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ORIGINAL CONTRIBUTION

Acute effects of cocoa flavanols on visual working memory: maintenance and updating

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Abstract

Background Consumption of cocoa flavanols may have acute physiological effects on the brain due to their ability to activate nitric oxide synthesis. Nitric oxide mediates vasodilation, which increases cerebral blood flow, and can also act as a neurotransmitter.

Objectives This study aimed to examine whether cocoa flavanols have an acute influence on visual working memory (WM).

Methods Two separate randomised, double-blind, placebo-controlled, counterbalanced crossover experiments were conducted on normal healthy young adult volunteers ($N_{Exp1} = 48$ and $N_{Exp2} = 32$, gender-balanced). In these experiments, 415 mg of cocoa flavanols were administered to test their acute effects on visual working memory. In the first experiment, memory recall precision was measured in a task that required only passive maintenance of grating orientations in WM. In the second experiment, recall was measured after active updating (mental rotation) of WM contents. Habitual daily flavanols intake, body mass index, and gender were also considered in the analysis.

Results The results suggested that neither passive maintenance in visual WM nor active updating of WM were acutely enhanced by consumption of cocoa flavanols. Exploratory analyses with covariates (body mass index and daily flavanols intake), and the between-subjects factor of gender also showed no evidence for effects of cocoa flavanols, neither in terms of reaction time, nor accuracy.

Conclusions Overall, cocoa flavanols did not improve visual working memory recall performance during maintenance, nor did it improve recall accuracy after memory updating.

Keywords Cocoa flavanols · Visual working memory · Mental rotation

Introduction

Even though cocoa consumption is not related to being granted a Nobel Award [1], its physiological and cognitive benefits, both acute (e.g., Sansone et al. [2]; Scholey et al. [3]) and long-term (e.g., Mastroiacovo et al. [4]; Francis et al. [5]), have been pointed out in several studies to date. Supposed benefits aside, people increasingly consume cocoa in their daily life simply because of its unique flavour. For instance, in The Netherlands, approximately 5.1 kg of chocolate per person is consumed per year [6]. In view of the ubiquitous consumption of cocoa, it is important to determine the possible effects on cognition that this might have.

Such potential effects are mediated by specific components that are found in cocoa. In general, cocoa beans include four main chemical components: alkaloids (methylxanthines, mainly theobromine and caffeine), polyphenols (flavanols, anthocyanins, and proanthocyanidins), proteins, and carbohydrates [7]. Among these, methylxanthines are known to have acute psychostimulant effects. For instance, Smit et al. [8] found that consumption of methylxanthines in chocolate decreased reaction time in cognitive tasks. However, of interest to the present study are primarily flavanols, which are also known as flavan-3-ols, or catechins, one of the subclasses in the polyphenols family [9–14], and which are also found elsewhere, such as in grapes, apples,

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blueberries, tea and wine [15]. Like methylxanthines, cocoa flavanols (CF) may also have acute psychostimulant effects.

Biochemical studies of CF have identified a potential mechanism that could generate acute effects on cognition, as CF have been found to increase nitric oxide (NO) synthesis [16, 17]. Heiss et al. [18] showed that CF increases nitric oxide bioactivity in human plasma. Additionally, Karim et al. [19] observed significant acute effects of cocoa extracts on nitric oxide synthase in an animal in vitro study. In a human study, Loke [20] found that the dietary flavonoids quercetin and epicatechin increases nitric oxide products acutely. NO has many biological functions, two of which may explain CF effects on cognition: (I) Increasing blood flow and, (II) enhancing neurotransmission [21]. With regard to (I), NO is diffused from the NO generator cells to NO target cells, and binds to guanylate cyclase. As a result, conformational changes occur in guanylate cyclase, increasing the concentration of guanosine monophosphate within the cells. This in turn relaxes the muscles, and induces vasodilation not only in blood vessels but also cerebral arteries [22]. As cerebral blood flow thus increases, even rather immediate cognitive benefits can be expected. With regard to (II), although NO does not act as a classic neurotransmitter like norepinephrine or dopamine, NO has functions that influence neural signalling pathways, as it works as an intercellular messenger. Guanylate cyclase, which is the NO receptor, can act pre- and post-synaptically at GABAergic and glutamatergic synapses, as a result of NO synthesis [23, 24]. NO is stored in synaptic vesicles and in dendrites, as well as pre- and postsynaptic areas of the neurons [25]. Functionally, there is accumulating evidence that neural NO synthesis in the central nervous system strengthens communication between neurons, and enhances synaptic plasticity, with consequences for memory, learning, smell, and pain perception [26, 27].

Beneficial acute effects of CF on various aspects of cognitive performance have indeed been reported in several studies over the years. Scholey et al. [3] studied the effect of CF consumption on mental fatigue and cognitive performance with a Rapid Visual Information Processing (RVIP) task, and with the serial threes and sevens task. In the RVIP task, participants were asked to monitor a series of digits for the occurrence of three consecutive odd or even digits, while in the serial tasks, participants had to count backwards from a random starting number between 800 and 999, in steps of three or seven, as fast and correctly as possible. Scholey and colleagues [3] showed that CF improved the number of correct responses in the serial threes task, decreased reaction time in RVIP, and decreased the number of errors in both the serial threes and sevens tasks. The authors also reported significant positive acute effects of CF on mental fatigue. Nonetheless, they found no significant change in the number of correct responses in the serial sevens and RVIP tasks.

More positive evidence was later obtained by Field et al. [28], who demonstrated that CF increases visual contrast sensitivity, visual spatial working memory, and, to a degree, speeded choice reaction time. A few years later, Massee et al. [29] found that CF had acute effects on mental fatigue and on performance in the serial sevens task, as well as sub-chronic effects on stress. However, neither acute nor sub-chronic effects could be found on cardiovascular functioning, on spatial working memory, on performance in the congruent/incongruent Stroop colour word task, or on contextual memory. In 2016, Grassi et al. did not find differences overall between treatment conditions in a psychomotor vigilance task and in an N-back memory task (see below for further discussion of this task). Interestingly, despite a relatively small sample size, CF did increase performance in the N-back task in their female subsample, but only when this group was sleep-deprived. Finally, most recently, Karabay et al. [21] observed that CF acutely benefitted spatial attention, improving visual search efficiency and reaction time, but also found no effects on temporal integration nor temporal attention.

In addition to these experimental reports, meta analyses and systematic reviews of the cognitive effects of flavanols also paint a mixed picture, perhaps even more so than the individual experimental studies. Some reviews tend towards the positive. For instance, Scholey and Owen [31] highlighted beneficial acute effects of CF on cognitive performance, and Socci et al. [32] mentioned that CF could increase cognitive functioning and generally enhance cognitive achievement. In a systematic review that came out this year, Barrera-Reyes et al. [33], indicated that CF has a medium to large effect on memory and executive function. However, there is also a review that is mostly negative on the possible cognitive effects of cocoa flavanols. Veronese et al. [34], in a paper that was designed as a systematic review of reviews, stated that chocolate consumption has no effects on mood or cognitive functions, although the authors stressed that more research is needed.

