The Effect of Flavanol-rich Cocoa on the fMRI Response to a Cognitive Task in Healthy Young People

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Abstract: Flavanols are the main flavonoids found in cocoa and chocolate, and can be especially abundant in certain cocoas. Research over the past decade has identified flavanols as showing diverse beneficial physiologic and antioxidant effects, particularly in context of vascular function. The present study employed functional magnetic resonance imaging based on blood oxygenation level-dependent (BOLD) contrast to explore the effect of flavanols on the human brain. Magnetic resonance imaging was used to measure BOLD responses to a cognitive task in 16 healthy young subjects. The data presented show an increase in the BOLD signal intensity in response to a cognitive task following ingestion of flavanol-rich cocoa (5 days of 150 mg of cocoa flavanols). This may arise either as a result of altered neuronal activity, or a change in vascular responsiveness, or both—the net effect then being dependent on which of the two effects is dominant. No significant effects were evident in behavioral reaction times, switch cost, and heart rate after consumption of this moderate dose of cocoa flavanols. A pilot study evaluated the relationship between cerebral blood flow and a single acute dose (450 mg flavanols) of flavanol-rich cocoa and showed that flavanol-rich cocoa can increase the cerebral blood flow to gray matter, suggesting the potential of cocoa flavanols for treatment of vascular impairment, including dementia and strokes, and thus for maintaining cardiovascular health.

Key Words: flavanols, fMRI, task switching, cerebral blood flow, cocoa

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Task switching paradigms involve subjects being asked to shift mental resources between different cognitive tasks. Under some circumstances, subjects when asked to perform a simple cognitive task as quickly as possible will respond substantially slower if before this they have recently performed a different cognitive task. In task-switching studies this increased delay in reaction time is often termed as the "switch cost."[1] These costs are assumed to reflect the executive control processes required for the coordination of multiple tasks. Here, we use functional magnetic resonance imaging (fMRI) to examine brain activation to a letter-digit pair task switching paradigm. This paradigm is similar to that of Rogers and Monsell[2] and that used in the fMRI study of Kimberg,[3] where letter-digit pairs are displayed and subjects must switch between consonant-vowel and odd-even judgment tasks.

In this paper we investigate the effect of a specific subclass of flavonoids—known as flavanols—on the fMRI blood oxygenation level dependent (BOLD) response[4] to task switching. Cocoa is derived from the seeds of the fruit of the *Theobroma cacao* tree, and certain cocoas can be manufactured to be extraordinarily rich in flavanols. However, cocoa containing high flavanol content is relatively difficult to obtain due to common postharvest handling and processing procedures used in the food industry that can dramatically reduce the flavanol content of chocolate and cocoa.[5]

The fMRI BOLD response reflects the change in blood oxygenation in active brain regions. This oxygenation change arises from a complex imbalance of increases in cerebral blood flow (CBF), cerebral blood volume, and cerebral metabolic rate of oxygen consumption. Flavanols have been suggested to cause an increase in blood flow.[6,7] If flavanols act to alter cerebral blood flow, then this effect alone will modulate the resting BOLD signal and potentially alter the magnitude of the BOLD response. Therefore, in this study we also perform an arterial spin labeling (ASL) magnetic resonance imaging study to produce quantitative measurements of CBF after ingestion of flavanols.

METHODS

Subjects

Sixteen young female subjects between the ages of 18 and 30 years participated in the study under the following exclusion criteria: no history of migraines, stroke, hypertension, diabetes, or any neurological or vascular disease, and no use of tobacco products. All subjects had normal vision and normal color vision, were not dyslexic, were right handed and their first language used the "Roman" alphabet. Subjects' average daily caffeine intake was estimated from their responses to a dietary questionnaire and they were all classified as low

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caffeine users (< 120 mg/day). All subjects were instructed to refrain from alcohol and caffeine, or from using any medication for 12 hours before each visit for the fMRI measurements. The local Medical School Research Ethics Committee approved this study and all subjects gave informed written consent before taking part in the study.

