



Influence Of 2-Weeks Ingestion Of High Chlorogenic Acid Coffee On Mood State, Performance, And Postexercise Inflammation And Oxidative Stress: A Randomized, Placebo-Controlled Trial

By: **David C. Nieman**, Courtney L. Goodman, Christopher R. Capps, Zack L. Shue, and Robert Arnot

Abstract

This study measured the influence of 2-weeks ingestion of high chlorogenic acid (CQA) coffee on postexercise inflammation and oxidative stress, with secondary outcomes including performance and mood state. Cyclists (N = 15) were randomized to CQA coffee or placebo (300 ml/day) for 2 weeks, participated in a 50-km cycling time trial, and then crossed over to the opposite condition with a 2-week washout period. Blood samples were collected pre- and postsupplementation, and immediately postexercise. CQA coffee was prepared using the Turkish method with 30 g lightly roasted, highly ground Hambela coffee beans in 300 ml boiling water, and provided 1,066 mg CQA and 474 mg caffeine versus 187 mg CQA and 33 mg caffeine for placebo. Plasma caffeine was higher with CQA coffee versus placebo after 2-weeks (3.3-fold) and postexercise (21.0-fold) (interaction effect, $p < .001$). Higher ferric reducing ability of plasma (FRAP) levels were measured after exercise with CQA coffee versus placebo ($p = .01$). No differences between CQA coffee and placebo were found for postexercise increases in plasma IL-6 ($p = .74$) and hydroxyoctadecadienoic acids (9 + 13 HODEs) ($p = .99$). Total mood disturbance (TMD) scores were lower with CQA coffee versus placebo ($p = .04$). 50-km cycling time performance and power did not differ between trials, with heart rate and ventilation higher with CQA coffee, especially after 30 min. In summary, despite more favorable TMD scores with CQA coffee, these data do not support the chronic use of coffee highly concentrated with chlorogenic acids and caffeine in mitigating postexercise inflammation or oxidative stress or improving 50-km cycling performance.

Nieman, D. C., et al. (2018). "Influence of 2-Weeks Ingestion of High Chlorogenic Acid Coffee on Mood State, Performance, and Postexercise Inflammation and Oxidative Stress: A Randomized, Placebo-Controlled Trial." *International Journal of Sport Nutrition and Exercise Metabolism* 28(1): 55-65. <https://doi.org/10.1123/ijsnem.2017-0198>. Publisher version of record available at: <https://journals.humankinetics.com/doi/full/10.1123/ijsnem.2017-0198>

Influence of 2-Weeks Ingestion of High Chlorogenic Acid Coffee on Mood State, Performance, and Postexercise Inflammation and Oxidative Stress: A Randomized, Placebo-Controlled Trial

David C. Nieman, Courtney L. Goodman, Christopher R. Capps, and Zack L. Shue
Appalachian State University

Robert Arnot
Academic Nutraceuticals LLC

This study measured the influence of 2-weeks ingestion of high chlorogenic acid (CQA) coffee on postexercise inflammation and oxidative stress, with secondary outcomes including performance and mood state. Cyclists ($N = 15$) were randomized to CQA coffee or placebo (300 ml/day) for 2 weeks, participated in a 50-km cycling time trial, and then crossed over to the opposite condition with a 2-week washout period. Blood samples were collected pre- and postsupplementation, and immediately postexercise. CQA coffee was prepared using the Turkish method with 30 g lightly roasted, highly ground Hambela coffee beans in 300 ml boiling water, and provided 1,066 mg CQA and 474 mg caffeine versus 187 mg CQA and 33 mg caffeine for placebo. Plasma caffeine was higher with CQA coffee versus placebo after 2-weeks (3.3-fold) and postexercise (21.0-fold) (interaction effect, $p < .001$). Higher ferric reducing ability of plasma (FRAP) levels were measured after exercise with CQA coffee versus placebo ($p = .01$). No differences between CQA coffee and placebo were found for postexercise increases in plasma IL-6 ($p = .74$) and hydroxyoctadecadienoic acids (9 + 13 HODEs) ($p = .99$). Total mood disturbance (TMD) scores were lower with CQA coffee versus placebo ($p = .04$). 50-km cycling time performance and power did not differ between trials, with heart rate and ventilation higher with CQA coffee, especially after 30 min. In summary, despite more favorable TMD scores with CQA coffee, these data do not support the chronic use of coffee highly concentrated with chlorogenic acids and caffeine in mitigating postexercise inflammation or oxidative stress or improving 50-km cycling performance.

Keywords: exercise, IL-6, oxidative stress

Coffee is one of the most commonly consumed beverages worldwide, and is the primary dietary source for polyphenols in most European regions (Zamora-Ros et al., 2016). In the United States, coffee is consumed by 75% of the adult population (mean intake, 417 ml/d), with 49% reporting daily intake (Loftfield et al., 2016).

Coffee is a good source of caffeine and chlorogenic acids (CQA), a group of nonflavonoid polyphenol compounds comprising hydroxycinnamates linked to quinic acid that form caffeoylquinic acids (CQA), feruloylquinic acids (FQA), and *p*-coumaroylquinic acids (*p*-CoQA) (Del Rio et al., 2010; Tajik et al., 2017). Coffee contains many isomers of these compounds, with

the principal CQA in coffee being 5-caffeoylquinic acid (5-CQA) (Monteiro et al., 2007). A typical cup of coffee delivers 15 to 325 mg CQAs (Richelle et al., 2001), and heavy coffee consumers may take in up to 1,000 mg CQA per day, depending on multiple factors including the specific coffee variety, the geographic location where it is grown, and the processes used in postharvest, washing/drying procedures, and roasting and grinding particle size of the coffee beans (Lang et al., 2013; Liang & Kitts, 2014, 2015; Ludwig et al., 2014).

Coffee consumption has been associated with multiple health benefits and reduced total mortality rates (Ding et al., 2015; Je & Giovannucci, 2014), and these have been primarily attributed to CQA antioxidant, anti-inflammatory, and blood vessel dilation effects (Kempf et al., 2010; Liang & Kitts, 2015; Lopez-Garcia et al., 2006; Tajik et al., 2017). In vitro indicate that CQAs have antioxidant and anti-inflammatory activity,

Nieman, Goodman, Capps, and Shue are with the Human Performance Lab, Appalachian State University, Kannapolis, NC. Arnot is with Academic Nutraceuticals LLC, Cheyenne, WY. Address author correspondence to David C. Nieman at niemandc@appstate.edu.

alleviate oxidative stress and inflammation in various animal disease models, and reduce related biomarkers in human clinical trials (Tajik et al., 2017). Acute coffee ingestion has been linked to a modest and transient increase in plasma antioxidant activity (Agudelo-Ochoa et al., 2016; Moura-Nunes et al., 2009; Natella et al., 2002). Limited data suggest that CQAs may improve mood state, but to a lesser extent than caffeine (Camfield et al., 2013).

