High flavonoid cocoa changes regional cerebral blood flow

L. M. Parkes[1,2], J. A. Goodwin[2], A. Irwin[2], R. van Kerckhoven[3], and R. Draijer[3]
[1]Imaging Sciences, University of Manchester, Manchester, United Kingdom, [2]MARIARC, University of Liverpool, Liverpool, United Kingdom, [3]Unilever Research & Development, Vlaardingen, Netherlands

Introduction: Cocoa flavonoids have been suggested as potential preventive agents for a range of pathologic conditions, such as hypertension, coronary heart disease, stroke and dementia[1]. They are attracting interest because of accumulating evidence that they can enhance endothelial function in a nitric oxide-dependent manner[2,] and induce peripheral vascular dilatation[3]. In addition there is some evidence that cocoa can improve endothelial function in cerebral vessels[4], increase cerebral blood flow (CBF)[4] and improve memory performance[5,6,7]. One study in rats showed angiogenesis in the hippocampus following increased flavonoid intake[7] along with improved memory performance. The primary objective of this study is therefore to investigate the effect of a high flavonoid cocoa test drink on CBF in a group of middle-aged subjects using arterial spin labeling. In addition we investigated whether there was a link between altered CBF and any changes in performance on a series of cognitive tests.

Methods: We used a double-blind, placebo-controlled randomised full-crossover design with 2 treatments: Test treatment: Cocoa soy-drink with 500 mg of cocoa flavonoids. Placebo treatment: Cocoa flavoured soy-drink without cocoa flavonoids. Each treatment was administered once each day for a period of 2 weeks with a one week washout period in between. Measurements of CBF and cognitive performance were performed at the end of each two week period. 15 healthy participants with an age range of 56 – 76 years took part.
CBF measurements were made using a QUIPSSII Arterial Spin Labeling sequence[1] at 3 Tesla. Acquisition parameters were: TR=2 s, TI_1=0.7 s, TI_2=1.4 s, TE=19 ms, in-plane resolution 3.5 mm, 75 label and control pairs. Two acquisitions were collected, each with 8 slices of 5 mm thickness and 1 mm gap, covering the entire cerebrum. An image with TR=10s was also collected in order to estimate equilibrium magnetization in order to produce quantitative CBF maps. The mean difference image was created from the control and labeled image pairs and quantitative CBF maps were produced through the use of a single blood compartment model, i.e. assuming that the labelled water does not cross the capillary wall or leave the voxel during the inversion time[2]. CBF maps following test and placebo were compared using voxel-based analysis within SPM8. CBF images were normalized to MNI space using the raw EPI images and 8 mm spatial smoothing was applied. A pair-wise t-test analysis was then performed over all voxels including the global CBF value as a regressor of no interest (such that the analysis is more sensitive to regional differences). We hypothesized that CBF is most likely to increase in the hippocampus, as angiogenesis has previously been reported in this region following increased flavonoid consumption in rats[7], and the hippocampus is most likely to be implicated in the observed memory improvements[5,6,7]. Hence, we also extracted the CBF measures from a region encompassing left and right hippocampus and parahippocampal gyrus, using the Wake Forest PickAtlas[8].
Cognitive testing was performed on 3 occasions, once several weeks before the trial began (to reduce practice effects), and once on each scanning day. Seven short tests were administered: verbal fluency, trail making tests (B-A), digit span backwards, a digit symbol test, (choice-simple) reaction time, digit vigilance test and a memory test for pictures in which subjects had to remember pictures that were presented 10 minutes earlier.

Results and Discussion:

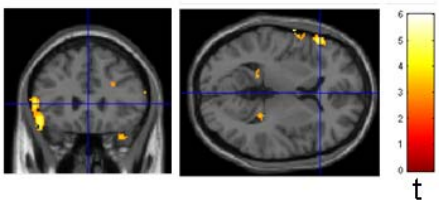

Figure 1: regions where cocoa>placebo

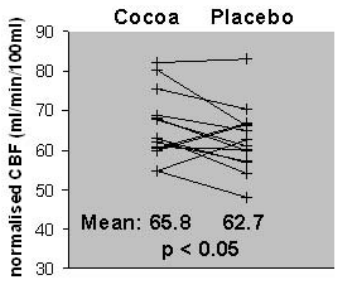

Figure 2: Signal from hippocampal region as defined by WFU PickAtlas[8]

Cocoa treatment showed significantly increased CBF compared to placebo in the left middle temporal lobe, significant at $p<0.01$ using cluster level statistics corrected for multiple comparisons. At a more lenient threshold of $p=0.01$ uncorrected, bilateral activation in the caudate tail and in bilateral hippocampus were also seen (Figure 1). The hippocampal regional analysis showed significantly increased CBF following cocoa in comparison to placebo (Figure 2). In addition, a number of regions were found where cocoa showed significantly decreased CBF in comparison to placebo, in the right dorso-lateral pre-frontal cortex and the precuneus. Cluster level statistics shows CBF in these regions to be significantly decreased at $p<0.05$, corrected for multiple comparisons. These results suggest that cocoa increases blood flow in structures underlying memory, such as the caudate, hippocampus and temporal lobe, but decreases blood flow in structures underlying attention and executive function, such as the anterior cingulate and DLPFC.

The cognitive test results revealed impaired performance for the digit span backwards test for cocoa in comparison to placebo (mean number of digits recalled was 8.7 ± 2.3 for placebo and 7.0 ± 2.5 for cocoa, $p=0.002$ paired t-test). However, performance was improved on the long-term memory task (sensitivity index 0.83 ± 0.18 for placebo and 0.90 ± 0.10 for cocoa, $p=0.03$ paired t-test). To determine any relationship between CBF change and altered performance on the cognitive tasks, we looked at the correlation across subjects between the extent of CBF change in the affected regions and change in performance on the cognitive tasks. No significant relationship was found between these values.

In summary, we find evidence that consumption of high flavonoid cocoa induces regional CBF changes, increasing blood flow to regions such as the hippocampus involved in long-term memory function and decreasing blood flow to regions involved in working memory. This is in good agreement with the observed impaired working memory performance (digit span backwards), but improved long-term memory performance.
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