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Impact of chlorogenic acids from coffee on urine metabolome in healthy human subjects

Francisco Madrid-Gambin [a], Mar Garcia-Aloy [a,1], Rosa Vázquez-Fresno [a,*,1], Esteban Vegas-Lozano [b], M. Carmen Ruiz de Villa Jubany [b], Koichi Misawa [c], Tadashi Hase [c], Akira Shimotoyodome [c], Cristina Andres-Lacueva [a,**]

[a] Biomarkers & Nutrimetabolomic Lab., Nutrition and Food Science Department, XaRTA, INSA, Campus Torribera, Pharmacy Faculty, University of Barcelona, Barcelona, Spain
[b] Department of Statistics, University of Barcelona, Barcelona, Spain
[c] Biological Science Laboratories, Kao Corporation, 2606, Akabane, Ichikai, Haga, Tochigi 321-3497, Japan

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ABSTRACT

Several studies suggest that coffee has some benefits for health; however, little is known about the specific role of the main polyphenol compounds of coffee, chlorogenic acids (CGAs), without caffeine interaction. A ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR)-based metabolomics approach was used to assess the effect of CGAs from coffee on the human urine metabolome. Ten male volunteers participated in a dietary crossover randomized intervention study with a rich CGAs coffee extract beverage (CEB: 223 mg/100 ml of CGAs). The study consisted of a daily intake of CEB or a control beverage with equal caffeine dose during 28 days. Fasting urines collected at the first and last days of each period of the study were analyzed using an CGAs untargeted ^{1}H-NMR approach. Additionally, 4-hour postpandrial urines after the first intake of each beverage were also analyzed. Uni- and multi-variate statistic approaches were used to strengthen the results. Multilevel partial least squares discriminant analysis (ML-PLS-DA) was used to paired comparisons across the crossover design. A further univariate analysis model for crossover studies was performed to assess the significant changes. Acute consumption of CEB resulted in high excretion of 2-furoylglycine, likewise endogenous compounds such as succinic, citric, 3-methyl-2-oxovaleric and isobutyric acids. Sustained consumption of CEB showed an increase of microbiota-derived compounds such as hippuric, 3-(3-Hydroxyphenyl)-3-hydroxypropionic and 3-hydroxyhippuric acids in urine. Moreover, trigonelline was found in urine after both acute and sustained intakes, as well as in the composition of the beverage exhibiting a direct excretion of this biomarker without any biotransformation, suggesting a non-interindividual variation.

1. Introduction

Coffee is one of the most widely consumed beverages worldwide, and therefore there are a huge number of studies concerning its benefits for health (Cowan et al., 2014; Guertin et al., 2015; R M van Dam & Hu, 2005). Coffee beans contain a large variety of biologically active compounds, the predominant ones being chlorogenic acids (CGAs) and caffeine (Johnston, Clifford, & Morgan, 2003). Several epidemiological researches suggest that coffee consumption may help prevent chronic diseases, including type 2 diabetes mellitus (Van Dam & Feskens, 2002), obesity and metabolic syndrome (Nordestgaard, Thomsen, & Nordestgaard, 2015), Parkinson's disease (Sääksjärvi et al., 2008) and liver disease (La Vecchia, 2005). Moreover, coffee is rich in many polyphenols and diterpenes (Urgert & Katan, 1997), which have antioxidant properties (Yanagimoto, Ochi, Lee, & Shibamoto, 2004) and may also mitigate harmful gut microbiota species with its regular consumption (Cowan et al., 2014). Although these beneficial effects are attributed to caffeinated coffee, high intakes of decaffeinated coffee have also been associated with a reduced risk of type 2 diabetes mellitus (Ding, Bhupathiraju, Chen, van Dam, & Hu, 2014; Huxley et al., 2009) and positive effects on cognition and psychomotor behavior (Shukitt-Hale, Miller, Chu, Lyle, & Joseph, 2013), indicating that some compounds in coffee other than caffeine may have a protective effect. Few

Abbreviations: CEB, coffee extract beverage; CGAs, chlorogenic acids; FID, free induction decay; HPHPA, 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid; LR, loading rank; ML-PLS-DA, multilevel partial least squares discriminant analysis; NMP, *N*-methylpyridinium; NMR, Nuclear Magnetic Resonance; PCA, principal component analysis; TCA, tricarboxylic acid; TSP, 3-(trimethylsilyl)-proprionate-2,2,3,3-d_4.

