A Prospective Randomized, Double-Blind, Two-Period Crossover Pharmacokinetic Trial Comparing Green Coffee Bean Extract—A Botanically Sourced Caffeine—With a Synthetic USP Control

Kayce Morton¹, Katelin Knight², Douglas Kalman¹, and Susan Hewlings²

Abstract

Coffee is a primary dietary source of the chlorogenic acids (CGAs) of phenolic compounds. Coffee contains caffeine and other phytonutrients, including CGAs. Caffeine on its own has been well characterized and described pharmacokinetically in the literature, less so for CGAs. The purpose of this double-blind crossover study was to determine the comparative pharmacokinetics of CGAs with caffeine (natural extract) with synthetic caffeine (US Pharmacopeia [USP] standard). Sixteen healthy male subjects were randomly assigned to take 1 dose of product 1, 60 mg of botanically sourced caffeine from 480 mg of green coffee bean extract, or product 2, 60 mg of synthetic USP caffeine, with 5 days between. Blood analysis was done to determine the levels of CGA compounds, more specifically 3-, 4-, and 5-caffeoylquinic acid (CQA), and serum caffeine. The natural caffeine extract exhibited mean peak concentrations (Cmax) of 3-CQA (11.4 ng/mL), 4-CQA (6.84 ng/mL), and 5-CQA (7.20 ng/mL). The mean systemic 4-hour exposure (AUC0–4 h) was 3-CQA (27.3 ng·h/mL), 4-CQA (16.1 ng·h/mL), and 5-CQA (15.7 ng·h/mL). The median tmax was 3-CQA (1.00 hour), 4-CQA (1.00 hour), and 5-CQA (1.50 hours). The tmax of caffeine was 0.75 hours (natural extract) and 0.63 hours (synthetic caffeine). Cmax and AUC0–4 h of serum caffeine were statistically equivalent between products. The geometric least-squares mean ratios (GMRs) of Cmax and AUC0–4 h of caffeine were 97.77% (natural extract) and 98.33% (synthetic caffeine). It would appear that CGA compounds from the natural caffeine extract are bioavailable, and 3-CQA may be the compound most absorbed. In addition, caffeine sourced from natural extract versus synthetic were statistically similar for pharmacokinetic parameters. There were no adverse events or safety concerns.

Keywords

chlorogenic acids, coffee bean extract, caffeine, pharmacokinetics, phenolic compounds

Data from the National Health and Nutrition Examination Survey indicate that 89% of Americans regularly consume caffeinated products.¹² The United States Department of Agriculture and Health Canada have concluded that consumption of caffeine < 400 mg/day is generally safe and may even confer some health benefits.³⁴ Caffeine consumption has been linked to a variety of health outcomes from a reduced rate of metabolic syndrome and diabetes and lowered serum triglyceride levels to enhanced athletic performance and improved memory and attention.³⁵ Negative side effects have also been noted and attributed to caffeine’s stimulation of the body’s adrenaline response, which has been associated with acute increases in blood pressure and heart rate, although habitual users usually develop tolerance to these effects.³⁵ Substances other than caffeine that have antioxidant properties

¹QPS Bio-Kinetic, Springfield, MO, USA
²Central Michigan University, Substantiation Sciences, Mt. Pleasant, MI, USA

