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RESEARCH ARTICLE

One-week cocoa flavanol intake increases prefrontal cortex oxygenation at rest and during moderate-intensity exercise in normoxia and hypoxia

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Decroix L, Tonoli C, Lespagnol E, Balestra C, Descat A, Drittij-Reijnders MJ, Blackwell JR, Stahl W, Jones AM, Weseler AR, Bast A, Meeusen R, Heyman E. One-week cocoa flavanol intake increases prefrontal cortex oxygenation at rest and during moderate-intensity exercise in normoxia and hypoxia. *J Appl Physiol* 125: 8–18, 2018. First published March 15, 2018; doi:10.1152/japplphysiol.00055.2018.—During exercise in hypoxia, O_2 delivery to brain and muscle is compromised, and oxidative stress is elicited. Cocoa flavanols (CF) have antioxidant capacities and can increase blood flow by stimulating endothelial function. We aimed to examine the effects of 7-day CF intake on oxidative stress, nitric oxide production, and tissue oxygenation in response to exercise in normobaric hypoxia (14.3% O_2). In a randomized, double-blind, cross-over study, 14 well-trained male cyclists completed four trials: exercise in normoxia or hypoxia, after 7-day CF or placebo intake. Flow-mediated dilation (FMD) was measured before intake of the last dose CF or placebo. One hundred minutes later, 20-min steady-state (SS; 45% $\dot{V}O_{2max}$) and 20-min time trial (TT) (cycling) were performed. Blood samples were taken. Prefrontal and muscular oxygenation was assessed by near-infrared spectroscopy. At baseline, FMD was increased by CF. Hypoxia increased exercise-induced elevations in lipid peroxidation and antioxidant capacity. CF suppressed exercise-induced lipid peroxidation but did not influence antioxidant capacity. At rest and during SS, prefrontal and muscular oxygenation was decreased by hypoxia. CF elevated prefrontal oxygenation but did not impact muscular oxygenation. During TT, hypoxia accelerated the exercise-induced decrease in prefrontal oxygenation, but not in muscular oxygenation. During TT, CF did not alter prefrontal and muscular oxygenation. CF did not change plasma nitrite, nitrate, and arginine:citrulline. During high-intensity exercise, CF improved neither tissue oxygenation nor performance in well-trained athletes. At rest and during moderate-intensity exercise, CF reduced exercise-induced lipid peroxidation and partially restored the hypoxia-induced decline in prefrontal oxygenation.

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NEW & NOTEWORTHY For the first time, we showed that CF had beneficial effects on endothelial function at rest, as well as on prefrontal oxygenation at rest and during moderate-intensity exercise, both in normoxia and hypoxia. Moreover, we showed that CF intake inhibited oxidative stress during exhaustive exercise in hypoxia.

altitude; cocoa; endothelial function; exercise; oxidative stress

INTRODUCTION

Several sports such as skiing, mountaineering, and sometimes cycling and running involve exercise at high altitude. The lower barometric pressure at high altitude reduces the partial pressure of inspired oxygen, which results in reductions of O_2 delivery to the active muscles and the brain (38) and elicits the formation of reactive oxygen species (ROS) (26). This leads to a faster development of peripheral and central fatigue, resulting in decreased exercise performance (43). Thus, enhancing O_2 delivery by improving blood flow at high altitude could improve tolerance to physical exercise and recovery thereafter.

One of the key molecules regulating blood flow is nitric oxide (NO). NO is endogenously produced by the conversion of arginine into citrulline by endothelial NO synthase (eNOS), in the presence of O_2. NO exerts its vasodilatory function via stimulating guanylate cyclase and relaxing smooth muscle cells. eNOS-dependent NO production can be limited in conditions of low O_2 availability and high levels of oxidative stress (29, 33). Besides, oxidative stress decreases NO availability by increased NO degradation through the reaction of NO with superoxide, the precursor of most other ROS, to form peroxynitrite (5). Both exercise (32) and hypoxia (26) independently elicit the formation of ROS. While ROS have important roles in cell signaling, apoptosis, gene expression and ion transport, excessive ROS leads to oxidative modification and damage of DNA, RNA, proteins, and lipids. In the context of

exercise, especially at altitude, the excessive ROS formation can lead to impaired muscle contractile and mitochondrial function, resulting in faster development of exercise-induced muscle fatigue and a decreased NO availability (32).

It has been reasoned that modulating NO metabolism by nutritional interventions may influence physiological responses to exercise and, thus, exercise performance in both normoxia and hypoxia (9). Dietary nitrate supplementation, for example, via beetroot juice, has beneficial effects on NO availability, muscle oxygenation, and exercise performance (9), but other supplements hold promise to increase NO availability, too. The intake of cocoa flavanols (CF), a subgroup of polyphenols with antioxidant capacities causes NO-mediated vasodilatation, clinically measured by flow-mediated dilation (FMD) (16, 19). In vitro and in vivo data showed that (−)-epicatechin, the main bioactive constituent of cocoa, increased nitrite concentration, an indirect marker of eNOS-dependent NO production (5, 23). Furthermore, CF and/or their metabolites are strong antioxidants, by directly scavenging superoxide, inhibiting NADPH oxidase (37), and/or by modulating the endogenous antioxidant defense (34).

Despite the existing evidence of the beneficial effects of CF on endothelial function and oxidative stress, few studies investigated the possibilities of CF to modulate exercise-induced oxidative stress and/or exercise performance (1, 8, 13, 15, 31, 41, 42, 44). Because of the large variety of study designs, subject samples, type of exercise, and timing and dosages of CF intake and used in these studies, results concerning the CF-induced decrease in oxidative stress after exercise are inconsistent. These studies were all performed at sea level. However, it may be conceivable that this nutritional strategy is more efficient in hypoxia, where O_2 delivery is reduced and where ROS formation is exaggerated.

Consequently, the objectives of this study were to investigate the effects of a 7-day CF intake on *1*) selected plasma markers of NO availability and oxidative stress, *2*) muscle and cerebral oxygenation in response to an acute exercise bout in normoxia (sea level) and normobaric hypoxia (simulated altitude of 3,000 m, 14.3% O_2), and *3*) its implications for exercise performance. We hypothesized that compared with placebo (PL), CF intake would increase NO availability, decrease oxidative stress, and increase cerebral and muscular oxygenation during exercise in normoxia and hypoxia and enhance exercise performance.

MATERIALS AND METHODS

Participants

A sample size calculation, based on the results of Allgrove et al. (1), Patel et al. (31), and Wiswedel et al. (44), indicated that 14 subjects were required to detect differences at a P value <0.05 with 90% power. The recruitment started in January 2016. Subjects were excluded if they *1*) were younger than 18 yr or older than 36 yr, *2*) smoked or smoked in the past, *3*) took antioxidant supplementation, *4*) trained less than 10 h per week, *5*) stayed at high altitude (>2,000 m) for more than 3 wk during the last 6 mo, or *6*) were hypertensive or had cardiovascular disease, as determined by medical examination before the experiment. Fifteen healthy well-trained male cyclists were selected for participation in this study. One subject dropped out because of an injury (knee injury). The study was approved by the University Hospital Brussel Ethics Committee and was in accordance with the Declaration of Helsinki. The experimental procedures and potential risks were explained to the participants, and written informed consent was provided and signed before the start of the study. This trial was registered at clinicaltrials.gov as NCT03135314, and this article is compliant with CONSORT (Consolidated Standards for Reporting Trials).