* Correspondence to: R. Vázquez-Fresno, Biomarkers & Nutrimetabolomic Lab., Nutrition and Food Science Department, Campus Torribera, Pharmacy Faculty, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain.
** Correspondence to: C. Andres-Lacueva, Biomarkers & Nutrimetabolomic Lab., Nutrition and Food Science Department, Campus Torribera, Pharmacy Faculty, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain.
E-mail addresses: rosavfr@gmail.com (R. Vázquez-Fresno), candres@ub.edu (C. Andres-Lacueva).
[1] These authors contributed equally to the work.

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interventional studies focused on the effect of bioactive compounds in coffee in both the short- and long-term have been able to provide an explanation for physiological mechanisms. However, in most epidemiological studies there is no distinction between caffeinated coffee consumers and newer or occasional decaffeinated coffee consumers (Higdon & Frei, 2006). Therefore the role of CGAs alone in this beverage is still unclear.

The application of metabolomics in the nutrition field can provide new information on dietary components, with the discovery of new biomarkers of food exposure, thereby revealing potential modifications in diet-related pathways in early disease stages likewise in healthy individuals (Puiggròs, Canela, & Arola, 2015; Scalbert et al., 2014). Nuclear Magnetic Resonance (NMR) in metabolomics is a fast, robust, reproducible, non-destructive technique (Wishart, 2008). Metabolomics allows a global description of metabolites that gives detailed information on metabolic pathways and in turn on biological processes, thereby clarifying associations with health benefits and elucidating underlying mechanisms (Brennan, 2014). Untargeted metabolomics is generally used for the discovery of metabolites and modifications in global metabolic pathways, whereas targeted metabolomics is focused on specific metabolic pathways (Xu, Wang, Ho, & Ong, 2014).

Several biomarkers of coffee intake have previously been proposed, including trigonelline, 2-furoylglycine, caffeine and several polyphenols (Heinzmann, Holmes, Kochhar, Nicholson, & Schmitt-Kopplin, 2015; Ito et al., 2005; Lang, Wahl, Stark, & Hofmann, 2011; Lloyd et al., 2013; Rothwell, Fillâ Tre, et al., 2014; Stalmach et al., 2009). Coffee is the main dietary source of CGAs (Clifford, 2000), but although CGAs and caffeic acid are the main phenolic compounds identified in urine after coffee intake in short-term (Ito et al., 2005), derived compounds of polyphenol metabolism are dependent on high interindividual variation in humans in long-term because of gut microbiota (Hervert-Hernández & Goñi, 2011).

The present study was conducted to determine whether CGAs from coffee may impact on the human urine metabolome through an untargeted metabolomic approach, and to identify the changes on the metabolome after both acute and sustained consumptions on healthy human volunteers.

2. Material and methods

2.1. Subjects and study design

Ten healthy male between 25 and 44 years old with a body mass index of 23.4 ± 2.1 kg/m^2 (mean ± SD) participated in a randomized, double-blind, placebo-controlled, crossover clinical trial. Exclusion criteria included caffeine intoxication, intake of CGAs supplements, serious illness (such as heart disease, kidney disease or diabetes) and food allergies. The study protocol was approved by the Human Research Ethics Committee of Biological Science Laboratories of the KAO Corporation (ref: 507-20131218). This clinical trial was registered as International Standard Randomized Controlled Trial Number 15516017.

