

Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions

Article

Accepted Version

Field, D. T. ORCID: https://orcid.org/0000-0003-4041-8404, Williams, C. M. ORCID: https://orcid.org/0000-0003-4452-671X and Butler, L. T. (2011) Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions. Physiology & Behavior, 103 (3-4). pp. 255-260. ISSN 0031-9384 doi:

https://doi.org/10.1016/j.physbeh.2011.02.013 Available at https://centaur.reading.ac.uk/19779/

It is advisable to refer to the publisher's version if you intend to cite from the work. See <u>Guidance on citing</u>.

Published version at: http://dx.doi.org/10.1016/j.physbeh.2011.02.013

To link to this article DOI: http://dx.doi.org/10.1016/j.physbeh.2011.02.013

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

www.reading.ac.uk/centaur



CentAUR

Central Archive at the University of Reading Reading's research outputs online

Consumption of cocoa flavanols results in an acute improvement in visual and cognitive function

David T Field^{a,*}, Claire M Williams^a, Laurie T Butler^a

^a School of Psychology and Clinical Language Sciences, University of Reading, UK

* Corresponding author. School of Psychology and Clinical Language

Sciences, University of Reading, Reading, RG6 6AL, United Kingdom. Tel

+441183785004; fax: +441183786715; email address: d.t.field@reading.ac.uk

ABSTRACT

Cocoa flavanols (CF) influence physiological processes in ways that suggest their consumption may improve aspects of neural function, and previous studies have found positive influences of CF on cognitive performance. In this preliminary study we investigated whether visual, as well as cognitive, function is influenced by an acute dose of CF in young adults. We employed a randomized, single-blinded, order counterbalanced, crossover design in which 30 healthy adults consumed both dark chocolate containing 720 mg CF and a matched quantity of white chocolate, with a one week interval between testing sessions. Visual contrast sensitivity was assessed by reading numbers that became progressively more similar in luminance to their background. Motion sensitivity was assessed firstly by measuring the threshold proportion of coherently moving signal dots that could be detected against a background of random motion, and secondly by determining the minimum time required to detect motion direction in a display containing a high proportion of coherent motion. Cognitive performance was assessed using a visual spatial working memory for location task and a choice reaction time task designed to engage processes of sustained attention and inhibition. Relative to the control condition, CF improved visual contrast sensitivity and reduced the time required to detect motion direction, but had no statistically reliable effect on the minimum proportion of coherent motion that could be detected. In terms of cognitive performance, CF improved spatial memory and performance on some aspects of the choice reaction time task. As well as extending the range of cognitive tasks that are known to be influenced by CF consumption, this is the first report of acute effects of CF on the efficiency of visual function. These acute effects can be explained by increased cerebral blood flow caused by CF, although in the case of contrast sensitivity there may be an additional contribution from CF induced retinal blood flow changes.

Keywords:

cocoa; flavanol; vision; cognition; brain

1. Introduction

Flavonoids are a class of natural compounds, several subcategories of which are common in the human diet, including flavanols, flavonols, isoflavones, flavones, and anthocyanidins. High levels of flavanols are found in cocoa as well as grapes, green and black tea, red wine, and apples [1]. Cocoa contains a particularly high concentration of flavanols [2], and in recent years there has been an increasing interest in the health benefits of flavanolcontaining foods.

Consumption of cocoa flavanols (CF) has previously been shown to influence hemodynamics, increasing both central and peripheral blood flow [3-7]. These effects are thought to be mediated by increased nitric oxide synthesis within blood vessels [8-10], and can occur after a few doses or even acutely [8, 11, 12]. One consequence of the effect of CF on cerebral blood flow might be to improve performance on visual and cognitive tasks. One study to date has confirmed that CF influences cerebral hemodynamics, causing a large increase in blood flow comparable to that produced by hypercapnia, peaking approximately 2 hours after ingestion [13]. An increase

in cerebral blood flow could improve performance on a wide range of tasks via a number of possible mediating mechanisms, including increased motivation, attention, or arousal. Alternatively, an increased supply of metabolic substrates to individual neurons involved in task specific processing might improve their efficiency. However, all of these potential mechanisms lead to the same prediction of a general performance improvement on cognitive and visual tasks.