Study Design

A randomized, placebo-controlled, counter-balanced, cross-over study design was used. The study was conducted at the Department of Human Physiology of the Vrije Universiteit Brussels (Brussels, Belgium) from March 2016 until July 2016. On the first laboratory visit, subjects underwent a complete medical screening (including skinfold measures) and performed a maximal incremental cycle test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Gröningen, The Netherlands). During this test, initial work rate was set at 80 W, and work rate was then increased every 3 min by 40 W until volitional exhaustion. Maximal oxygen uptake ($\dot{V}O_{2max}$) was determined using the Metalyzer cortex (Biophysik, Leipzig, Germany), and peak power output (PPO) was determined.

Subsequently, subjects visited the laboratory once every 2 wk for 8 wk (four visits): each visit was preceded by a 1-wk washout (except for the first visit) and a 1-wk nutritional intervention (PL or CF). The sequence of the four nutritional interventions was randomly assigned for each participant by using a computer-based randomly permutated block method. The allocation list was generated by C. Tonoli), recruitment of participants was conducted by L. Decroix, and allocation of participants was conducted by E. Lespagnol. Participants and researchers involved in data collection, outcome assessment, and statistical analysis were blinded to the nutritional intervention. Participants and all researchers, except for L. Decroix, were blinded for $F_{I_{O_2}}$.

Subjects performed four interventional trials in randomized order: *1*) exercise in (normobaric) hypoxia (H) (3,000 m; 14.3% O_2) preceded by 7 days of CF intake (H-CF), *2*) exercise in H (3,000 m; 14.3% O_2) preceded by 7 days of PL intake (H-PL), *3*) exercise in normoxia (N) (0 m; 21.0% O_2) preceded by 7 days of CF intake (N-CF), and *4*) exercise in N (0 m; 21% O_2) preceded by 7 days of PL intake (N-PL). All experimental trials were conducted in 20°C, and relative humidity was kept between 30 and 40%.

Supplementation

Subjects were asked to consume the provided supplements (PL or CF; Naturex, Avignon, France) every morning at breakfast during the 6 days before the testing day. On the testing day, subjects consumed the last dose of supplements upon arrival in the laboratory. The daily dose of CF consisted of four capsules, containing a total of 1,765 mg cocoa extract of which there was 100 mg of epicatechin (EP), 23 mg of catechin, 119 mg of theobromine, and 17 mg of caffeine (Table 1). The dose and duration of the supplementation were based on the finding that 1 wk of CF intake enhances vascular function in a dose-dependent manner, with an optimal effect of 100 mg EP (16) and

Table 1. *Composition of cocoa flavanol and placebo supplementation (Naturex, daily dose)*

Content in Four Pills	PL	CF
Cocoa extract, mg	0	1,764
Maltodextrin, mg	1,764	136
Total flavanols, mg	0	530
Total monomers, mg	0	121
(−)-Epicatechin, mg	0	100
(+)-Catechin, mg	0	21
Theobromine, mg	119	119
Caffeine, mg	17	17

CF, cocoa flavanol; PL, placebo.

on the pooled results from a recent meta-analysis where 1 wk of polyphenol intake increases performance (36). The PL capsule contained 1,765 mg maltodextrin and was matched with the CF capsule in color and shape, theobromine, and caffeine content. From the blinding check, it was clear that subjects were unable to distinguish between the two interventions. The nutritional intervention was double-blinded and counter-balanced. Subjects were provided with a list of foods rich in polyphenols that they should avoid throughout the 8-wk study. They were asked to abstain from caffeine during the last 24 h before each intervention trial and to repeat the same nutritional regimen during the last 24 h before each intervention trial. Subjects completed a 24-h food recall on three random days during the study, to check for a potential influence of polyphenol intake on the measurements.

Four Interventional Trials

Subjects were asked to keep a training diary for the entire duration of the study and to repeat the same weekly training regimen (volume and intensity) for the duration of the study. They were instructed to abstain from intensive training the last 24 h before each intervention trial. On each visit, subjects arrived at the laboratory at the same time of the day in a 3-h fasted state. The entire protocol is depicted in Fig. 1. First, a baseline FMD measurement took place. Subsequently, a catheter was placed in a forearm vein, and the first venous blood sample was collected. Subjects then consumed the last dose of their supplementation, together with a carbohydrate-rich meal, which was carefully selected by a nutritionist to contain 600 kcal, 85% carbohydrates, 10% proteins, and 5% fat. After the meal, subjects entered the isobaric hypoxic chamber, which was preset at the desired percentage of O_2. Subjects were asked to sit down and relax. It was shown that the maximal concentration of plasma flavanols is reached 100 min after acute CF intake, and the plasma concentration of flavanols remains at a maximum for 50 min (35). Therefore, 95 min after the last intake of CF, a second blood sample was taken. Subsequently, the participants started a 20-min steady-state (SS) cycling exercise, 100 min after the last dose. During the SS, power output was fixed at 45% of their PPO. SS was followed by 5-min passive rest in a seated position, and a blood sample was taken. The 20-min timed trial (TT) then started at 75% of PPO, but subjects were free to increase or decrease their power output as desired from the outset. The goal was to perform as much as possible during 20 min. Subjects received information on the time lapsed but did not receive any feedback regarding power output or heart rate (HR). HR and saturation (Sa_{O_2}) were recorded continuously during the experimental trial using a chest belt and Polar HR monitor and a pulse oximeter, which was positioned on the participants' left index finger (Medlab, Stutensee, Germany). Rate of perceived exertion (RPE) was measured at the start and after 5, 10, 15, and 20 min of the SS and the TT. Blood lactate was enzymatically determined in a capillary blood sample from the ear lobe (Ekf, Biosen 5030, Magdeburg, Germany), at the start, after 10 min, and after 20 min of the SS and the TT. During the 20-min TT, the completed work (kJ) was used as the main outcome parameter of exercise performance. The occurrence of acute mountain sickness (AMS) was assessed using the Lake Louise Questionnaire at the end of each trial, but none of the subjects experienced any symptoms of AMS.

Measurements

Primary outcome measures were muscle and cerebral oxygenation, markers of oxidative stress, and exercise tolerance and performance. Secondary outcome measures included flavanol metabolites, markers of NO availability, FMD, and mean arterial pressure.

Flow-Mediated Dilation and Mean Arterial Pressure

Upon arrival at the laboratory, subjects were instructed to relax in a supine position for 10 min, during which, mean arterial pressure (MAP) was measured automatically (BU510; Medisana, Kerkrade, Netherlands). Then, arterial endothelial function was assessed by FMD. The operator, blinded to PL or CF condition, took images of the vessel upon arrival of the participants (baseline measure) with a 5–10-Hz linear array probe. The scan of the right brachial artery, ~2–7 cm above the antecubital fosse (marked on the first visit, ensuring that measurement occurred at the same place for each scan), was evaluated in the longitudinal image. The sphygmomanometer placed around the forearm (distal) was inflated 50 mmHg above the systolic blood pressure for 5 min. A continuous scan of the brachial artery was then performed during 90 s after the rapid deflation of sphygmomanometer, which induced shear stress and endothelium-dependent dilation. Each brachial artery diameter was manually measured three times at end of diastole, 60 s after the cuff deflation. The FMD (%) was calculated as [(hyperemic diameter – preinflation diameter)/preinflation diameter]·100.

Muscle and Cerebral Oxygenation during Exercise

Near-infrared spectroscopy (NIRS) [Portalite continuous-wave NIRS system (Artinis, Elst, Netherlands)], a noninvasive optical imaging technique, was used to assess changes in oxygenation status of the prefrontal cerebral cortex and the vastus lateralis during exercise. The use of NIRS to assess tissue oxygenation, including its limitations, has been extensively described (11).