CQAs are poorly and incompletely absorbed in the upper gastrointestinal tract, with most entering the large intestine unmetabolized (Farah et al., 2008; Monteiro et al., 2007; Renouf et al., 2014; Stalmach et al., 2010). CQAs are metabolized by colon bacteria, with cleavage of the ester link between quinic acid and caffeic acid. Caffeic acid may be absorbed through the colon back into the body, with conjugation into O-methylated, sulphated and glucuronidated derivatives, and transformation into dihydro metabolites such as dihydrocaffeic acid and dihydroferulic acid (Ludwig et al., 2014). CQA metabolites absorbed through the small intestine are conjugated and have an early appearance in the plasma at low concentrations (~1–2 hours), with CQA metabolites formed by intestinal microflora appearing late (>6 hours) at high concentrations and persisting in the plasma, but with wide variation between individuals (Lang et al., 2013; Renouf et al., 2014). CQAs act as prebiotics in the colon due to the selective metabolism and expansion of specific bacterial populations related to human health (Mills et al., 2015).

Caffeine in moderate to high doses (3–13 mg/kg body mass) ingested prior to and during prolonged, intensive exercise has been linked in many studies to improved performance and decreased ratings of perceived exertion (Doherty & Smith, 2005; Ganio et al., 2009; Goldstein et al., 2010; McLellan et al., 2016; Spriet, 2014; Tarnopolsky, 2008). The principal mechanism of caffeine's ergogenic effects is through its ability to act as an adenosine receptor antagonist to induce effects on the central and peripheral nervous system to reduce pain and exertion perception, improve motor recruitment, and excitation-contraction coupling (Tarnopolsky, 2008). Although most studies have used anhydrous caffeine, moderate but inconsistent evidence supports the use of coffee as an ergogenic aid to improve performance in endurance cycling and running (Church et al., 2015; Goldstein et al., 2010; Hodgson et al., 2013).

Given the bioactive effects of CQAs and caffeine, we hypothesized that 2-weeks chronic ingestion of caffeinated coffee high in CQAs (including the morning of the exercise challenge bout) would mitigate exercise-induced inflammation and oxidative stress while supporting endurance performance. The primary purpose of this study was to investigate the influence of 2-weeks ingestion of high CQA coffee on postexercise (50-km cycling time trial) inflammation and oxidative stress, with secondary outcomes including performance and mood state.

Methods

Participants

The research procedures were conducted at the Human Performance Laboratory operated by Appalachian State University at the North Carolina Research Campus (NCRC) in Kannapolis, NC. Male ($n=10$) and female ($n=5$) cyclists ages 19 to 51 years were recruited that were healthy, non-smokers, habitually consumed less than 3 cups of coffee per day during the previous month, did not regularly consume tablets containing caffeine or high amounts of energy drinks or other beverages containing caffeine, regularly competed in road races (category 1 to 5), and were capable of cycling 75 km in a laboratory setting. Study participants agreed to train normally, maintain weight, and avoid the regular use of large-dose vitamin and mineral supplements, herbs, and medications that had the potential to influence inflammation and immune function for the duration of the study. Participants signed informed consent, and study procedures were approved by the Institutional Review Board at Appalachian State University.

Research Design

This study used a double-blind, placebo-controlled, randomized crossover design. Study participants ($N=15$) were randomized to coffee or placebo conditions for two weeks, participated in a 50-km cycling time trial, and then crossed over to the opposite condition after a 2-week washout period. Participants completed the two arms of the study, and data were analyzed using repeated measures analysis of variance (ANOVA), with participants operating as their own controls.

One to two weeks prior to the first 50-km cycling time trial, participants reported to the NCRC Human Performance Lab for orientation/baseline testing. Participants were screened for inclusion and exclusion criteria, and provided voluntary consent. Demographic and training histories were acquired with questionnaires. Maximal power, oxygen consumption, ventilation, and heart rate were measured during a graded exercise test (25 W increase every 2 min, starting at 150 W) with the Cosmed Quark CPET metabolic cart (Rome, Italy) and the Lode cycle ergometer (Lode Excaliber Sport, Lode B.V., Groningen, Netherlands). Body composition was measured with the Bod Pod body composition analyzer (Cosmed, Rome, Italy).

Study participants were randomized to ingest one cup per day of high chlorogenic coffee (CQA coffee) or placebo for two weeks using double-blind procedures. Following the 2-week supplementation period, cyclists participated in a 50-km cycling time trial using their bicycles on CompuTrainer devices. Blood samples were collected pre- and post-2-weeks supplementation, and immediately following the 50-km cycling time trial. Cyclists crossed over to the opposite beverage, and repeated all procedures following a 2-week washout period.

Participants returned to the lab within one to two weeks after baseline testing in an overnight fasted state to provide a blood sample. Participants received a 2-week supply of high CQA coffee or placebo coffee with written and video instructions to prepare using Turkish methods, and ingested 1 cup per day.

During the 3-day period prior to each 50-km cycling trial, participants tapered exercise training (as if preparing for a race) and ingested a moderate-carbohydrate diet using a food list restricting high fat foods and visible fats. Participants were instructed to keep the food record current by listing items immediately after they were eaten, to measure and record the volume using household measures (tablespoons, cups, slices, ounces), to provide sufficient detail about the method of preparation and include condiments, sugar, oils, butter, and other visible fats, and to avoid the tendency to eat less or under-report because of the recording process. The 3-day food records were analyzed for nutrient and flavonoid content using the Food Processor v. 11.1 (ESHA Research, Salem, OR). ESHA's port utility (v. 4.0) was used to upload the Flavonoid Values for USDA Survey Foods and Beverages (FNDDS) 2007–2010 database (Sebastian et al., 2016), and each food/beverage was assessed for macro- and micro-nutrients, and total flavonoids.

For each trial, participants came to the lab at 7:00 am in an overnight fasted state (at least 9 h with no food or beverage other than water) and completed the 50-cycling time trials. Participants first provided a blood sample, and then according to the randomized schedule ingested either one cup of hot CQA coffee or placebo. At approximately 7:30 am, participants started the 50-km cycling time trial. Participants used their own bicycles on CompuTrainer Pro Model 8001 trainers (RacerMate, Seattle, WA), with a flat 50-km course controlled by the CompuTrainer MultiRider software system (RacerMate, Seattle, WA). Heart rate and rating of perceived exertion (RPE) were recorded every 30 minutes, and workload (watts) continuously monitored. Oxygen consumption, carbon dioxide production, and ventilation were measured using the Cosmed CPET metabolic system every 30 minutes. Participants consumed water ad libitum, but did not exceed 3 ml/kg per 15 minutes, and no other food or beverages were ingested during the 50-km cycling time trials. A blood sample was collected immediately postexercise. Plasma was aliquoted and flash-frozen in liquid nitrogen, and stored at -80°C before analysis.