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Corresponding Author:
Dr. Susan Hewlings, Central Michigan University College of Education and Human Services, 1200 S. Franklin St., Mt. Pleasant, MI 48859
(e-mail: sue.hewlings@gmail.com)
in natural sources such as coffee and green tea have been suggested as the mechanism behind the positive health outcomes.6,7 This is supported by evidence showing that the benefits are similar in caffeinated and decaffeinated beverages.8 For example, coffee contains phenolic compounds called hydroxycinnamates, which are made primarily of chlorogenic acids (CGAs). CGAs are formed by the esterification of hydroxycinnamic acids, such as caffeic, ferulic, and p-coumaric, with quinic acid. The CGA major subclasses in coffee are caffeoylquinic acids (CQAs), dicaffeoylquinic acids (diCQAs), and feruloylquinic acids (FQAs) with several isomers per group.9 Among these compounds, 5-CQA alone accounts for about 56%–62% of total CGAs in green coffee beans and approximately 35% of total CGAs in roasted coffee, with all CQA and diCQA isomers together responsible for 92%–95% of CGAs.10,11 Derivatives and phenolic metabolites of CGAs, like caffeic (CA) or ferulic (FA) acid have been studied for their potential biological efficacy because of their potential health benefits and their documented antioxidant, nitrite-scavenging, and anticarcinogenetic activities. CA is abundant in berries, fruits, and coffee in relatively high concentrations. Because of prevalent intake of coffee in most cultures, CGAs are among the most abundant polyphenols in the diet.12 A single serving of coffee provides between 20 and 675 mg of CGAs, depending on the type of roast and the volume consumed, and regular coffee consumers can easily have an intake in excess of 1 g per day.9,13

It appears that the major CGA compounds present in coffee are differentially absorbed and/or metabolized in humans, with a large interindividual variation.14 CGAs are most likely not absorbed as whole compounds but first metabolized to smaller phenolics prior to absorption. Renouf et al reported that caffeic and ferulic acid derivatives were detected in plasma or urine after coffee ingestion. They observed differences in urinary excretion or the plasma appearance of the smaller phenolics, suggesting that some absorption of phenolic acids takes place in the small intestine but that the colon and the microflora play a major role in the metabolism of chlorogenic acids.15 The evidence that the colon is a primary site for CGA absorption has been supported by other studies.16,17 It has been shown that commensal gut bacteria such as Bifidobacterium and Lactobacillus are involved in the release of bioactive hydroxycinnamic acids, primarily CGAs, in the human colon.18 Furthermore, there appears to be a synergistic relationship whereby CGA inhibits noncommensal intestinal bacteria such as opportunistic pathogens.19,20 The known interindividual differences in gut microflora suggest that individual variations in the bioactivity of CGA are secondary to this.21

Overall data suggest that there may be advantages to a plant source of caffeine compared with a synthetic form; although the caffeine structure is the same, the additional polyphenols from plant sources may differentiate them. A recent study by Krieger et al examined a dose of 200 mg of caffeine (similar to the amount in many popular commercial coffee beverages in the United States) and demonstrated that natural caffeine extracts behave like synthetic caffeine with respect to effects on the cardiovascular system and absorption.22 Specifically, the Krieger et al study demonstrated that over a 240-minute postingestion period (4 hours), relative changes from baseline for serum caffeine did not differ between the botanical caffeine extract and standardized synthetific caffeine.22 More pointedly, the prior published data show that the 4-hour bioequivalence ratios of the log-transformed Cmax, AUC0–4 h, and AUC0–4 h values relative to the control were within the Food and Drug Administration’s standard equivalence range of 80%–125% for log-transformed data.23

Caffeine is rapidly absorbed, with 99% absorbed within 45 minutes of ingestion. In a study of adult men, a dose of 4 mg/kg (280 mg/70-kg human, or about 2–3 cups of coffee) had a caffeine half-life of 2.5–4.5 hours and was not affected by age.23 The mean half-life of caffeine in plasma of healthy individuals is about 5 hours, ranging from 1.5 to 9.5 hours because of individual variation.24 Less is known about the pharmacokinetics of caffeine at lower doses, and considering that the effects of caffeine are dose dependent, studies comparing synthetic to natural sources of caffeine at different doses are needed.24 The current study sought to replicate the postingestion duration of 240 minutes (4 hours) to be consistent with prior published research that also examined natural caffeine extract and a synthetic comparator.22 With the aforementioned in mind, we sought to undertake this comparative pharmacokinetic study of natural caffeine extract (containing polyphenols as inherent in coffee) versus a standard synthetic source of caffeine to learn about the relative activity and kinetics of CGA and caffeine, respectively, in healthy adult humans.