In summary, the outcomes of experimental studies as well as those of reviews about the effects of cocoa consumption on cognitive performance vary. Generally, the extent and nature of acute effects of CF on cognitive performance are still not apparent. One reason for this state of affairs may be that the tasks used to measure cognitive functions have varied themselves also. It is conceivable that some tasks may be more sensitive than others. Additionally, some of the tasks used to date are at times 'over-interpreted' to reflect

exclusively on one cognitive function, while in reality they might not. An example of such a task is the N-back task, which is often seen as measuring working memory performance (e.g., Jaeggi et al. [35]. In this task participants are asked to compare the currently presented stimulus with the one presented N (e.g., 2 or 3) trials before. Stimuli are shown in an ongoing fashion, so participants need to update and manipulate remembered information continuously. The N-back task certainly taxes working memory, but it also requires constant attention to ongoing stimuli, and creates a lot of interference between the successive items to be maintained for later and those to be manipulated instantaneously; interference that has to be dealt with to perform well. These latter aspects of the task would seem to require a considerable amount of attention and/or executive control, not just working memory.

This might complicate the interpretation of potential flavanols effects, because not all cognitive functions may be affected similarly by cocoa flavanols. The purpose of the current study was to isolate and test the acute effects of cocoa flavanols on working memory specifically. To this end, two visual working memory tasks were implemented as randomised, double-blind, placebo-controlled, crossover trials. In the first experiment, the maintenance of information in working memory was examined, and in the second experiment, the updating of information in working memory was tested.

Experiment 1

In the first experiment, participants were asked to remember the orientation of one to three visual gratings, which were simultaneously presented. After a brief retention (maintenance) interval, memory performance was assessed by means of a response probe, which consisted of another grating that the participants could adjust to match their memory.

Methods

Participants

Forty-eight healthy adults (24 female, 24 male) participated in this experiment ($\overline{X} = 22.15 \pm 1.86$ years, range = 18–26). The mean body mass index (BMI) of male participants was 21.56 ± 2.13, and the mean BMI of female participants was 23.33 ± 2.13. None of the participants were excluded from the analysis on the basis of overall performance criteria. A power analysis was run using G*Power software [36] for F test, effect size, $d = 0.24$; $\alpha = 0.05$; actual power = 0.957; sample size = 48; critical $F = 3.09$. Effect size and its corresponding sample size were conservatively based on a previous study from our group, namely Karabay et al. [21]. Karabay et al. [21] reported a medium effect size ($\eta_p^2 = 0.09$, effect size $f = 0.31$) for CF (274 mg of cocoa flavanols) on visual search reaction time.

Participants had no history of medical, neurological, or psychiatric disorders, they were not following a medically restricted diet, and they were not pregnant or breastfeeding. Also, all subjects had a (corrected) visual acuity of 20/20 (Snellen) at a test distance of 45 cm.

Experimental product

The placebo drink that was given to the participants included 7.5 g alkalised cocoa powder, while the drink in the CF condition comprised 5 g high-flavanols powder, which contained 415 mg flavanols, and 2.5 g alkalised cocoa powder to mask colour differences. The composition details are given in Table 1. These powders were provided free of charge by the Barry Callebaut company. This company was not involved in any part of the study and did not sponsor it otherwise. These powders were mixed with 10 g of sugar, and either 200 ml lactose-free low-fat milk (Experiment 1),

Table 1 Nutritional composition of the study treatments

	CF			Placebo
	5 g high-flavanols cocoa powder	2.5 g alkalized cocoa powder	CF-total	7.5 g alkalized cocoa powder
Flavanols (mg)	415	0	415	0
Energy (kcal)	17.2	7.6	24.8	22.8
Protein (mg)	1120	555	1675	1665
Fat (mg)	700	275	975	825
Caffeine (mg)	10	5	15	15
Theobromine (mg)	105	52.5	157.5	157.5
Sugar (g)	10			
[a]Lactose-free low-fat milk (ml)/water (ml)	200			

[a]Lactose-free low-fat milk in Experiment 1, water in Experiment 2

or 200 ml tap water (Experiment 2). The dosage was determined based on findings of a recent study focused on acute effects of CF on attention [21]. Methylxanthines (caffeine and theobromine) in the CF and placebo conditions were matched in order to avoid possible positive confounding effects of methylxanthines on cognition, and to target CF effects exclusively [8].

Apparatus and stimuli

At each session, participants were individually seated in sound-attenuated testing cabins with dimmed lighting, at 60 cm viewing distance from the computer screen. The experimental task was presented on a 22″ CRT monitor (Iiyama MA203DT) with a refresh rate of 100 Hz at 32-bit colour depth. WM maintenance task was programmed in OpenSesame 3.1.9 [37], and executed under the Windows 7 operating system. Responses were collected with a Logitech Attack 3 joystick.

Screen resolution was set to 1024 × 768 pixels. All of the visual stimuli were displayed on a light grey background (RGB 192, 192, 192). Each of the orientation gratings was shown at 2.75° of visual angle from the fixation dot. The white fixation dot (0.61° of visual angle) was presented in the centre of the screen. Memory items were Gabor patches (sine-wave gratings) of 2.2º of visual angle, and a spatial frequency of 1.8 cycles per degree. Memory items were presented on an invisible circle with a diameter of 6.46º of visual angle. The locations of the memory items on the circle were always random, but in the two memory items condition, the items were presented symmetrically, such that the fixation dot was always in the centre of them. In the three items condition, the items were located on an equilateral triangle (still on the circle), again such that the centre of the triangle coincided with the fixation dot. Following the participant's response, a feedback smiley was presented in white 32 pt mono font type, in the centre of the screen.

Procedure

All participants visited the labs on three different days, separated by at least five days (the wash-out period), of which the first visit was always the control condition. Their second and third visits were designated randomly so that one of them was the CF condition and the other the placebo condition. The overall distribution of these conditions was counterbalanced between participants. In contrast to the CF and placebo conditions, in the control condition participants were not asked to drink any experimental product. Each data collection session lasted approximately 90 min, and participants were compensated with course credits.

Before all sessions, including the control condition, participants were asked not to drink or eat food that contained caffeine, alcohol, or high concentrations of flavonoids or theobromine in the 24 h preceding the experiment (cf. [28]). In all conditions, there were at least five days between sessions, serving as a wash-out interval. Participants attended the experiment at fixed times on each day: at 10:00, 11:30, 13:30 or 14:00, to avoid diurnal effects. To provide a double-blind experimental procedure, all drinks were served by a researcher 2 h before the experiment sessions, and another researcher started the experiment. Thus, neither the experimenter nor the participants knew what they drank (i.e., placebo or CF. After consuming the drink, participants were asked not to eat or drink anything other than water until the actual experiment commenced.

Participants were informed about the experiment and the tasks before the sessions, and a consent form was signed at each visit. The research was approved by the Departmental Ethics Committee (approval number:17460-SP-NE), and was conducted in accordance with the declaration of Helsinki.