High-Chlorogenic Acid Coffee and Placebo

Lightly roasted coffee beans high in chlorogenic acids (CQA coffee) were selected from the Hambela Estate in Ethiopia and ground to a very small particle size. Study participants were instructed to use the Turkish method of preparation, and consume one 300 ml cup of coffee each morning for two weeks. Participants measured 30 grams of coffee grounds using a drip scale, and then transferred the coffee and 300 ml water into a Turkish copper coffee

pot (supplies, written instructions, and video were provided for each study participant). With the stove top burner on, the coffee and water were stirred periodically until boiling occurred. The heat was turned off after three minutes of boiling, with care to avoid the coffee foaming over the top. The coffee grounds were allowed to settle for one to two minutes, with the coffee supernatant poured into a cup and then consumed black (without milk, cream or similar products). High Performance Liquid Chromatography (HPLC) analysis of the Turkish brewed coffee (30 grams in 300 ml boiled water) was conducted by the Green Mountain Laboratories, Inc. (Montpelier, VT), and results showed 1,066 mg of caffeoylquinic acids (CQA) (specifically, 545 mg 3-CQA, 298 mg 4-CQA, 223 mg 5-CQA), 474 mg caffeine, and 1,533 mg total phenolics per 300 ml Turkish boiled coffee. For the placebo coffee, decaffeinated coffee beans were darkly roasted and finely ground, and HPLC analysis showed that 30 grams in 300 ml boiled water (Turkish method as described) provided 187 mg total CQA and 33 mg caffeine. The CQA and placebo coffee grounds were packaged in coded coffee bags, with the double blind code held until the study was completed.

Plasma Caffeine Analysis

Plasma caffeine concentration was analyzed by a Waters Acquity UPLC-Quattro Premier XE Mass Spectrometry (Waters Corp., Milford, MA). Chromatographic separation of caffeine and the internal standard caffeine-d9 was performed on a UPLC BEH C18 1.7 μm analytical column (2.1×100 mm, Waters Corp., Milford, MA) with a gradient elution using mobile phase A water and mobile phase B acetonitrile, both containing 0.1% formic acid. The separation was achieved by the following gradients: 0–0.5 min (5% B), 0.5–2 min (5–80% B), 2–2.1 min (80–100% B), 2.1–3.5 min (100% B), 3.5–3.6 min (100–5% B), and 3.6–4 min (5% B). The flow rate was 0.4 ml/min. Detection was performed using a Waters Quattro Premier XE mass spectrometer that was operated in positive ion mode with electrospray ionization. The analyses were performed in the multiple reaction monitoring (MRM) mode (caffeine $195 > 138$ and caffeine-d9 $204 > 144$). Instrument control and data acquisition was performed using Masslynx software package (Waters Corp., Milford, MA). The optimized parameter settings were as follows: capillary 3 KV, sampling cone 35 V, extraction cone 4V, source temperature 120°C , desolvation temperature 350°C , desolvation gas flow 800 L/Hr.

Sample preparation was performed by protein precipitation with methanol. Fifty microliter aliquots of plasma samples were transferred to 2 mL microcentrifuge tubes. 200 μL of methanol containing 40 ng/mL of internal standard caffeine-d9 was added to each tube. Samples were vortexed for 5 minutes and then centrifuged at 12,000 g for 10 min at 4°C . The supernatant of each tube was transferred to an autosampler vial for LC-MS analysis.

Plasma FRAP Analysis

Total plasma antioxidant potential/capacity was determined by the ferric reducing ability of plasma (FRAP) assay as previously described (Benzie & Strain, 1996). This assay utilizes water-soluble antioxidants in the plasma to reduce ferric iron to the ferrous form identifiable at 593 nm. Samples and standards were expressed as ascorbate equivalents based on an ascorbate standard curve. The intra-assay coefficient of variation (CV) for FRAP was 2.6%.

Plasma 9 + 13-HODE Analysis

Nine- and 13-hydroxy-octadecadienoic acids (9-HODE and 13-HODE) were measured as previously described (Nieman et al., 2014). Briefly, 10 μ L of 500 ng/mL 9-HODE-d4 and 10 μ L of 50 mM BHT were added to 200 μ L of plasma sample. Then 1.0 mL (10% v/v acetic acid in water)/2-propanol/hexane (2/20/30, v/v/v) and 2.0 mL hexane were used for extraction. After centrifugation, the upper layer was removed, dried and reconstituted with 100 μ L of 85% methanol in water (v/v, containing 0.1% acetic acid) for LC-MS analysis. Chromatographic separation of 9-HODE and 13-HODE was achieved using an UPLC system (Acquity UPLC, Waters, Milford, MA) equipped with Waters BEH C18 1.7 μ m analytical column (2.1 \times 100 mm). The mobile phases consisted of (A) 0.2% acetic acid in water and (B) 0.2% acetic acid in acetonitrile. The gradient used was: 0–0.5 min (80% B), 0.5–3 min (80–100% B), 3–6 min (100% B), 6–6.1 min (100–80% B), 6.1–7 min (80% B). For detection of 9-HODE and 13-HODE, the UPLC system was coupled with Quattro Premier XE MS (Waters, Milford, MA) and the system was operated in electrospray ionization (ESI) negative mode. The multiple reaction monitoring (MRM) was performed with optimized transitions (295 > 171 for 9-HODE; 295 > 195 for 13-HODE; and 299 > 172 for 9-HODE-d4). All peaks were integrated using TargetLynx Application Manager (Waters, Milford, MA), and the peaks were quantified using calibration curves of peak areas of native compounds divided by the internal standard peak area.

Plasma IL-6 Analysis

Total plasma concentration of IL-6 was determined using an electrochemiluminescence based solid-phase sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD, USA). All samples and provided standards were analyzed in duplicate, and the inter-assay CV was 4.5%, with the intra-assay CV ranging from 4 to 6%. Preexercise and postexercise samples for the cytokines were analyzed on the same assay plate to decrease inter-kit assay variability.

Profile of Mood States

An abbreviated 40-item version of the Profile of Mood States was administered prior to and after the 2-week coffee/placebo supplementation period, 30 minutes after

ingesting 1 cup of coffee/placebo (before the exercise bout), and immediately following the 50-km cycling time trial (Grove & Prapavessis, 1992). Since short-term effects on mood were being measured, the “right now” response was employed. All responses were based on a five-point scale anchored by “not at all” (score of 0) and “extremely” (score of 4). Scores for the seven subscales were calculated by summing the numerical ratings for items that contributed to each subscale, with the total mood disturbance (TMD) calculated by summing the totals for the negative subscales (tension, depression, anger, fatigue, confusion) and then subtracting the total for the positive subscales (vigor, esteem-related affect), and adding 100 to eliminate negative scores.

Statistical Analysis

Data are presented as mean \pm SE. Our power analysis showed that at an effect size of 0.7 and alpha of 0.05, $N=15$ in a randomized, crossover clinical trial group design will provide a power of 0.90 for plasma IL-6 (a variable for which sufficient data are available). Subject characteristics were compared between genders using independent *t*-tests, and performance data were compared between CQA coffee and placebo groups using dependent *t*-tests. All other data were analyzed using a 2 (CQA coffee, placebo trials) \times 3 or 4 (time points) repeated measures ANOVA, within subjects' approach. When interaction effects were significant ($p \leq .05$), changes from prestudy values within trials were compared between trials using dependent *t*-tests.

