beverage*

Introduction

Previous studies have demonstrated improvements in brain activity following acute consumption of commercial drink products containing a combination of caffeine, taurine and glucuronolactone (Seidl et al. 2000). However, studies have questioned the biological activity and utility of individual ingredients such as taurine, an intracellular amino acid. Specifically, taurine has been believed to have positive inotropic effects; however, this claim is not supported by empirical research (Clayson et al. 2008). Further, recent studies have emphasized concern regarding the safety of taurine supplements particularly in terms of cardiac toxicity (Berger and Alford 2009; Steinke et al. 2009; Wiklund et al. 2009; Worthley et al. 2010). For these reasons beverages containing high levels of caffeine, taurine and glucuronolactone have been banned in several countries over the past decade and are the focus of renewed scrutiny regarding their safety. A recent study also indicated that taurine may

have synergistic effects when combined with caffeine and potential serious adverse effects in individuals under the age of 25 (Seifert 2011).

Recent commercially available products developed to support brain function do not combine caffeine, taurine and gluconolactone in liquid (examples include Brain Toniq, Nawgan and Brain Storm). However, studies have not examined whether these products are efficacious. One particular example (Nawgan) contains citicoline (cognizin[®]), choline, lycopene and vitamin E. The former ingredients are of particular interest as they have shown in previous studies to increase overall levels of choline, a brain chemical that occurs naturally in the body. Choline is a key ingredient that has been used for Alzheimer's disease and other types of dementia, stroke and age-related memory loss (Gatti et al. 1992; Silveri et al. 2008). Citicoline is a safe and well-tolerated chemical that has shown no adverse systemic cholinergic effects

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(Secades 2011). In Europe, citicoline is frequently prescribed for cognitive dysfunction associated with circulation problems in the brain.

Most previous work on citicoline supplementation has focused on 500 mg to 2 g daily consumption (Babb et al. 2002; Silveri et al. 2008), with no increase in response associated with doses above 500 mg per day (Babb et al. 2002). No published studies have examined lower doses of citicoline supplementation either alone or in combination with other ingredients important for brain health. In the present study, we examined a beverage that contains 250 mg of citicoline as well as other ingredients known to be associated with supporting brain integrity. Quantitative electroencephalogram (EEG) was examined using a double-blind, placebo-controlled, crossover study with the prediction that the citicoline-enhanced beverage would result in significant changes in EEG relative to placebo.

Methods

Participants

Ten healthy individuals (five men and five women) aged 25–32 (mean age 28.1) were included in the study and completed a written informed consent. All participants were college graduates with a mean of 17.9 years of education. Participants were included if they had a minimal caffeine intake equal to one can of soda per day (35 mg of caffeine) and a maximum caffeine intake of 200 mg of caffeine per day. Thus, individuals who abstain from caffeine and individuals who consume high levels of caffeine daily were excluded. Before testing, all participants had refrained from caffeine for at least 6 hours. In addition, participants were excluded if they had a personal history of: (1) physical brain injury defined by loss of consciousness lasting more than 30 min; (2) brain tumour or stroke; (3) any medical condition that might put them at an increased risk if exposed to caffeine (including cardiac rhythm disorder, prior myocardial infarction, angina, congestive heart failure, hypertension, active peptic ulcer; all unlikely in the target demographic); (4) severe impediment to vision, hearing and/or hand movement; (5) addiction to illicit drugs; (6) current illicit substance use of any amount; (7) participants who are smokers (or who have smoked/used nicotine products within the 6 months before the study entry); and (8) participants who consume two or more standard alcoholic drinks per day.

Experimental design

A double-blind, placebo-controlled crossover design was implemented for this study. The study was approved by a university institutional review board. Participants were randomized to receive either placebo or the brain supplement on the first visit. The placebo

condition was identical in quantity (eight-ounce), carbohydrates (sugars) and flavour of the supplement drink minus the active ingredients (choline and citicoline (cognizin[®]), lycopene, caffeine and vitamin E). Measures of electrical brain activity using EEG were collected 30 min after consuming the beverage using a double-blind procedure. Seven days after the initial assessment participants returned and completed the remaining condition (placebo or supplement drink) at approximately the same time of day to control for circadian fluctuations.

Procedures

Participants undertook a non-invasive EEG. The brain measures, undertaken using recording discs placed on the scalp during resting and a range of cognitive tasks (event related potentials, ERPs), provide a profile of automatic information processing over a fraction of a second time scale. The experimental activation tasks were designed to reflect a profile of the brain's core adaptive competencies and underlying neural networks. This battery of activation tasks took approximately 45 min to complete.

