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In vivo study of the bioavailability and metabolic profile of (poly)phenols after *sous-vide* artichoke consumption

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ABSTRACT

Artichokes are a rich source of (poly)phenols, mainly caffeoylquinic acids, but little is known about their bioavailability from this source. This study investigated the absorption, metabolism and excretion of (poly)phenols after *sous-vide* artichoke consumption (5776 μmol of (poly)phenols) by healthy volunteers. Seventy-six (poly)phenol metabolites were identified by UHPLC-MS/MS using authentic standards, including acyl-quinic acids plus C_6–C_3, C_6–C_1, C_6–C_2, C_6–C_1–N, C_6–C_0 metabolites, and their phase-II conjugates. The major metabolites were 3'-methoxy-4'-hydroxycinnamic acid, 3'-methoxycinnamic acid-4'-sulfate, and 4'-hydroxycinnamic acid-3'-sulfate, which appeared early in plasma (T_{max} < 4 h); plus 3-(3'-methoxy-4'-hydroxyphenyl)propanoic acid, 3-(4'-methoxyphenyl)propanoic acid-3'-glucuronide, 3-(3'-hydroxyphenyl) propanoic acid and hippuric acids, which appeared later (T_{max} > 6 h). The 24 h urinary recovery averaged 8.9% (molar basis) of the (poly)phenols consumed. Hepatic beta-oxidation of 3',4'-dihydroxycinnamic acid and methylated conjugates occurred, but was limited (<0.04%). 3'-Methylation exceeded 4'-methylation and interindividual variability was high, especially for gut microbial metabolites (up to 168-fold).

1. 1. Introduction

Globe artichoke *(Cynara scolymus L.)* is cultivated worldwide but especially in Europe. In some municipalities of Navarra (Spain) the variety Blanca de Tudela (*Cynara scolymus L.* cv. Blanca de Tudela) is sold as "Alcachofa de Tudela" and protected under the "Geographical Indication" tag. Artichokes are a rich source of (poly)phenols, particularly acyl-quinic acids, commonly known as chlorogenic acids, and especially caffeoylquinic acids (CQAs) (D'Antuono, Garbetta, Linsalata, Minervini, & Cardinali, 2015). Artichokes are commonly eaten cooked, which can affect content and bioaccessibility of their (poly)phenols (Bento-Silva et al., 2020). A recent study reported that the novel *sous-vide* cooking applied to artichokes was the heat treatment that best preserved (poly)phenols compared with boiling and microwaving (Domínguez-Fernández, Ludwig, De Peña, & Cid, 2021).

Many epidemiological studies (Rienks, Barbaresko, & Nöthlings, 2017) supported by an increasing number of clinical trials have observed associations between higher (poly)phenol intake and reduced risk of chronic diseases (Lin et al., 2016). In particular, consumption of acyl-quinic acid sources, such as coffee, has been associated with cardiovascular and metabolic health benefits (Li et al., 2020; Williamson, 2020). A few clinical trials have investigated the effects of artichoke leaf extract consumption on biomarkers of cardiometabolic risk (Ceccarelli et al., 2010; Rondanelli et al., 2013). However, little is known about their mechanism of action (Clifford, Jaganath, Ludwig, & Crozier, 2017). Additionally, most of the *in vitro* studies on the bioactivity of acyl-quinic acids, and (poly)phenols in general, have used the native compounds present in food in concentrations much higher than the physiological (Clifford et al., 2017; Williamson, 2020). However, it is well known that acyl-quinic acids are extensively metabolized after

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absorption in the small intestine and specially by the gut microbiota in the colon, yielding many lower molecular weight metabolites with hardly any unmetabolized acyl-quinic acid present in plasma or urine (Clifford, Kerimi, & Williamson, 2020). Such transformations and the bioaccessibility of acyl-quinic acids from Tudela artichoke have been demonstrated *in vitro* (Domínguez-Fernández, Ludwig, et al., 2021), but not *in vivo*.

Previous studies investigating the acyl-quinic acid bioavailability have focussed on interventions with coffee (Clifford et al., 2020). However, it is known that the food matrix can affect the bioavailability of hydroxycinnamic acids (Bento-Silva et al., 2020). While two studies have investigated the (poly)phenol metabolite profile after consumption of artichoke extract (Rechner, Pannala, & Rice-Evans, 2001; Wittemer, Ploch, Windeck, Muller, Drewelow, Derendorf, & Veit, 2005), only one study has investigated the bioavailability and metabolism of (poly) phenols after consumption of steamed artichokes (Azzini et al., 2007). These three studies quantified only a maximum of five metabolites using either enzymatic hydrolysis or tentative identification with relative quantifications because of the lack of standards. Both approaches can lead to inaccuracies because of variabilities in enzymatic hydrolysis efficiencies, or important underestimations or overestimations of compound quantities because of different response factors (Ottaviani, Fong, Borges, Schroeter, & Crozier, 2018).