At the start of the first session, their daily flavanols intake, BMI, and age were assessed with a short questionnaire. In the questionnaire, participants were asked how much they consumed each of the following flavanols-containing products in the last three months: dark chocolate, red wine, green tea, and apples. These amounts were converted and summed as total daily flavanols intake in mg for analysis purposes.

The experiment consisted of 360 trials in total (120 for each load condition), preceded by 36 practice trials that were excluded from the analysis. Missing responses were also excluded from analysis (10.5%).

Figure 1 illustrates the trial procedure of the working memory maintenance task with two memory items. Each trial started with a fixation dot shown at the center of the screen for 600–800 ms. After a 250 ms blank interval, the memory array was shown for 250 ms. The memory array

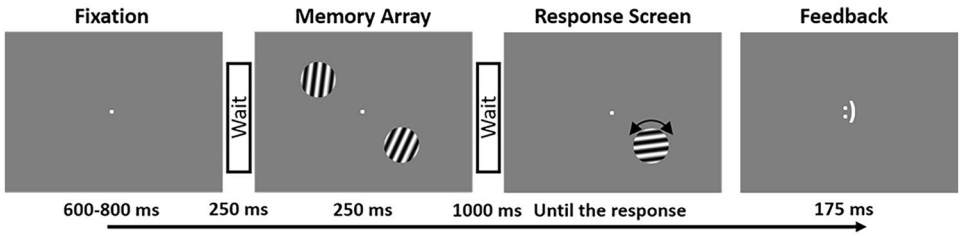

Fig. 1 WM maintenance task trial procedure. One, two, or three memory items (circular orientation gratings) could appear on the memory array

consisted of one, two or three orientation gratings, depending on the memory load condition. Orientations for each item were chosen randomly for each trial. The number of items on each trial was randomised, but equally distributed across all trials. After a one second delay following the memory array, one of the previously shown item locations was probed, and participants had to reproduce the corresponding orientation as accurately as possible by adjusting a probe grating. The initial probe orientation was randomly selected, with the constraint that probe orientation differed at least 15° from the orientation of target memory item. Visual feedback was given afterwards on each trial for 175 ms. Positive feedback was given if the error was less than 15°, negative feedback was given otherwise.

Design and analysis

Performance on the WM maintenance task was transformed from degrees to percent correct. Differences between the target orientation and the response item ranged from 0° to 90°, and these values were then transformed to percent correct by means of the following formula: $100 \times [(90 - \text{precision})/90]$.

Performance was further modelled with guess rate and precision parameters using MemToolBox [38] in Matlab2018a. The logic is as follows. First, if responses are not correlated with the target value, they should form a uniform distribution (guess rate). Guess rates reflect the proportion of trials in which the items are assumed not to be in memory at all. Accordingly, lower guess rate indicates better performance. Second, if responses are given that relate, even if slightly, to the actual target values, such errors should form a distribution around those values, for which the standard deviation of the distribution indicates the relative precision of the responses [39, 40]. Similar to guess rates, the lower the precision is, the better the quality of the memory.

A 3 (treatment: control, placebo and CF) by 3 (memory load: 1, 2, or 3 items) design was used in the ANOVA. A priori, the expectations were straightforward: Flavanols consumption should increase recall accuracy. To assess this in the simplest (and most powerful) manner possible, in a second step, the analysis was simplified by averaging across memory load and excluding the control condition. Thus, a paired-sample *t*-test was used to compare overall task performance between flavanols and placebo conditions. Furthermore, a potentially beneficial flavanols effect might interact with the load condition, just because the most difficult trials may leave more room for improvement. To check this contingency, another *t*-test was run on the hardest conditions exclusively (i.e., load 3), again comparing between placebo and flavanols condition.

In addition to these planned analyses, gender was added as a factor to the ANOVA post-hoc, to explore possible interactions with the treatment conditions. Lastly, two separate ANCOVA analyses were conducted, which included the continuous variables of daily flavanols intake and BMI. These variables were not systematically manipulated in the experiment, and the analyses should therefore be considered exploratory as well.

Bayesian analysis with the default Cauchy prior (center = 0; $r = 0.707$) was applied to each analysis next to the frequentist tests. All tests were run as two-tailed unless otherwise specified. In addition to classic ANOVA, an inclusion Bayes factor ("Baws factor"), based on matched models, was calculated to test if there was any evidence in favour of one of the independent variables or of an interaction [41]. Bayes factors (BF) were interpreted in accordance with Aczel et al. [42]. Bayesian post-hoc tests were corrected for multiple comparisons by fixing prior probability to 0.5 [43].

ANOVAs, *t*-tests and Bayesian statistics were run in JASP (Version 0.9.1.0). Greenhouse–Geisser correction was applied when appropriate. Partial eta-squared (η_p^2) and Cohen's *d* (*d*) were used to assess effect sizes. Bonferroni (HSD) correction was used for pair-wise comparisons to characterise interaction effects.

Pre-registration and data availability

In the interest of scientific transparency, the present study was fully pre-registered on the Open Science Framework with the identifier krhm4 (osf.io/krhm4). This public pre-registration comprised the design, hypotheses, analysis approach, randomisations, and the experimental programs (including instructions). The data of both experiments is also available there.

Results

Descriptive statistics for all measures in Experiment 1 are shown in Table 2.

Accuracy

Working memory maintenance performance is shown in Fig. 2. The ANOVA showed a significant main effect for memory load, $F(2, 94) = 593.87$, MSE = 22.33, $p < 0.01$, $\eta_p^2 = 0.93$; $BF_{10} > 1000$, and for treatment, $F(2, 94) = 22.36$, MSE = 8.70, $p < 0.01$, $\eta_p^2 = 0.32$; $BF_{10} > 1000$. Post-hoc comparisons showed that participants had better performance in the one item condition than in the two, $t(47) = 17.37$, $p < 0.001$, $d = 2.5$; $BF_{10} > 1000$, and three items conditions, $t(47) = 28.35$, $p < 0.001$, $d = 4.10$; $BF_{10} > 1000$. Also, their performance was better in the two items condition than in the three items condition, $t(47) = 22.37$, $p < 0.001$, $d = 3.25$; $BF_{10} > 1000$. Accuracy, compared to control, was higher in

Table 2 Descriptive statistics for all measures in Experiment 1

WM maintenance task	Measure	Control		Placebo		CF	
		Mean	SD	Mean	SD	Mean	SD
Accuracy (%)	One item	90.53	2.39	91.67	2.23	91.70	2.42
	Two items	80.99	5.21	83.14	5.83	83.19	5.24
	Three items	70.33	6.12	73.04	6.49	73.02	6.93
	Overall	80.75	4.09	82.68	4.45	82.68	4.39
Precision (%)	One item	11.20	2.73	10.24	2.57	10.05	2.65
	Two items	15.47	4.69	14.09	3.04	14.45	3.26
	Three items	17.91	5.70	15.33	3.78	15.68	3.60
Guess rate (%)	One item	0.83	1.18	0.41	0.69	0.53	1.42
	Two items	9.02	5.71	7.75	6.56	7.14	5.75
	Three items	21.50	8.56	19.66	7.91	19.31	8.93