Each subject underwent two fMRI sessions that were repeated at least 14 days apart. Subjects were randomized to receive a high flavanol cocoa drink (172 mg flavanols per drink) (Cocoapro™ cocoa, Mars, Incorporated) for 5 days before one fMRI session and a low flavanol cocoa drink (13 mg flavanols per drink) for 5 days before the other session in a double blind counterbalanced manner. These will be referred to hereafter as "high flavanols" and "low flavanols." Subjects consumed one drink per day at a set time for the 5 days before each scan session, with the final drink being consumed approximately 1.5 hours before the fMRI scan.

Study Design

Subjects were pretrained to perform 2 tasks, a letter task of odd-even judgment and a number task requiring consonant-vowel judgment. In the letter task subjects learnt to respond to single letter stimuli depending on whether the letter displayed was a consonant (G, K, M, R) or a vowel (A, E, O, U). Subjects were trained to press a left button in response to a vowel and right button for a consonant. In the number task, subjects were trained to respond to digits that were either odd (3, 5, 7, 9) or even (2, 4, 6, 8), using left and right button responses, respectively.

Once subjects were familiarized with the rules of the letter and number tasks, they were trained on the letter-digit pairs task to be performed in the fMRI study. The letter-digit pairs task consisted of a letter and a digit displayed simultaneously on a computer screen (for the fMRI scanning a projector and screen were used). The letter-digit pairs were either red or blue. When letter-digit pairs were presented in red, subjects were instructed to attend to the letter and respond by pressing the appropriate button as trained (ie, applying the rule for categorizing as vowel or consonant). If the letter-digit pair was blue they responded to the digit (odd-even judgment) in a similar manner.

In this study the definition of the "switch" task is the changing between the 2 sets of rules, 1 for the letters (consonant-vowel judgment) and 1 for digits (odd-even judgment). To create a paradigm comprising of "switch" and "nonswitch" conditions, the letter-digit pairs were grouped into blocks. A block of 5 letter-digit pairs, all of the same color is a "nonswitch" block. A block of 5 letter-digit pairs alternating between red and blue stimuli (and so reconfiguring task judgement) is a "switch" block. The gap between each letter-digit pair within the block was 3 seconds, giving a total block length of 15 seconds, this was then followed by a 12 second fixation cross (baseline condition). The blocks were presented alternately (ie, "switch" block, "nonswitch" block, "switch" block, "nonswitch" block, etc.). The presentation of letter-digit pairs within a block helps to increase the switch cost and so also increase the magnitude of the fMRI BOLD response, while the 12 second interval between the blocks allows the BOLD response to return to baseline.

Before the fMRI study the subjects were trained to a competent level (error rates below 5%) in the task by performing 5 blocks of "switch" and "nonswitch" trials. During the fMRI study 20 blocks of "switch" trials and 20 blocks of "nonswitch" trials were performed in the study, resulting in a total study duration of 18 minutes.

fMRI Scanning

A 3.0 T purpose-built scanner was used with TEM head coil and insert head gradient coil. T_2*-weighted coronal echo-planar images (EPI) with a 128×64 matrix size, 3 mm in-plane resolution and 9 mm slice thickness were acquired using MBEST acquisition sequence with 30 ms echo time and 1.9 kHz gradient switching frequency. Sixteen contiguous coronal slices were acquired every 3 seconds (repetition time = 3 s). Throughout the study subjects' reaction time and error rate data were recorded. Further the subjects' heart rate was monitored. After the fMRI study, a 64 slice EPI set was acquired to aid anatomic localization.

fMRI Data Processing

The fMRI data was processed using SPM99[8] (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, UK). The raw data from the scanner was motion corrected to realign all functional slices to the first volume of the data set, and spatially normalized to the standard EPI template. Eight millimeters full-width half-maximum spatial smoothing and 128 seconds high-pass filter cut-off were applied.