Upon entrance in the isobaric hypoxic chamber, one emitter/receptor optode pair was positioned over the left prefrontal cortical area between Fp1 and F3, according to the modified international EEG

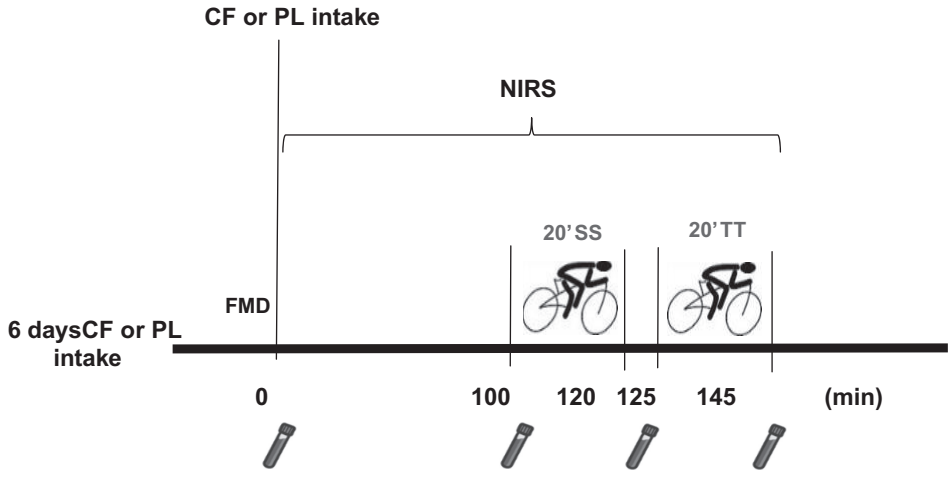

Fig. 1. Interventional exercise protocol, twice executed in hypoxia and twice in normoxia, following 7 days of cocoa flavanol (CF) or placebo (PL) intake. FMD, flow-mediated dilation; NIRS, near infrared spectroscopy at vastus lateralis and prefrontal cerebral cortex; SS, steady state; TT, time trial.

10-20 system. One emitter/receptor optode pair was attached to the (shaved) skin on the lower one-third of the belly of the right vastus lateralis (middle between the lateral epicondyle and trochanter). Skinfold measures during the medical screening assured that the adipose tissue thickness was well below 1.5 cm to allow the NIRS photons to penetrate into the muscle (11). The interoptode distance for both probes was 4 cm, and the probes were covered with a black cloth to minimize intrusion of extraneous light. A dark elastic band was wrapped around the head and the leg to keep the NIRS-optode pairs in place.

NIRS data collection was started 20 min before the start of exercise. To collect a baseline NIRS value, the mean of a 2-min period during which subjects sat still without speaking or moving, was calculated. The tissue saturation index (TSI), which was determined by spatially resolved spectroscopy, offers a surrogate measure of the fraction of O_2 saturated hemoglobin and myoglobin, reflecting a tissue oxygenation status in percentage (%) (11). Data were collected at a sampling frequency of 5 Hz and were down-sampled with factor of 5 for analysis. During exercise, mean TSI was calculated per 30-s window. NIRS values of the following 30-s epochs were used for data analysis: 0, 5, 10, 15, and 20 min of the SS and TT.

Blood Analyses

Venous blood samples were collected at baseline and at the start and end of the SS and the TT. Blood was collected into 5-ml EDTA tubes, 5-ml heparinized tubes, and 8-ml anticoagulant-free tubes and were centrifuged immediately to obtain plasma or after 30 min at room temperature to allow clotting to obtain serum (10 min at 704 *g*, 4°C). Plasma and serum were aliquoted and stored at −80°C until further analyses. Values were corrected for changes in plasma volume using the hematocrit and hemoglobin concentration, according to Dill and Costill (7). Hemoglobin concentration was measured in duplicate using an azidemethemoglobin double-wavelength photometer method (Hemocue Hb201+, Angelholm, Sweden), while hematocrit was determined by microcentrifugation in triplicate (Heraeus Pico 17).

Serum Flavanols

Serum samples were analyzed for EP and catechin concentrations, as described by Neukam et al. (27). In detail, 0.5 ml of serum was mixed with 1.0 ml phosphate buffer [100 mM, pH 5, containing ascorbic acid (20 mg/ml), EDTA (1.5 mg/ml)] and 20 μl glucuronidase/sulfatase (100,000 and 7,500 units/ml, respectively) and incubated at 37°C for 30 min to hydrolyze glucuronate and sulfate conjugates of epicatechin and catechin. Then, 5 ml *tert*-butyl methyl ether was added and vortexed for 1 min. For phase separation, the mixture was centrifuged at 10°C for 5 min at 1,957 *g*. The organic phase was transferred to a new tube, and after a second extraction with 5 ml *tert*-butyl methyl ether, the combined extracts were dried under a stream of nitrogen and stored at −80°C. For HPLC analyses, the dry residues were reconstituted in 200 μl methanol, vortex-mixed, and centrifuged at 5°C at 15,339 *g* for 5 min. Twenty microliters of the supernatant were injected onto the HPLC column. For HPLC analysis, a reversed-phase RP18 end-capped column (LiChrospher 100, 5 μm, 250 × 4 mm; Merck, Darmstadt, Germany) coupled with a guard column (LiChrospher 100, 5 μm, 4 × 4 mm; Merck) was used. Detection was accomplished using an excitation wavelength of 280 nm and an emission wavelength of 310 nm. Data were recorded by HPLC-System Manager software (Merck/Hitachi). Samples were eluted from the column at 20°C using a step-gradient as follows: from 0 to 18 min 53% acetonitrile/H_2O/acetic acid (150:846:4)/47% H_2O/acetic acid (1,000:5) and from 18 to 27 min 80% acetonitrile/H_2O/acetic acid (150:846:4/20% H_2O/acetic acid (1,000:5). To elute retained compounds, the column was flushed from 27 to 60 min with 100% acetonitrile/acetic acid (1,000:4), and subsequent equilibration was performed from 60 to 80 min with starting conditions. The flow rate was 1.5 ml/min. Under these conditions, the analytes elute with retention times of 15.4 min for catechin and 24.9 min for epicatechin. Peak areas of catechin and epicatechin were used to calculate the concentrations applying the external standard method. Standard curve linearity was observed in the range of 0.125–20 μM for both compounds.

Plasma Nitrite and Nitrate

Plasma nitrite (NO_2^-) and nitrate (NO^-) were analyzed by gas-phase chemiluminescence analysis. Plasma was deproteinized with ice-cold ethanol. For NO_2^- analysis, samples were injected into a glass purge vessel containing 5 ml glacial acetic acid and 1 ml NaI solution, which reduces NO_2^- to nitric oxide (NO) gas, which is carried into the NO detector in inert nitrogen. For NO_3^- analysis, samples were reduced in a solution of vanadium (III) chloride in 1 M hydrochloric acid (0.8% wt/vol). Quantification of NO was enabled by the detection of light emitted during the production of nitrogen dioxide formed upon reaction of NO with ozone. Luminescence was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i; Analytix, Durham, UK). The concentrations of NO_2^- and NO_3^- were determined by plotting signal area (mV) against a calibration plot of 25 nM to 1 μM sodium nitrite and 100 nM to 10 μM sodium nitrate, respectively. The NO_3^- concentration was then corrected by deduction of the NO_2^- value, since the vanadium chloride solution also reduces nitrite.

