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Randomized Control Trials

Mediation of coffee-induced improvements in human vascular function by chlorogenic acids and its metabolites: Two randomized, controlled, crossover intervention trials

Charlotte E. Mills [a, 1], Andreas Flury [b], Cynthia Marmet [c], Laura Poquet [c], Stefano F. Rimoldi [b], Claudio Sartori [d], Emrush Rexhaj [b], Roman Brenner [b], Yves Allemann [b], Diane Zimmermann [c], Glenn R. Gibson [a], Don S. Mottram [a], Maria-Jose Oruna-Concha [a], Lucas Actis-Goretta [c, 2], Jeremy P.E. Spencer [a, *]

[a] Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, RG2 6AP, Reading, UK
[b] Department of Cardiology and Clinical Research, Inselspital, University Hospital Bern, CH-3010, Bern, Switzerland
[c] Nestlé Research Centre, Route du Jorat 94, Lausanne, 1000, Switzerland
[d] Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

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SUMMARY

Background & aims: Polyphenol intake has been linked to improvements in human vascular function, although data on hydroxycinnamates, such as chlorogenic acid (CGA) have not yet been studied. We aimed to investigate the impact of coffee intake rich in chlorogenic acid on human vascular function and whether CGAs are involved in potential effects.

Methods: Two acute randomized, controlled, cross-over human intervention trials were conducted. The impact of coffee intake, matched for caffeine but differing in CGA content (89, and 310 mg) on flow-mediated dilation (FMD) was assessed in 15 healthy male subjects. In a second intervention trial conducted with 24 healthy male subjects, the impact of pure 5-caffeoylquinic acid (5-CQA), the main CGA in coffee (5-CQA; 450 mg and 900 mg) on FMD was also investigated.

Results: We observed a bi-phasic FMD response after low and high polyphenol, (89 mg and 310 mg CGA) intake, with increases at 1 (1.10 ± 0.43% and 1.34 ± 0.62%, respectively) and 5 (0.79% ± 0.32 and 1.52% ± 0.40, respectively) hours post coffee consumption. FMD responses to coffee intake was closely paralleled by the appearance of CGA metabolites in plasma, notably 3-, 4- and 5-CQA and ferulic-4′-O-sulfate at 1 h and isoferulic-4′-O-glucuronide and ferulic-4′-O-sulfate at 5 h. Intervention with purified 5-CQA (450 mg) also led to an improvement in FMD response relative to control (0.75 ± 1.31% at 1 h post intervention, $p = 0.06$) and concomitant appearance of plasma metabolites.

Conclusions: Coffee intake acutely improves human vascular function, an effect, in part, mediated by 5-CQA and its physiological metabolites.

Study registration: The National Institutes of Health (NIH) on ClinicalTrials.gov NCT01813981 and NCT01772784.

Abbreviations: 3-CQA, 3-Caffeoylquinic acid; 3-FQA, 3 feruloylquinic acid; 4-CQA, 4-caffeoylquinic acid; 4-CQ15L, 4-caffeoylquinic-1,5-lactone; 4-FQA, 4-feruloylquinic acid; 4-MeCinA, 4-methoxycinnamic acid; 5-CQA, 5-caffeoylquinic acid; 5-FQA, 5-feruloylquinic acid; Ach, acetylcholine; ANOVA, analysis of variance; C3S, caffeic-3′-O-sulfate; CA4S, caffeic-4′-O-sulfate; CGA, chlorogenic acid; CV, coefficient of variance; CE, collision cell entrance potential; CPX, collision cell entrance potential; DP, declustering potential; ESI, electrospray ionization; EDTA, ethylene-diamine-tetra-acetic acid; FA, ferulic acid; F4G, ferulic-4′-O-glucuronide; F4S, ferulic-4′-O-sulfate; FMD, flow mediated dilatation; HDL, high density lipoprotein; HSD, highest significant difference; HPC, high polyphenol coffee; IAUC, incremental area under the curve; iFA, isoferulic acid; iF3G, isoferulic-3′-O-glucuronide; iF3G, isoferulic-3′-O-glucuronide; iF4S, isoferulic-3′-O-sulfate; LDI, laser Doppler imaging; LC, liquid chromatography; LDL, low density lipoprotein; LPC, low polyphenol coffee; MS, mass spectrometry; mCo3S, m-coumaric acid-3′-O-sulfate; mCo3G, m-coumaric-3′-O-glucuronide; mCo3G, m-courmaric-3′-O-glucuronide; MeFA, methylferulic acid; NIH, National Institute of Health; NO, nitric oxide; PDBP, peripheral diastolic blood pressure; PSBP, peripheral systolic blood pressure; POC, proof of concept; SNP, sodium nitroprusside; SD, standard deviation; SEM, standard error of the mean.