A general linear model design matrix was created within SPM99 that modeled the paradigm. "Switch" and "nonswitch" blocks were modeled as 12 second box-car-top-hat functions. The paradigm time course was then convolved with the canonical hemodynamic response function and its temporal derivative. Statistical parametric maps (SPMs) of the "switch" condition and "nonswitch" condition versus baseline were formed. In addition, direct comparison between the 2 activation conditions (ie, "switch" vs. baseline and "nonswitch" vs. baseline) was also performed at a corrected significance level of $P < 0.05$, with the respective activation versus baseline comparisons ("switch" vs. baseline and "nonswitch" vs. baseline) being used as a mask.

CBF Measurements and Analysis

In this initial study on 4 subjects (24 to 31 y) we assessed the time course of the effect of flavanol-rich cocoa on brain-blood flow. A CBF map was acquired using an Echo-Planar MR Imaging and Signal Targeting with Alternating Radiofrequency ASL sequence on 5 multi-slice axial 7 mm slices. Diffusion weighting ($b = 5\,mm^2/s$) was applied for suppression of intra-arterial spins.

A hyperbolic secant pulse was used for the labeling, and the tag and control slabs were 9 cm in width with an inversion time of 1400 ms. The Echo-Planar MR Imaging and Signal Targeting with Alternating Radiofrequency sequence was implemented with TR of 3 seconds between tag and control images, and a total of 60 tag and control pairs were acquired. Each subject underwent CBF imaging before and at 2, 4, and 6 hours after ingestion of a high flavanols cocoa drink (516 mg flavanol) or a low flavanols cocoa drink (39 mg flavanol), on two separate occasions. In this study, in contrast to the fMRI study, a single dose of the drinks was consumed on only one occasion. Each CBF measurement was followed by the acquisition of a T_1 map for segmentation of brain tissue types and gray matter territories.

Cerebral blood flow data were first segmented into gray and white matter regions using masks generated from the T_1 map. Gray matter CBF maps were then further segmented into territories fed by major vessels.[9] Mean cerebral blood flow values were then calculated for white and gray matter regions. Whole gray matter perfusion values are presented here.

RESULTS

Behavioral Results for Task Switching Paradigm

Robust switch costs in response times were observed. Each of the subjects was numerically slower for the "switch" condition than "nonswitch" condition, both for the letter and number tasks, the constant switching from one task rule to the other proving difficult. The mean reaction times across the group for the "switch" and "nonswitch" blocks are shown in Figure 1. The significance of the switch cost was $P = 5 \times 10^{-6}$ for "low flavonols" and $P = 1 \times 10^{-6}$ for "high flavonols". There was no significant difference in the switch cost between the two drinks ($P = 0.30$). The average switch cost was 224 ± 25 ms.

The possibility that during the 40 blocks of "switch" and "nonswitch" trials of the fMRI study the subjects would either make more or less errors as they either became fatigued or improved due to learning effects was also investigated. Analysis of the reaction time responses revealed that no significant fatigue/learning effects occurred over the course of the fMRI sessions ($P = 0.74$). Also the drink order was randomized and a comparison of the reaction time responses between first and second scanning sessions made, this again revealed no significant differences ($P = 0.73$).

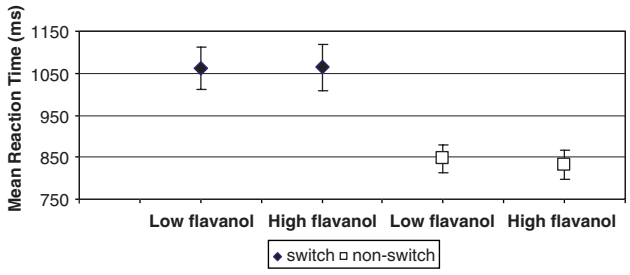

FIGURE 1. Mean reaction time (± SEM) for the letter-digit task. Reaction times were averaged over all subjects. Reaction times and the switch cost of the letter-digit pair task were not significantly altered by the repeated dose of a high flavanols drink.