Methods

Prior to enrollment in the study, written informed consent was obtained from each subject. The protocol and the informed consent form were reviewed and approved by the Bio-Kinetic Clinical Applications Institutional Review Board (IRB) on January 16, 2017. The IRB is constituted and operates in accordance with the principles and requirements in 21 CFR Part 56. The study was executed at QPS-Bio-Kinetics, Springfield, Missouri.

The goals of this study were to determine the relative and comparative pharmacokinetics of CGA from
green coffee beans (as a standardized natural extract), as measured by standardized laboratory techniques, as well as to determine if different sources of caffeine (naturally vs synthetically derived) would have the same or similar relative pharmacokinetic characteristics. Additional objectives were to assess and determine the safety profile of caffeine derived from green coffee beans via monitoring of vital signs (blood pressure and heart rate), adverse events, and subjective remarks compared with the standardized control.

Sixteen healthy male subjects were randomly assigned to 1 of the 2 test groups in a double-blind crossover design. Product 1 (natural extract) was 60 mg of botanically sourced caffeine derived from 480 mg of green coffee bean extract from Applied Food Sciences, Inc. (Austin, Texas); see Table 1 for CGA analysis for product 1. Product 2 (synthetic caffeine) was 60 mg of synthetic US Pharmacopeia caffeine. Fifteen finishers participated for approximately 2 weeks, with the pharmacokinetic (PK) test visits separated by a minimum of 5 days. In each PK test period (1 and 2), subjects were given the treatment in an 8-ounce liquid form (in a premade bottle of the green coffee bean extract or the synthetically derived caffeine mixed in water with flavorings [the beverages were in appearance the same]; subjects were also instructed to add 2 ounces of water into the bottle after they finished the initial 8 ounces, swish the added water, and drink to ingest any potential residue) in period 1 and the alternative/opposite treatment in period 2.

The subjects did not consume caffeine or caffeine-containing products for at least 24 hours prior to testing. They fasted overnight for at least 10 hours prior to each test period and until after the final PK sample collections. Subjects were instructed to consume the entire 8 ounces of beverage within a 5-minute period. Blood collections were done to determine CGA levels (3-, 4-, and 5-CQA) along with serum caffeine (methylxanthine) approximately 1 hour prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, and 4 hours postadministration. The study purposely only used 4 hours postdosing to collect blood samples for caffeine. This was done to be in concert with prior work in our laboratory. Caffeine has a known half-life of ~5 hours, with, more importantly, a tmax of ~60 minutes. Knowing the tmax of caffeine is approximately 60 minutes, the 4-hour post-ingestion period is appropriate. Blood pressure and heart rate were measured approximately 1 hour predose and approximately 4 hours postadministration (±30 minutes) for safety monitoring. Additional analysis was done for serum CGA compounds approximately 1 hour predose and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, and 4 hours postadministration for caffeine derived from green coffee beans only (product 1). Subjects were provided a standard snack or meal following the 4-hour postdose blood sample collection.

**Pharmacokinetic Methods**

PK analyses of CGAs (3-, 4-, and 5-CQA) and serum caffeine were conducted using the concentration–time data, and they were further analyzed/calculated by the noncompartmental model of Phoenix WinNonlin version 6.3 (linear trapezoidal with linear interpolation; Pharsight Corporation, St. Louis, Missouri). Actual elapsed times from dosing were used to estimate all individual serum PK parameters for evaluable subjects; the end points included Cmax, tmax, and AUC0-4 h.

The PK variables and PK end points were summarized by time and caffeine source (natural vs synthetic). The descriptive statistics of the PK parameters and concentration data were summarized as arithmetic mean, standard deviation, number of subjects, median, minimum, maximum, geometric mean, and related standard procedures.