To assess the effects of CF we employed a battery made up of tests of visual and cognitive ability. Performance on any of the tests in our battery could be subject to general improvement arising from a cerebral blood flow effect of CF, which leads in turn to increased motivation, attention, arousal, or perhaps neural efficiency. However, performance on one of our visual tests, which measured the minimum detectable luminance contrast (contrast sensitivity, CS) could potentially be influenced both by general improvement and by the known peripheral blood flow effects of CF, which might include an effect on retinal blood flow. Retinal function is a critical determinant of CS because it is in the intraretinal neural pathways that the pattern of luminance values encoded by photoreceptors is first converted to a signal encoded in terms of spatial contrast, and so logically the neural retina is the first place to seek an explanation for changes in CS. A further indication that retinal function is a critical determinant of CS is that a large part of the decline in CS caused by aging is attributable to changes in retinal function [14-18]. That CS is influenced by variation in retinal blood flow has been demonstrated experimentally by having participants' breathe air with an increased percentage of CO2, which induces mild hypercapnia resulting in vasodilatation and an increase in blood flow [19]. This manipulation produced improved visual contrast sensitivity, which the authors attributed to an increase in retinal blood flow.

Although this is the first study of CF to include measures of visual processing, several recent studies have investigated whether the hemodynamic effects of CF are accompanied by changes in cognitive performance. While Francis [13] demonstrated large cerebral hemodynamic effects of CF, they did not find accompanying effects on cognitive performance, although this failure was possibly attributable to a ceiling effect. Another study found no chronic effects of CF on cognitive function in older adults, but in this case the lack of effect may have been due to the over representation in the sample of cognitively high functioning older adults whose normal diets were rich in flavonoids [20]. A recent study performed by Scholey et al. avoided ceiling effects by subjecting young adults to a fatiguing cognitive demand battery aimed at producing a progressive decline in performance on rapid information processing and serial subtraction tasks, and succeeded in demonstrating positive effects of CF on performance and subjective mental fatigue, which were more evident for a 520 mg acute dose than a 994 mg acute dose [21]. It would be helpful to know if the effects of CF on cognition generalise to a wide range of tasks tapping multiple cognitive functions, and so in this study as well as testing visual function we investigated the effect of CF on two tasks not previously used by Scholey et al [21]: visual spatial working memory for location and a choice reaction time task designed to require sustained attention and response inhibition.

2. Methods

2.1 Experimental design

This study was conducted in the School of Psychology and Clinical Language Sciences at The University of Reading. Each participant was tested in a high CF condition and a low CF condition, with testing days separated by one week. Order of testing was counterbalanced. On both study days participants reported to the lab at 9.00 am to eat 35 g of chocolate. Cognitive and visual testing began at 11.00 am and lasted approximately 45 minutes. The two hour interval was chosen to coincide the test battery with the peak of the blood flow effects of CF detected by Francis et al. [13]. During the two hour interval participants were allowed to return to their normal daily student routine, while adhering to the dietary restrictions described below. For each of the tests described below short practice versions were used to ensure participants understood the procedure. In all cases the order of tests in the battery was visual spatial working memory, choice reaction time, motion coherence threshold, contrast sensitivity, and finally motion integration time threshold.

2.2 Participants

Thirty participants (twenty-two female), aged between 18 and 25 took part in the study. All participants read and signed an informed consent document approved by the local Research Ethics Committee. Participants were recruited from the School Undergraduate Research Panel and received

course credit in return for their participation. We excluded participants who reported having a medically restricted diet or any kind of ongoing illness. All participants had normal visual acuity, or acuity corrected to normal by glasses. For 24 hours prior to study days and during the 2 hour interval between eating chocolate and testing participants were asked to avoid consuming a list of food and drinks high in flavonoids, as well as alcohol and caffeine. To disguise the purpose of the study, for every high flavonoid item on the list we also asked them to avoid a high fat food. We also provided a list of acceptable foods. To increase compliance we informed participants that a cheek swab would be collected and that we would be able to analyze the cheek swab to check conformity to the restrictions. The cheek swab was collected, but not analyzed. Participants were instructed to eat a light breakfast before arriving at the lab, again subject to dietary restrictions.