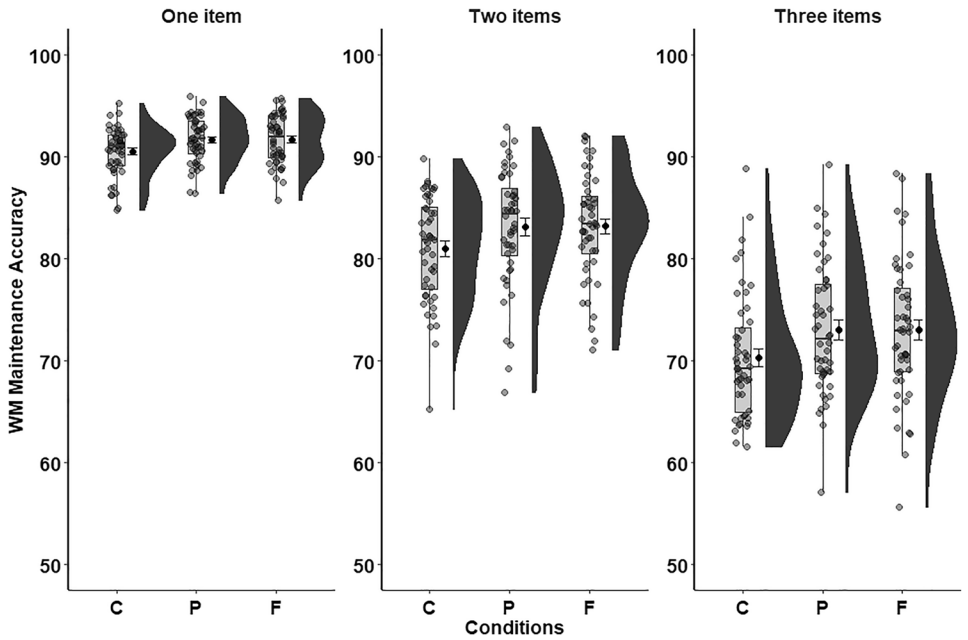

Fig. 2 Working memory maintenance accuracy (percent correct) by treatment (control, placebo, and flavanols) in Experiment 1. Means with SE are shown in dot plots, boxplots indicate quartiles, with lines denoting the 95% confidence intervals. Translucent dots behind the boxplots show individual data points and violin plots display the distribution

the CF, $t(47) = 5.60$, $p < 0.001$, $d = 0.82$; $BF_{10} > 1000$, and in the placebo condition, $t(47) = 5.41$, $p < 0.001$, $d = 0.77$; $BF_{10} > 1000$. There was no reliable difference between CF and placebo, $t(47) = 0.09$, $p = 0.93$, $d = 0.01$; $BF_{10} = 0.09$; providing strong support for H0. The interaction between treatment and memory load was not reliable either, $F(3, 147) = 2.11$, MSE = 0.001, $p = 0.09$, $\eta_p^2 = 0.04$; $BF_{10} = 0.05$, again providing strong support for H0. Thus, although the memory task showed the expected performance profile with accuracy decreasing for higher memory loads, there was no evidence for an effect of flavanols. The only differences between the treatment conditions were due to the control condition, which might have comprised both learning and placebo effects.

The one-tailed paired samples *t*-test between the placebo (M = 82.68, SD = 4.45) and flavanols (M = 82.68, SD = 4.39) conditions revealed no significant difference, $t(47) = 0.20$, $p = 0.58$, $d = 0.02$; $BF_{10} = 0.14$, indicating moderate support for H0. The one-tailed *t*-test on the means of the 3 items load conditions also revealed no difference between flavanols and placebo conditions, $t(47) = 0.07$, $p = 0.53$, $d = 0.01$; $BF_{10} = 0.15$, again showing moderate support for H0.

In the first exploratory analysis that included gender, the interaction of gender and treatment did not influence accuracy, $F(2, 92) = 0.09$, MSE = 8.87, $p = 0.91$, $\eta_p^2 = 0.002$; $BF_{10} = 0.05$, providing strong support for H0. Similarly, the interaction of gender, treatment, and number of items did not influence performance, $F(4, 184) = 0.81$, MSE = 3.57, $p = 0.52$, $\eta_p^2 = 0.017$; $BF_{10} = 0.05$, again providing strong support for H0.

In a second exploratory step, daily flavanols intake was added to the model (without gender) as a covariance variable, and the ANCOVA showed no interaction effect of daily flavanols intake and treatment $F(2, 92) = 1.05$, MSE = 8.69, $p = 0.36$, $\eta_p^2 = 0.022$; $BF_{10} < 0.01$, providing strong support for H0. There was also no reliable interaction between daily flavanols intake, treatment, and number of items $F(4, 184) = 0.96$, MSE = 4.34, $p = 0.43$, $\eta_p^2 = 0.020$; $BF_{10} = 0.03$, again providing strong support for H0. Similarly, adding BMI to the model showed no interaction effect of BMI and treatment $F(2, 92) = 0.73$, MSE = 8.75, $p = 0.49$, $\eta_p^2 = 0.016$; $BF_{10} = 0.09$, providing strong support for H0, and no interaction effect of BMI, treatment, and number of items either $F(4, 184) = 1.51$, MSE = 4.29, $p = 0.20$, $\eta_p^2 = 0.032$; $BF_{10} = 0.031$, again providing strong support for H0.

Guess rate and precision

In terms of guess rate (see Fig. 3 and Table 2 for means and SDs), the ANOVA showed a significant main effect for the number of memory items, $F(2, 94) = 280.69$, MSE = 50.09, $p < 0.001$, $\eta_p^2 = 0.857$; $BF_{10} > 1000$. Post-hoc comparisons with Bonferroni correction showed that guess rates were lower in the one item condition than in the two, $t(47) = -9.86$, $p < 0.001$, $d = -1.42$; $BF_{10} > 1000$, and three items conditions, $t(47) = -18.85$, $p < 0.001$, $d = -2.72$; $BF_{10} > 1000$. Also, guess rates were lower in the two items condition than in the three items condition, $t(47) = -18.17$, $p < 0.001$, $d = -2.62$; $BF_{10} > 1000$. In addition, a significant main effect of treatment existed on guess rate, $F(2, 94) = 5.8$, MSE = 14.82, $p < 0.01$, $\eta_p^2 = 0.110$; $BF_{10} = 1.04$, although the Bayes Factor provided only anecdotal support for H1. Guess rate was lower in the flavanols condition than in the control condition, $t(47) = 3.12$, $p < 0.01$, $d = 0.45$; $BF_{10} = 26.73$. There was no significant difference between guess rates in the placebo and control conditions, $t(47) = 2.14$, $p = 0.11$, $d = 0.308$; $BF_{10} = 2.21$, even though there was anecdotal support for H1. There was also no significant difference between the placebo and flavanols conditions, $t(47) = 0.89$, $p = 1$, $d = 0.12$; $BF_{10} = 0.13$, with the Bayes Factor corresponding to moderate support for H0. Lastly, the interaction of treatment and number of memory items did not influence guess rate significantly, $F(4, 188) = 1.30$, MSE = 10.35, $p = 0.271$, $\eta_p^2 = 0.027$; $BF_{10} = 0.031$, providing strong support for H0.

