Acute cocoa flavanols intake improves cerebral hemodynamics while maintaining brain activity and cognitive performance in moderate hypoxia.

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ORIGINAL INVESTIGATION

Acute cocoa flavanols intake improves cerebral hemodynamics while maintaining brain activity and cognitive performance in moderate hypoxia

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Abstract

Introduction Acute cocoa flavanols (CF) intake has been suggested to modulate cognitive function and neurovascular coupling (NVC). Whether increased NVC is solely driven by improved vascular responsiveness or also by neuronal activity remains unknown. This study investigated the effects of acute CF intake on cognitive performance, NVC, and neuronal activity in healthy subjects in normoxia and hypoxia (4000 m simulated altitude; 12.7% O_2).

Methods Twenty healthy subjects (age 23.2 ± 4.3 years) performed four trials. Participants performed a Stroop task and "cognition" battery 2 h after acute CF (530 mg CF, 100 mg epicatechin) or placebo intake, and 30 min after initial exposure to hypoxia or normoxia. Electroencephalogram and functional near-infrared spectroscopy were used to analyze hemodynamic changes and neuronal activity.

Results CF enhanced NVC in the right prefrontal cortex during several tasks (risk decision making, visual tracking, complex scanning, spatial orientation), while neuronal activity was not affected. CF improved abstract thinking in normoxia, but not in hypoxia and did not improve other cognitive performances. Hypoxia decreased accuracy on the Stroop task, but performance on other cognitive tasks was preserved. NVC and neuronal activity during cognitive tasks were similar in hypoxia vs. normoxia, with the exception of increased β activity in the primary motor cortex during abstract thinking.

Conclusions Acute CF intake improved NVC, but did not affect neuronal activity and cognitive performance in both normoxia and hypoxia. Most cognitive functions, as well as NVC and neuronal activity, did not decline by acute exposure to moderate hypoxia in healthy subjects.

Keywords Electroencephalogram · Functional near-infrared spectroscopy · Cocoa flavanol · Cognitive performance

Introduction

Nutritional supplements are popular not only for their potential beneficial effects on general health but also on brain health (Socci et al. 2017). In this context, there is increasing interest in flavonoids, a subgroup of polyphenols, which are a class of natural compounds found in the human diet and include subcategories of flavanols, flavonols, iso-flavones, flavones, and anthocyanidins (Del Rio et al. 2013). Flavanols are found in grapes, tea, red wine, apples, and especially cocoa (Manach et al. 2004). Previous human clinical studies showed that cocoa flavanols (CFs) have antioxidant and anti-inflammatory properties and improve vascular function (Andújar et al. 2012). Moreover, evidence exists that CFs have neuroprotective and neuromodulatory effects (Francis et al. 2006; Scholey et al. 2010; Nehlig 2013; Scholey and Owen 2013). It was suggested that long-term CF intake enhances neuronal function by interacting with neuronal intracellular signaling pathways involved in neuronal survival and differentiation, memory, and long-term potentiation (Spencer 2008), while it seems that cognitive enhancements observed upon acute CF

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supplementation are associated with its vasodilatory actions (Socci et al. 2017). Previous research has shown nitric oxide (NO)-mediated vasodilation in response to acute CF intake, resulting in improved blood flow in the periphery (Grassi et al. 2015) and the brain (Lamport et al. 2015). It has been shown that acute CF intake increased the neurovascular coupling (NVC) during cognitive tasks, assessed by function near-infrared spectroscopy (fNIRS) (Decroix et al. 2016) and functional magnetic resonance imaging (fMRI) blood oxygenation level dependent (BOLD) response (Francis et al. 2006). NVC reflects the local increase in CBF to match oxygen (O_2) supply to neuronal demand during neuronal activation (Ogoh 2017). Several processes, including vasodilation, blood volume, blood oxygenation, neuronal activity, and synaptic activity, underlie NVC (Steinbrink et al. 2006). An altered hemodynamic response can arise as a result of altered neuronal activity or as a change in vascular responsiveness (or both) (Murta et al. 2015).

While acute CF intake has been shown to improve NVC (Francis et al. 2006; Lamport et al. 2015; Decroix et al. 2016), it remains unclear whether this is solely caused by improved vascular function, or also by altered neuronal activity. The increased BOLD response after acute and 5-day CF intake, in the absence of behavioral effects, was thought to be of neurovascular origin and not related to changes in brain activity (Francis et al. 2006). Camfield et al. found that chronic CF intake (500 mg CF) decreased steady-state visually evoked potential (SSVEP) amplitude at several centro-frontal sites during the spatial working memory test in the absence of improved cognitive performance, which was interpreted by the authors as increased neural efficiency (Camfield et al. 2012). Thus, it has been proposed that a longer period of CF intake is needed to alter brain activity (Camfield et al. 2012) and that short-term CF intake can alter vascular responsiveness but not brain activity (Socci et al. 2017). However, whether acute CF intake may enhance neural efficiency (i.e., reducing the neuronal activation required to perform cognitive tasks) has not been investigated so far. Therefore, we aimed to simultaneously investigate the effects of acute CF intake on neuronal activity and the hemodynamic response (changes in oxyhemoglobin (ΔHbO_2) and deoxyhemoglobin (ΔHHb)) during cognitive tasks. Changes of cortical excitability can be assessed by multichannel electroencephalogram (EEG), and standardized low-resolution brain electromagnetic tomography (sLORETA) can be utilized to localize regional changes in cerebrocortical activity associated with cognitive performance (Schneider and Strüder 2009). Understanding the relationship between EEG and fNIRS signals should give additional insights into the neuronal substrate of the increased NVC signal, measured by fNIRS (Murta et al. 2015).