**Chlorogenic Acids and Caffeine Analysis**

CGAs (3-, 4-, and 5-CQA) were analyzed using high-pressure liquid chromatography (HPLC) ultraviolet technology. Caffeine was analyzed using the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. All laboratory analysis was conducted by Keystone Bioanalytical, Inc (North Wales, Pennsylvania: https://www.keystonebioanalytical.com/), a licensed contract analytical laboratory. Keystone Bioanalytical Inc. developed a method, M161202, for the quantification of several caffeoylquinic acids (listed below) in K2-ethylenediaminetetraacetic acid (EDTA) human plasma using LC-MS/MS.

Samples are thawed on an ice bath and aliquoted to test tubes. The samples separated from plasma by protein precipitation using methanol. A 5-μL sample...
was injected into the LC-MS/MS system for analysis. The standard curve range was 2.5–250 ng/mL, and the lower limit of quantitation (LLOQ) was 2.5 ng/mL. A 100-μL aliquot of plasma was used for analysis. Keystone maintains its own detailed standard analytical procedure for the quantification of caffeoylquinic acids in K2-EDTA human plasma (M161202.02). Caffeoylquinic acid is used in the calibration standard.

Caffeine was also analyzed by the contract research laboratory Keystone Bioanalytical, Inc. (https://www.keystonebioanalytical.com/). Keystone has also developed a method (M130705.00) for the quantification of caffeine in human serum using LC-MS/MS technology (MDS Sciex API 4000 with TurboIonSpray source; Applied Biosystems). In summary, samples were aliquoted to test tubes and then spiked with the internal standard (caffeine-13C3). The samples with caffeine and the internal standard were isolated using liquid–liquid extraction (acetonitrile used as the solvent). After vortexing and centrifugation, 50 μL of supernatant was transferred to a clean plastic injection vial that contained 400 μL of reconstitution solution (2 mM ammonium acetate in 40:60 acetonitrile/water), and a 10-μL injection volume was used for LC-MS/MS analysis. The standard curve range is 0.05–20 μg/mL, with an LLOQ of 0.05 μg/mL. Fifty microliters of human serum is required for analysis.

The chromatography was acceptable in terms of column efficiency, linearity, and sensitivity. Endogenous interference was found in blank human serum. No endogenous interference was found in the blank control (4% bovine serum albumin in phosphate-buffered saline). Precision and accuracy of this method were found to be satisfactory (Dr. Allan Xu, Keystone, internal communication). The HPLC used was a Synergi Polar-RP 50 × 2 mm, 4 μm supplied by Phenomix. The blank control used was 4% bovine serum albumin. The primary standard stock solution for the caffeine reference standard had a purity of 96.9%, with the primary internal standard stock solution being caffeine-13C3 (Cerilliant) with a purity of 99.8%.

**Statistical Methods**

Statistical software used for this study included SAS v9.3 (SAS Institute Inc, Cary, North Carolina). PK variables (AUC0–4 h and Cmax) were analyzed using the SAS Mixed procedure for the log-transformed values. The analysis of variance model included sequence, period, and treatment as fixed effects and subject within sequence as a random effect. Back-transformed statistics and inferential results were reported for PK parameters. The 90% CIs were generated for the GMR of AUC0–4 h and Cmax for botanically sourced caffeine to the synthetic USP control.

To assess the for comparative pharmacokinetics and characterization (comparison of the botanically sourced caffeine with the synthetic) within the 240-minute period (4 hours postingestion), 90% CIs for the GMRs of AUC0–4 h and Cmax were analyzed. This comparison was made solely within the design of the study for the 240-minute (4-hour) postingestion period.

The type 1 error rate was specified at an α level of 0.05. Each efficacy end point was considered an independent question of interest and was tested independently at the 0.05 α level (P ≤ .05) required for a conclusion of statistical significance.