During the washout and the study periods, the subjects were forbidden to consume coffee and tea beverages other than test drink. Subjects were not allowed to consume alcoholic beverages and to practice exercise from 2 days before the beginning of the intervention. After a 14-day washout period, subjects were asked to consume a coffee extract beverage (CEB) containing 223 mg/100 ml of CGAs (KAO Corporation, Japan) or a control beverage (caffeine-containing beverage). The daily dose of caffeine was similar between the intervention and control groups (see Table S1, Supporting information). Then, the participants consumed the corresponding beverage every day for the next 28 days (period I). The same procedure was repeated switching the individuals between the groups (period II) after a second 14-day washout period, in accordance with the crossover design. To analyze acute consumption, urine samples were collected during the first 4 postprandial hours after beverage intake on the first day of the intervention. For the analysis of sustained consumption, fasting urines on the first and last days of each period of the study were collected. All urine samples were stored in aliquots at −80 °C prior to analysis.

2.2. Sample preparation

Both urine and beverage samples were thawed, vortexed and centrifuged at 13,200 rpm for 5 min. The supernatant (600 μl) from each sample was mixed with an internal standard solution [120 μl, consisting of 0.1% 3-(trimethylsilyl)-proprionate-2,2,3,3-d_4 (TSP), chemical shift reference), 2 mM of sodium azide (NaN_3, bacteriostatic agent) and 1.5 M KH_2PO_4 in 99% deuterium water (D_2O)]. The optimized pH of the buffer was set at 7.0, with a potassium deuteroxide solution, to minimize variations in the chemical shifts of the NMR resonances. This mixture was transferred to a 5 mm NMR tube.

2.3. ^{1}H-NMR data acquisition and processing

The ^{1}H-NMR urinary spectra were acquired on a Varian-Inova-500 MHz NMR spectrometer with presaturation of the water resonance using a NOESYPRESAT pulse sequence. During the acquisition, the internal temperature was kept constant at 298 K. An exponential window function was applied to the free induction decay (FID) with a line-broadening factor of 0.3 Hz prior to the Fourier transformation. For each sample, FIDs were collected into 32 K data points, 128 scans, with a spectral width of 14 ppm, an acquisition time of 2 s, a relaxation delay of 5 s and a mixing time of 100 ms (Vázquez-Fresno et al., 2012).

A CEB sample was acquired on a Bruker Avance III 400 MHz NMR spectrometer equipped with a cryoprobe with presaturation of the water resonance using a NOESYPRESAT pulse sequence. The internal temperature was kept constant at 298 K and each sample was processed with a line-broadening factor of 0.3 Hz, 64 scans with a spectral width of 15 ppm, an acquisition time of 3 s, a relaxation delay of 5 s and a mixing time of 10 ms.

NMR spectra were phased, baseline corrected and referenced (TSP, 0.0 ppm) using TopSpin software (version 3.0, Bruker, BioSpin, Germany). After baseline correction, original spectral data were bucketed in intelligent bucketing domains of 0.005 ppm with ACD/NMR Processor 12.0 software (Advanced Chemistry Development, Toronto, Canada). The water signal and the regions above 9.5 ppm and below 0.5 ppm were excluded from the analysis.

2.4. Data pre-processing and statistical analysis

Data from acute and sustained interventions were submitted individually to MetaboAnalyst 3.0 for filtering and normalization purposes (Xia, Sinelnikov, Han, & Wishart, 2015). To exclude data points showing little variance across experimental conditions, the matrix was interquartile range filtered. Data were row-wise normalized (rows were samples) by the sum of the intensities of the spectra and column-wise normalized (columns were metabolites) using Pareto scaling and cube root transformation. Both data sets were used for further statistical analyses.

All the statistical analyses were performed with R version 3.1.2. Principal component analysis (PCA) was performed to detect the presence of outliers and to evaluate a potential carryover effect. Multilevel partial least squares discriminant analysis (ML-PLS-DA) was used to paired comparisons of the effects of CEB versus control beverage exploiting the crossover design with the R package mixOmics (Lê Cao, González, & Déjean, 2009). ML-PLS-DA is an extension of ordinary PLS-DA described by Van Velzen et al. (2008) which allows separation of the within-subject variation from the inter-subject variation that could obscure nutrition-related metabolic effects. A “leave-one-subject-out” cross-validation was performed to assess the ML-PLS-DA model and the classification error rate was determined by comparing the predicted class with the original one. Discriminant variables were determined