Increasing the provision of metabolic substrates by enhancing CBF can result in cognitive benefits (Scholey and Owen 2013). Although there is compelling evidence that acute CF intake increases the cerebrovascular response, the effect of CF on cognitive performance is still ambiguous (Socci et al. 2017). It seems that the quantity and bioavailability of the consumed CF and the subject population used in the studies highly influence the observed beneficial effects (Scholey et al. 2010; Field et al. 2011; Pase et al. 2013; Massee et al. 2015). Pase et al. (2013) and Francis et al. (2006) failed to find beneficial effects of 500 and 150 mg CF. The authors stated that the healthy subjects were already performing at a high level of cognitive ability, which would be very difficult to be improve upon. However, several authors (Scholey et al. 2010; Field et al. 2011; Massee et al. 2015) detected improved cognitive performance on working memory tasks and visual function after different dosages of CF in healthy persons (750, 994, and 250 mg). In a recent review, it was noted that in healthy young subjects, acute CF intake mainly improved cognitive performance in cognitive demanding environments, such as during sustained mental efforts or in fatigued state (Grassi et al. 2016). Acute exposure to hypoxia (12.7% O_2) also forms a cognitive demanding environment. Optimal brain function requires adequate O_2 supply, and the decreased arterial pressure of oxygen (PaO_2) and arterial saturation of O_2 (SaO_2) in hypoxia may compromise cerebral O_2 supply and decrease cerebral oxygenation. Previous studies indeed reported cognitive impairments during acute hypoxia, which were related to an insufficient cerebral O_2 supply (Ando et al. 2013). Yet, there appear to be regional differences in the vulnerability/sensitivity of neurons to hypoxia. With the exception of central oxygen-chemosensitive neurons that regulate changes in cardiovascular and respiratory responses, most neurons decrease their metabolic demand, which is directly related to level of neuronal activity, in response to hypoxia (Neubauer and Sunderram 2004). While some cognitive domains, such as short-term and spatial memory, cognitive flexibility, and motor speed, are impaired by hypoxia, other cognitive domains such as long-term memory and perception, are not affected (Wilson et al. 2009).

In the present study, we combined simultaneous recording of hemodynamic changes (fNIRS) with EEG to unmask the physiological origin of NVC, namely vascular responsiveness and/or neuronal activity. We hypothesized that acute CF supplementation would increase NVC (by facilitating NO-mediated vasodilation), without altering neuronal activity, as suggested by Socci et al. (2017).

Moreover, we hypothesized that acute CF intake might (partially) counteract hypoxia-induced cognitive impairments as a consequence of increased NVC. Thus, the aim of this study was to investigate the effects of acute CF intake on cognitive function, neuronal activity and NVC during exposure to normobaric hypoxia (4000 m altitude; 12.7% O_2).

Materials and methods

A randomized, placebo (PL)-controlled, counter-balanced, double-blinded, cross-over study design was used.

Participants

Twenty healthy young (male and female) students were selected for participation in this study (age 23.2 ± 4.3 years). The number of subjects was chosen after a power calculation based on the results of Scholey et al. (2010), examining the acute effect of CF on cognitive performance in normoxia. Subjects were not acclimatized to a hypoxic environment. Subjects were excluded if they (1) were younger than 18 years or older than 35 years, (2) had severe head injuries in the past, (3) took neuromodulating medication (psychotropic drugs, beta adrenergic blockers, steroids,…), (4) were hypertensive, (5) had cardiovascular disease, (6) were smokers, (7) had other diseases which can alter cognitive function (diabetes, depression, etc.), and (8) had a G6PD-deficiency. The experimental procedures and potential risks were explained to the participants and a written informed consent was provided and signed before the start of the study. The study protocol was approved by the Ethical Committee of the Brussels University hospital and was carried out in accordance with the Declaration of Helsinki.

Intervention

On the first visit, subjects familiarized with the cognitive task battery by performing the battery three times. Subsequently, subjects performed four interventional trials, with a wash-out period of 1 week in between the trials. Subjects were asked not to change their regular sleeping pattern and eating behavior during the entire study and especially the two nights preceding each interventional trial. They were also asked to abstain from caffeine, alcohol, other psychoactive substances, and polyphenol-rich foods (green tea, red wine, dark chocolate (cocoa), and grape (juice)) in the last 24 h prior to each intervention trial.

The interventional trials consisted of (1) CF intake before cognitive performance in normobaric hypoxia (4000 m; 12.7% O_2), (2) PL intake before cognitive performance in hypoxia, (3) PL intake before cognitive performance in normoxia (0 m; 21.0% O_2), and (4) CF intake before cognitive performance in normoxia (0 m; 21.0% O_2). All experimental trials were conducted in a normobaric hypoxic chamber, set at 20 °C and with a relative humidity between 30 and 40%.

Subjects reported to the lab in a 3-h fasted state at the same time of the day for each experimental trial (between 8 am and 4 pm). Subjects then consumed the provided food supplement (four capsules) (PL or CF, Naturex, Avignon, France), together with a carbohydrate rich meal (which was carefully selected by a nutritionist to contain 600 kcal, 85% carbohydrates, 10% proteins, and 5% fat) to increase CF absorption (Schramm et al. 2003). The CF supplement was a 1765 mg cocoa extract which contains 530 mg flavanols (of which 100 mg epicatechin, 23 mg catechin), 119 mg theobromine, and 17 mg caffeine. This dose was based on previous research showing that acute intake of 450 mg CF (Francis et al. 2006) and 494 mg CF (Lamport et al. 2015) increased cerebral blood flow during cognitive performance. Capsules with maltrodextrin, matched in theobromine, caffeine, color, shape, and texture served as PL. The nutritional intervention was double-blinded. Supplements were matched in color, shape, and texture. The capsules were named "A" and "B" and samples were prepared (after randomization) by an external researcher. Blinding was maintained until the end of the statistical analysis.

Ninety minutes after supplement intake, subjects entered the climatic chamber, which was set at the desired altitude. NIRS equipment was applied and a pulse oximeter was positioned on the participants' left index finger (Medlab, Germany) to record heart rate (HR) and indirectly measure arterial O_2 saturation (SpO_2). Subjects were blinded to the hypoxic or normoxic environment. Only one subject experienced symptoms of acute mountain sickness. The subject was instructed to immediately leave the hypoxic chamber, and his results were excluded from data analysis.