2.3 Acute supplementation

In the high CF condition participants consumed 35 g of the commercially available dark chocolate, CHOXI+, which contained 178 kcal and 773 mg of CF [22], a quantity falling midway between the high and low doses used by Scholey et al. [21]. An enquiry to the manufacturer was made to determine the quantities of caffeine (38 mg), and theobromine (222 mg) in 35g of CHOXI+ dark chocolate. In the control condition participants consumed 35 g of white chocolate (Waitrose own brand), which contained 196 kcal and only trace amounts of CF, caffeine, and theobromine. The experimenters were blind to which of the two types of chocolate a participant had consumed. Participants knew which of the two chocolates they had eaten, and may

conceivably have been influenced by this knowledge. To minimise the likelihood that they would guess the study hypothesis they were informed when signing up that the study investigated the effects of different types of fats on test performance. We reasoned they would probably infer that the two types of chocolate contained different types of fats, but this speculation would not easily lead to forming an opinion about which type of chocolate was likely to improve performance.

2.4 Visual tests

2.4.1 Contrast sensitivity

Participants viewed a series of two digit numbers on a low contrast display with both eyes and reported the number to the Experimenter. The test began with stimuli above threshold and the contrast was gradually reduced until participants could not detect the numbers at all, at which point testing was discontinued. The test was self paced and for the stimuli where participants were able to give a response they scored one point for each digit correct, e.g. if the correct answer was 49 and a participant responded 47 they scored 1 point. Individual digits subtended approximately 0.8 deg of visual angle horizontally at their widest point. Mean stimulus luminance averaged across the digits and their background was 25.6 candela per meter², falling in the photopic range, in which vision depends upon mainly upon the cone system. The percentage Michelson contrast of the digits with the background was varied in 6 steps selected to vary between easily visible and below threshold (1.946, 1.590, 1.354, 0.963, 0.576, and 0.230). There were 6 trials at each

contrast, resulting in a score out of 12 at each contrast step. Two alternative versions of this test were produced and half of the participants were tested with each version on visit one, switching to the other version for visit two.

2.4.2 Motion coherence threshold

On each trial two patches of moving dots were presented simultaneously for 100 msec, one either side of a fixation cross. One patch, which the participant had to identify, was made up of a mixture of signal dots moving horizontally and dots moving in random directions. The other patch contained only dots moving in random directions. To ensure the task required neural integration of motion signals across visual space, and prevent the strategy of tracking the motion of individual dots the lifetime of each dot was limited to 60 msec. The contrast of individual dots with the background was set well above detection threshold at 46.5%. At this level of stimulus contrast, variation in CS is unlikely to be a factor influencing performance, but performance variation will reflect the motion integration process in visual cortex [14].

A descending staircase procedure was used to determine the minimum proportion of signal dots that a participant could successfully detect. The first trial of the staircase began with 100% signal dots, and each time a correct answer was given the proportion of signal dots was reduced, initially by a large amount, and then by smaller amounts as the threshold was approached. In the final phase the percentage of signal dots was reduced by 1% for each correct answer, and increased by 2% each time an incorrect answer was given. The test stopped after the staircase reversed directions 6 times, and the threshold was estimated as the average of the percentage of signal dots

present in the stimulus at the last 5 reversal points. Details of the visual stimulus were as follows. The two patches subtended 5.98 by 4.48 deg, and the closest point of each patch was 3.86 deg from fixation. Each patch consisted of 100 dots, which were redrawn in randomly generated locations if they moved off the edge of the patch or exceeded their 60 msec lifetime. Individual dots subtended 0.15 deg and moved at 10.22 deg per sec.

2.4.2 Motion integration time threshold

On each trial a single patch of moving dots was presented. The participant decided whether the patch contained a mixture of 80% horizontally moving signal dots plus 20% dots moving in random directions, or just dots moving in random directions. A descending staircase procedure was used to determine the minimum viewing time the participant needed to make this judgment successfully. The first trial of the staircase began with display duration of 3000 msec, and each time a correct answer was given the display duration was reduced, initially by a large amount, and then by smaller amounts as the threshold was approached. In the final phase the display duration was reduced by 60 msec for each correct answer, and increased by 120 msec each time an incorrect answer was given. The test stopped after the staircase reversed directions 6 times, and the threshold was estimated as the average of the stimulus duration at the last 5 reversal points. Details of the visual stimulus were the same as 2.4.1 except as follows. The patch subtended 20.22 by 15.17 deg. Individual dots subtended 0.52 deg and moved at 34.58 deg per sec.