Results

Characteristics for the male ($n=10$) and female ($n=5$) study participants are compared in Table 1. Age, body mass index (BMI), and training distance were comparable between gender groups, with maximum cycling power (watts) and ventilation (L/min) higher in the males compared to females. No differences were found between male and female cyclists when comparing the contrast in 50-km trial time performances between coffee and placebo trials (gender difference, $p=.19$). Additionally, no significant between-subjects gender effects were found using the GLM model for repeated measures for TMD ($p=.56$), plasma caffeine ($p=.14$), plasma IL-6 ($p=.10$), and HODEs ($p=.99$), and supports similar findings in our lab from a metabolomics-based study (Nieman et al., 2013). Thus gender groups were combined for all data analyses.

The performance measures for the coffee and placebo 50-km cycling time trials are summarized in Table 2. Performance time, and absolute and relative power and oxygen consumption did not differ between the coffee and placebo trials. Average heart rate and ventilation were higher with coffee compared to placebo, especially at the 30-minute time point during the 50-km

Table 1 Subject Characteristics ($M \pm SE$)

| Variable | Males ($n = 10$) | Females ($n = 5$) | p -value |
|---|--------------------|---------------------|------------|
| Age (years) | 36.1 \pm 3.3 | 40.0 \pm 4.5 | .51 |
| Height (m) | 1.79 \pm 0.01 | 1.67 \pm 0.02 | .001 |
| Weight (kg) | 77.1 \pm 2.1 | 63.4 \pm 4.1 | .01 |
| Body mass index (kg/m ²) | 24.2 \pm 0.7 | 22.6 \pm 0.9 | .20 |
| Body fat (%) | 15.3 \pm 2.0 | 24.6 \pm 3.0 | .02 |
| VO _{2max} (ml · kg ⁻¹ · min ⁻¹) | 48.2 \pm 2.3 | 40.4 \pm 2.9 | .06 |
| Maximum power (watts) | 280 \pm 8.2 | 184 \pm 13.0 | <.001 |
| Maximum heart rate (beats/min) | 174 \pm 4.6 | 167 \pm 6.4 | .46 |
| Maximum ventilation (L/min) | 139 \pm 7.3 | 91.7 \pm 4.5 | .001 |
| Maximum respiratory exchange ratio | 1.17 \pm 0.02 | 1.13 \pm 0.04 | .39 |
| Maximum respiratory rate (breaths/min) | 53.0 \pm 3.4 | 44.4 \pm 3.0 | .12 |
| Training distance (km/wk) | 120 \pm 25.2 | 98.2 \pm 34.6 | .62 |

Table 2 Performance Measurements During the Coffee and Placebo 50-Km Cycling Time Trials ($M \pm SE$)

| Variable | Coffee | Placebo | p -value |
|---|-----------------|-----------------|------------|
| Time to complete 50-km cycling (minutes) | 98.8 \pm 3.2 | 99.2 \pm 3.5 | .71 |
| Average power (watts) | 169 \pm 13.3 | 171 \pm 13.3 | .60 |
| % maximal watts | 67.2 \pm 2.1 | 68.1 \pm 2.3 | .58 |
| Average oxygen consumption (ml/min) | 2533 \pm 123 | 2516 \pm 118 | .80 |
| % VO _{2max} | 76.9 \pm 1.9 | 77.5 \pm 2.1 | .74 |
| Average heart rate, 50-km (beats/min) | 154 \pm 3.9 | 150 \pm 4.0 | .003 |
| 30 minutes | 156 \pm 4.2 | 148 \pm 4.3 | <.001 |
| 60 minutes | 154 \pm 4.0 | 151 \pm 4.0 | .16 |
| % maximal heart rate, 50-km | 90.2 \pm 1.6 | 87.5 \pm 1.7 | .004 |
| Average ventilation, 50-km (L/min) | 70.2 \pm 2.9 | 65.0 \pm 2.9 | .07 |
| 30 minutes | 74.3 \pm 3.3 | 64.9 \pm 3.4 | .01 |
| 60 minutes | 67.3 \pm 3.3 | 65.8 \pm 2.5 | .60 |
| Average respiratory rate, 50-km (breaths/min) | 36.6 \pm 2.1 | 35.5 \pm 2.1 | .45 |
| 30 minutes | 36.2 \pm 2.2 | 33.6 \pm 2.3 | .16 |
| 60 minutes | 36.0 \pm 2.2 | 36.3 \pm 2.1 | .88 |
| Average respiratory exchange ratio, 50-km | 0.84 \pm 0.01 | 0.84 \pm 0.01 | .71 |
| 30 minutes | 0.87 \pm 0.01 | 0.85 \pm 0.01 | .15 |
| 60 minutes | 0.83 \pm 0.01 | 0.84 \pm 0.01 | .10 |
| Average rating of perceived exertion, 50-km | 13.0 \pm 0.4 | 13.3 \pm 0.4 | .44 |
| 30 minutes | 12.1 \pm 0.5 | 12.9 \pm 0.4 | .06 |
| 60 minutes | 13.5 \pm 0.5 | 13.4 \pm 0.5 | .73 |

cycling trials. The rating of perceived exertion (RPE) tended to be lower at the 30-minute time point in the coffee compared to placebo trial.

Three-day food records did not show any significant differences in macro- and micro-nutrient intake during the 3-day period prior to the 50-km cycling time trials under coffee and placebo conditions (data not shown). Energy intakes were 8.29–0.76 MJ/day and 8.64–0.54 MJ/day, with carbohydrate representing 45.7–3.1% and 47.0–

2.2%, protein 19.0–0.9% and 16.9–0.9%, and fat 35.1–2.9% and 36.3–1.5% of total energy for the coffee and placebo trials, respectively (all $p > .05$). Caffeine intake (irrespective of the coffee supplement) averaged 28.3–13.7 mg/day and 22.7–11.6 mg/day for the coffee and placebo trials, respectively ($p = .39$). Total flavonoid intake (irrespective of the coffee supplement) averaged 130–70.0 mg/day and 135–77.6 mg/day for the coffee and placebo trials, respectively ($p = .64$).

Plasma caffeine was significantly higher in the coffee compared to placebo condition after 2-weeks ingestion and immediately postexercise (interaction effect, $p < .001$) (Figure 1). TMD scores were lower in the coffee compared to placebo condition (interaction effect, $p = .04$), but contrasts did not differ significantly at any particular time point (Figure 2). The pattern of change in plasma FRAP between coffee and placebo conditions differed significantly (interaction effect, $p = .01$), especially immediately after the 50-km cycling trials (Figure 3). No differences between coffee and placebo trials were found for postexercise increases in

plasma IL-6 (interaction effect, $p = .74$) and plasma HODEs ($p = .99$; Figures 4 and 5).