In terms of precision (see Fig. 4 and Table 2 for means and SDs), the ANOVA again showed a significant main effect for the number of memory items, $F(2, 94) = 8.21$, MSE = 10.14, $p < 0.001$, $\eta_p^2 = 0.731$; $BF_{10} > 1000$. Post-hoc comparisons with Bonferroni correction showed that precision was higher in the one item condition than in the two, $t(47) = 12.62$, $p < 0.001$, $d = -1.82$; $BF_{10} > 1000$, and three items conditions $t(47) = 13.62$, $p < 0.001$, $d = -1.97$; $BF_{10} > 1000$. Also, precision was higher in the two items condition than in the three items condition, $t(47) = 4.52$, $p < 0.001$, $d = -0.65$; $BF_{10} = 536.02$. A main effect of treatment on precision existed as well, $F(2, 94) = 10.42$, MSE = 11.22, $p < 0.001$, $\eta_p^2 = 0.181$; $BF_{10} = 1.04$, but the Bayes Factor yielded only anecdotal support for H1. Precision was higher in the placebo condition compared to the control condition, $t(47) = 3.67$, $p = 0.002$, $d = 0.529$; $BF_{10} = 247.91$. In addition, precision was higher in the flavanols condition than in the control condition, $t(47) = 3.30$,

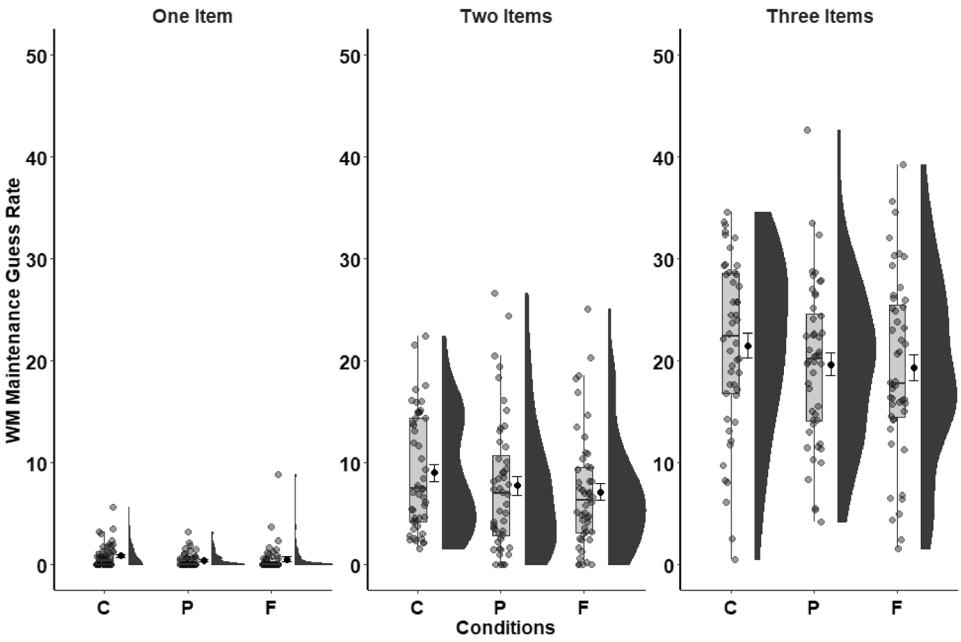

Fig. 3 WM maintenance guess rate means, medians, and standard errors (in percent of trials) for each treatment condition in Experiment 1. Figure conventions follow Fig. 2

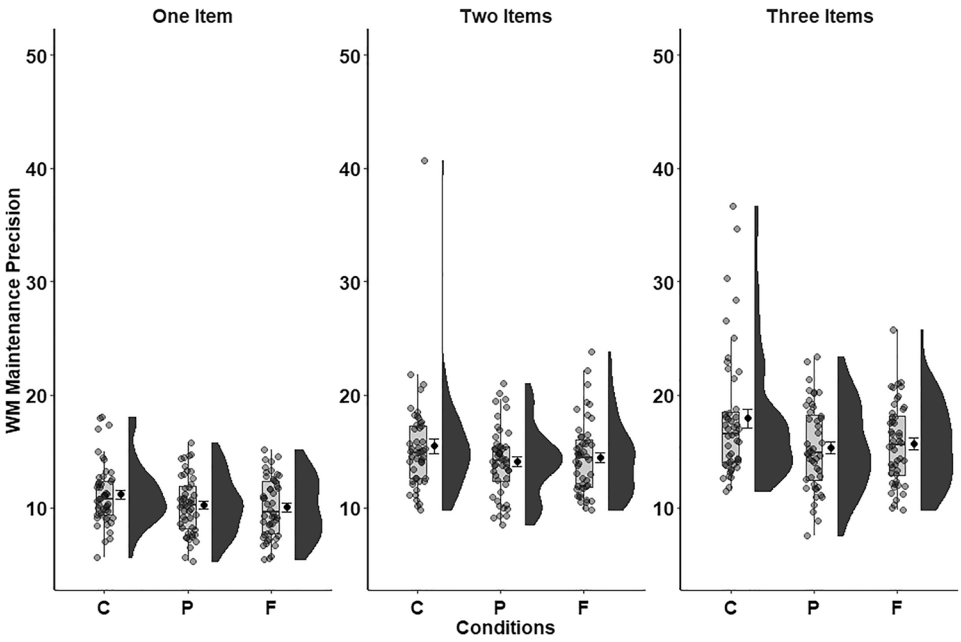

Fig. 4 WM maintenance precision means, medians, and standard errors (in degrees) for each treatment condition in Experiment 1. Figure conventions follow Fig. 2

$p = 0.006$, $d = 0.476$; $BF_{10} > 116.71$. There was no difference in precision between the flavanols and placebo conditions, $t(47) = -0.65$, $p = 1.00$, $d = -0.093$; $BF_{10} = 0.11$, with moderate support for H0. Lastly, the interaction of treatment and number of memory items did not influence precision significantly, $F(4, 188) = 1.32$, MSE = 9.258, $p = 0.262$, $\eta_p^2 = 0.027$; $BF_{10} = 0.031$.

Experiment 2

Contrary to our initial expectations, Experiment 1 showed no evidence for acute effect of CF on WM maintenance. A possible reason for this negative finding is that WM maintenance does not require active processing. Although WM maintenance has been attributed to ongoing neural activity in the past (e.g., Curtis and D'Esposito [44]), recent proposals have suggested that maintenance may be achieved by means of activity-silent processes, such as short-term synaptic plasticity [45, 46]. If WM maintenance is indeed associated with little to no specific neural activity, it follows that CF might not have the opportunity to facilitate this process, as its physiological effects (i.e., enhancing blood flow, neurotransmitter function) are related to such activity.