TABLE 1. The Mean Heart Rate (± SEM) (beats per minute) in Response to "Switch" and "Non-switch" Conditions for Low and High Flavanols Drinks

	Low Flavanols	High Flavanols
Switch	66.8 ± 2.6	67.6 ± 2.6
Nonswitch	63.0 ± 2.5	64.2 ± 3.0

Heart Rate Results for Task Switching Paradigm

The mean heart rate for the "switch" and "nonswitch" conditions for the low and high flavanol cocoa drinks was measured as shown in Table 1. Paired *t*-tests were performed to determine any differences in heart rate between the "switch" and "nonswitch" conditions; for both drinks there was a significant increase in heart rate for the "switch" condition compared with "nonswitch" condition ("low flavonols": "switch" > "nonswitch" $P = 0.01$; "high flavonols": "switch" > "nonswitch" $P = 0.0009$). No significant difference in heart rate was found between the low and high flavanols drinks.

fMRI Results for Task Switching Paradigm

Figure 2A shows the group statistical parametric map for the switch task versus baseline at a corrected probability of $P < 0.05$. The "switch" and "nonswitch" versus baseline conditions revealed activation in the medial and lateral prefrontal cortex (including the dorsolateral prefrontal cortex), parietal cortex, anterior cingulate cortex (ACC), and cerebellum.

The brain areas outlined above have previously been shown to be associated with task switching.[10–12] Further, a number of cognitive neuroimaging studies aside from task-switching have found similar patterns of activation. These include working memory,[13,14] memory retrieval,[15,16] and arithmetic problem solving tasks.[17,18] All such tasks demonstrate strong prefrontal-parietal interconnections,[19,20] suggesting that these 2 areas may serve complementary roles in high-level cognition.

Figure 2B shows the group statistical map of areas of activation which show significantly increased BOLD response during the "switch" task relative to the "nonswitch" task. From this comparison, it can be seen that those brain areas activated preferentially to the "switch" condition are largely localized in the right hemisphere, in the dorsolateral prefrontal and parietal cortices, as well as the ACC and cerebellum.

In task-switching it is thought that the ACC detects conflict in a task-setting,[21] the right frontal cortex plays a role related to the inhibition of irrelevant (preceding)

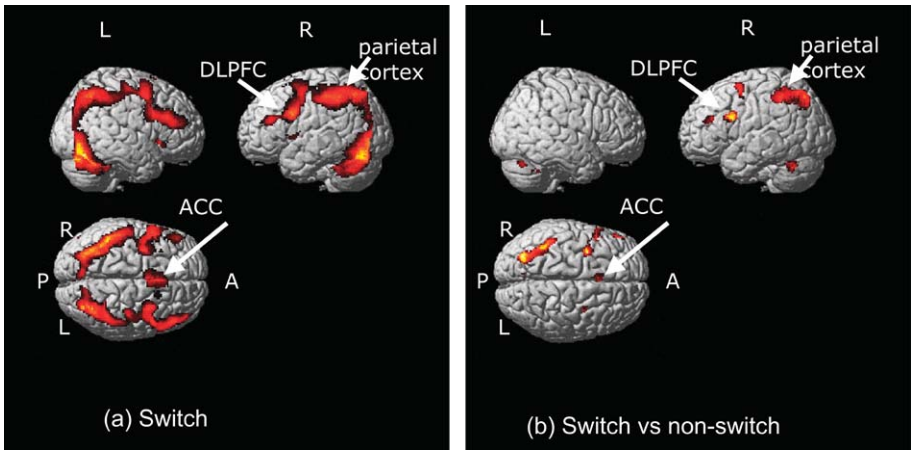

FIGURE 2. Task-related activity in the ''switch'' versus baseline condition for ''high flavonols''. Statistical parametric maps thresholded at $P<0.05$ (corrected) for height and spatial extent. Task-related activity for the comparison between ''switch'' and ''nonswitch'' conditions (''switch'' vs. baseline>''nonswitch'' vs. baseline) for ''high flavonols''. Statistical parametric maps thresholded at $P<0.05$ (corrected) for height and spatial extent. Significant activation can be seen to be localized to the right hemisphere in the medial and lateral prefrontal cortex (including the dorsolateral prefrontal cortex), parietal cortex, cerebellum, and anterior cingulate cortex (ACC). These areas have previously been shown to be associated with switching into a response suppression mode, attention allocation, internal timing, and processing of response conflict, respectively.