**Results**

**Safety Results**

There were no statistically significant differences in age, body mass index, vital signs, or any metabolic parameter between participants in either group throughout the study. There were no safety concerns in this study. No adverse event were reported by any subject or observed by the research staff.

**Pharmacokinetic Results**

The comparative PK analysis of CGA compounds provides insight into their relative rate of appearance in the blood (rate of recovery or crude availability) and characteristics. Focusing on the 3 main CGA compounds (3-, 4-, and 5-CQA), as described in Figure 1, the rise from baseline values is sharp and strongest by the
1-hour postingestion period (1 over the 4 hours tested). This peak at the first hour mark over the 4 hours tested for the individual CGA compounds and for the total of the 3 indicates rapid overall absorption or uptake in the blood. The fast absorption or rate of appearance is supported by the t\text{max} results for the individual CGA compounds, which were equal for 3- and 4-CQA (mean, 1.00 hour [0.75–4.00 hours]), whereas the 5-CQA t\text{max} was slightly longer and differed from the other compounds (mean, 1.50 hours [0.75–2.50 hours]). Thus, the time to maximum absorption over the 4 hours tested (as indicated by rate of appearance in the blood) was approximately 1 hour after ingestion, at least for the 3 and 4-CQA CGA compounds. Further bolstering the relatively fast absorption/rate of appearance profile was that the total CQA compounds combined also had a mean t\text{max} of 1.00 hour (0.75–4.00 hours), indicating the time to maximal concentration over the 4 hours tested was generally reached within the first hour after ingestion.

When examining the data for how well any of the CGA compounds were absorbed (as viewed by rate of appearance and blood concentrations), the C\text{max} analysis indicated that the 3-CQA reached a maximum concentration of almost double that of either 4-CQA or 5-CQA (11.4 ± 4.52 ng/mL vs 6.84 ± 3.13 and 7.20 ± 3.66 ng/mL, respectively). The order of magnitude for the AUC appeared to be directly related to the dosing or concentration of each of the 3-, 4-, and 5-CQA compounds. The dosing of the 3-CQA was ~103 mg, whereas the 4- and 5-CQA were ~90 mg, making them similar in net weight delivered. In relative terms, the 3-CQA compound reached a concentration that was 60% and 58% greater than the 4-CQA and 5-CQA compounds, respectively. The greatest apparent rate of appearance appeared to be for the 3-CQA compound. This was upheld when examining the AUC data for the 4-hour period measured. The relative AUC\textsubscript{0–4 h} was 27.3 ± 14.7 ng·h/mL for 3-CQA, 16.1 ± 9.8 ng·h/mL for 4-CQA, and 15.7 ± 10.4 ng·h/mL for 5-CQA, indicating that 3-CQA was most absorbed of the CGA compounds evaluated. The 3-CQA compound was in fact ~41% greater than 4-CQA and 42.5% more noticeable in the blood test measures than 5-CQA within the tested period.

Overall when examining the comparative pharmacokinetics of dietary CGA compounds, it was apparent that they were absorbed relatively quickly (peak concentration was within the first hour) and that by the end of the 4-hour study period, the 3-, 4-, and 5-CQA compounds all remained elevated in the blood at a degree greater than baseline (see Figure 1). This indicates early-phase metabolism with slow excretion or clearance time. Orally, the natural caffeine extract product contained 53.5% 3-CQA, 24% 4-CQA, and 22.6% 5-CQA, and the rate of appearance (apparent absorption) also reflected the hierarchy of oral dosing—meaning that the PK values (C\text{max} and AUC\textsubscript{0–4 h}) also followed this hierarchy for CGA appearance in the blood.