* Corresponding author. Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading, RG6 6AP, UK.
E-mail address: j.p.e.spencer@reading.ac.uk (J.P.E. Spencer).
[1] Present address: King's College London, Division of Diabetes and Nutrition Sciences, Franklin Wilkins Building, 150 Stamford St, London, SE1 9NH, UK.
[2] Present address: Nestlé Research Centre, 818802, Singapore.

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1. Introduction

Moderate coffee consumption (3–5 servings per day) has been associated with reductions in both stroke and heart disease risk [1,2], something that may be related to its high levels of phenolic acids. A serving of coffee may contain between 27 and 121 mg of phenolic acids (200 mL), most notably the hydroxycinnamate chlorogenic acid (CGA), which is high in dietary terms [3]. Previous clinical trials indicate that the intake of phenolic-rich foods such as berries [4], grape/wine [5], tea and cocoa [6], which all contain structurally related phenolics, improve endothelium-dependent vascular reactivity, suggesting coffee, may also be capable of similar vascular benefits. Despite this, controlled human intervention studies with coffee are few and most have focused on the effects of caffeine, rather than that of the phenolics present, yielding data ambiguous as the overall influence of caffeine and/or coffee on vascular function [8–10]. However, previous studies with CGA in both humans [11] and animals [12] have indicated a potential for coffee to influence vascular endpoints, such as blood pressure, plasma cholesterol levels [13,14] and flow mediated dilatation (FMD), a measure of endothelial dysfunction [15].

The CGA content of coffee is highly variable, depending to a large degree on its processing into the final consumed form, particularly bean roasting, which is detrimental to CGA levels [16]. Thus, presently, it is unclear as to the levels of CGAs sufficient to induce physiological effects on the endothelium. Furthermore, the impact of coffee intake on vascular function over prolonged periods is also unclear. Studies using coffee and pure chlorogenic acid indicate that CGA absorption/metabolism may occur partly in the small intestine and also in the large intestine [17,18], where the microbiota activity cleaves the quinic acid moiety to yield caffeic acid which may then undergo further metabolism and/or absorption in the large intestine [19]. This absorption profile of CGA in humans and the inherent differences in CGA content of differently processed coffees, provides an opportunity to investigate whether CGA is linked to the vascular effects of coffee intake in humans, via interactions of circulating CGA metabolites on the vascular epithelium.

As such, the current investigations were designed to test the hypothesis that CGA is causally related to improvements in endothelial function, measured here as flow-mediated dilation (FMD), by conducting two clinical trials: firstly a randomized, controlled, single blinded, crossover intervention trial with two coffees differing in CGA content and controlled against caffeine (*efficacy study*); and a second supporting doubled blinded intervention trial using pure 5-caffeoylquinic acid (5-CQA; the major CGA in coffee) isolated from coffee (*proof of concept study (POC)*). Vascular measures are linked to measures plasma CGA metabolites in order to build cause-and-effect relationships between CGA intake, individual vascular responses and circulating levels of CGA metabolites.