Discussion

This study utilized a randomized crossover design, and showed that 2-weeks ingestion of high CQA (1,066 mg/day CQA) versus placebo (187 mg/day CQA) coffee prepared using the Turkish method did not alter increases in inflammation and oxidative stress after participating in a 50-km cycling time trial. Ingestion of one cup CQA coffee (474 mg caffeine) versus

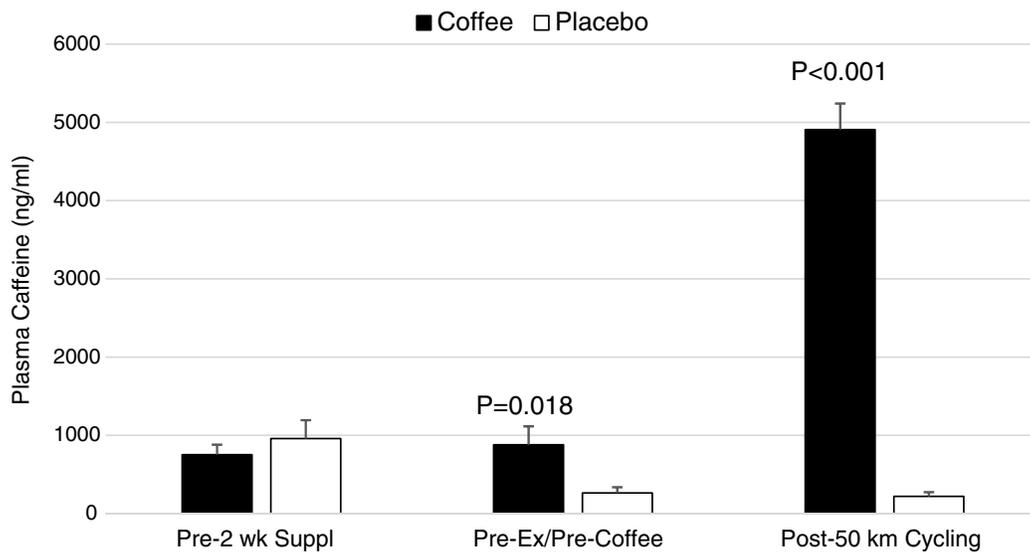


Figure 1 — Plasma caffeine in CQA coffee and placebo trials (interaction effect, $p < .001$). P -values above bars represent contrast in change from prestudy between trials.

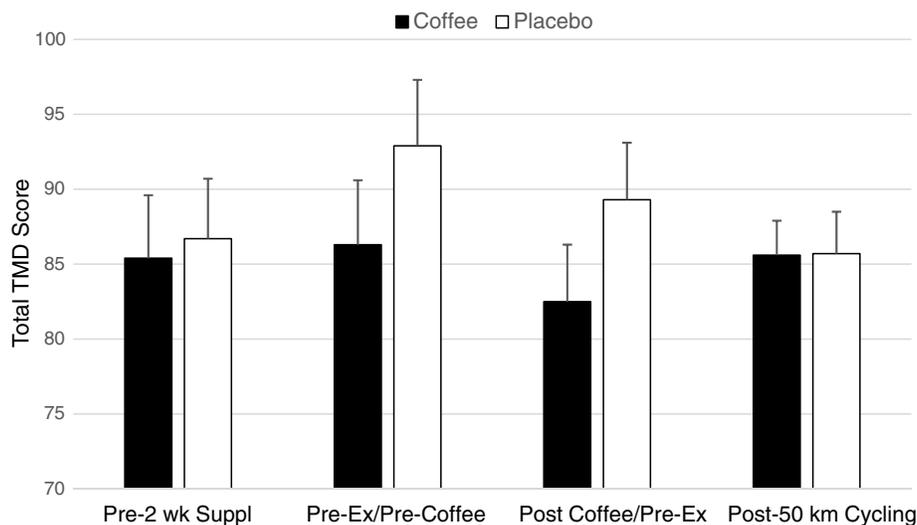


Figure 2 — Total mood disturbance (TMD) scores in CQA coffee and placebo trials (interaction effect, $p = .04$).

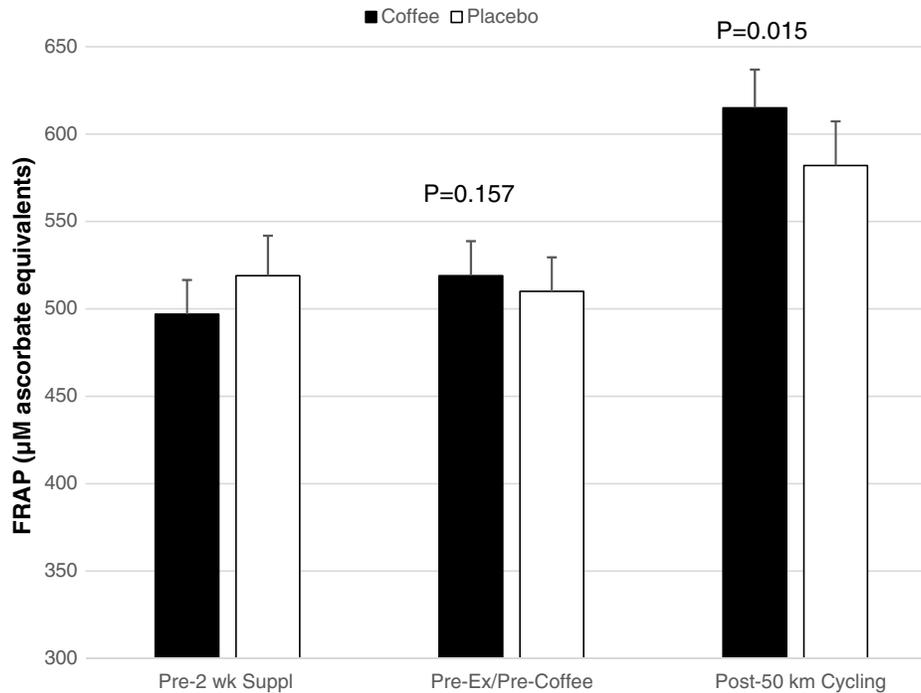


Figure 3 — Plasma FRAP in CQA coffee and placebo trials (interaction effect, $p = .01$). P -values above bars represent contrast in change from prestudy between trials.

placebo (33 mg caffeine) 30 minutes prior to exercise was associated with significant trial differences for plasma caffeine (901% increase postexercise) and FRAP, and improved mood state, but no difference in 50-km cycling time performance or power.

The 50-km cycling bout induced significant increases in plasma IL-6 (650%) and 9+13 HODEs (94%). Postexercise plasma IL-6 is an indicator of inflammation, with highest levels seen after prolonged and intensive endurance exercise (Nieman et al., 2014). Linoleic acid is the direct precursor to the stable oxidized metabolites 9+13 HODEs that function as biomarkers for both oxidative stress and inflammation (Nieman et al., 2014). 9+13 HODEs are secreted by a variety of cells including macrophages, endothelial cells, platelets, and smooth muscle cells, and exert biological and signaling activities as proliferator-activated receptor (PPAR) and G protein coupled receptor 132 (G2A) ligands (Nieman et al., 2014). High versus low CQA intake for two weeks was associated with higher FRAP as reported by others (Agudelo-Ochoa et al., 2016; Moura-Nunes et al., 2009; Natella et al., 2002), but did not lower postexercise plasma levels of IL-6 and 9+13 HODEs. Several explanations are possible, including the dosing regimen (e.g., duration, daily dose and timing), and issues related to CQA biotransformation.