We thus reasoned that for CF to affect WM performance, an active component needs to be implicated. In Experiment 2, we therefore asked participants to not only maintain information, but also to manipulate it, via mental rotation. We expected that this active updating of WM contents could provide a window of opportunity for acute CF effects to arise.

Methods

Participants

Using the same criteria as in Experiment 1, 32 healthy individuals (16 female, 16 male) participated in this experiment ($\overline{X} = 22.34 \pm 3.30$ years, range = 18–31). The mean BMI of male participants was 23.34 ± 3.19, and the mean BMI of female participants was 22.73 ± 3.85. Six additional participants were excluded from the analyses because their performance was at or below chance level. A power analysis was run using G*Power software [36] for F test, effect size, $d = 0.30$; $\alpha = 0.05$; actual power = 0.961; sample size = 32; critical $F = 3.15$. To estimate effect and sample size, we included not only the previous study of Karabay et al. [21], as in Experiment 1, but also Field et al. [28] and data from Massee et al. [29]. Field et al. [28] found that CF consumption increases visual spatial working memory performance with medium effect size (effect size $f = 0.35$). Also, as cited in Massee et al. [29], effect size ranged between 0.30 and 0.53 for the effects of CF on serial subtraction task performance.

Apparatus and stimuli

The apparatus and stimuli were identical to those of Experiment 1 with the following exceptions. The experimental products were the same as in Experiment 1 (see Table 1),

but in the control condition participants were now asked to drink a sugar-water mixture, and in the experimental conditions, the drinks were mixed with water instead of milk. The experiment was run in the same lab and computer environment as Experiment 1, but responses were now collected with a standard USB keyboard. The stimuli were a pair of orientation gratings, shown at 2.75° from the centre on the horizontal axis. The retro-active cue (<< or >>; 1.01° of visual angle) was shown in white. The rotation instructions (0°, 30° or 60°) were rendered in the same font and size, and were accompanied by an arrow symbol of 1.9° of visual angle, appearing above the text.

Procedure

The procedure of Experiment 2 was the same as that of Experiment 1, with the following changes. As in Experiment 1, participants were informed about the experiment and the tasks before the sessions, and a consent form was signed at their first visit. The research was approved by the Departmental Ethics Committee (approval number: 17460-SP-NE), and was conducted in accordance with the declaration of Helsinki. In Experiment 2, session order was randomised and counterbalanced across all three conditions (control, placebo and CF). Each data collection session lasted approximately 70 min, and participants were compensated with 10 euros for each session.

Participants completed 304 trials in total, after completion of 16 practice trials; the latter trials were again excluded from analysis, as were missing responses (9.5%). The working memory updating task used in Experiment 2 is illustrated in Fig. 5. Two memory items were shown for 250 ms at the start of each trial after a fixation period of 250–400 ms. The memory items consisted of two randomly oriented gratings, to the left and right of the fixation dot. The memory array was followed by a 500 ms blank interval. A retro-cue then appeared, pointing either to the left or right, indicating which memory item would be probed at the end of the trial for 200 ms. The direction of the cue was randomly chosen with an equal distribution across the experiment. Participants were asked to remember the cued item and forget the uncued one, as uncued memory items were task-irrelevant from thereon. After another delay of 1000 ms, the rotation instruction (0°, 30°, or 60°, clockwise or counter clockwise) was shown. Participants were asked to mentally rotate the cued memory item with the degrees stated on the rotation instruction, in clockwise or counter-clockwise direction. Rotation examples were given to the participants before the experiment started to ensure they understood what the rotation instructions meant. 1200 ms after the offset of the rotation instruction in the trial, the probe was then shown for 250 ms. The probe deviated 0° (50% of trials), 25°–30°, or 50°–55° (25% each) from the rotated memory item. Participants were instructed to indicate whether the orientation of the probe was the same or different from the rotated memory item. Responses were submitted by pressing the "m" key with the right index finger, or by pressing the "c" key with the left index finger. The response keys were counterbalanced; participants with an even subject number were instructed to press "c" to indicate "same" and "m" for "different", and vice versa for odd numbers. Responses had to be given within 800 ms, and reaction time was measured.

Design and analysis

Performance on the working memory updating task was assessed by analysing the percentage of correct responses, as well as probe reaction times. A 3 (treatment: control, placebo and CF) by 3 (difficulty; degree of rotation) design was used. Except for the addition of probe reaction times, and for the omission of guess rate and precision estimates, the analysis structure was the same as in Experiment 1.

Results

Descriptive statistics for all measures in Experiment 2 are shown in Table 3.

Accuracy

Working memory updating accuracy is shown in Fig. 6. The ANOVA showed a significant main effect for the degrees of rotation required, $F(2, 62) = 96.76$, MSE = 32.58, $p < 0.001$, $\eta_p^2 = 0.757$; $BF_{10} > 1000$. Post-hoc comparisons showed that participants had better performance in the 0° condition than in the 30°, $t(31) = 9.61$, $p < 0.001$, $d = 1.70$; $BF_{10} > 1000$, and 60° conditions, $t(31) = 12.82$, $p < 0.001$, $d = 2.26$; $BF_{10} > 1000$. Also, their accuracy was higher in the 30°

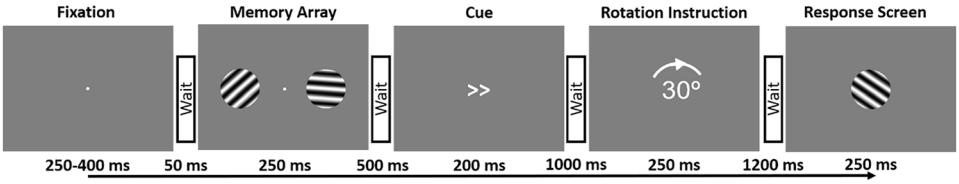

Fig. 5 Experiment 2 trial procedure

Table 3 Descriptive statistics for all measures in Experiment 2

WM Updating Task	Measure	Control		Placebo		CF	
		Mean	SD	Mean	SD	Mean	SD
Accuracy (%)	0°	73.87	10.37	75.26	9.01	75.42	10.25
	30°	67.32	10.67	68.12	8.75	65.52	10.20
	60°	63.08	10.18	64.88	9.63	63.14	9.56
	Overall	68.08	9.69	69.43	8.31	68.02	8.73
Reaction Time (ms)	0°	385.30	68.33	384.99	78.75	382.96	62.41
	30°	419.80	81.12	406.91	82.77	405.47	72.44
	60°	424.95	79.55	415.53	92.12	409.37	69.45
	Overall	406.48	72.60	401.31	81.39	395.29	61.24