responses,[22] and the active maintenance of information that is newly loaded into working memory.[23] The right posterior parietal cortex has widely been shown to be responsible for spatial or visual attention,[24] while the cerebellum is thought to be primarily activated with timing irregularity in the switch task, consistent with its role as an internal timing system.[25] These are areas that have previously been shown to be associated with switching into a response suppression mode, attention allocation, and processing of response conflict as well as overcoming prior response suppression, respectively.[10–12]

Statistical comparison of the BOLD signal change between the ''low flavonols'' and ''high flavonols'' conditions revealed that the ''high flavanol'' generated a significantly greater BOLD signal change for both the activation (''switch'' and ''nonswitch'') versus baseline conditions and for the comparison of ''switch'' versus baseline with ''nonswitch'' versus baseline condition. Table 2 shows the average percentage signal change for the ''switch'' BOLD response relative to baseline, for selected regions of interest, after ingestion of ''low'' and ''high'' flavanols drinks.

TABLE 2. The Average Percentage Signal Change (± SEM) of the BOLD Response Relative to Baseline for the ''Switch'' Condition Following Ingestion of a Repeated Dose of Low and High Flavanols Drinks

	Low Flavanols	High Flavanols
Dorsolateral prefrontal cortex (DLPFC)	2.3 ± 0.2	3.0 ± 0.2
Parietal cortex	2.1 ± 0.3	2.5 ± 0.2
Anterior cingulate Cortex (ACC)	1.7 ± 0.1	2.1 ± 0.3

The high flavanols drink revealed a marked increase in the BOLD response.

CBF Timecourse Results

Figure 3 shows the time course of the mean cerebral blood flow response across gray matter after ingestion of the low and high flavanols drinks. It can be seen that there was an increase in cerebral blood flow in response to the high flavanols drink, with a peak in the cerebral blood flow response occurring at approximately 2 hours postingestion, and CBF returning to baseline after approximately 6 hours. It should be noted that in this study an acute dose of flavanol-rich cocoa was given, in contrast to the repeated dose given in the fMRI study.

ASL studies have previously been performed to measure the effects of hypercapnia, which induces cerebral vasodilation and CBF increases. Such studies have shown a CBF increase from 30% up to 87% in response to an expiration breath hold, which leads to an instantaneous increase in $PaCO_2$.[26–28] Direct effects of

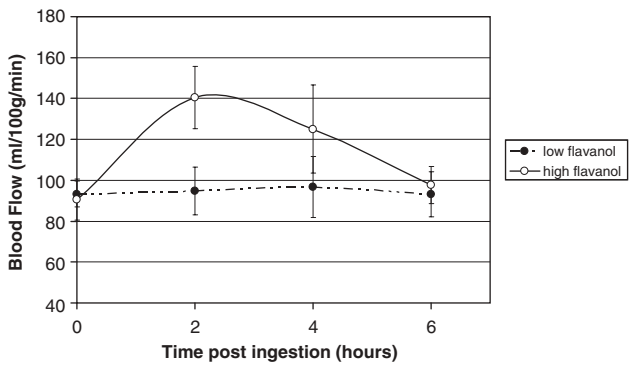

FIGURE 3. Time course of the mean cerebral blood flow responses (± SEM) across gray matter (n = 4) after ingestion of an acute dose of high flavanols drink and low flavanols drink.

inhalation of 5% CO_2 as a vasodilative stimulus have revealed global CBF increases of approximately 87%.[29] The 60% CBF changes shown here at 2 hours postingestion of the high flavanols drink are of a similar order to these effects.