2.5 Cognitive tests

2.5.1 Visual spatial working memory

Participants viewed a screen displaying cartoon depictions of a number of common objects, drawn from a set of eight. Participants were allowed 3 sec to view the objects and form an impression of their relative locations before the display was replaced by a blank screen for 1 sec. After the blank screen the same objects were displayed again but two of them had switched locations. Participants circled which two objects they thought had changed location on a response sheet. One point was scored for each correctly circled object. To avoid floor and ceiling effects 6 easy, 6 medium, and 6 hard trials were included. Easy trials were based on 4 objects, medium trials on 6 objects, and hard trials used the full set of 8 objects. Note the purpose of the blank screen was to prevent apparent motion of the two objects that switched location, which would have made the task trivial and removed the need to remember the object locations. Two alternative versions of this test were produced using different sets of cartoon objects and different location switches. Half of the participants were tested with each version on visit one, switching to the other version for visit two.

2.5.2 Choice reaction time

Participants were instructed that they were to press one of 3 buttons on the computer keyboard as quickly as possible in response to letters or digits that appeared on the screen. The 3 response buttons were labelled "X", "Y" and "N", where "X" was to be pressed if "X" appeared on the screen, "Y" was to be

pressed if "Y" appeared, and "N" was to be pressed if any single digit number appeared on the screen. Inter stimulus intervals (ISI) varied randomly between 2000 and 7000 msec. This relatively large range of ISIs was intended to require participants to sustain attention in the ISI periods in order to avoid missing stimuli or making late responses. The task was made up of 60 trials divided into two phases. In the first phase, which was made up of 18 trials, stimuli alternated between "X" and "Y" in a predictable sequence. In the second phase the sequencing of "X" and "Y" became unpredictable and single digit numbers appeared as stimuli with a low frequency (12% of second phase trials). It was intended that the unpredictability of the sequence in the second phase would additionally require participants to engage inhibitory processes in order to prevent incorrect responses. The measures produced by this test were reaction time (rtm) in the predictable phase, rtm to the stimuli "X" and "Y" in the unpredictable phase, and the overall percentage of correct responses. Incorrect trials were excluded from rtm calculations. Rtm to the single digit stimuli was not calculated due to the small number trials, which was further reduced by the high error rate on this trial type. Two alternative versions of this test were produced and half of the participants were tested with each version on visit one, switching to the other version for visit two.

2.6 Statistical analysis

Performance on the contrast sensitivity test was converted to percent correct at each stimulus contrast. An overall percentage correct was calculated for visual spatial working memory. We used mixed ANOVA to assess the main effect of CF consumption versus control (one tailed), as well as the interaction

of treatment order with CF consumption versus control. Treatment order was a between subjects factor. The distributional assumptions of parametric statistical tests were violated by the data from the motion integration time and choice reaction time measures. For these tests we describe the central tendency of the inter-participant distribution using medians and analyse the main effect of CF consumption versus control with non-parametric Wilcoxon t tests (one tailed). While it is not possible to analyse the interaction with treatment order using non parametric tests, inspection of the data revealed no evidence of such an interaction for motion integration time or choice reaction time.

3.0 Results

Means, medians, and standard deviations for all measures in both the high CF and control conditions are given in Table 1. Measures that revealed significant effects of treatment condition are highlighted in bold.

TABLE 1 ABOUT HERE

3.1 Contrast sensitivity

Mean Percentage of digits read correctly after consuming dark chocolate containing 773 mg CF or white chocolate over the 6 levels of stimulus contrast are shown in Fig. 1. At the highest stimulus contrast all digits were read correctly by all participants, while at the lowest contrast percent correct approaches zero. There was no effect of CF at stimulus contrast 1.354%, at which performance was very high but not at ceiling. However, for the two