Cell culture studies indicate that CQAs (in particular, 5-CQA) exert antioxidant and anti-inflammatory influences through multiple pathways, and downregulate

pro-inflammatory cytokines through modulation of key transcription factors (Liang & Kitts, 2014, 2015). However, a relatively low percentage of CQAs are absorbed in the small intestine, and these metabolites appear in low concentrations in the plasma 1- to 2-hours post-ingestion in conjugated form (i.e., linked to sulfate, methyl, and glucuronide molecules) (Del Rio et al., 2010). There is a growing realization that the colon plays a major role in CQA metabolism. Most CQAs pass through the small intestine unabsorbed, and then are extensively transformed by intestinal microbes. Substantial quantities of these colonic catabolites can be absorbed into the portal vein and pass through the liver into the circulation prior to excretion in the urine. Most CQA metabolites formed by intestinal transformation also become methylated, sulfated, or glucuronated (Lang et al., 2013; Stalmach et al., 2010). Some of these compounds have been studied and found to exert anti-inflammatory and antioxidant effects, but results from human intervention trials are unclear (Martini et al., 2016). More information is needed to understand underlying mechanisms, biochemical pathways, optimal dosing regimens, appropriate biomarkers, and reasons for the large variation between study participants (Lang et al., 2013). Martini et al. (2016) reviewed human coffee intervention studies and concluded that long-term exposure to coffee and its bioactive, metabolically transformed metabolites may be needed before measurable influences are experienced. In support, two epidemiological studies reported an inverse, modest

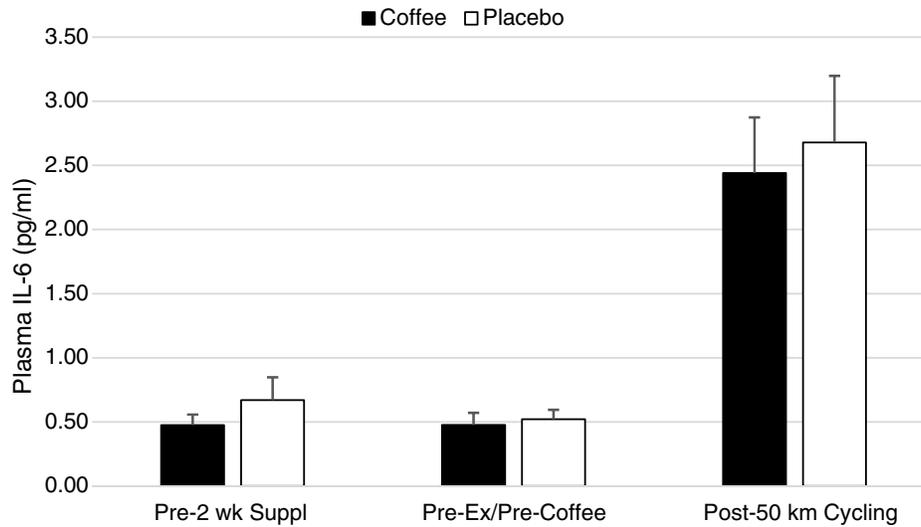


Figure 4 — Plasma IL-6 in CQA coffee and placebo trials (interaction effect, $p = .74$).

association between habitual coffee consumption and various measures of inflammation including CRP, IL-6, soluble tumor necrosis factor-receptor 2, E-selectin, and soluble vascular adhesion molecule-1 (Kempf et al., 2010; Lopez-Garcia et al., 2006).

In this study, 50-km cycling performance and power did not differ between coffee and placebo trials, with coffee ingestion associated with significantly increased heart rate and ventilation at the 30-minute time point, and reduced RPE ($p = .06$). Most performance studies have utilized anhydrous caffeine, and although results are not entirely consistent, reviewers have concluded that ingestion of 3–6 mg/kg caffeine is associated with

reduced RPE during exercise and improved time-trial performance (Doherty & Smith, 2005; Goldstein et al., 2010; McLellan et al., 2016). Fewer exercise performance studies have been published with caffeinated coffee, and results are mixed and in general less impressive than when anhydrous caffeine is consumed (Goldstein et al., 2010). One review showed that five of nine studies observed significant improvements in endurance performance, and three of six showed reduced RPE with caffeinated coffee ingestion (Higgins et al., 2016). Comparisons between studies are difficult due to varying research designs, the lack of randomized placebo control trials, insufficient information regarding the

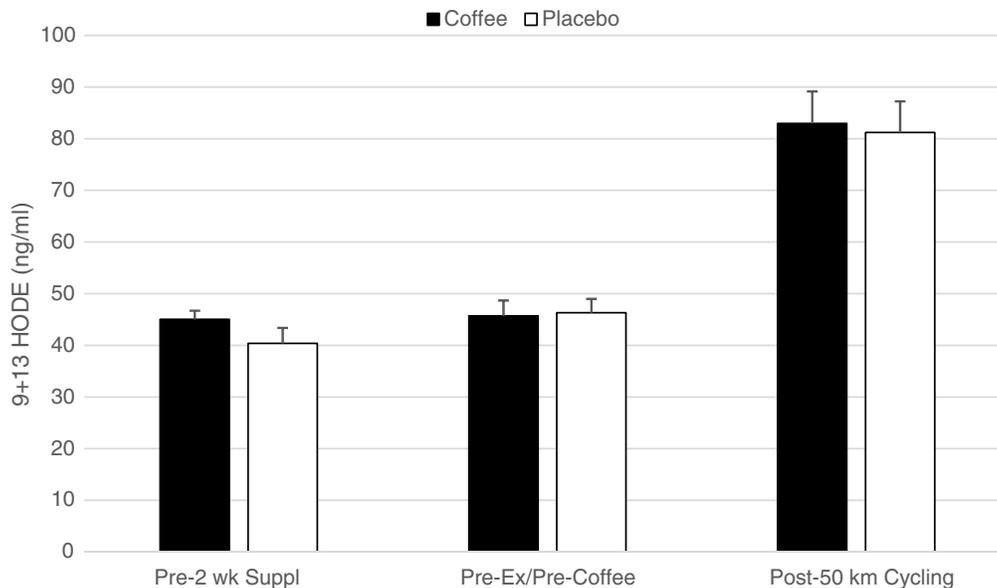


Figure 5 — Plasma 9 + 13 HODEs in CQA coffee and placebo trials (interaction effect, $p = .99$).

caffeine and CQA concentrations in the coffee doses, and the use of time-to-exhaustion exercise challenges instead of the preferred exercise time trials (Goldstein et al., 2010; Higgins et al., 2016). Although not a consistent finding, other investigators have reported higher exercise heart rates during caffeine compared to placebo trials (Hunter et al., 2002), and this may be due in part to increased release of the neurotransmitter noradrenaline that has been linked to intake of high caffeine doses (McLellan et al., 2016).