Comparative pharmacokinetic analysis of the 2 caffeine-containing products revealed the C\text{max} of product 1 to be 1.91 ± 0.876 μg/mL, whereas product 2 had a C\text{max} of 2.09 ± 1.49 μg/mL. The t\text{max} for product 1 was 0.75 hours (with a minimum and maximum of 0.50 and 1.50, respectively), whereas product 2 t\text{max} was determined to be 0.63 hours (with a minimum and maximum of 0.50 and 1.50 hours, respectively). Furthermore, the area under the 4-hour curve (AUC\textsubscript{0–4 h} for product 1 was 6.35 ± 3.34 μg·h/mL, whereas for product 2, the AUC\textsubscript{0–4 h} was 6.99 ± 5.45 μg·h/mL). The GMRs of the C\text{max} and AUC\textsubscript{0–4 h} of caffeine between product 1 and product 2 were 97.77% and 98.33%, respectively. The 90%CIs of GMRs of C\text{max} and AUC\textsubscript{0–4 h} for caffeine between products 1 and 2 were not considered different. The median t\text{max} of caffeine after administration of products 1 and 2 was 0.75 and 0.63 hours, respectively. See Figure 2 for mean changes from baseline for caffeine between the groups. Expression includes from 0 baseline as to examine the comparative changes in this biomarker. See Table 2 for mean comparative pharmacokinetic parameters and Table 3 for statistical comparisons.

**Discussion**

In this human double-blind crossover study, there were no adverse events reported after consumption of 60 mg...
Table 2. Summary (Mean and SD) of Pharmacokinetic Parameters for Caffeine After Administration of Products 1 and 2 in Healthy Male Subjects

| PK Parameters          | Product 1 |          | Product 2 |          |
|------------------------|-----------|----------|-----------|----------|
|                        | n | Mean (SD) | n | Mean (SD) |
| Cmax, µg/mL            | 15 | 1.91 (0.876) | 16 | 2.09 (1.49) |
| tmax, h               | 15 | 0.75 (0.50, 1.50) | 16 | 0.63 (0.50, 1.50) |
| AUC0-4 h, µg·h/mL     | 15 | 6.35 (3.34) | 16 | 6.99 (5.45) |

Product 1, botanically sourced caffeine 60 mg derived from 480 mg of green coffee bean extract; product 2, synthetic US Pharmacopeia caffeine 60 mg.

Table 3. Statistical Comparisons of Serum Pharmacokinetic Parameters of Caffeine After Administration of Products 1 and 2 to Healthy Male Subjects

| PK Parameters          | Product 1 |          | Product 2 |          | Products 1 and 2 |          |
|------------------------|-----------|----------|-----------|----------|------------------|----------|
|                        | n | GM        | n | GM    | GMR (90%CI)     |
| Cmax,a, µg/mL         | 15 | 1.71 | 16 | 1.75 | 97.77 (81.90–116.71) |
| AUC0-4 h,a, µg·h/mL   | 15 | 5.51 | 16 | 5.60 | 98.33 (82.11–117.74) |

*aBack-transformed least-squares mean and confidence interval from ANOVA model performed on log-transformed values. GM, geometric least-squares mean; GMR, geometric least-squares mean ratio; CI, confidence interval. GMR and 90%CI are reported as percentages. Product 1, botanically sourced caffeine 60 mg derived from 480 mg of green coffee bean extract; product 2, synthetic US Pharmacopeia caffeine 60 mg.

Table 4. Summary (Mean and SD) of Pharmacokinetic Parameters for 3-CQA, 4-CQA, 5-CQA, and All After Administration of Product 1 in Healthy Male Subjects

| PK Parameters          | 3-CQA |          | 4-CQA |          | 5-CQA |          | All (3-CQA, 4-CQA, and 5-CQA) |
|------------------------|--------|----------|--------|----------|--------|----------|-------------------------------|
|                        | n | Mean (SD) | n | Mean (SD) | n | Mean (SD) | n | Mean (SD) |
| Cmax, ng/mL            | 15 | 11.4 (4.92) | 15 | 6.84 (3.13) | 15 | 7.20 (3.66) | 15 | 25.1 (10.2) |
| tmax, h               | 15 | 1.00 (0.75, 4.00) | 15 | 1.00 (0.75, 4.00) | 15 | 1.50 (0.75, 2.50) | 15 | 1.00 (0.75, 4.00) |
| AUC0-4 h, ng·h/mL    | 15 | 27.3 (14.7) | 15 | 16.1 (9.80) | 15 | 15.7 (10.4) | 15 | 59.1 (31.1) |