Plasma Arginine and Citrulline

One-hundred-and-fifty microliters of internal standard (50 μM arginine, methanol mixture) was added to 10 μl of heparinized plasma and centrifuged (13,000 rpm, 10 min, 4°C) to remove the precipitated proteins. Supernatant was collected and dried under a stream of nitrogen at 70°C. The dried extract was dissolved in 100 μl of a butanol solution containing 3 N HCl and kept at 70°C for 40 min. The solvent was removed by evaporation under nitrogen flow at 70°C. The sample was then dissolved in 2.5 ml of water-methanol (90:10, vol/vol) containing 0.1% formic acid, and 5 μl was injected into an analytical column [Kinetex C18 (5 μm, 2.1 × 100 mm)]. Mass spectrometric analysis was performed using an UFLC-XR Shimadzu coupled with a QTRAP 5500 hybrid system, equipped with a Turbo VTM ion197 source (AB Sciex, Foster City, CA). Multiple reaction monitoring (MRM) measurement was performed using optimal cone and collision energy values. Each run was performed at a flow rate of 500 μl/min at 30°C, lasting 9 min in total. A gradient profile consisted of *solution A* (water with 0.1% (vol/vol) formic acid) and *solution B* (methanol with 0.1% (vol/vol) formic acid). The percentage of organic solution B was gradually changed as follows: 0 min, 2%; 4 min, 7%; 6 min, 50%; 7 min, 2%; 9 min, 2%. Data were acquired using Analyst Software version 1.5.2. Calibration curves, performed in water, were obtained by adding increasing concentrations of arginine and citrulline from 12.5 to 125 μM.

Quantification of Total Antioxidant Capacity, Uric Acid, and Malondialdehyde in Plasma

Plasma antioxidant capacity was quantified as Trolox equivalent antioxidant capacity (TEAC), according to Fischer et al. (12). In this procedure, the decolorization of the preformed green-blue 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical by the heparinized plasma within a fixed time reflects the antioxidant capacity of the sample. To correct plasma TEAC values for individual differences in uric acid (UA) concentrations, the most abundant antioxidant in blood, UA plasma concentrations were quantified by HPLC (34). Malondialdehyde (MDA), a marker of lipid peroxidation resulting from oxidative stress, was quantified in EDTA-plasma samples after derivatization with thiobarbituric acid by using HPLC with fluorometric detection, as described by Lepage et al. (22).

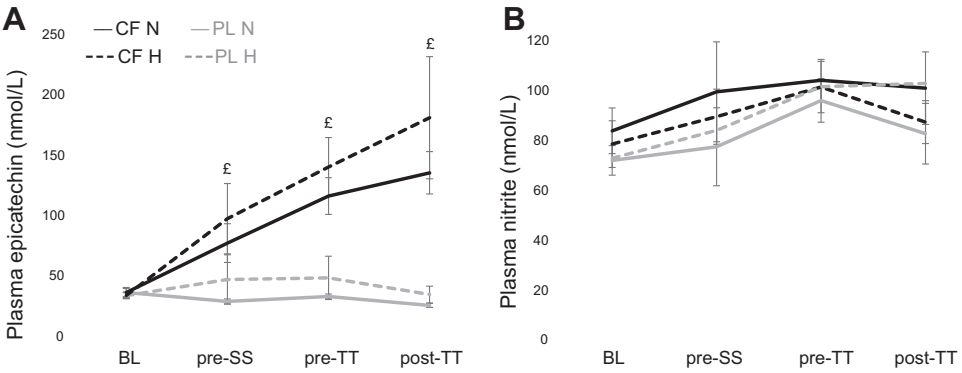

Fig. 2. Effect of 7-day cocoa flavanol (CF; black lines) or placebo (PL; gray lines) supplementation in hypoxia (H; dashed lines and normoxia (N; solid lines) on plasma epicatechin (*A*) and plasma nitrite (*B*) concentrations. BL, baseline, before intake of the last dose; Pre-SS, at the start of the 20-min steady-state exercise (45% of peak power output); pre-TT, at the start of the 20-min time trial; post-TT, at the end of the 20-min time trial. Values are expressed as means ± SE. £$P < 0.05$, main effect of supplement.

Statistical Analyses

A power calculation to determine the minimal sample size required to determine whether CF intake would affect exercise-induced markers of oxidative stress and exercise performance (TT) ($n = 15$, $P = 0.05$, power = 0.8) was based on the results of Allgrove et al. (1), Patel et al. (31), and Wiswedel et al. (44).

Statistical analyses were performed with IBM SPSS Statistics (version 22; IBM, Armonk, NY) and were considered significant at $P < 0.05$. Data are presented as means ± SD, as proposed by Curran-Everett and Benos (6), for $n = 13$, except when otherwise indicated. Normality and sphericity of the data were assessed via the Kolmogorov-Smirnov test and Mauchly's test. To follow the absorption and metabolism of EP and catechin after 6-day CF intake and after intake of the final dose of CF, a three-way repeated-measures ANOVA [FI_{O_2} × supplement × time (baseline, start SS, start TT, and end TT)] was used. Two-way, repeated-measures ANOVA (fraction of inspired O_2 (FI_{O_2}) × supplement) at baseline was used to assess the baseline differences in nitrite, nitrate, arg:citr, TEAC, UA, MDA, MAP, and FMD between 6-day CF and PL intake, with interpretation of the main effect of supplement. Two-way repeated-measures ANOVAs (FI_{O_2}) × supplement) were used to assess differences in nitrite, nitrate, arg:cit, TEAC, UA, MDA, and muscular and prefrontal TSI between CF and PL in H and N at start of SS (acute effect supplement and FI_{O_2}) and start of TT. For significant interactions between FI_{O_2} and supplement, pairwise comparisons were performed using the post hoc Bonferroni correction. Two three-way repeated-measures ANOVAs (FI_{O_2} × supplement × time) were used to assess differences between CF and PL in H and N during exercise (1 for SS, 1 for TT) for the following outcome parameters: nitrite, nitrate, arginine:citrulline ratio (arg:citr ratio), TEAC, UA, MDA, work performance during TT, muscular and prefrontal TSI. The effects of H and CF intake on TT performance (work performed after 20 min) were assessed by a two-way repeated-measures ANOVA (FI_{O_2} × supplement). Significant interactions in the three-way repeated-measures ANOVA were further analyzed by two-way repeated-measures ANOVA with subsequent paired *t*-tests to interpret the effect of the supplement over time at each FI_{O_2} (N and H) and the effect of FI_{O_2} over time after CF or PL intake. If no significant interaction effects were observed, main effects were immediately interpreted through pairwise comparisons with the Bonferroni correction. Significant interactions in the two-way repeated-measures ANOVA (supplement × FI_{O_2}) were further analyzed by paired *t*-tests to interpret the effect of the supplement at each FI_{O_2} (N and H) and the effect of FI_{O_2} after each supplementation. RPE was not normally distributed and was, therefore, analyzed by Friedman tests and Wilcoxon signed-rank tests. A Pearson correlation was used to assess correlations between baseline concentrations of nitrite and nitrate and relative increases in nitrite and nitrate concentration after 6 days of CF intake.

RESULTS

Subject Characteristics

The 14 well-trained athletes included in this study were 30.7 ± 3.1 yr old, had a height of 1.80 ± 0.05 m, weight of 73.4 ± 7.4 kg, and a body mass index of 22.5 ± 1.5. They had a $\dot{V}O_{2max}$ of 62.9 ± 5.8 $ml \cdot min^{-1} \cdot kg^{-1}$ and PPO of 366 ± 45 W.