2. Subjects and methods

2.1. Clinical trial ethics

Both clinical studies were conducted in line with the guidelines in the Declaration of Helsinki and study protocols were approved by the University of Reading Research Ethics Committee, UK (reference: 11/31) and Kantonale Ethikkommission Bern, Switzerland (reference 039/12). The trials were registered with the National Institutes of Health (NIH) records on ClinicalTrials.gov website (NCT01813981; *efficacy study*, NCT01772784; *POC study*).

2.2. Subjects

During year 2011,16 healthy male volunteers were recruited and enrolled onto *efficacy study*, a three arm, randomized, controlled, sinlge-blinded, crossover clinical trial and in year 2012 24 male volunteers were enrolled on *POC study*, a four arm, randomized, double-blinded, crossover clinical trial; all volunteers gave written informed consent prior to their participation (Fig. 1).

For both studies, volunteers were screened before to the start of the trial to ensure they were in good health and were selected according to the inclusion criteria in Table 1. Note, that using the same inclusion criteria for *POC study* as for *efficacy study* resulted in some volunteers in this study having basal FMD values higher than 8.5%. As such, in order to maximize our chances of observing increases in % FMD following CGA intake, we introduced an inclusion criteria of moderate smoking in order to recruit a population with basal % FMD at rest of between 5 and 6%. Those selected for the study were asked not to change their usual dietary or fluid intake and asked to refrain from consumption of polyphenols rich foods including fruits, vegetables, cocoa, chocolate, coffee, tea and wine, all alcoholic beverages in addition to refraining from vigorous exercise such as running, swimming and other high aerobic forms of exercise for 24 h prior to, and during, the study.

2.3. Interventions

For *efficacy study* a low polyphenol soluble coffee (LPC): roasting to internal bean temperature of 225 °C), containing 89 mg CGA per 3.6 g of coffee and a high polyphenol coffee (HPC) derived from combining 50% roasted and 50% green beans (165 °C), containing 310 mg CGA (3.6 g ground coffee) were utilized. Both coffees were prepared by addition of 3.6 g of ground coffee to 50 mL of hot (90 °C), nitrate/nitrite free water. The control intervention was 110 mg caffeine and 0 mg CGA in nitrate/nitrite free hot water, whilst 28 mg of caffeine was added to LPC in order to match with both the control and the HPC (*efficacy study*) (Table 2).

For *POC study* 450 mg purified 5-CQA + 1 g maltodextrin; 900 mg purified 5-CQA + 1 g maltodextrin, doses of CGA shown to decrease cardiovascular disearse risk [21]; 1 g maltodextrin (negative control) and 200 mg purified (−)-epicatechin + 1 g maltodextrin (positive control). Each treatment was reconstituted in 200 mL of warm, nitrate/nitrite free water (~60 °C) and stirred until completely dissolved.

2.4. Study design

Both studies were randomized controlled crossover trials. *Efficacy study* was 3 armed and single blinded (researcher blinded; participant not due to water control), where subjects consumed a LPC, a HPC, or a caffeine control. The two intervention coffees were indistinguishable in appearance and taste. *POC study* was 4 armed and doubled blinded where volunteers consumed two doses of 5-CQA, a negative and positive control; interventions were indistinguishable. In both studies participants were assigned unique sequential random numbers, which was previously allocated to one sequence of the study products according to a computer generated paper list produced by a researcher not otherwise involved in the study. All study personnel involved in the assessment of study outcomes, including nurses, care providers, researchers and the principle investigator, were blinded to intervention and intervention order.

On arrival at the Hugh Sinclair Unit for Human Nutrition, University of Reading, UK (*efficacy study*) or Department of Cardiology and Clinical Research, Inselspital, University Hospital Bern, Switzerland, *POC study*, subjects were rested for 30 min in a quiet, temperature controlled room (~21 °C) before they were cannulated

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