Cognitive test

Two hours after CF intake and 30 min upon entrance in the climatic chamber, subjects started the cognitive test battery. This timeframe was chosen because the peak of plasma concentration of epicatechin occurs 2 h post CF intake (Schramm et al. 2003) and arterial oxygen saturation and oxygenation of the prefrontal cortex plateau after 20 and 30 min, respectively, of hypoxic exposure (Rupp et al. 2013). During the cognitive testing, subjects were seated, had earplugs inserted, and had been instructed to minimize head movement and eye blinking, to avoid frowning and maintain the same posture, in order to minimize movement artifacts in the NIRS data.

First, subjects performed a Stroop task (approximately 9 min) which was immediately followed by the Joggle cognition test battery.

Stroop test The Stroop test was programmed and performed on E-prime 2.0 software (Psychology Software Tools, Inc., Pittsburg, PA) and is commonly known as a tool to measure selective attention, cognitive flexibility, and response inhibition (MacLeod and MacDonald 2000). This test assesses the ease with which a person can maintain a goal in mind and suppress a habitual response in favor of less familiar ones. The words "green, blue, yellow, and red" were shown in

matching (congruent) or non-matching (incongruent) colors. Participants had to press the colored button on the keyboard in which the color names were printed, disregard their reading content. Eighty congruent and 80 incongruent stimuli were given, the interval response–stimulus onset was set at 500 ms and the stimulus appeared on the screen until the subject responded. The stimuli were displayed in the middle of the computer screen. Outcome measures were accuracy and reaction time (RT) of the decision-making process.

Cognition test battery The computerized cognitive test battery "*Cognition*" was used because of its sensitivity to multiple cognitive domains at high-level cognitive performance (Basner et al. 2015). *Cognition* consists of eight neuropsychological tests known to engage specific brain regions evidenced by functional neuroimaging. In particular, the battery consists of the motor praxis test (MPT, measure of sensorimotor speed), visual object learing test (VOLT, measure of spatial learning and memory), abstract matching (AM, measure of abstraction), line orientation test (LOT, measure of spatial orientation), digit symbol substitution test (DSST, measure of complex scanning and visual tracking), balloon analog risk test (BART, measure of risk decision making), NBACK (measure of working memory) and psychomotor vigilance test (PVT, measure, or vigilant attention) and takes approximately 18 min in total.

In the MPT, participants are instructed to click on squares that appear randomly on the screen, each successive square smaller and thus more difficult to track. The MPT is a measure of sensorimotor speed. In the VOLT, participants are asked to memorize 10 sequentially displayed three-dimensional figures. Later, they are instructed to select those objects they memorized from a set of 20 such objects also sequentially presented, some of them from the learning set and some of them new. The VOLT assesses spatial learning and memory. In the AM, the test paradigm presents subjects with two pairs of objects, varied on perceptual dimensions and subjects must classify a target object as more belonging with one of the two pairs, based on a set of implicit, abstract rules. AM measures abstractation and concept formation. The LOT format consists of presenting two lines, one stationary and the other can be rotated by clicking an arrow. Participants rotate the movable line until it is parallel to the stationary line. LOT assesses spatial orientation. DSST, assessing complex scanning and visual tracking, requires participants to refer to a displayed legend relating each of the digits one through nine to specific symbols. One of the nine symbols appears on the screen and the participant must select the corresponding number as quickly as possible. BART, which assesses risk decision making, requires participants to either inflate an animated balloon or collect a reward. Participants are rewarded in proportion to the final size of each balloon, but a balloon will pop after a hidden number of pumps, which changes from trial to trial. The NBACK test is a test of working memory and consists of a sequential presentation of a set of figures, each potentially repeated multiple times. Participants have to respond when the current stimulus matches the stimulus displayed two figures ago. In the PVT, assessing vigilant attention, subjects are instructed to monitor a box on the screen and hit the button once a millisecond counter appears in the box and starts incrementing. Outcome measures on all tests were accuracy, RT, and overall score (/1000). For more detailed information, we refer to Basner et al. (2015).

fNIRS measurements

Functional NIRS, a non-invasive optical imaging technique, was used to assess acute neuronal hemodynamic changes (Octamon continuous-wave NIRS (CW-NIRS) system (Artinis Medical Systems B.V., The Netherlands). By introducing near-infrared light through the skull, oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb) absorb light at 800–940 and 600–750 nm, respectively, allowing the measurement of their relative concentrations in the cerebral blood (Perrey 2008). This device uses the modified Beer-Lambert law to monitor concentration changes in ΔHbO_2 and ΔHHb (in μM cm) relative to first datum. Concentration changes are the result of the interplay between regional CBF, blood volume and metabolic rate of O_2. NVC is reflected by an increase in ΔHbO_2 and decrease in ΔHHb (Steinbrink et al. 2006).

A six-channel fNIRS system was used to measure ΔHbO_2 and ΔHHb using an age-dependent constant differential path-length factor given by $4.99 + 0.0067 \times (\text{age } 0.814)$ (Duncan et al. 1996). The unit consisted of a headband with six light emitters and two light detectors, with an interoptode distance of 3.5 cm. The device placement was replicated between trials by positioning the bottom of the headband 1 cm above the eyebrows, and the middle of the headband in the center of the forehead. Thus, the six fNIRS optodes (six emitters and two detectors) were placed symmetrically over the anterior and medial prefrontal cortex (PFC) (Brodmann areas 46 and 10) (Okamoto et al. 2004). Data were acquired at a sampling frequency of 10 Hz and down sampled by factor 10 for data analysis.

Data were normalized to a 30-s resting period, during which participants sat still and did not speak, 30 min after entering the climatic chamber and prior to the start of the cognitive test battery. This normalization was done in order to reflect changes from this 30 s-reference measurement to express the magnitudes of changes. Mean concentration changes (ΔHbO_2 and ΔHHb) during the last 30 s of each bout of the cognitive test (durations between 40 s and 5 min) were calculated for each cognitive test. Data from the channels on the left (T1, T2, T4) and right (T6, T7, T8) PFC were averaged for each cognitive task. The

use and limitations of NIRS for monitoring cerebral regional hemodynamic changes and oxygenation have been extensively reviewed (Rooks et al. 2010).