Effects of CF Intake on (−)-Epicatechin and (+)-Catechin

At baseline, there was no significant difference between 6-day CF and PL intake on serum EP concentration. Three-way repeated-measures ANOVA showed a significant supplement × time interaction ($F = 14.70$, $P < 0.001$). Post hoc analysis showed that 100 min after acute CF intake, serum EP was elevated compared with baseline (+238 ± 43%, H and N pooled, $P < 0.05$). After CF intake, serum EP further increased during SS exercise (+359 ± 32%, H and N pooled, $P < 0.001$ compared with baseline), while a plateau phase in serum EP was reached during the TT (no further increase post-TT compared with pre-TT) (Fig. 2*A*). After PL intake, EP did not change over time. Serum catechin values were not affected by CF intake, compared with PL intake, at any time points and in both N and H (Table 2 for baseline values, other data not shown).

Table 2. *Baseline measures following 6-day (before intake of last dose) CF or PL intake*

	PL	CF
(−)-Epicatechin, nM	35.4 ± 10.8	35.3 ± 9.2
(+)-Catechin, nM	19.7 ± 10.8	21.7 ± 14.7
Nitrite, nM	69.9 ± 17.5	81.4 ± 19.9
Nitrate, nM	43.8 ± 22.9	47.5 ± 21.5
MDA, μmol/l	1.17 ± 0.25	1.19 ± 0.21
Arg:Citr ratio	1.93 ± 0.56	1.92 ± 0.27
TEAC	442.8 ± 28.7	447.4 ± 49.3
UA, μmol/l	302.0 ± 39.3	303.5 ± 40.4
MAP, mmHg	95.5 ± 7.1	94.0 ± 7.1
FMD, %	0.56 ± 2.26	2.15 ± 2.19*

Values are expressed as means ± SD; $n = 14$. Epicatechin and catechin were measured in serum, nitrite, nitrate, malondialdehyde (MDA), arginine (Arg), citrullin (Citr), uric acid (UA), and Trolox equivalent antioxidant capacity (TEAC) were measured in plasma. CF, cocoa flavanol; PL, placebo; MAP, mean arterial pressure; FMD, flow-mediated dilation. *$P < 0.05$ between CF and PL.

Effect of CF Intake on NO Availability during Exercise in H

eNOS-dependent NO production. eNOS-dependent NO synthesis was reflected by plasma nitrite and nitrate concentrations and by the plasma ratio of arg:citr (39). Plasma nitrite and nitrate did not change during exercise and were not affected by H (Fig. 2*B*). Arg:citr ratio significantly decreased at the end of exercise compared with preexercise (main effect of time: $F = 14.1$, $P = 0.003$) and was significantly higher in H compared with N (main effect of FI_{O_2}: $F = 10.0$, $P = 0.008$) (Fig. 3*A*). CF intake did not significantly change plasma nitrite, nitrate, and arg:citr ratio, either before (thus at "baseline") or after the final dose. CF intake did not change plasma nitrite, nitrate, and arg:citr ratio after exercise. However, a significant negative correlation was found between baseline nitrite (after 6 days of PL intake) and the relative increase in nitrite concentration after 6 days of CF intake ($R^2 = 0.67$, $P < 0.001$, Pearson correlation).

Oxidative Stress and Antioxidant Capacity

Two-way, repeated-measures ANOVAs at baseline and preexercise showed that MDA was neither affected by 6-day CF intake (Table 2) nor by the final dose of CF in resting conditions. Three-way repeated-measures ANOVA during exercise revealed a significant interaction effect of time × supplementation ($F = 7.95$, $P = 0.018$): the significant exercise-induced increases in plasma MDA concentrations after PL intake (+12.2 ± 5.5%, $P = 0.047$ in N and +19.0 ± 6.8%, $P = 0.016$ in H) were suppressed by CF intake in both N (+2.9 ± 4.4%, NS) and H (+2.0 ± 4.4%, NS) (Fig. 3*B*).

Two-way, repeated-measures ANOVAs at baseline and preexercise showed that total plasma antioxidant capacity, measured as TEAC, was affected neither by the intake of 6 days of CF nor by the last dose of CF in N and H during rest. Three-way repeated-measures ANOVA revealed that the exercise-induced increase in TEAC was larger in H than in N (time × FI_{O_2}: $F = 4.83$, $P = 0.05$), but was not affected by CF intake (Fig. 3*C*). To correct for individual differences in UA—the most abundant plasma antioxidant that contributes to plasma TEAC—concentrations of UA were quantified in every sample. Two-way, repeated-measures ANOVAs showed that UA was neither affected by 6-day CF intake, nor by intake of the final dose of CF at rest. Three-way, repeated-measures ANOVA showed that UA concentrations were elevated after exercise, and in H compared with N (main effect of time: $F = 25.26$, $P < 0.001$; main effect of FI_{O_2}: $F = 6.48$, $P = 0.026$), while CF did not influence this response (Fig. 3*D*).

Vasoreactivity

At baseline, MAP was not different between 6-day CF and PL intake (Table 2). FMD was significantly increased after 6 days of CF intake compared with PL (main effect of supplement: $F = 5.59$, $P = 0.042$) (Table 2). The change in FMD after 6 days of CF intake compared with 6 days of PL was not correlated with the relative increases in nitrite and (−)-epicatechin concentration after 6 days of CF intake compared with PL.