stimulus contrasts where performance fell either side of the absolute detection threshold, conventionally defined as 50% correct, participants identified more digits correctly after consuming high CF chocolate. The mean performance improvement of 13.3% at 0.576% stimulus contrast was assessed using mixed ANOVA and found to be significant (F(1,28) = 4.28, p < 0.05). There was no between subject effect of testing order (F(1,28) = 0.34, p = 0.56), or interaction of testing order and treatment condition (F(1,28) = 0.27, p = 0.61). At 0.963% stimulus contrast the mean performance improvement was 8.9%, which approached significance (F(1,28) = 2.25, p = 0.07). The group of participants who consumed high CF chocolate in week 1 performed better on average than the group who consumed white chocolate in week 1 (F(1,28) = 6.12, p < 0.05). This was the only significant main effect of testing order on any part of the test battery, and therefore we suspect this result is a type 1 error. The significant effect of testing order does not complicate the interpretation of our results because it does not interact with treatment condition (F(1,28) = 0.32, p = 0.58). Testing performance averaged across the two near threshold contrast levels produced a significant effect of high CF chocolate versus control (F(1,28) = 5.66, p < 0.05), but no effect of testing order (F(1,28) = 0.51, p = 0.48), or interaction of testing order and treatment condition(F(1,28) = 2.06, p = 0.16).

FIGURE 1 ABOUT HERE

3.2 Motion coherence threshold

In the high CF condition the mean motion coherence threshold was slightly better than in the low CF condition (48.5 versus 52.0% signal dots), but this difference was not significant (F(1,28) = 0.78, p = 0.39). There was no between subject effect of testing order (F(1,28) = 0.34, p = 0.56), or interaction of testing order and treatment condition (F(1,28) = 1.29, p = 0.27).

3.2 Motion integration time threshold

In the high CF condition the median motion integration time threshold was 172 msec better than in the low CF condition (417 versus 619 msec required to judge whether the motion patch contained signal dots), which was a significant difference, (z = 1.68, p < 0.05).

3.3 Visual spatial working memory

In the high CF condition the mean percent correct was 3.6% better than in the low CF condition (87.1 versus 83.5% correct), which was a significant difference (F(1,28) = 3.41, p < 0.05). There was no between subject effect of testing order (F(1,28) = 1.19, p = 0.28), but there was an interaction of testing order and treatment condition (F(1,28) = 4.14, p = 0.05). The two treatment order groups performed similarly in the high CF condition (86.9% high CF first versus 87.4% control first), and the high CF first group maintained this performance level in the second week when they consumed white chocolate (87.2%). The source of the interaction was that the control condition first group performed significantly worse in week one than in the second week when they consumed high CF chocolate (79.8% versus 87.4%, t(14) = 2.21, p < 0.05)).

3.4 Choice reaction time

In the high CF condition the median of the inter-participant rtm distribution during the predictable phase of the task was faster by 40 msec than in the low CF condition (517 versus 557 msec), which was a significant difference, (z = -2.33, p < 0.01). There were no statistically reliable effects on the measures relating to the unpredictable phase of the task. In the high CF condition median reaction time during the unpredictable phase was 8 msec faster (593 versus 601 msec, z = -1.31, p = 0.19). The median percentage of correct responses was slightly lower in the high CF condition (60 versus 62.5 %, z = -0.94, p = 0.35, two tailed).

4. Discussion

The results of the current study demonstrate for the first time that performance on tests of visual system function in healthy young adults can be improved by the acute consumption of CF. Improvements in visual function were observed approximately 2.5 hours after CF consumption, and if the improvement depends upon increased blood flow it is likely that the effect seen here lasted several hours [13, 21]. We also demonstrated improvements in cognitive performance due to CF, consistent with a recent report by Scholey et al. [21]. Because CF improved performance on several different tests, these results are indicative of a general mechanism underlying the improvements seen. As such we propose that increased cerebral blood flow may be producing an increase in motivation or attentiveness on the tasks.