EEG and Sloreta

Before and during the cognitive test battery, brain activity was continuously measured by 32 active Ag/AgCl electrodes attached on the subject's head (Acticap, Brain Products, Munich, Germany), according to the 10–20 International System. Electrode impedance was kept below 10 kΩ during the entire recording. During a 2-min baseline measurement, 30 min after entering the climate room, subjects were instructed to sit still, not speak, and minimize eye movement. During the cognitive test battery, events were created in the software to indicate start and endings of each part of the cognitive battery.

Brain Vision Analyzer (version 2.1) was used to preprocess and process the data. First, raw data were downsampled to 256 Hz, filtered (high pass 1 Hz, low pass 45 Hz and Notch 50 Hz, Slope 48 dB/oct) with a Butterworth filter design and re-referenced to an average reference. Then, data were segmented in segments of interest (baseline and each cognitive task) and artifacts were manually removed by raw data inspection. For the segments of the baseline measurement and each part of the cognitive task, artifacts were further removed by using ICA and inverse ICA upon manual artifact removal. Subsequently, data were segmented and averaged to a 4 s window (1024 data points, frequency resolution: 0.25 Hz) for analysis in sLORETA. sLORETA is a source localization method which analyses whether brain frequency bands differ between 2 conditions and where these differences take place. This analysis attempts to solve the inverse problem by assuming related orientations and strengths of neighboring neuronal sources (Pascual-Marqui 2002). EEG files were converted to cross spectra files and the classical frequency bands of interest (i.e., α1 (8.5–10 Hz), α2 (10.5–12 Hz), β1 (12.5–18 Hz), β2 (18.5–21 Hz), β3 (21.5–30 Hz), δ (1.5–6 Hz), θ (6.5–8 Hz)) were selected and the sLORETA program computed the corresponding 3D distribution of the electric neuronal generators. The latter were computed for each subject and dataset, for each aforementioned frequency band.

Statistics

Statistical analysis was carried out by using the Statistical Package for the Social Sciences, version 22 (SPSS Inc., Chicago, IL, USA), with significance set at 0.05 for all analyses. Data are presented as means ± standard deviation (SD), unless stated otherwise.

Normality of the data was tested by using one-sample Kolmogorov-Smirnov test, while sphericity was verified by the Mauchly's test. When assumption of sphericity was not met, the significances of F-ratios were adjusted with the Greenhouse-Geisser procedure. When normality was violated, non-parametric testing was used.

For accuracy and RT on congruent and incongruent stimuli of the Stroop task, a three-way repeated measure ANOVA was used to assess the effects of stimuli (congruent vs. incongruent), supplement (CF vs. PL), and environment (hypoxia vs. normoxia). For cognitive performance on each task of the Joggle Cognition battery, the effects of supplement (CF vs. PL) and environment (hypoxia vs. normoxia) were analyzed by two-way repeated measure (2 × 2) ANOVAs. The effects of environment, supplement, as well as time (before/after Stroop and before/after cognition battery) on physiological measures HR and SpO_2 were examined by three-way repeated measure ANOVAs. For the fNIRS-data, three-way repeated measure ANOVAs were employed to investigate the effects of side (left or right), environment and supplement on ΔHbO_2 and ΔHHb. If significant interaction effects were found in the three-way or two-way repeated measures ANOVAs, two-way repeated measure ANOVAs (per side) or paired *t* tests (per environment) were respectively performed in order to interpret the effects of supplement in each environment. If no significant interaction effects were observed in the three-way or two-way repeated measures ANOVAs, main effects of environment and supplement were immediately observed and further interpreted through pairwise comparisons with the Bonferroni correction.

For sLORETA, paired sampled *t* tests were computed at each voxel for each cognitive test between the following conditions: PL N vs PL H to assess the effect of hypoxia, PL N vs CF N and PL H vs CF H to assess the influence of the supplement. Non-parametric randomization tests were performed at all voxels simultaneously, since no "a priori" hypotheses existed. To correct for these multiple comparisons, the statistical program of sLORETA was based on Fisher's permutation test and relied on a bootstrap method with 5000 randomizations. A nonparametric single-threshold test was assessed, defining a critical threshold (t critical), to correct for multiple comparisons. Voxels with statistic values exceeding this threshold have their null hypothesis, i.e., no difference in EEG power between two conditions, rejected. The omnibus hypothesis (that all the voxel hypotheses are true) was rejected if a voxel value exceeded the critical threshold for $p < 0.05$ defined by 5000 randomizations. The statistical non-parametric map method provided voxel information of the results (i.e., Montreal Neurological Institute/Talairach coordinates, Brodmann area (BA), lobe, and structure).

Results

Physiological measures

Acute exposure to hypoxia significantly lowered SpO_2 compared to normoxia (hypoxia 83.8 ± 2.1%; normoxia 98.0 ± 1.0%; main effect of environment ($F(1,19) = 1135.51$, $p < 0.001$)). CF supplementation and time did not affect SpO_2. HR was significantly elevated during the cognitive test, compared to before the test (main effect of time ($F(1,19) = 27.11$, $p < 0.001$)) and was 4.3% higher in hypoxia (81.99 ± 2.9 bpm) than in normoxia (78.58 ± 2.41 bpm) (main effect of environment ($F(2,34) = 4.47$, p 0.046)). HR was not affected by CF supplementation.