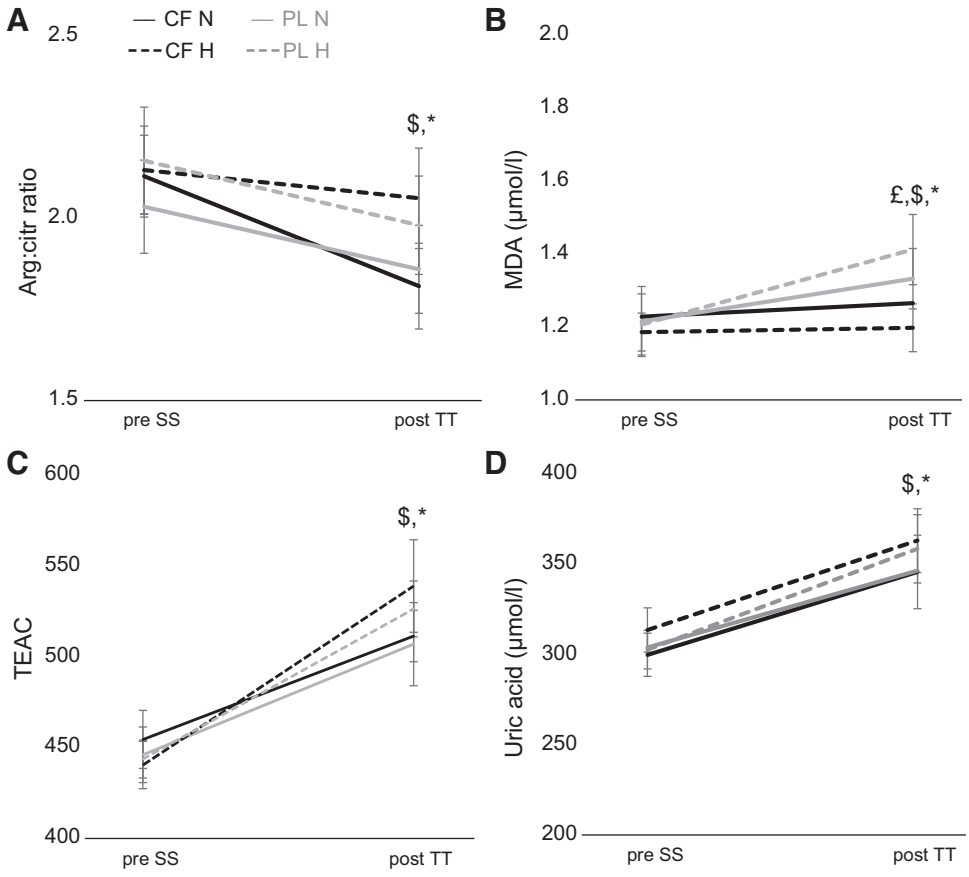

Fig. 3. Effect of 7-day cocoa flavanol (CF; black lines) or placebo (PL; gray lines) supplementation in hypoxia (H, dashed lines) and normoxia (N, solid lines) on exercise-induced changes in plasma arginine:citrulline (Arg:citr) ratio (*A*), plasma malondialdehyde concentration (MDA) (μmol/l) (*B*), plasma Trolox equivalent antioxidant capacity (TEAC) (*C*) and plasma uric acid concentration (UA) (μmol/l) (*D*). Pre-SS, at the start of the 20-min steady-state exercise (45% of peak power output); Post-TT, at the end of the 20-min time trial. Values are expressed as means ± SE. *$P < 0.05$, main effect of FI_{O_2}; £$P < 0.05$, main effect of supplement; \$$P < 0.05$, main effect of exercise.

Muscle Oxygenation during Exercise

At the start of SS exercise, the TSI in the vastus lateralis was not affected by the supplement and H. During SS, a significant interaction time × FI_{O_2} effect was found for TSI ($F = 11.95$, $P < 0.001$(Fig. 4*A*). Post hoc Bonferroni corrections showed that TSI decreased during the first 5 min and then stabilized. This exercise-induced decrease was aggravated in H, compared with N, while CF intake had no effect.

At the start of the TT, TSI was significantly lower in H than in N (main effect of FI_{O_2}: $F = 6.96$, $P = 0.02$). Three-way, repeated-measures ANOVAs showed a main effect of time during the TT for TSI ($F = 71.65$, $P < 0.001$): TSI significantly decreased during the first 5 min and stabilized during the last 15 min. H and CF intake did not influence TSI during the TT.

Prefrontal Cortex Oxygenation during Exercise

At the start of SS exercise, both H and CF influenced TSI (main effect of FI_{O_2}: $F = 7.05$, $P = 0.02$, main effect of supplement: $F = 7.66$, $P = 0.017$) (Fig. 4*B*). TSI was significantly lower in H compared with N. TSI was significantly higher after CF intake compared with PL (Fig. 4*B*). During SS, three-way repeated-measures ANOVA showed a significant main effect of supplement ($F = 12.28$, $P = 0.004$) and a significant FI_{O_2} × time interaction for TSI ($F = 24.10$, $P < 0.0001$). CF intake significantly increased prefrontal TSI during SS exercise. TSI significantly decreased in H, but not in N.

At the start of the TT, TSI was significantly lower in H than in N (effect of FI_{O_2}: $F = 6.43$, $P = 0.026$), while CF intake had no significant effect. Three-way, repeated-measures ANOVA showed a significant FI_{O_2} × supplement × time interaction effect for TSI during the TT ($F = 4.11$, $P = 0.016$). In N, TSI decreased significantly for the entire duration of the TT after both PL and CF intake. However, a larger decrease was observed after CF intake, compared with PL (significant supplement × time interaction effect ($F = 6.38$, $P < 0.001$). In H, TSI decreased enormously during the first 5 min but did not change significantly during the remaining 15 min after both PL and CF intake, and no interaction effect of supplement × time was found.

Exercise Tolerance and Performance

Steady state. Two-way, repeated-measures ANOVA showed that at the start of SS, Sa_{O_2} was significantly lower in H than in N (Table 3). At rest, CF intake did not alter Sa_{O_2}. HR and lactate were similar in H and N and were not different after CF intake, compared with PL. Three-way, repeated-measures ANOVAs showed a significant FI_{O_2} × time interaction effect for Sa_{O_2}, HR, and lactate during SS. An exercise-induced decrease in Sa_{O_2} occurred in H, but not in N. The exercise-induced increase in HR was larger in H than N. In N, lactate decreased during SS, but in H, lactate increased during SS. During SS, RPE was significantly higher in H than in N. The (significant) difference in Sa_{O_2} between CF and PL intake during SS exercise in H (−1.21 ± 0.48% in CF vs. PL) was smaller than the accuracy range (2–3%) claimed by the distributor of the pulse oximeter used and, thus, might not be reliable. CF did not influence HR, lactate, and RPE during SS exercise.

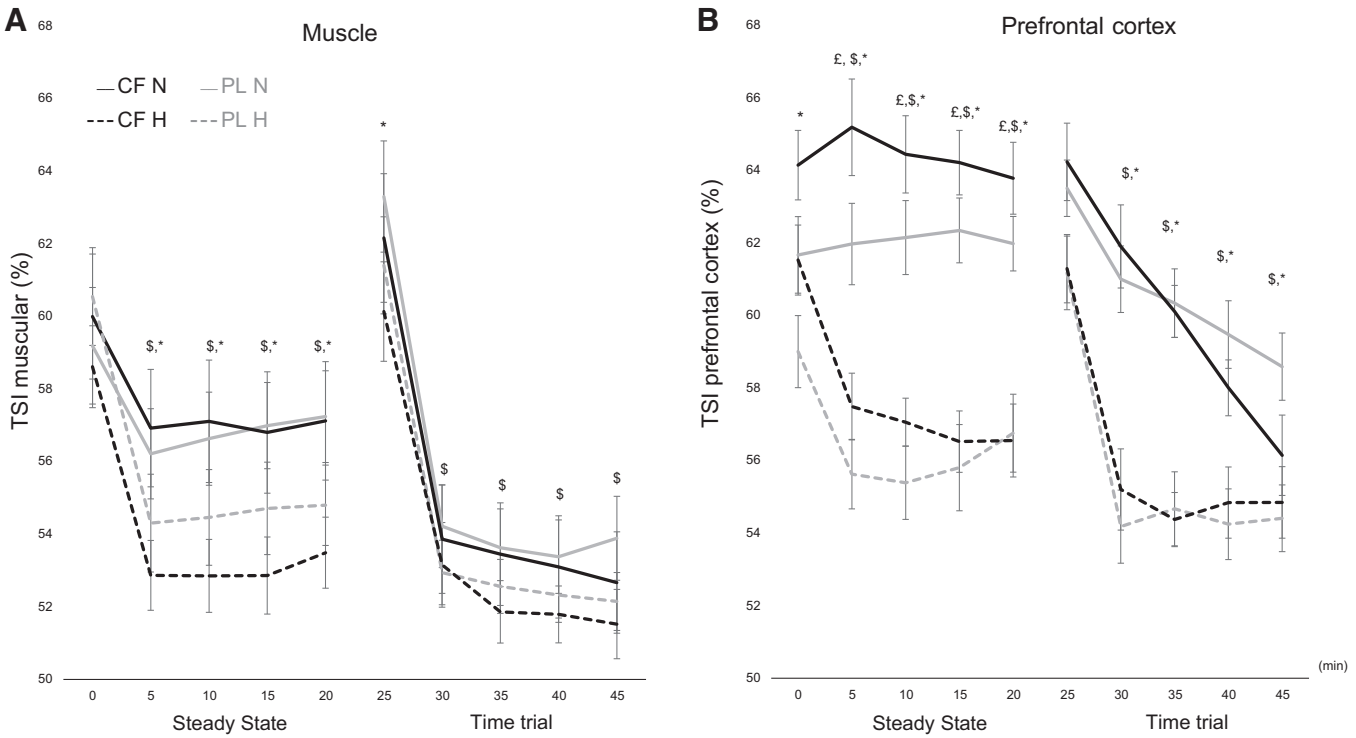

Fig. 4. Effect of 7-day cocoa flavanol (CF; black lines) or placebo (PL; gray lines) supplementation in hypoxia (H; dashed lines) and normoxia (N; solid lines) on tissue oxygenation (TSI; %) in the vastus lateralis (*A*) and prefrontal cerebral cortex (*B*). 0–20 min, steady-state exercise (45% of peak power output); 20–25 min, passive rest in seated position on the bike; 25–45 min, time trial. Values are expressed as means ± SE. *$P < 0.05$, main effect of FI_{O_2}; £$P < 0.05$, main effect of supplement; \$$P < 0.05$, main effect of exercise.