In the case of contrast sensitivity, the performance improvement we found might reflect a general improvement in task performance, or a more specific effect due to changes in retinal blood flow produced by CF, or a combination of the two mechanisms. It is not possible to distinguish between these possibilities on the basis of the current data, but a number of previous findings are suggestive of some role for a retinal mechanism. Firstly, and most directly, is the observation that increasing retinal blood flow by means of hypercapnia improves CS [19]. Secondly, in glaucoma, changes in CS can be explained by changes in retinal blood flow [27]. Furthermore, gingko biloba, which contains a high level of flavonoids, has shown some promise as a treatment for glaucoma. Gingko biloba extract increases ocular blood flow in glaucoma patients [28], as well as reducing pre-existing visual field damage in normal tension glaucoma [29]. These findings, as well as those mentioned in the introduction point towards the hypothesis that flavonoids are able to influence the function of retinal neurons.

The acute improvement due to CF supplementation in motion integration time threshold, the small improvement in spatial working memory, and the faster responses in the initial phase of the choice reaction time task must be mediated by cortical effects. Most likely this reflects an improvement in the supply of metabolic substrates to cortical neurons produced by increased blood flow. A reduction in the time required to integrate visual motion could be beneficial in time critical everyday tasks, such as driving. The effect on the simpler early phase of the choice reaction time task suggests that CF can increase response speed in simple tasks. The stimulus sequence was predictable in the phase of the task where a treatment effect occurred,

and therefore it is also possible that the response speed improvement was mediated by an improvement in procedural memory. There was no reliable effect of CF when the number of response categories increased and the sequence became unpredictable, introducing to the task a requirement to inhibit incorrect responses. This choice reaction time task employed long time intervals between responses, and it may be speculated that the effects of CF manifested themselves here by improving the ability to sustain attention during the long inter trial intervals. This proposal could be tested using a simple reaction time task with a single response key and systematically varying the inter trial interval.

The only test to show an interaction of treatment with treatment order was the spatial working memory task. Participants who consumed CF in week 1 maintained their performance level in the subsequent testing week, while participants who consumed white chocolate in week 1 and high CF chocolate in week 2 did much better in the second week. A possible explanation of this interaction is that consumption of CF produced more highly motivated participants who consequently developed better strategies for remembering the locations of test items. Such participants might then make use of the same strategy in the control condition week. Further experimentation would be required to test this hypothesis.

Turning to the applied value of these results, arguably the most important finding is the influence of CF supplementation on visual contrast sensitivity. Using Fig. 1 to estimate the average absolute thresholds (50% detection rates) in the two experimental phases, and then converting these to contrast sensitivity values as used in clinical practice by calculating the

inverse of the physical stimulus contrast at threshold, results in a contrast sensitivity of 161 when supplemented and 137 when not. This represents a 17% improvement, and could potentially improve the performance of young adults in challenging visual environments such as when working in low light levels, or where there is a need to spot small low contrast targets. Aircraft pilots routinely face this situation, and one study has compared CS and Snellen acuity as predictors of pilots' performance on the challenging visual task of spotting other aeroplanes. One experiment assessed the ability of pilots to spot aircraft in a simulator, and another looked at the ability to detect real aircraft from the ground in a variety of weather conditions [23]. Both experiments found that individual differences in CS predicted successful spotting, while Snellen acuity did not predict success. Given these correlations, it seems likely that an intervention that improves CS would also improve the performance of some visually challenging everyday tasks.

Older adults typically experience a considerable decline in CS, particularly at medium and high spatial frequencies, and in more severe cases everyday functional vision is impaired [14, 24-26]. In this context, a 17% improvement such as we achieved here would be of considerable practical value if it were replicated in older adults. If our hypothesis that the improvement in contrast sensitivity due to CF supplementation is caused by a combination of increased blood flow to the retina and visual cortex is correct, then we might expect a similar effect size in older adults provided CF increases blood flow in older adults to the same extent as it does in young adults. In fact, a recent study indicated that the peripheral blood flow effects of CF in older adults are actually larger than in young controls [9], suggesting

that the percentage improvement in elderly CS could be larger than in young adults.

There are a number of reasons to predict that the chronic effects of CF supplementation on visual function will be larger than the acute effects reported here. Dietary flavonoids are neuroprotective, suppress inflammatory processes, and enhance endothelial function [30], all of which are likely to be beneficial for the retina and visual cortex. Perhaps the most encouraging findings regarding a possible chronic effect of dietary flavonoids on visual function are rat models suggesting that epigallocatechin gallate, a flavonoid derived from green tea, has a protective effect on the neural layer of the retina, and when injected or administered orally accelerates recovery from various insults to the retina [31-34].