Cognitive performance

Stroop task For accuracy on the Stroop task, main effects of environment ($F(1,15) = 4.71$, p 0.046) and stimuli ($F(1,15) = 30.71$, $p < 0.001$) were found; post hoc analyses showed that accuracy was significantly lower in hypoxia (90.6 ± 4.5%) compared to normoxia (92.5 ± 4.3%) and that accuracy was significantly lower on the incongruent stimuli (88.9 ± 2.6%) compared to the congruent stimuli (94.2 ± 1.3%) (Tables 1 and 2). CF intake did not influence accuracy. RT was not slower in hypoxia compared to normoxia and was not affected by CF intake. The significant main effect of stimuli ($F(1,17) = 81.41$, $p < 0.001$) indicated that RT was significantly higher on the incongruent stimuli (627.0 ± 70.7 ms) than congruent stimuli (571.1 ± 65.3 ms).

Joggle cognition battery Performances on the LOT, MPT, NBACK, BART, and DSST were neither affected by hypoxia (compared to normoxia) nor by CF intake (compared to PL) (Table 1). The score (expressed as a number/1000) on the PVT tended to be lower in hypoxia (762.3 ± 148.4) compared to normoxia (793.6 ± 160.6) (main effect of environment $F(1,19) = 3.19$, p 0.075)). RT on the VOLT tended to be slower in hypoxia (1844.6 ± 567.7 ms) compared to normoxia (1753.0 ± 585.6 ms) (main effect of environment ($F(1,19) = 3.23$, p 0.080)). A two-way interaction effect *environment* × *supplement* ($F(1,19) = 4.99$, p 0.038)) was found for RT on the AM. Post hoc t tests showed that in normoxia, RT was faster after CF intake (2085.2 ± 171.4 ms) compared to PL intake

Table 1 Cognitive performance in normoxia and hypoxia after acute cocoa flavanol or placebo intake. MPT: motor praxis task, VOLT: Visual Object Learning Task, AM: abstract matching, LOT: line orientation test, DSST: digit symbol substitution test, BART: balloon analog risk test, PVT: psychomotor vigilance test, RT: reaction time. $p < 0.05$

$N = 20$ Task		Placebo		Cocoa flavanol	
	Outcome	Normoxia	Hypoxia	Normoxia	Hypoxia
Stroop-congruent	Accuracy (%)	95.1 ± 2.6	93.8 ± 4.2[a]	94.8 ± 3.9	93.1 ± 3.9[a]
	RT (ms)	566.4 ± 66.3	575.2 ± 59.7	567.8 ± 58.9	575.1 ± 76.3
Stroop-incongruent	Accuracy (%)	89.7 ± 5.8	87.2 ± 11.1[a]	90.3 ± 6.7	88.4 ± 6.4[a]
	RT	621.8 ± 75.5	631.5 ± 66.8	631.5 ± 68.7	623.2 ± 74.8
LOT	Score (/1000)	815.5 ± 94.7	841.6 ± 62.1	813.0 ± 95.7	820.3 ± 68.3
	RT (ms)	5981.5 ± 1570.5	5491.1 ± 1045.4	5861.2 ± 1925.7	5798.5 ± 1217.6
	Accuracy (%)	61.1 ± 13.7	62.2 ± 12.5	62.7 ± 13.7	60.0 ± 17.6
MPT	Score (/1000)	815.5 ± 94.7	841.6 ± 62.1	813.0 ± 95.7	820.3 ± 68.3
	RT (ms)	5981.5 ± 1570.5	5491.1 ± 1045.5	5861.2 ± 1925.7	5798.5 ± 1217.6
NBACK	Score (/1000)	724.8 ± 142.2	748.0 ± 158.5	687.4 ± 172.3	731.4 ± 179.8
PVT	Score (/1000)	809.0 ± 153.5	765.6 ± 151.9	778.1 ± 158.4	759.0 ± 179.8
	RT (ms)	243.6 ± 24.3	247.4 ± 23.7	248.0 ± 27.2	257.5 ± 43.9
VOLT	Score (/1000)	736.4 ± 114.8	699.5 ± 112.7	728.4 ± 117.3	720.6 ± 112.7
	RT (ms)	1680.2 ± 493.4	1906.5 ± 698.4	1825.7 ± 676.4	1782.7 ± 547.0
AM	Score (/1000)	575.6 ± 85.2	586.4 ± 51.1	557.8 ± 79.4	611.7 ± 51.1
	RT (ms)	2257.2 ± 836.8	1967.6 ± 664.6	2085.2 ± 746.9[b]	1981.2 ± 679.4
BART	Score (/1000)	920.0 ± 92.2	908.6 ± 110.2	930.0 ± 85.4	914.6 ± 93.4
DSST	Score (/1000)	973.9 ± 21.2	973. 1 ± 13.5	971.7 ± 26.6	960.9 ± 43.5
	RT (ms)	932.0 ± 70.0	918.3 ± 54.0	917.8 ± 67.1	919.9 ± 85.3
	Accuracy (%)	99.1 ± 0.0	99.0 ± 0.0	99.1 ± 0.0	98.5 ± 0.0

[a] Main effect of hypoxia in two-way repeated measures ANOVA

[b] Main effect of supplement in two-way repeated measures ANOVA

Table 2 Summary of results. CF: cocoa flavanols, ΔHHb: change in deoxy-hemoglobin, assessed by near-infrared spectroscopy, MPT: motor praxis task, VOLT: Visual Object Learning Task, AM: abstract matching, LOT: line orientation test, DSST: digit symbol substitution test, BART: balloon analog risk test, PVT: psychomotor vigilance test, BA: Brodmann area

TEST	Cognitive performance		ΔHHb (prefrontal cortex)		Neuronal activity	
	Hypoxia	CF	Hypoxia	CF	Hypoxia	CF
Stroop	↓	=	=	=	=	=
MPT	=	=	↑	=	=	=
VOLT	↓	=	=	=	=	=
NBACK	=	=	=	=	=	=
AM	=	↑	=	=	↑ β2 in BA4	=
LOT	=	=	=	↑	=	=
DSST	=	=	=	↑	=	=
BART	=	=	=	↑	=	=
PVT	↓	=	=	=	=	=

(2257.2 ± 192.0 ms) ($t = -2.75$, p 0.013), while there was no significant difference between CF and PL intake in hypoxia.

fNIRS

The typical hemodynamic response to neural activity is characterized by a concomitant increase in ΔHbO_2 and decrease in ΔHHb. One-sample t tests compared ΔHbO_2 and ΔHHb with zero for each test in each condition, showing significant changes (and thus hemodynamic coupling) during all cognitive tests (Fig. 1).