Table 3. *Effect of 7-day cocoa flavanol intake on physiological changes during moderate and high intensity exercise in hypoxia and normoxia*

	PL-N	CF-N	PL-H	CF-H	Two-Way RM ANOVA (start)	Three-Way RM ANOVA
Sa_{O_2}-SS						
Start	97 ± 4	98 ± 1	89 ± 3*	88 ± 6*†	S: NS	$O_2 \times S$: $F = 5.11$, $P = 0.05$
5′	97 ± 2	95 ± 4	79 ± 5*	78 ± 5*†	O_2: $F = 122.00$, $P < 0.001$	$O_2 \times T$: $F = 26.90$, $P < 0.0001$
10′	97 ± 2	97 ± 1	79 ± 4*	76 ± 4*†		S: $F = 6.48$, $P = 0.027$
15′	95 ± 2	96 ± 1	79 ± 4*	76 ± 3*†		O_2: $F = 624.55$, $P < 0.0001$
End	96 ± 2	96 ± 2	80 ± 3*	78 ± 3*†		T: $F = 25.20$, $P < 0.0001$
Sa_{O_2}-TT						
Start	97 ± 1	97 ± 2	88 ± 1*	89 ± 4*	S: NS	$T \times O_2$: $F = 34.92$, $P < 0.001$
5′	93 ± 2	94 ± 2	79 ± 3*‡	78 ± 2*	O_2: $F = 100.3$, $P < 0.001$	S: NS
10′	93 ± 2	93 ± 2	79 ± 3*	79 ± 3*		O_2: $F = 102.61$, $P < 0.0001$
15′	93 ± 2	93 ± 2	80 ± 3*	79 ± 3*		T: $F = 113.13$, $P < 0.0001$
End	93 ± 2	94 ± 2	80 ± 2*	80 ± 3*		
HR-SS						
Start	72 ± 5	69 ± 3	72 ± 3	68 ± 3	S: NS	$T \times O_2$: $F = 14.54$, $P < 0.001$
5′	122 ± 3	123 ± 3	136 ± 3	136 ± 3	O_2: NS	S: NS
10′	127 ± 3	129 ± 3	141 ± 3	143 ± 3		O_2: $F = 44.19$, $P < 0.0001$
15′	130 ± 3	132 ± 3	142 ± 2	146 ± 4		T: $F = 530.72$, $P < 0.0001$
End	131 ± 3	134 ± 2	146 ± 3	148 ± 3		
HR-TT						
Start	91 ± 3	95 ± 4	93 ± 2	94 ± 3	S: NS	$T \times O_2$: $F = 8.06$, $P < 0.001$
5′	162 ± 2	165 ± 2	168 ± 3	168 ± 2	O_2: NS	S: NS
10′	170 ± 2	173 ± 2	172 ± 2	171 ± 2		O_2: NS
15′	175 ± 2	176 ± 2	173 ± 2	171 ± 2		T: $F = 140.60$, $P < 0.0001$
End	180 ± 2	181 ± 2	177 ± 1	176 ± 1		
Lactate-SS						
Start	1.4 ± 0.2	1.4 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	S: NS	$T \times O_2$: $F = 34.34$, $P < 0.001$
10′	1.1 ± 0.1	1.0 ± 0.1	1.9 ± 0.2	1.8 ± 0.2	O_2: NS	S: NS
End	0.9 ± 0.1	0.8 ± 0.1	1.9 ± 0.2	2.0 ± 0.3		O_2: $F = 30.57$, $P < 0.0001$
						T: NS
Lactate-TT						
Start	0.9 ± 0.1	0.8 ± 0.1	1.7 ± 0.2	1.7 ± 0.2	S: NS	$T \times O_2$: $F = 4.24$, $P = 0.026$
10′	4.2 ± 0.6	4.5 ± 0.4	6.9 ± 0.8	7.2 ± 0.7	O_2: $F = 53.02$, $P < 0.001$	S: NS
End	6.5 ± 0.8	7.2 ± 0.8	8.9 ± 0.6	7.8 ± 0.7		O_2: $F = 22.90$, $P < 0.0001$
						T: $F = 109.44$, $P < 0.0001$
RPE-SS						
5′	10 ± 1	11 ± 2	11 ± 2	11 ± 2	Wilcoxon	Wilcoxon
10′	10 ± 1	11 ± 2	12 ± 2*	12 ± 2*		
15′	11 ± 1	11 ± 2	12 ± 2*	12 ± 2*		
End	11 ± 1	11 ± 2	12 ± 2*	12 ± 2*		
RPE-TT						
5′	14 ± 1	14 ± 1	16 ± 1*	16 ± 1*	Wilcoxon	Wilcoxon
10′	15 ± 1	16 ± 1	17 ± 1*	17 ± 1*		
15′	17 ± 1	17 ± 1	18 ± 1	18 ± 1		
End	18 ± 1	19 ± 1	19 ± 1	19 ± 1		
Work performed, kJ						
5′	79.1 ± 9.2	79.5 ± 10.2	75.2 ± 10.0	74.7 ± 9.1	S: NS	$T \times O_2$: $F = 46.74$, $P < 0.001$
10′	160.9 ± 20.9	162.8 ± 22.8	147.7 ± 18.7*	147.7 ± 19.1*	O_2: $F = 46.67$, $P < 0.0001$	S: NS
15′	244.0 ± 34.0	246.2 ± 36.0	216.9 ± 27.5*	217.2 ± 28.4*		O_2: $F = 35.98$, $P < 0.0001$
End	327.5 ± 46.7	330.9 ± 49.9	287.2 ± 37.7*	288.3 ± 37.4*		T: $F = 651.3$, $P < 0.0001$

Values are expressed as means ± SD; $n = 14$. CF, cocoa flavanol; H, hypoxia; HR, heart rate; N, normoxia; NS, not significant; PL, placebo; RPE, rate of perceived exertion; Sa_{O_2}, peripheral oxygen saturation; SS, steady-state; TT, time trial. *$P < 0.05$; main effect of $FI_{O_2}(O_2)$ (H-PL compared with N-PL and H-CF compared with N-CF); †$P < 0.05$; main effect of supplement (S) (CF compared with PL); ‡$P < 0.05$; main effect of time (T) (compared with previous time point).