*aMedian (Min, Max).

of caffeine from either green coffee bean extract or that which was synthetically derived. These results are also in agreement with a similar study that reported no safety concerns with an acute dose of 200 mg of caffeine from the same products.22

Phenolic compounds found in coffee, specifically CGAs, were analyzed. In this study, we compared 3-CQA, 4-CQA, and 5-CQA because CQA is the major CGA subclass found in coffee, with 5-CQA accounting for approximately 35% of the total CGA in roasted coffee.10,11 We measured these compounds both individually and grouped together to denote their relative comparative pharmacokinetics (PK), rate of appearance in the blood, and estimated absorption profiles within the confines of this study design. Our results indicated that 3-CQA was absorbed to the greatest degree compared with both 4-CQA and 5-CQA. Our results indicate that of the 3 CGA compounds analyzed, 3-CQA demonstrated the greatest rate of appearance and concentration in the blood, as indicated by the Cmax values. This is apparent when examining the data in Figure 1 and Table 4. The tmax (time it takes to get the maximal amount of the compound in the blood) did not significantly differ between CGA compounds (tmax ranged from 1 to 1.5 hours), whereas it appeared that 3-CQA was absorbed in the greatest concentration compared with the other CGA compounds in the study product. Note that prior research found that 5-CQA accounts for about 56%–62% of total CGA in green coffee beans and approximately 35% of total CGA in roasted coffee.10,11 In this study, the study product contained a total of 238 mg of CGA per serving, with
3-CQA, 4-CQA, and 5-CQA comprising 193.1 mg of the total (81%). Of this, 5-CQA comprised 18% of the total CGA compounds. It should be noted that CGA levels can be significantly impacted by crop conditions, agricultural processes, genetics, variety of the plant, maturation of the plant, and roasting and processing, which can challenge comparisons across products.

A study by Farah et al (2008) evaluated the pharmacokinetic profile and bioavailability of CGA in plasma and urine of 10 healthy adults for 8 hours after the consumption of a decaffeinated green coffee extract containing 170 mg (451 mmol) of CGA (including lactones and caffeoyltryptophan), and CQA represented 71.2% of CGAs in the capsules (compared with 81% in our study), with 3-CQA, 4-CQA, and 5-CQA contributing to 23%, 21.6%, and 6.6% (wt:wt), respectively. In contrast to our study, in which 3-CQA was the major CGA, in the Farah et al study, 5-CQA was the major CGA identified in the plasma of all subjects at all times after green coffee extract consumption, as indicated by both its \( C_{\text{max}} \) and AUC. Based on the AUC, 3-CQA, 4-CQA, and 5-CQA comprised 5.2%, 7.5%, and 31.3%, respectively, of the total phenolic compounds in the plasma. They reported considerable variability of apparent bioavailability of CGAs among subjects, not influenced by sex, age, or body composition. The authors concluded that that the main CGA compounds present in the green coffee matrix were highly bioavailable in humans. Large interindividual variation clearly exists in CGA absorption, metabolism, and kinetics in humans.