Stroop task ΔHbO_2 and ΔHHb during the Stroop task were not affected by hypoxia, nor by CF supplementation. Three-way repeated measures ANOVA showed a main effect of side ($F(1,13) = 5.36$, p 0.046) for ΔHbO_2 and ΔHHb with a larger increase in ΔHbO_2 and larger decrease in ΔHHb at the right side of the PFC compared to the left side.

Joggle cognition battery During the PVT, AM, NBACK, and VOLT, ΔHbO_2, and ΔHHb were not altered by CF intake nor by hypoxia. During DSST, BART, and LOT, CF intake enlarged the decrease in ΔHHb in the right PFC but did not influence ΔHbO_2 (ΔHHb: supplement × side effect: BART ($F(1,9) = 7.79$, p 0.025), DSST ($F(1,9) = 5.20$, p 0.049) and LOT ($F(1,9) = 5.14$, p 0.049)). ΔHbO_2 and ΔHHb were not altered by hypoxia. During MPT, hypoxia increased ΔHbO_2 compared to normoxia (main effect of environment: $F(1,9) = 8.03$, p 0.020), but ΔHHb was not altered. CF intake did not alter ΔHbO_2 nor ΔHHb during MPT.

sLORETA

sLORETA confirmed that the different cognitive tasks lead to activation of specific brain regions in several frequency bands, compared to the baseline condition. During AM, increased β2 activity was found in hypoxia compared to normoxia, in the frontal lobe precentral gyrus (BA4; $p < 0.05$, $t = 5.33$* (t_{crit} for $p < 0.05 = 5.09$)), which was of highest significance at MNI coordinate $x, y, z = -35, -20, 55$ (Fig. 2). No further significant changes were obtained for the other frequency bands. During all other cognitive tests, there was no significant difference in brain activity in any of the frequency bands, between hypoxia and normoxia, in any brain region.

Statistical overall analysis by means of the omnibus significance test in sLORETA revealed no differences in brain activity in none of the frequency bands during all the cognitive tests, between CF and PL intake in normoxia. Similarly, during all the cognitive tests, brain activity in each frequency band was not significantly different in any of the brain regions between CF and PL intake in hypoxia.

Discussion

The main findings of this study were as follows: (i) acute CF intake enhanced NVC in the right PFC during tasks known to activate the PFC (risk decision making, visual tracking and complex scanning and spatial orientation). This was not associated by altered neuronal activity. Acute CF intake did not affect any outcome measure of cognitive performance, with the exception of improved RT on abstract thinking in normoxia. (ii) Although selective attention and response inhibition were reduced in hypoxia, other cognitive domains were preserved. Exposure to hypoxia did neither change NVC nor brain activity, with the exception of increased β2 activity in the frontal lobe during abstract thinking.

Effects of CF intake

To the best of our knowledge, this was the first study simultaneously investigating the effects of CF on the cerebrovascular response and neuronal activity during cognitive performance, both in normoxia and hypoxia. Acute CF intake increased the hemodynamic response in normoxia and hypoxia: ΔHHb was larger in the right PFC during several cognitive