Time trial. TT performance (work performed during the 20 min TT) decreased in H compared with N, but CF intake did not influence TT performance (Table 3). Two-way, repeated-measures ANOVAs at the start of the TT showed that Sa_{O_2} and lactate were significantly lower ($-8 \pm 1\%$, $P < 0.001$) and higher ($+0.9 \pm 0.2$ mmol/l, $P < 0.001$), respectively, in H compared with N. No significant difference between N and H was observed for HR. CF did not influence Sa_{O_2}, lactate, or HR. Three-way, repeated-measures ANOVAs showed a significant $FI_{O_2} \times$ time interaction effect for Sa_{O_2}, HR, and lactate during the TT. Post hoc analysis showed a larger drop in Sa_{O_2} and a larger increase in lactate during the TT in H compared with N. Post hoc analysis showed a faster elevation of HR, but lower HR_{max} at the end of the TT in H than in N. RPE was significantly higher in H than in N during the first half of the TT, but there was no difference during the second half. CF intake did not influence any of these physiological changes.

DISCUSSION

The important novel findings of this study were that in well-trained cyclists, 1-wk CF intake can *1*) increase prefrontal

oxygenation at rest and during moderate-intensity exercise and, thus, can partially restore the hypoxia-induced decline in oxygenation during exercise at altitude and *2*) reduce exercise-induced oxidative stress, which is substantially higher in hypoxia than in normoxia. CF does not improve exercise performance in normoxia and hypoxia.

It is well documented that CF intake leads to improved endothelial function, as reflected by FMD, in individuals with and without cardiovascular risks (16, 17). We found that this beneficial effect also occurs in well-trained athletes who already have an enhanced endothelial function by regular exercise training. Studies using either NO-synthase inhibitor (L-NMMA) (18) or parallel measure of circulating nitrites (17) suggested that the CF-induced improvement in FMD can originate from an effect of CF on NO metabolism. However, in the current study, nitrite concentration and arg:citr ratio, two indirect markers of eNOS-dependent NO production and NO availability (5, 10), were not altered by CF intake, and no correlation between the change in FMD and change in nitrite concentration was found. One hypothesis to explain this result is that CF could also act on other molecules than NO, which could play a role in smooth muscle relaxation. As for example, Grassi et al. (16) showed that 7-day CF supplementation improved FMD and decreased concentrations of endothelin 1, a substance known to act directly on smooth muscles by inducing vasoconstriction in healthy volunteers. Despite a greater CF-induced increase of plasma nitrites in subjects with lower initial levels of nitrite, we did not find greater FMD improvements in those subjects. This result might be an additional argument for a putative role of other dilator substances, besides NO, in CF effects.

CF intake did not affect nitrite, nitrate, and the arg:citr ratio in response to exercise and hypoxia. Moreover, nitrite and nitrate were altered neither by acute hypoxia nor by exercise. Similarly, nitrite was similar to preexercise levels after a 3-h cycling race in the study of Sureda et al. (40) and Kelly et al. (21) found no effect of exercise in normoxia and hypoxia on nitrite after PL intake. However, the interpretation of these data is not straightforward, since plasma nitrite is likely to reflect the dynamic balance between NOS-derived NO production and the reduction from nitrate to nitrite and further NO, which is expected to be facilitated in hypoxia (21). Furthermore, in the current study, the arg:citr ratio was lowered after exercise, and the magnitude of this decrease was smaller in hypoxia compared with normoxia. This seems consistent with the notion that enzymatic production of NO depends on the availability of O_2 (29) and that hypoxia triggers superoxide anion generation, causing depletion of tetrahydrobiopterin, the essential eNOS cofactor, which results in eNOS uncoupling and decreased eNOS-dependent NO production (30).

Previously, it has been shown that CF intake leads to inhibition of NADPH oxidase (20). The generation of the superoxide anion radicals by NADPH oxidase results in scavenging of NO, eNOS uncoupling, and reduced NO availability, but also triggers oxidative stress. CF intake may decrease oxidative stress after different types and durations of exercise in humans at sea level (1, 8, 13, 44). At altitude, the magnitude of exercise-induced oxidative stress is elevated compared with at sea level (26). For the first time, we demonstrated that 7-day CF intake can inhibit the exercise-induced increase in lipid peroxidation in N, but also in H. Lipid peroxidation, which is the result of a multistep chain reaction in which ROS attack lipids in cell membranes (3), was affected by CF intake. However, CF affected neither plasma UA concentrations nor the total plasma antioxidant capacity measured as TEAC, in response to exercise and hypoxia. The plasma antioxidant capacity does not necessarily correlate with changes in lipid peroxidation since hydrophilic antioxidants are not efficient against lipid peroxidation (28). Previous in vitro studies showed that CF can directly scavenge free radicals, act as a chain-breaking antioxidant in lipid peroxidation, and/or regulate ROS-related enzymes (2, 20, 24). Our results propose that during exercise in hypoxia, CF mainly reduces oxidative stress in the environment of membranes and lipoproteins. The diminished oxidative stress raises the possibility for CF to prevent muscle damage and, thus, have a beneficial effect on exercise recovery.

Consistent with previous research, the exercise-induced drops in tissue oxygenation were larger in hypoxia than in normoxia during moderate-intensity exercise (14, 25). The decreased muscular oxygenation in hypoxia was paralleled by the elevated blood lactate concentration, indicating a higher reliance on anaerobic glycolysis, but it was not affected by CF intake. Thus, the effects of hypoxia to inhibit oxidative energy production during moderate-intensity exercise were not suppressed by CF. In contrast, CF intake beneficially impacted cerebral oxygenation during rest and during moderate-intensity exercise in hypoxia. Although no other studies have examined muscular or prefrontal oxygenation changes in response to CF intake, we might speculate that there is a tissue-specific reaction to CF supplementation. Using another supplement (beetroot) during moderate-intensity exercise in hypoxia, Masschelein et al. (25) found a tissue-specific reaction, but with opposite results, with improved muscular oxygenation, but no difference in prefrontal oxygenation. However, beetroot is known to influence the NO metabolism, while we found no differences in nitrate and nitrite concentrations after CF intake. Thus, the specific tissue responsiveness to CF supplementation merits further investigation.

The beneficial effects of CF on prefrontal oxygenation vanished during high-intensity exercise, indicating that the physiological alterations in response to exhaustive exercise largely overruled any beneficial effects of CF. CF intake could not increase muscular oxygenation and could not prevent greater reliance on anaerobic glycolysis during the TT in hypoxia, as evidenced by the higher blood lactate concentration. Moreover, CF intake did not have ergogenic effects in hypoxia and normoxia.

Future research may address some of the potential limitations of the current study. The measured markers of NO availability and oxidative stress in plasma might not exactly reflect changes in the endothelium, brain, and muscle. While NIRS is currently the only method that allows the measurement of muscular and cerebral blood flow and oxygenation continuously during whole body exercise, it only provides indirect information.

For the first time, we have shown that CF intake inhibited oxidative stress during exhaustive exercise in hypoxia. CF had beneficial effects on endothelial function at rest, as well as on prefrontal oxygenation at rest and during moderate-intensity exercise. This is not only relevant for athletes exposed to altitude, but also for hypoxemic patients who suffer from a

reduced blood oxygenation, as well as for patients suffering from chronic diseases involving increased levels of oxidative stress.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.D., C.T., A.B., and R.M. conceived and designed research; L.D., E.L., M.J.D.-R., J.R.B., W.S., and A.M.J. performed experiments; L.D., C.B., and A.D. analyzed data; L.D. interpreted results of experiments; L.D. prepared figures; L.D. drafted manuscript; L.D., C.T., A.M.J., A.R.W., A.B., R.M., and E.H. edited and revised manuscript; R.M. and E.H. approved final version of manuscript.

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