Roasted coffee (standard coffee) contains chlorogenic acids, as do green coffee beans (and their extract). In a study by Monteiro et al,14 6 subjects were given 190 mL of standard coffee. The authors reported that 5-CQA, the major CGA in the standard coffee, was alone responsible for 40% of total hydroxycinnamates identified in plasma during the 4 hours of the study, with a considerable contribution of other CGAs to total plasma hydroxycinnamates. Together, 3-CQA and 4-CQA were responsible for \( \sim 18\% \), and diCQA was responsible for \( \sim 28\% \). The authors note that plasma caffeic acid contributed only 14% of the total plasma hydroxycinnamates, and no nonesterified caffeic acid was present in the standard coffee, and during the analytical recovery test in plasma, \( \sim 7\% \) of 5-CQA was hydrolyzed into caffeic acid. These processes may explain why the 3-CQA was the most abundant in our study—it may have been because of 5-CQA hydrolyzation. In addition, they noted large interindividual variation in CGA absorption and/or metabolism, which could also explain differences across studies.

Studies on the comparative and sole bioavailability of CGAs are few and difficult to compare and interpret because of differences in study design and dosage and varying types of CGAs analyzed.14 In a recent study on healthy subjects ingesting 412 \( \mu \)mol of CGA in 200 mL of coffee, maximum \( C_{\text{max}} \) ranged from 6 nmol/L for 5-FQA to 385 nmol/L for dihydroferulic acid, with the duration for \( t_{\text{max}} \) extending from 0.6 hours (ferulic acid-4-O-sulfate, 3-CQA-O-sulfate) to 5.2 hours (dihydroferulic acid). The compounds detected in the highest concentrations in plasma were free and sulfated conjugates of dihydroferulic acid and dihydrocaffeic acid, with \( C_{\text{max}} \) ranging from 41 to 385 nmol/L. The \( t_{\text{max}} \) for these compounds was in a narrow range from 4.7 to 5.2 hours, indicating absorption in the large intestine. Much shorter \( t_{\text{max}} \) values, of 0.6 to 1 hour, which is comparable to that reported in our study and is indicative of some small intestinal absorption, were obtained with 5-CQA, 2 CQA-sulfates, and 3 FQAs, all of which had relatively low \( C_{\text{max}} \) values.13,17 The potential individual differences as a result of differences in large intestinal microbial balance add an additional challenge to defining the PK of the various CGAs and their multiple isomers.

In terms of caffeine pharmacokinetics over a 4-hour test period, this study revealed the comparative lack of differences of the green coffee bean extract versus that synthetically derived at a 60-mg dose. In a prior study, this same botanical extract (dosed at 200 mg of caffeine) was found to be comparatively bioequivalent to synthetic caffeine for caffeine (versus USP caffeine).22 The cumulative data now reveal that the comparative pharmacokinetics exist at 60 and 200 mg for caffeine, which also demonstrates the consistency of the botanical extract studied, at least as measured over a 4-hour postigestion period. Therefore, caffeine from the natural green coffee bean extract was considered comparatively not different to the synthetic US Pharmacopeia caffeine.23 Similarly, in a study by Krieger et al using a 200-mg dose, there were no significant differences in absorption between the natural and synthetic caffeine sources.22 Caffeine concentration reached its peak plasma concentration for both sources on average between 63 and 75 minutes postigestion, which is consistent with other studies of oral doses of caffeine varying from 160 to 200 mg.27,28

**Conclusion**

The pharmacokinetics of CGA and its constituent CQA compounds, as well as the comparative pharmacokinetics of caffeine, were determined. In the blood 3-CQA had the greatest rate of appearance compared with 4- and 5-CQA; all were apparently readily bioavailable. Caffeine at a dose of 60 mg was not found to differ between the natural caffeine extract and the synthetic reference source (for the 4 hours postigestion tested). There were no safety concerns.
observed in this study, including no changes related to blood pressure or heart rate. It would be valuable for future studies to compare various CGA sources to determine optimal bioavailability and potentially for those studies to last longer than 240 minutes beyond ingestion. Future studies are needed to clarify the bioavailability and absorption of the various CGA compounds to determine ideal recommendations for foods naturally containing them or supplemented with them for their polyphenolic-related health benefits.

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