Following ingestion, caffeine is rapidly absorbed, with plasma levels rising to peak levels within one hour (Blanchard & Sawers, 1983). Our cyclists consumed one cup of coffee (474 mg caffeine) 30 minutes prior to the 50-km cycling time trial (99-minute average completion time), ensuring that plasma caffeine levels peaked during exercise. Caffeine plasma half-lives vary from 2.7 to 9.9 h, and behavioral effects are in part due to antagonism of A₁ and A_{2a} adenosine receptors in the CNS and interactions with dopamine receptors, and increased release of a variety of neurotransmitters including dopamine and serotonin (McLellan et al., 2016). Most studies support that ingestion of moderate caffeine doses improves vigilance, learning, and mood (Childs & de Wit, 2008; McLellan et al., 2016). One meta-analysis of 21 studies concluded that caffeine ingestion reduced RPE during exercise by 5.6%, with an equivalent RPE effect size of -0.47 (Doherty & Smith, 2005), and is consistent with our data showing a reduction in RPE at 30 minutes during the 50-km cycling time trial. Our data also support a significant trial difference for mood state as reported in other similar studies using both anhydrous caffeine and caffeinated coffee (Church et al., 2015; McLellan et al., 2016). Limited data suggest that CQA ingestion has a modest, positive effect on some aspects of mood state, but this has not been studied within an exercise context and potential underlying mechanisms have not been adequately explored (Camfield et al., 2013).

In summary, 2-weeks ingestion of coffee versus placebo prepared using the Turkish method and supplying 1,066 mg/day CQA and 474 mg/day caffeine increased plasma antioxidant capacity but did not offset increases in IL-6 and 9 + 13 HODEs following a 50-km cycling time trial. Plasma caffeine was higher and TMD scores lower with CQA coffee versus placebo, but 50-km cycling time performance and power did not differ between trials. These data do not support the strategy of ingesting coffee highly concentrated with chlorogenic acids and caffeine for two weeks prior to and the morning of a 50-km cycling time trial in lessening postexercise inflammation or oxidative stress.

Acknowledgments

We acknowledge the assistance of Drs. Kevin Knagge and Huiyuan Chen for their analysis of samples for plasma caffeine concentrations. Funding was provided by Academic Neutraceuticals LLC. Robert Arnot, MD, is the founder and CEO for

Academic Neutraceuticals. This study had prior approval from the IRB of Appalachian State University, and informed consent was obtained in writing from participants. Independent verification of the composition of the product was conducted.

References

- Agudelo-Ochoa, G.M., Pulgarín-Zapata, I.C., Velásquez-Rodríguez, C.M., Duque-Ramírez, M., Naranjo-Cano, M., Quintero-Ortiz, M.M., . . . Muñoz-Durango, K. (2016). Coffee consumption increases the antioxidant capacity of plasma and has no effect on the lipid profile or vascular function in healthy adults in a randomized controlled trial. *Journal of Nutrition*, *146*, 524–531. PubMed doi:10.3945/jn.115.224774
- Benzie, I.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power'; The FRAP assay. *Analytical Biochemistry*, *239*, 70–76. PubMed doi:10.1006/abio.1996.0292
- Blanchard, J., & Sawers, S.J. (1983). The absolute bioavailability of caffeine in man. *European Journal of Clinical Pharmacology*, *24*, 93–98. PubMed doi:10.1007/BF00613933
- Camfield, D.A., Silber, B.Y., Scholey, A.B., Nolidin, K., Goh, A., & Stough, C. (2013). A randomized placebo-controlled trial to differentiate the acute cognitive and mood effects of chlorogenic acid from decaffeinated coffee. *PLoS ONE*, *8*(12), e82897. PubMed doi:10.1371/journal.pone.0082897
- Childs, E., & de Wit, H. (2008). Enhanced mood and psychomotor performance by a caffeine-containing energy capsule in fatigued individuals. *Experimental and Clinical Psychopharmacology*, *16*, 13–21. PubMed doi:10.1037/1064-1297.16.1.13
- Church, D.D., Hoffman, J.R., LaMonica, M.B., Riffe, J.J., Hoffman, M.W., Baker, K.M., . . . Stout, J.R. (2015). The effect of an acute ingestion of Turkish coffee on reaction time and time trial performance. *Journal of the International Society of Sports Nutrition*, *12*, 37. PubMed doi:10.1186/s12970-015-0098-3
- Del Rio, D., Stalmach, A., Calani, L., & Crozier, A. (2010). Bioavailability of coffee chlorogenic acids and green tea flavan-3-ols. *Nutrients*, *2*, 820–833. PubMed doi:10.3390/nu2080820
- Ding, M., Satija, A., Bhupathiraju, S.N., Hu, Y., Sun, Q., Han, J., . . . Hu, F.B. (2015). Association of coffee consumption with total and cause-specific mortality in 3 large prospective cohorts. *Circulation*, *132*, 2305–2315. PubMed doi:10.1161/CIRCULATIONAHA.115.017341
- Doherty, M., & Smith, P.M. (2005). Effects of caffeine ingestion on rating of perceived exertion during and after exercise: A meta-analysis. *Scandinavian Journal of Medicine & Science in Sports*, *15*, 69–78. PubMed doi:10.1111/j.1600-0838.2005.00445.x
- Farah, A., Monteiro, M., Donangelo, C.M., & Lafay, S. (2008). Chlorogenic acids from green coffee extract are highly bioavailable in humans. *Journal of Nutrition*, *138*, 2309–2315. PubMed doi:10.3945/jn.108.095554