The present study was an initial investigation with a focus on the potential influence of CF on visual function in young adults. The findings presented here are being extended in a new study of CF effects in older adults. As practiced by Scholey et al., the follow up study include caffeine and theobromine in the control condition to rule out the possibility that these ingredients of cocoa contribute substantially to the positive effects of cocoa. We have also included baseline performance measures on each day, and a double blind procedure to rule out the possibility of placebo effects.

References

- [1] Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., et al. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. J Nutr. 2004,134:613-7.
- [2] Lazarus, S. A., Hammerstone, J. F., Schmitz, H. H. Chocolate contains additional flavonoids not found in tea. Lancet. 1999,354:1825.
- [3] Engler, M. B., Engler, M. M., Chen, C. Y., Malloy, M. J., Browne, A., Chiu, E. Y., et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr. 2004,23:197-204.
- [4] Flammer, A. J., Hermann, F., Sudano, I., Spieker, L., Hermann, M., Cooper, K. A., et al. Dark chocolate improves coronary vasomotion and reduces platelet reactivity. Circulation. 2007,116:2376-82.
- [5] Heiss, C., Finis, D., Kleinbongard, P., Hoffmann, A., Rassaf, T., Kelm, M., et al. Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. J Cardiovasc Pharmacol. 2007,49:74-80.
- [6] Heiss, C., Kleinbongard, P., Dejam, A., Perre, S., Schroeter, H., Sies, H., et al. Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. J Am Coll Cardiol. 2005,46:1276-83.
- [7] Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C. L., Hollenberg, N. K., et al. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A. 2006,103:1024-9.

- [8] Balzer, J., Rassaf, T., Heiss, C., Kleinbongard, P., Lauer, T., Merx, M., et al. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial. J Am Coll Cardiol. 2008,51:2141-9.
- [9] Fisher, N. D., Hollenberg, N. K. Aging and vascular responses to flavanol-rich cocoa. J Hypertens. 2006,24:1575-80.
- [10] Heiss, C., Dejam, A., Kleinbongard, P., Schewe, T., Sies, H., Kelm, M. Vascular effects of cocoa rich in flavan-3-ols. Jama. 2003,290:1030-1.
- [11] Davison, K., Coates, A. M., Buckley, J. D., Howe, P. R. Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. Int J Obes (Lond). 2008,32:1289-96.
- [12] Faridi, Z., Njike, V. Y., Dutta, S., Ali, A., Katz, D. L. Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial. Am J Clin Nutr. 2008,88:58-63.
- [13] Francis, S. T., Head, K., Morris, P. G., Macdonald, I. A. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. J Cardiovasc Pharmacol. 2006,47 Suppl 2:S215-20.
- [14] Spear, P. D. Neural bases of visual deficits during aging. Vision Res. 1993,33:2589-609.
- [15] Lee, J. Y., Holden, L. A., Djamgoz, M. B. Effects of ageing on spatial aspects of the pattern electroretinogram in male and female quail. Vision Res. 1997,37:505-14.
- [16] Morrison, J. D., McGrath, C. Assessment of the optical contributions to the agerelated deterioration in vision. Q J Exp Physiol. 1985,70:249-69.

- [17] Muir, J. A., Barlow, H. L., Morrison, J. D. Invariance of the pattern electroretinogram evoked by psychophysically equivalent stimuli in human ageing. J Physiol. 1996,497 (Pt 3):825-35.
- [18] Porciatti, V., Burr, D. C., Morrone, M. C., Fiorentini, A. The effects of aging on the pattern electroretinogram and visual evoked potential in humans. Vision Res. 1992,32:1199-209.
- [19] Huber, K. K., Adams, H., Remky, A., Arend, K. O. Retrobulbar haemodynamics and contrast sensitivity improvements after CO2 breathing. Acta Ophthalmol Scand. 2006,84:481-7.
- [20] Crews, W. D., Jr., Harrison, D. W., Wright, J. W. A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. Am J Clin Nutr. 2008,87:872-80.
- [21] Scholey, A. B., French, S. J., Morris, P. J., Kennedy, D. O., Milne, A. L., Haskell, C. F. Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. J Psychopharmacol. 2009.
- [23] Ginsburg, A. P., Evans, D. W., Sekule, R., Harp, S. A. Contrast sensitivity predicts pilots' performance in aircraft simulators. Am J Optom Physiol Opt. 1982,59:105-9.

