

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 Cocoa Flavanol (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. . 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 , a quantity falling midway between the high and low doses used by Scholey et al. 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 ***Details in this section removed as they are regarding tests that we are not currently concerned about***

2.5 Cognitive tests

2.5.1 ***Details in this section removed as they are regarding tests that we are not currently concerned about***

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 (RT) in the predictable phase, RT to the stimuli “X” and “Y” in the unpredictable phase, and the overall percentage of correct responses. Incorrect trials were excluded from RT calculations. RT 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.

**Reference:** Field, D. T., Williams, C. M., & Butler, L. T. (2011). Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions. *Physiology & Behavior, (103)* 3-4, 255-260.