- Ganio, M.S., Klau, J.F., Casa, D.J., Armstrong, L.E., & Maresh, C.M. (2009). Effect of caffeine on sport-specific endurance performance: A systematic review. *Journal of Strength & Conditioning Research*, 23:315–324. [PubMed](#) doi:10.1519/JSC.0b013e31818b979a
- Goldstein, E.R., Ziegenfuss, T., Kalman, D., Kreider, R., Campbell, B., Wilborn, C., ... Antonio, J. (2010). International society of sports nutrition position stand: Caffeine and performance. *Journal of the International Society of Sports Nutrition*, 7, 5. [PubMed](#) doi:10.1186/1550-2783-7-5
- Grove, B., & Prapavessis, H. (1992). Preliminary evidence for the reliability and validity of an abbreviated profile of mood states. *International Journal of Sport Psychology*, 23, 93–109.
- Higgins, S., Straight, C.R., & Lewis, R.D. (2016). The effects of preexercise caffeinated coffee ingestion on endurance performance: An evidence-based review. *International Journal of Sport Nutrition and Exercise Metabolism*, 26, 221–239. [PubMed](#) doi:10.1123/ijnsnem.2015-0147
- Hodgson, A.B., Randell, R.K., & Jeukendrup, A.E. (2013). The metabolic and performance effects of caffeine compared to coffee during endurance exercise. *PLoS ONE*, 8(4), e59561. [PubMed](#) doi:10.1371/journal.pone.0059561
- Hunter, A.M., St Clair Gibson, A., Collins, M., Lambert, M., & Noakes, T.D. (2002). Caffeine ingestion does not alter performance during a 100-km cycling time-trial performance. *International Journal of Sport Nutrition and Exercise Metabolism*, 12, 438–452. [PubMed](#) doi:10.1123/ijnsnem.12.4.438
- Je, Y., & Giovannucci, E. (2014). Coffee consumption and total mortality: A meta-analysis of twenty prospective cohort studies. *British Journal of Nutrition*, 111, 1162–1173. [PubMed](#) doi:10.1017/S0007114513003814
- Kempf, K., Herder, C., Erlund, I., Kolb, H., Martin, S., Carstensen, M., ... Tuomilehto, J. (2010). Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: A clinical trial. *American Journal of Clinical Nutrition*, 91, 950–957. doi:10.3945/ajcn.2009.28548
- Lang, R., Dieminger, N., Beusch, A., Lee, Y.M., Dunkel, A., Suess, B., ... Hofmann, T. (2013). Bioappearance and pharmacokinetics of bioactives upon coffee consumption. *Analytical & Bioanalytical Chemistry*, 405, 8487–8503. [PubMed](#) doi:10.1007/s00216-013-7288-0
- Liang, N., & Kitts, D.D. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanisms of action. *Molecules*, 19, 19180–19208. [PubMed](#) doi:10.3390/molecules191119180
- Liang, N., & Kitts, D.D. (2015). Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients*, 8(1), 16. doi:10.3390/nu8010016
- Lofffield, E., Freedman, N.D., Dodd, K.W., Vogtmann, E., Xiao, Q., Sinha, R., & Graubard, B.I. (2016). Coffee drinking is widespread in the United States, but usual intake varies by key demographic and lifestyle factors. *Journal of Nutrition*, 146, 1762–1768. [PubMed](#) doi:10.3945/jn.116.233940
- Lopez-Garcia, E., van Dam, R.M., Qi, L., & Hu, F.B. (2006). Coffee consumption and markers of inflammation and endothelial dysfunction in healthy and diabetic women. *American Journal of Clinical Nutrition*, 84, 888–893. [PubMed](#)
- Ludwig, I.A., Mena, P., Calani, L., Cid, C., Del Rio, D., Lean, M.E., & Crozier, A. (2014). Variations in caffeine and chlorogenic acid contents of coffees: What are we drinking? *Food & Function*, 5, 1718–1726. [PubMed](#) doi:10.1039/C4FO00290C
- Martini, D., Del Bo, C., Tassotti, M., Riso, P., Del Rio, D., Brighenti, F., & Porrini, M. (2016). Coffee consumption and oxidative stress: A review of human intervention studies. *Molecules*, 21(8), 979. doi:10.3390/molecules21080979
- McLellan, T.M., Caldwell, J.A., & Lieberman, H.R. (2016). A review of caffeine's effects on cognitive, physical and occupational performance. *Neuroscience & Biobehavioral Reviews*, 71, 294–312. [PubMed](#) doi:10.1016/j.neubiorev.2016.09.001
- Mills, C.E., Tzounis, X., Oruna-Concha, M.J., Mottram, D.S., Gibson, G.R., & Spencer, J.P. (2015). In vitro colonic metabolism of coffee and chlorogenic acid results in selective changes in human faecal microbiota growth. *British Journal of Nutrition*, 113, 1220–1227. [PubMed](#) doi:10.1017/S0007114514003948
- Monteiro, M., Farah, A., Perrone, D., Trugo, L.C., & Donangelo, C. (2007). Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. *Journal of Nutrition*, 137:2196–2201. [PubMed](#)
- Moura-Nunes, N., Perrone, D., Farah, A., & Donangelo, C.M. (2009). The increase in human plasma antioxidant capacity after acute coffee intake is not associated with endogenous non-enzymatic antioxidant components. *International Journal of Food Sciences and Nutrition*, 60(Suppl. 6), 173–181. doi:10.1080/0963748090315889
- Natella, F., Nardini, M., Giannetti, I., Dattilo, C., & Scaccini, C. (2002). Coffee drinking influences plasma antioxidant capacity in humans. *Journal of Agricultural and Food Chemistry*, 50, 6211–6216. [PubMed](#) doi:10.1021/jf025768c
- Nieman, D.C., Shanely, R.A., Gillitt, N.D., Pappan, K.L., & Lila, M.A. (2013). Serum metabolic signatures induced by a three-day intensified exercise period persist after 14 h of recovery in runners. *Journal of Proteome Research*, 12, 4577–4584. [PubMed](#) doi:10.1021/pr400717j
- Nieman, D.C., Shanely, R.A., Luo, B., Meaney, M.P., Dew, D.A., & Pappan, K.L. (2014). Metabolomics approach to assessing plasma 13- and 9-hydroxy-octadecadienoic acid and linoleic acid metabolite responses to 75-km cycling. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 307, R68–R74. [PubMed](#) doi:10.1152/ajpregu.00092.2014
- Renouf, M., Marmet, C., Giuffrida, F., Lepage, M., Barron, D., Beaumont, M., ... Dionisi, F. (2014). Dose-response plasma appearance of coffee chlorogenic and phenolic acids in adults. *Molecular Nutrition & Food Research*, 58, 301–309. [PubMed](#) doi:10.1002/mnfr.201300349

- Richelle, M., Tavazzi, I., & Offord, E. (2001). Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *Journal of Agricultural and Food Chemistry*, *49*, 3438–3442. [PubMed](#) doi:10.1021/jf0101410
- Sebastian, R.S., Wilkinson Enns, C., Goldman, J.D., Steinfeldt, L.C., Martin, C.L., & Moshfegh, A.J. (2016). *Flavonoid Values for USDA Survey Foods and Beverages 2007–2010*. Beltsville, MD: U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group. Available from www.ars.usda.gov/nea/bhnrc/fsrg
- Spriet, L.L. (2014). Exercise and sport performance with low doses of caffeine. *Sports Medicine*, *44*(Suppl. 2), 175–184. doi:10.1007/s40279-014-0257-8
- Stalmach, A., Steiling, H., Williamson, G., & Crozier, A. (2010). Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Archives of Biochemistry and Biophysics*, *501*, 98–105. [PubMed](#) doi:10.1016/j.abb.2010.03.005
- Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. *European Journal of Nutrition*, *56*, 2215–2244. [PubMed](#) doi:10.1007/s00394-017-1379-1
- Tarnopolsky, M.A. (2008). Effect of caffeine on the neuromuscular system—potential as an ergogenic aid. *Applied Physiology, Nutrition, and Metabolism*, *33*, 1284–1289. [PubMed](#) doi:10.1139/H08-121
- Zamora-Ros, R., Knaze, V., & Rothwell, J.A. (2016). Dietary polyphenol intake in Europe: The European Prospective Investigation into Cancer and Nutrition (EPIC) study. *European Journal of Nutrition*, *55*, 1359–1375. doi:10.1007/s00394-015-0950-x