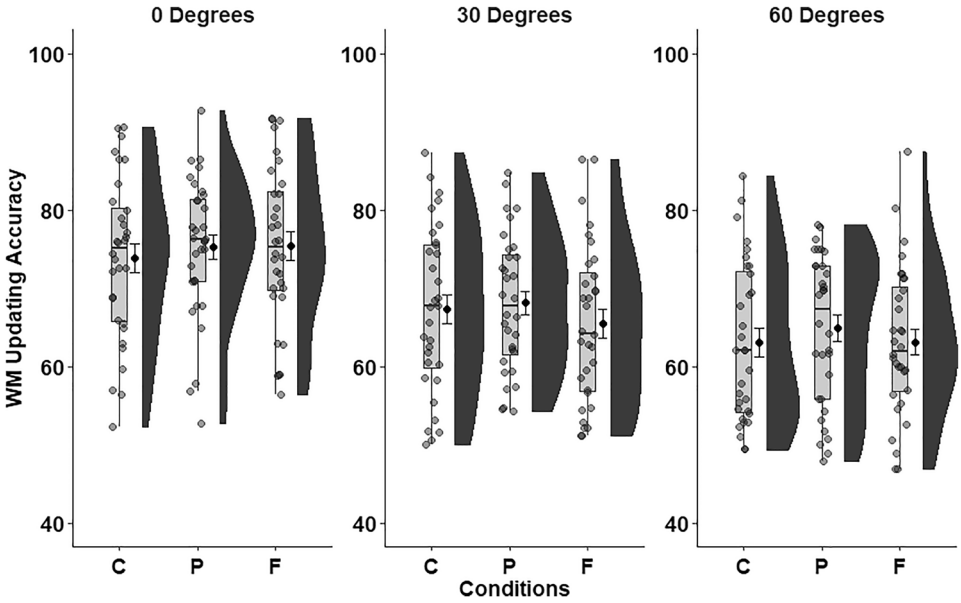

Fig. 6 Working memory updating accuracy (percent correct) for each treatment condition in Experiment 2. Figure conventions follow Fig. 2

condition than in the 60° condition, $t(31) = 4.21$, $p < 0.001$, $d = 0.74$; $BF_{10} = 647.8$. However, there was no significant main effect for treatment, $F(2, 62) = 0.65$, MSE = 92.45, $p = 0.53$, $\eta_p^2 = 0.02$; $BF_{10} = 0.13$, providing moderate support for H0, and there was no interaction effect between treatment and degrees of rotation either, $F(4, 124) = 1.15$, MSE = 23.29, $p = 0.34$, $\eta_p^2 = 0.036$; $BF_{10} = 0.05$, with strong support for H0.

The one-tailed *t*-test showed no significant difference in accuracy between the flavanols (M = 68.03, SD = 8.73), and placebo (M = 69.43, SD = 8.31) conditions, $t(31) = 0.94$, $p = 0.82$, $d = 0.16$; $BF_{10} = 0.28$, with moderate support for H0. The *t*-test on the most difficult condition (60° rotation) also showed no effects of flavanols on accuracy $t(31) = 0.90$, $p = 0.81$, $d = 0.09$; $BF_{10} = 0.05$, providing strong support for H0.

The exploratory repeated measures ANOVA with gender as between-group factor revealed no significant interaction between gender and treatment on accuracy, $F(2, 60) = 0.84$, MSE = 92.95, $p = 0.44$, $\eta_p^2 = 0.027$; $BF_{10} = 0.33$, providing moderate support for H0, and neither between gender, treatment, and rotation condition, $F(4, 120) = 0.11$, MSE = 23.98, $p = 0.98$, $\eta_p^2 = 0.004$; $BF_{10} = 0.06$, with strong support for H0. The ANCOVA analysis with daily flavanols intake as covariate also showed no interaction of daily flavanols intake and treatment, $F(2, 60) = 0.40$, MSE = 92.28, $p = 0.67$, $\eta_p^2 = 0.013$; $BF_{10} = 0.11$, providing moderate support for H0, and no interaction between daily flavanols intake, treatment, and rotation condition, $F(4, 120) = 1.88$, MSE = 22.65, $p = 0.12$, $\eta_p^2 = 0.059$; $BF_{10} = 0.006$, providing strong support for H0. The ANCOVA with BMI did not change the results; the interaction of BMI and treatment, $F(2, 60) = 0.25$, MSE = 94.74, $p = 0.78$, $\eta_p^2 = 0.008$; $BF_{10} = 0.04$, provided strong support for H0, and so did that of BMI, treatment, and rotation condition, $F(4, 120) = 1.23$, MSE = 23.12, $p = 0.30$, $\eta_p^2 = 0.040$; $BF_{10} = 0.007$.

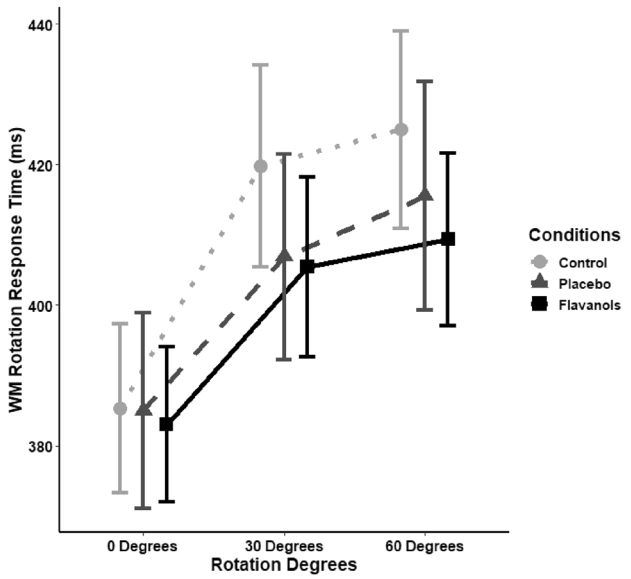

Fig. 7 Probe reaction time means and standard errors (ms) in Experiment 2, as a function of rotation degrees

Probe reaction time

Probe reaction time is shown in Fig. 7. The ANOVA showed a significant main effect of rotation degrees $F(2, 62) = 18.76$, MSE = 1504.1, $p < 0.001$, $\eta_p^2 = 0.38$; $BF_{10} > 1000$. Post-hoc comparisons showed that participants responded faster in the 0° condition than in the 30°, $t(31) = -4.35$, $p < 0.001$, $d = -0.77$; $BF_{10} > 1000$, and 60° conditions $t(31) = -4.82$, $p < 0.001$, $d = -0.85$; $BF_{10} > 1000$. However, there was no reliable difference between the 30° and 60° conditions, $t(31) = -1.65$, $p = 0.33$; $BF_{10} = 0.49$, providing anecdotal support for H0. There was no significant main effect for treatment, $F(2, 62) = 0.68$, MSE = 4268.3, $p = 0.51$, $\eta_p^2 = 0.022$; $BF_{10} = 0.17$, providing moderate support for H0. There was also no interaction between the treatment and rotation conditions, $F(4, 124) = 1.01$, MSE = 542.8, $p = 0.41$, $\eta_p^2 = 0.03$; $BF_{10} = 0.03$, providing strong support for H0.