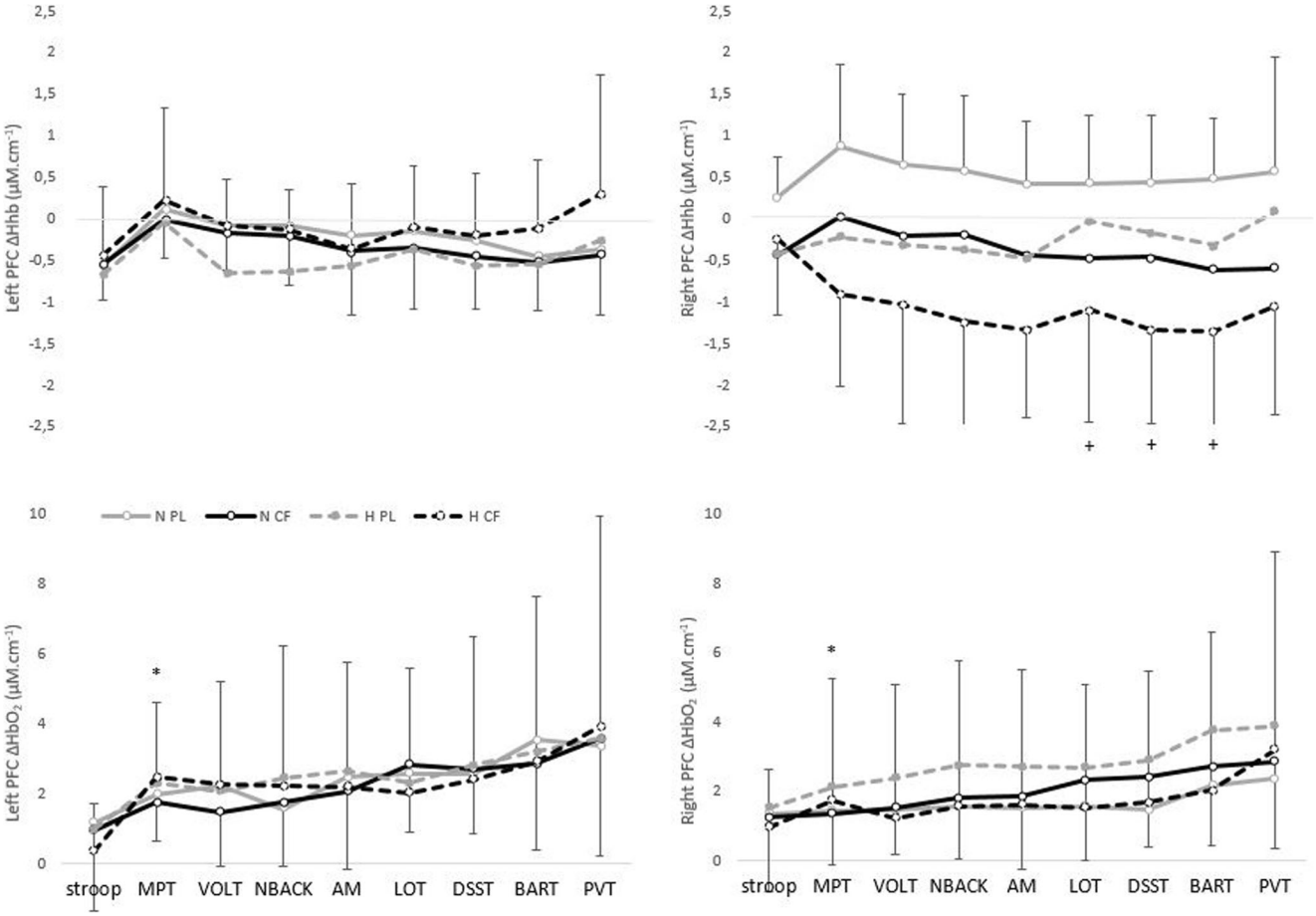

Fig. 1 Hemodynamic changes in deoxyhemoglobin (ΔHHb, **a**) and oxyhemoglobin ($\Delta Hb0_2$, **b**) in the left and right prefrontal cortex (PFC) during a cognitive test battery at 4000 m simulated altitude (hypoxia (H), dashed lines) and at sea level (normoxia (N), full lines) after acute cocoa flavanol (CF) supplementation (black lines) or placebo (PL, gray lines). *Significant main effect of environment (altitude) ($p < 0.05$); $^{+}$Significant difference between CF and PL in post hoc test (significant *supplement* × *side* interaction effect ($p < 0.05$)). MPT motor praxis task, VOLT Visual Object Learning Task, AM abstract matching, LOT line orientation test, DSST digit symbol substitution test, BART balloon analog risk test, PVT psychomotor vigilance test

tasks activating the PFC (tests assessing risk decision making, working memory and spatial orientation) after CF intake than PL. A hemispheric lateralization (i.e., greater ΔHHb and ΔHbO_2 in the right compared to left side of PFC) during cognitive tasks was previously also seen in the study of Medvedev et al., pointing to the primary involvement of the right hemisphere in resolving these cognitive tasks (Medvedev et al. 2011). Thus, CF intake induced a larger

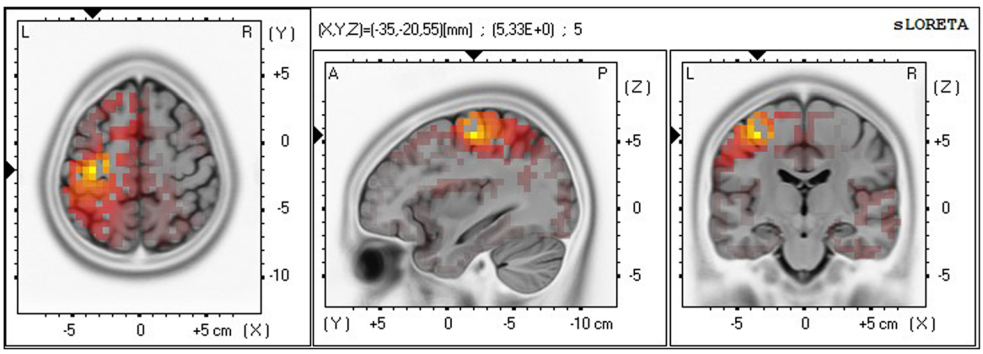

Fig. 2 Statistical parametric maps (SPM) of sLORETA during the abstract thinking test after 30 min exposure to hypoxia (4000 m simulated altitude), compared to normoxia. Red and yellow colors indicate increased activity in the β1-frequency range, which was found to be significant in the frontal lobe precentral gyrus. SPMs are based on voxel-by-voxel t-values of differences. Structural anatomy is shown in grayscale. L left, R right, A anterior, P posterior

ΔHHb in the hemisphere which was most involved in solving the cognitive task.

It has been shown that ΔHHb has the highest correlations with the BOLD fMRI response (Steinbrink et al. 2006) and that ΔHHb can be considered as a better indicator of cerebral hemodynamic changes in hypoxic conditions (Davranche et al. 2016). Thus, the lack of a significant change in ΔHbO_2 following CF intake might be explained by interference of hypoxia-induced extracortical changes (e.g., increased HR) with cerebral hemodynamic ΔHbO_2. Alternatively, the effect of CF on ΔHHb, without altering ΔHbO_2 could theoretically also reflect a decreased metabolic rate of O_2 consumption during neuronal activation (Perrey 2008). While global cerebral rate of O_2 consumption can be assessed by magnetic resonance (Scholey 2017), this has not yet been applied to acute CF intake.

The hemodynamic response (decrease in ΔHHb) to neuronal activity originates from an exaggerated regional increase in CBF in response to neuronal activity and O_2 extraction. Evidence exists that acute CF intake stimulates vasodilation and CBF by increasing eNOS-dependent NO production (Schroeter et al. 2006). While we found an increased NVC upon acute CF intake, we could not detect any changes in neuronal activity, as measured by EEG and sloreta. The lack of change in neuronal activity in this study contradicts a "direct" neuromodulatory effect of *acute* CF intake and points towards a purely vascular factor as underlying mechanism of the improved NVC (Spencer 2009; Socci et al. 2017). Besides, this shows that acute CF does not improve neuronal efficiency.