[22] http://www.acticoa.com/en/howdoesacticoawork/antioxidants#inDepth

- [24] Haegerstrom-Portnoy, G., Schneck, M. E., Brabyn, J. A. Seeing into old age: vision function beyond acuity. Optom Vis Sci. 1999,76:141-58.
- [25] Owsley, C., Sekuler, R., Siemsen, D. Contrast sensitivity throughout adulthood. Vision Res. 1983,23:689-99.

- [26] Owsley, C., Sloane, M. E. Contrast sensitivity, acuity, and the perception of 'real-world' targets. Br J Ophthalmol. 1987,71:791-6.
- [27] Harris, A., Evans, D. W., Cantor, L. B., Martin, B. Hemodynamic and visual function effects of oral nifedipine in patients with normal-tension glaucoma. Am J Ophthalmol. 1997,124:296-302.
- [28] Chung, H. S., Harris, A., Kristinsson, J. K., Ciulla, T. A., Kagemann, C., Ritch, R. Ginkgo biloba extract increases ocular blood flow velocity. J Ocul Pharmacol Ther. 1999,15:233-40.
- [29] Quaranta, L., Bettelli, S., Uva, M. G., Semeraro, F., Turano, R., Gandolfo, E. Effect of Ginkgo biloba extract on preexisting visual field damage in normal tension glaucoma. Ophthalmology. 2003,110:359-62; discussion 62-4.
- [30] Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., Spencer, J. P. The neuroprotective potential of flavonoids: a multiplicity of effects. Genes Nutr. 2008,3:115-26.
- [31] Costa, B. L., Fawcett, R., Li, G. Y., Safa, R., Osborne, N. N. Orally administered epigallocatechin gallate attenuates light-induced photoreceptor damage. Brain Res Bull. 2008,76:412-23.
- [32] Zhang, B., Osborne, N. N. Oxidative-induced retinal degeneration is attenuated by epigallocatechin gallate. Brain Res. 2006,1124:176-87.
- [33] Zhang, B., Safa, R., Rusciano, D., Osborne, N. N. Epigallocatechin gallate, an active ingredient from green tea, attenuates damaging influences to the retina caused by ischemia/reperfusion. Brain Res. 2007,1159:40-53.
- [34] Zhang, B., Rusciano, D., Osborne, N. N. Orally administered epigallocatechin gallate attenuates retinal neuronal death in vivo and light-induced apoptosis in vitro. Brain Res. 2008,1198:141-52.

Table 1. Descriptive statistics for each test battery measure. Measures for which the treatment effect was statistically significant are highlighted in bold.

Measure	High CF	Control	High CF	Control	High CF	Control
	mean	mean	median	median	SD	SD
Michelson contrast 1.95 %	100	100	100	100	0.00	0.00
(% correct)						
Michelson contrast 1.59 %	98.89	98.89	100	100	4.228	4.228
(% correct)						
Michelson contrast 1.35 %	95.00	94.44	100	100	18.65	11.85
(% correct)						
Michelson contrast 0.96 %	84.44	75.56	100	83.33	25.12	26.16
(% correct)						
Michelson contrast 0.58 %	43.89	30.56	50	16.67	34.60	36.38
(% correct)						
Michelson contrast 0.23 %	1.11	3.89	0.00	0.00	4.23	10.43
(% correct)						
Motion coherence threshold	48.50	51.97	45.00	45.50	16.35	17.40
(% coherence)						
Motion integration time	727	1108	447	619	645	1222
msec						
Visual spatial	87.13	83.51	88.89	84.72	9.32	11.16
(% correct)						
Reaction time msec	538	557	516	557	149	85
(predictable)						
Reaction time msec	593	604	591	601	73	69
(unpredictable)						
Accuracy	58.49	59.86	60	62.5	9.66	9.75
(% correct)						

Figure caption

Figure 1. The effect on visual contrast sensitivity of acute supplementation with high CF chocolate relative to low CF chocolate. The mean percentage of digits read correctly is plotted as a function of the contrast with the background the digits were presented on.