The one-tailed paired samples *t*-test on the overall means between flavanols (M = 395.30, SD = 61.24) and placebo (M = 401.3, SD = 81.39) conditions showed no significant difference, $t(31) = 0.71$, $p = 0.24$, $d = 0.13$; $BF_{10} = 0.36$, although there was only anecdotal support for H0. In the 60° rotation condition, the *t*-test also showed no evidence for an effect of flavanols on probe RTs $t(31) = 0.51$, $p = 0.31$, $d = 0.31$; $BF_{10} = 0.29$, this time providing moderate support for H0.

In the exploratory analyses, gender did not show an interaction effect with neither treatment, $F(2, 46) = 0.86$, MSE = 3670.7, $p = 0.40$, $\eta_p^2 = 0.027$; $BF_{10} = 0.37$, with anecdotal support for H0, nor with treatment and rotation condition, $F(4, 120) = 0.76$, MSE = 547.0, $p = 0.55$, $\eta_p^2 = 0.025$; $BF_{10} = 0.07$, providing strong support for H0. The first ANCOVA showed no significant interaction of daily flavanols intake and treatment, $F(2, 48) = 0.23$, MSE = 5542.3, $p = 0.79$, $\eta_p^2 = 0.007$; $BF_{10} = 0.09$, providing strong support for H0. There was also no interaction between daily flavanols intake, treatment and rotation degrees, $F(4, 120) = 1.41$, MSE = 535.7, $p = 0.23$, $\eta_p^2 = 0.045$; $BF_{10} = 0.11$, providing strong support for H0. Similarly, the second ANCOVA showed no significant interaction effect of BMI and treatment, $F(2, 47) = 0.74$, MSE = 5548.7, $p = 0.45$, $\eta_p^2 = 0.024$; $BF_{10} = 0.07$, providing strong support for H0, and no interaction between BMI, treatment and rotation degrees, $F(4, 120) = 1.65$, MSE = 531.7, $p = 0.17$, $\eta_p^2 = 0.052$; $BF_{10} = 0.08$, again providing strong support for H0.

Discussion

In this study, we hypothesised that cocoa flavanols might acutely improve working memory performance. To put this idea to the test, two randomised, double-blind, placebo-controlled, crossover trials were run. Contrary to expectations, the results showed that there was no evidence for any effect of cocoa flavanols on working memory, neither on memory maintenance, nor on working memory updating. Additionally, these negative results were consistent even when taking into account gender, BMI, and daily flavanols intake. Thus, at least for healthy young adults, consuming cocoa flavanols does not seem to improve working memory performance acutely.

Contrary to our findings, Field et al. [28] previously reported acute effects of CF on visual spatial working memory maintenance performance, although even these effects were small (3.6% difference in accuracy). Additionally, their participants performed better in the high CF condition than in the control condition only in the first experimental session; there were no reliable differences in the second session, suggesting that task familiarity might have played a role. Further complicating the interpretation of the findings of this study is the possibility that a placebo effect might have mediated the outcomes; the placebo product used in this study consisted of white chocolate, which was clearly identifiable as such. Indeed, Barrera-Reyes et al. [33] cited this study in their systematic methodological review, and reported a high bias risk, based on the Cochrane Risk of Bias Tool.

A second study that reported CF effects on WM performance was conducted by Grassi et al. [30]. In this study, N-back task performance was enhanced acutely, but only for a female subsample of their participants, and only when comparing task performance between sleep-deprived and

well-rested conditions. Limited sample size (4 per group) is a concern here, but the findings may still indicate that a lack of sleep and/or fatigue may be alleviated by CF consumption. In turn, 'preserved' WM performance may be one outcome. In the present study, there was no overall effect of gender (nor any interaction), but fatigue could not be well assessed. Nevertheless, an exploratory analysis of performance as a function of block sequence did not show evidence of clear trends in performance over time. Future research may focus on that issue, in particular to assess the cognitive specificity of this potential CF effect.

Experimental trials on the acute effects of CF on working memory are still relatively scarce, but studies on longer-term effects may also be related to the present results. For instance, Francis et al. [5] have considered the effects of consuming CF over a term of 5 days on cognitive tasks. They found that there were no effects of CF consumption on behavioural reaction times, switch cost, and heart rate in healthy young adults. Similarly, Camfield et al. [47] have pointed out there were no behavioural effects from long-term (30 days) consumption of CF on spatial working memory in middle-aged (40–65) healthy individuals, although they observed neural activation during memory encoding in posterior parietal and centro-frontal areas. In the same age population, Pase et al. [48] also observed that there were no differences in cognitive function between CF conditions after 30 days of treatment, however, participants did report a better mood. These results would seem to fit well to the present outcomes.

In contrast, for older participants, positive effects of CF on cognitive functioning were reported in several experimental studies [4, 49, 50]. It is conceivable that age might play a crucial role in CF effect mechanisms. The current negative results could be related to the tested population, namely university students, who are young and already have relatively high cognitive abilities, unlike older participants from the general population [51].

Apart from individual experimental studies, cues as to the possible nature of CF effects on WM can also be obtained from systematic reviews and meta-analyses. As indicated, such reviews also paint a mixed picture. Barrera-Reyes et al. [33] reported that consumption of CF in average doses (500–700 mg/day) improved memory and executive function. However, according to Veronese et al. [34], there was no association with better mood or improved cognitive function. It is important to note here that because the original empirical work typically used complex (compound) tasks, it is possible that any effects observed in such tasks are due to more than one cognitive functions, not WM alone (e.g., attention), even if this is not explicitly stated in these studies.

Before accepting the present outcomes as definite, it is important to point out some limitations of our study. Firstly, as alluded to above, the participants were all young adult university students. It is possible that other subgroups in the population, in particular the elderly, may respond differently to CF consumption. Secondly, the use of soy milk may have affected the outcomes. Soy milk contains isoflavones, which have been found to improve verbal working memory performance [52]. However, negative results also exist about the effects of flavonoids other than CF on verbal and numeric working memory performance. In their review, Macready et al. [53] reported that from 14 studies with 22 different outcome assessments that investigated the effects of flavonoids on working memory and executive function in various samples, 10 of these 22 measures came out positive. Future studies could test various sources of flavonoids more systematically to assess the different contributions. Thirdly, we used a control session that was separate from the other treatment conditions (placebo and CF) in the experiment, and as such did not constitute a typical baseline. Typically, baselines measures are taken immediately before or on the same day as the treatment sessions. As such, although our approach controls for possible learning- or fatigue-related differences between the control session and the others, we could not control for day-to-day fluctuations in performance.

In conclusion, the current study, featuring two crossover experimental trials that were both placebo controlled, randomised, gender-balanced and double-blinded, provided no evidence for positive effects of CF on WM performance. On the contrary, Bayesian statistics showed that it is likely that there is in fact no such effect. This finding contrasts with a previous study on visual attention that we conducted [21], which was set up and executed in a very similar fashion, and which showed positive evidence for a CF effect on spatial attention. This contrast suggests that there may be a fundamental difference between attention and WM in the brain, at least as far as sensitivity to CF is concerned.

Declarations

Conflict of interest Ahmet Altınok, Aytaç Karabay and Elkan G. Akyürek have no conflict of interest.

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