DISCUSSION

This study investigated the effect of flavanol-rich cocoa on both the behavioral and fMRI response to a cognitive task switching paradigm. Flavanol-rich cocoa had no significant effect on the reaction time, switch cost or error rate for the letter-digit pair switching task. However, a moderate dose (5 days of 150 mg flavanols per day) of flavanol-rich cocoa did modify the BOLD response to task switching. The BOLD response was significantly increased for the high flavanols drink when compared with low flavanols drink. No change in heart rate was found in the presence of flavanol-rich cocoa.

The BOLD response is a function of both neural activity and changes in vascular tone through coupling of neural activity to hemodynamic responses (alterations in cerebral blood flow and volume).[13,14] Thus any physiologic condition that influences either of these components will cause a change in the BOLD signal. The increase in BOLD response in the presence of the high flavanols drink could therefore arise either as a result of altered neuronal activity, or a change in vascular responsiveness, or both—the net effect then being dependent on which of the two effects is dominant.

Results of the pilot cerebral blood flow study show that an acute dose of flavanol-rich cocoa causes an increase in cerebral blood flow. This finding supports previous research studies in vitro in animal models,[15] and in humans[6,7] which have provided evidence for an action of cocoa flavanols on endothelial dysfunction with a boost in the synthesis of nitric oxide by blood vessels. In studies of ingestion of flavanol-rich cocoa, Heiss et al[6] showed an increase in flow-mediated vasodilation of the brachial artery after 5 minutes of ischemia, a response that correlated with biochemical evidence of increased nitric oxide bioavailability. Further, work by Fisher et al, also described in this supplement shows that flavanol-rich cocoa induced dilatation of the vessels of the finger in the normal volunteers after flavanol-rich cocoa, an effect which was reversed completely by an arginine analog that blocks nitric oxide synthesis.[7]

fMRI studies have investigated the effects of known vasoconstrictors and vasodilators on the BOLD signal. Morton et al used the vasoconstrictor theophylline in rats, and showed a decrease in the resting state BOLD signal, while the BOLD response was increased.[16] Several studies of the effects of caffeine (a cerebral vasoconstrictor) have shown an increase in the BOLD response,[17–19] although this effect has not been consistently demonstrated.[20] The vasodilator acetazolamide has been shown to increase the resting state BOLD signal and decrease the BOLD response in humans.[21]

In this study, it might be thought that in the absence of behavioral correlates (as evident by the lack of modulation of the switch cost), the pharmacological effects of flavanol-rich cocoas on the BOLD signal are of cerebrovascular origin. However, fMRI might be more informative than behavior as an indicator of underlying neurocognitive function.[22] BOLD changes might be related to cognitive changes, such as adaptations of strategy formation or cognitive effort, which are not manifest in standard behavioral measures such as response accuracy or latency. Furthermore, it is possible that in the present study the healthy young subjects were already performing at a high level of cognitive ability, which would be very difficult to improve upon. Thus no improvement in reaction time was seen. This could be tested in a subsequent investigation by assessing the effects of the cocoa flavanols in people whose cognitive performance was impaired temporarily due to fatigue.

CONCLUSIONS

This study demonstrates that the BOLD response to a cognitive task switching paradigm is significantly increased in the presence of flavanol-rich cocoa. No effects on behavioral responses or heart rate were evident. Arterial spin labeling cerebral blood flow measurements revealed an increase in blood flow after ingestion of flavanol-rich cocoa, with a peak in this response occurring 2 hours after ingestion. The effect of flavanol-rich cocoa on the BOLD response could be mediated by effects on the cerebral vasoactivity rather than direct effects on neurons, or by adaptations of strategy formation or cognitive effort, or a combination of these effects. The fact that flavanol-rich cocoa has been shown to increase blood flow to key areas of the brain suggests the potential to use cocoa flavanols for treatment of vascular impairment, including dementia and strokes, and thus for maintaining cardiovascular health.

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