Participants were seated in a sound and light attenuated room, set with an air-conditioned ambient temperature of $24 \pm 1^\circ\text{C}$. An electrode cap (Quikcap) was used to acquire data from Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, FC3, FCz, FC4, T3, T4, T5, T6, Pz, P3, P4, O1, O2 and Oz electrode sites (32 channels; Compumedics Neuroscan Nuamps; 10-20 International System). Horizontal eye movement potentials were recorded using two electrodes, placed 1.5 cm lateral to the outer canthus of each eye. Vertical eye movement potentials were recorded using two electrodes placed 3 mm above the middle of the left eyebrow and 1.5 cm below the middle of the left bottom eyelid. Gratton procedure and electro-oculogram (EOG) thresholding for contaminated epochs (exceeding $\pm 100 \mu\text{V}$) allowed correction/rejection. The sampling rate was 500 Hz and a 70 Hz low-pass filter was applied to the signals before digitization.

The EEG/ERP paradigm consisted of multiple tasks tapping resting state EEG and ERP activation. For the purpose of the present study, we focused on resting EEG and the Auditory Oddball Task. The details of each test are outlined as follows:

- (1) Resting EEG: participants were asked to rest quietly and focus on the red dot (eyes open), and then repeat with eyes closed. The baseline EEG measure allows for comparison between resting and active states of the brain.
- (2) Auditory Oddball: participants were presented with a series of high and low tones, at 75 dB and lasting for 50 ms (with rise and fall times of 5 ms). Participants were instructed to ignore the low ('background') tones (presented at 500 Hz) and to press, with the index finger of each hand, a

response button only when they hear high infrequent ('target') tones, which are presented at 1000 Hz. The auditory oddball task allows for assessment of processing novel task relevant, while ignoring task irrelevant, information.

Data analysis

EEG scores

All EEG data were uploaded through a secured server and transferred via the Internet to an independent company for analyses (Brain Resource, Ltd.). These data were encrypted and personal identifiers were removed from the data before uploading. Average power spectra were computed for eyes open, eyes closed and pre-stimulus target auditory oddball epochs. For eyes open and eyes closed paradigms, 2 min of EEG were acquired. To facilitate focus and data reduction only data from the eyes open condition were analysed in the current study. These 2 min of EEG were divided into adjacent intervals of 4 s. Power spectral analyses were performed on each 4 s interval by first applying a Welch window to the data, and then performing a Fast Fourier Transform. The resulting power spectra were averaged for each paradigm, yielding a single eyes open and a single eyes closed average power spectrum for each electrode position. For the pre-stimulus target auditory oddball epochs, the same procedure was followed, except that the epochs subject to power spectral analysis were 1 s in duration, from 1 s prior to each target until the target presentation. Again, the pre-stimulus power spectra were averaged across targets, yielding a single pre-stimulus target auditory oddball power spectrum for each electrode.

For each average power spectra, the power was calculated in frequency bands. For the purpose of the present study we focused on frontal alpha and gamma EEG measures as previous studies have found that EEG activity in the alpha and gamma range can be modulated by attention (Kubicki et al. 1979; Klimesch 1997; Vidal et al. 2006; Dockree et al. 2007; Jann et al. 2010). These power data were then square-root transformed in order that it might better approximate the normal distributional assumptions required by parametric statistical methods. Aggregate EEG scores for both alpha and gamma were derived by summing values across five frontal regions including Fp1, Fp2, Fp3, Fp4 and Fz.

ERP scores

Average ERPs were calculated for target and background auditory oddball stimuli. In each of these cases the individual single-trial epochs were filtered with a low-pass Tukey (cosine taper) filter function that attenuates frequencies above 25 Hz. The single-trials were then averaged to form conventional ERPs.

Amplitude and latency scores were obtained for target ERP with a focus on N100 and N200 amplitude. N100 amplitude was chosen as it has been found to be an index of attention selection and arousal (Maclean et al. 1975; Bruce et al. 1992; Georgiev et al. 2006), while N200 amplitude was chosen as it has been shown to be associated with cognitive processing (Hoffman 1990). The FCz site was isolated for both N100 and N200 amplitudes as inspection of the data revealed optimum signal to noise at this location.

Statistical analyses

Effects of the brain supplement beverage on EEG and ERP scores were evaluated using an independent samples *t*-test using SPSS 18.0 statistical software package. The level of significance was set at $p < .05$. Change scores were obtained by subtracting the raw scores of the citicoline supplement condition with the raw scores of the control condition.