While CF intake enhanced NVC, it did not result in better cognitive function. Only during abstract thinking, acute CF intake improved the speed of processing while maintaining the overall accuracy in normoxia. Since this was neither accompanied by a change in hemodynamic response in the PFC nor by changed brain activity in any region, we could not unveil the underlying mechanism. Likewise, Francis et al. also found no behavioral changes despite the increased NVC after 5-day CF (150 mg) intake (Francis et al. 2006). Since impaired NVC is associated with poorer cognitive functions (Sinn and Howe 2008), it was previously hypothesized that improving NVC may enhance cognitive function (Wong et al. 2016). However, our results indicate that in subjects with a healthy circulation, improving NVC by CF intake does not result in better cognitive performance. Alternatively, the question rises whether this cognitive test battery was sensitive enough to detect effects of dietary interventions, such as CF, on human cognitive function to fully assess their efficacy in healthy young subjects (Macready et al. 2009).

Effect of hypoxia

The brain's high energy demand compared to the low energy stores makes the brain critically dependent on adequate glucose and oxygen supply. This renders the brain particularly vulnerable to hypoxic conditions (Ogoh 2017). In contrast to our hypothesis, most aspects of cognitive function were preserved when acutely exposed to normobaric hypoxia (12.7%) in young healthy subjects. During the Stroop task, accuracy was decreased in hypoxia, while speed of information processing, measured as RT, did not decline. RT on VOLT and the score on PVT tended to be deteriorated in hypoxia ($p = 0.078$, $p = 0.081$), suggesting that spatial learning and memory and vigilant attention were slightly decreased. We did not observe any hypoxia-induced changes on sensory-motor speed, abstraction component of executive function, spatial orientation, working memory (complex scanning and visual tracking), and risk-taking behavior. Cognitive function deteriorates with increasing altitude (Taylor et al. 2016) and exposure to more severe hypoxia (simulated hypoxia of 5500 m) showed severely reduced verbal and visual memory, processing speed, executive function, psychomotor speed, cognitive flexibility, and complex attention (Turner et al. 2015). Moreover, NVC was similar in hypoxia compared to normoxia during all cognitive tasks, except during the PVC where the increase in ΔHbO_2 was elevated in hypoxia. This most likely reflects the hypoxic cerebral vasodilation (Davranche et al. 2016). Similarly, Davranche et al. (2016), Lefferts et al. (2016), and Shannon et al. (2017) found that acute hypoxia did not change the hemodynamic response during a cognitive task. A larger drop in SpO_2 may be necessary to disturb the preservation of cognitive function and hemodynamic response (Taylor et al. 2016). Moreover, neuronal activity was not altered in hypoxia, with the exception of increased β activity, thus increased brain activity (Thompson et al. 2008), in the frontal lobe precentral gyrus during abstract thinking. Schneider et al. (Schneider and Strüder 2009) likewise observed increased β activity at rest following hypoxic exposure in the frontal gyrus. Frontal lobe precentral gyrus is involved in planning, control and executive function and is known to be recruited during this abstract matching task (Trans Cranial 2012; Basner et al. 2015). As abstract thinking was not deteriorated, the larger activation of the frontal lobe precentral gyrus was required to preserve cognitive performance in hypoxia and thus reflects a declined neuronal efficiency (Richard and Benjamin 1988).

Limitations and future perspectives

This study was performed in a young, healthy population with a high cognitive level. This study provides insight in how CF intake can affect the healthy brain in normoxia and hypoxia, which is relevant for mountaineers, people moving to high altitude and aircraft pilots. However, we cannot apply these findings to other population with restricted vascular function such as elderly, people with mild cognitive impairment, dementia, and Alzheimer's

disease (Nehlig 2013). It seems that both age and the "baseline" quality of endothelial function influence the degree in which subjects can benefit from CF consumption; CF induced larger improvements in endothelial function in older adults relative to young adults (Fisher et al. 2006) and CF increased *global* perfusion in younger adults (Francis et al. 2006), while it increased *regional* perfusion (Lamport et al. 2015) in elderly. Therefore, future studies should include subjects with restricted vascular function to examine the beneficial effects of CF on NVC and its influence on cognitive function is larger in such populations.

In this study, we simultaneously used NIRS on the PFC and multichannel EEG, because of its usefulness to correlate hemodynamic responses to neural activity. In interpreting these results, we must keep in mind that despite the excellent temporal resolution to directly measure cortical activity of EEG, its spatial resolution is limited (Thompson et al. 2008). Moreover, another limitation was that we used NIRS only in the PFC and not in the entire cortex. By combining multichannel NIRS with multichannel EEG, more information could be gained between the distinct interplay of neuronal activity and NVC. Both NIRS and EEG are limited to observe changes on the superficial cortex and cannot assess the deeper brain regions, which is only possible with fMRI.

Another limitation of this study is that we did not assess ventilation nor arterial carbon dioxide tension. It is known that CBF changes in hypoxia are not only determined by hypoxic cerebral vasodilation but also by hyperventilation-induced hypocapnic cerebral vasoconstriction (Ogoh 2017). Furthermore, we used a laboratory setting of acute normobaric hypoxia, which was relatively short. Although this setting provided an excellent framework to study neurophysiological changes in response to hypoxia and the fact that normobaric and hypobaric hypoxia provoke similar physiological responses (Mounier and Brugniaux 2012), a longer exposure to hypobaric hypoxia would provide more insight into the mechanisms which play a role in real-life exposure to high altitude.

While we focused on neurophysiological effects of acute CF intake, positive effects on cognition and NVC after chronic CF intake have been shown in aging and clinical populations (Socci et al. 2017). Accordingly, it seems possible that chronic CF intake results in both electrophysiological and neurovascular changes in healthy subjects.

Conclusion

In healthy subjects, acute CF intake enhanced the hemodynamic response in the PFC during cognitive tasks activating the PFC, but did not alter neuronal activity and did not result in improved cognitive function. Acute hypoxic exposure of 12.7% (simulated altitude of 4000 m) barely disturbed normal functioning of the healthy brain, as cerebrovascular responsiveness, neuronal activity, and cognitive performance were maintained, with the exception of increased β2 activity in the frontal lobe during abstract thinking and reduced selective attention and response inhibition in hypoxia.

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Compliance with ethical standards

The study protocol was approved by the Ethical Committee of the Brussels University hospital and was carried out in accordance with the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflicts of interest. LD has a grant "Lotto Sport Science Chair."

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