Results

It was hypothesized that 30 min after consumption of the citicoline-enhanced beverage participants would exhibit an improvement in EEG coherence and EEG markers of attention compared to placebo. Within subject comparisons were conducted by creating difference scores of the frontal alpha and gamma EEG scores by subtracting the placebo condition EEG and ERP from the brain supplement condition. Independent sample *t*-tests showed significant differences were found with alpha EEG scores ($t(9) = 2.34, p < .05$). No differences were found with respect to gamma EEG scores. For ERP, N100 was found to be significantly improved for the brain supplement condition compared to placebo, ($t(9) = 2.39, p < .05$). No differences were found with N200 scores (see Table I).

Table I. Means and standard deviations for EEG and ERP indices (citicoline supplement vs. placebo control).

	Citicoline supplement (<i>N</i> = 10)	Placebo (<i>N</i> = 10)
	Mean (SD)	Mean (SD)
EEG		
Fp1 alpha	(-.085 (.851)	.087 (1.07)
Fp2 alpha	(-.062 (.845)	.265 (1.13)
F3 alpha	(-.149 (1.08)	.087 (1.14)
F4 alpha	(-.054 (.870)	.346 (1.92)
Fz alpha	(-.199 (1.13)	.063 (1.14)
Fp1 gamma	.480 (1.19)	.697 (1.38)
Fp2 gamma	.482 (.930)	.696 (1.34)
F3 gamma	(-.196 (.903)	.342 (1.65)
F4 gamma	(-.212 (1.11)	.134 (1.25)
Fz gamma	(-.351 (.920)	(-.078 (1.25)
ERP		
Fcz N100	(-10.29 (3.89)	(-11.53 (3.06)
Fcz N200	(-1.57 (2.58)	(-2.12 (4.10)

Discussion

Results indicate that alpha EEG and ERP indices were significantly modified 30 min after consuming the citicoline-enhanced beverage compared with the placebo condition. Implications for these findings suggest that the unique combination of the chemicals may assist in increasing the overall levels of attention in healthy adults and is consistent with previous findings on these compounds.

Citicoline is a unique compound, consisting of cytidine and choline. In a study by Babb et al. (2002), the administration of citicoline resulted in measurable increases in phosphodiesterases (PDEs), compounds involved in phospholipid biosynthesis and breakdown. As PDE has been shown to decrease with age and be associated with cognitive memory loss, increase in this substance may improve overall cognitive skills. In fact, Babb et al. found a significant correlation between increased PDE concentrations and improved verbal learning in healthy older subjects, which suggests that the administration of citicoline may be of use in reversing age-related cognitive changes. In another recent study citicoline supplements were also found to increase PDE concentrations especially in the frontal lobes, which the authors suggest that citicoline 'may therefore help to mitigate cognitive declines associated with aging by increasing energy reserves and utilization, as well as increasing the amount of essential phospholipid membrane components needed to synthesize and maintain cell membranes' (Silveri et al. 2008).

A second compound included in the beverage investigated in this trial is naturally occurring choline, which is an important compound for brain function. Dietary consumption of choline results in a rapid release of plasma choline (Gatti et al. 1992). Choline is important for acetylcholine synthesis in the brain, leading to increased acetylcholine production (Trabucchi et al. 1986). Thus, choline has been hypothesized to exert potential therapeutic effects in Alzheimer's disease (Moreno 2003) and has been used as a dietary supplement to enhance memory and cognition.

Collectively, the choline concentrations available from both citicoline and the L-Alpha Glycerylphosphorylcholine (A-GPC) in each serving of the beverage amounts to 95.5 mg, which is recognized by the FDA as a good source of choline. When combined in the beverage, the citicoline dose and the additional choline provide a strong foundation of phospholipids. As such, while previous studies have focused on higher doses of citicoline, the results of the present study suggest that 250 mg of citicoline, at least when combined with choline and caffeine in the beverage, results in significant improvements in EEG markers of alertness.

Limitations to this preliminary study include a small sample size, as well as the utilization of a young adult

sample. Studies with an older adult sample may assist in the generalizability of these findings to a larger age range. Further, future studies should also incorporate the addition of cognitive data to further elucidate any differences found with this citicoline beverage. It is also unclear how these findings may differ from studies that have examined the benefits of caffeine alone on improvements in attention (Bruce 1992) as this study was not designed to assess the specific mechanisms of action of each of the ingredients included in the supplement.

Conclusions

Results from this study suggest that this new supplemental drink is associated with improvements in electrophysiological indices of attention and mental alertness. Importantly, these potentially beneficial effects were evident in the absence of taurine, glucuronolactone and guarana. Additional studies are needed to validate the results and ensure appropriate generalization.

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