Accordingly, this study of the bioavailability, metabolism and excretion of *sous-vide* artichoke (poly)phenols uses a validated method with authentic standards to accurately identify and quantify metabolites in plasma and urine. Note that this study uses the metabolite nomenclature designed to facilitate automatic retrieval of data for review and meta-analysis, recommended by Kay et al. (Kay, Clifford, Mena, McDougall, Andres-Lacueva, & Cassidy, 2020), and acyl-quinic acids are referred to using IUPAC numbering (IUPAC, 1976), and cited literature has, if necessary, been adjusted accordingly.

2. Material and methods

2.1. Chemical and reagents

Fresh Tudela artichokes (*Cynara Scolymus* cv. Blanca de Tudela) were obtained from a local market in Tudela, Spain. The 25 analytical standards used for the analysis of *sous-vide* cooked artichokes are stated in the Supplementary Information. Methanol for LC-MS analysis was from Panreac (Barcelona, Spain), and acetonitrile and formic acid (LC-MS grade) were purchased from Scharlau (Barcelona, Spain).

For the analysis of urine and plasma samples obtained after artichoke consumption, 119 analytical standards were used, and their manufacturing information is stated in the Supplementary Information. OASIS HLB μElution plates (2 mg sorbent per well, 30 μm) were obtained from Waters Corporation (Waters, Eschborn, Germany). Acetic and formic acid were obtained from Thermo Fisher Scientific (Loughborough, UK). Phosphoric acid was obtained from Yorlab (Fluka, York, UK). Unless otherwise stated, all other reagents and solvents were obtained from Sigma-Aldrich Co (Steinheim, Germany).

2.2. Preparation of sous-vide artichokes and characterisation of their (poly)phenolic content by HPLC-MS/MS

Five artichokes (approximately 200 g) were packaged in a cooking vacuum bag from Bolsamack S.L (Alicante, Spain), containing 20 mL of tap water, by using a vacuum sealer from AK-Ramon (Barcelona, Spain). Samples were prepared in duplicate. Bags were immersed in 2 L of boiling water for 20 min. Afterwards, samples were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain) and stored at −18 °C until analysis.

(Poly)phenols from *sous-vide* artichokes were extracted in triplicate as previously described (Domínguez-Fernández, Ludwig, et al., 2021). Quantitative analyses of (poly)phenolic compounds were carried out using an Agilent 1200 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a triple quadrupole linear ion trap mass spectrometer (3200 Q-TRAP, AB SCIEX). Samples (5 μL) were injected into a CORTECS® C18 column (3x75 mm, 2.7 μm; Waters), kept in a thermostatic oven at 30 °C. Chromatographic analysis was performed with an elution flow rate of 0.6 mL/min and using 0.1% (v/v) formic acid in water as mobile phase A and acetonitrile as mobile phase B. Gradient elution was as follows: 5% B (0–1 min), 5–10% B (1–5 min), 10–14% B (8–8.5 min), 14–20% B (8.5–10.5 min), 20–30% B (10.5–16 min), 30–100% B (16–17.6 min), 100% B (17.6–25.6 min) and then return to 5% B in 5 min and maintained isocratic until the end of the analysis (35 min).

The mass spectrometer (MS) run in negative ionization mode, with the turbo heater maintained at 600 °C and Ion Spray voltage set at −3500 V. Nitrogen was used as nebulizing, turbo heater and curtain gas, and it was set at the pressure of −60, −65 and −35 psi, respectively. Declustering potential (DP) and entrance potential (EP) were set at −20 V and −10 V, respectively. Authentic standards in blank solvent were directly infused into the mass spectrometer at a constant flow rate of 10 μL/min for optimization of the collision energy (CE) using a syringe pump (1001 TLL SYR, Hamilton, Giarmata, Romania) (Supplementary Information Table S1). Chromatograms and spectral data were acquired using Analyst software 1.6.3 (AB SCIEX). Quantification of (poly)phenols was performed using calibration curves of their corresponding authentic standards for each compound except for six compounds due to the lack of authentic standard. Thus, 1-CQA and *p*-coumaroylquinic acid were quantified as 5-CQA equivalents, 1,4-diCQA and diCQA-glucoside were quantified as 1,3-diCQA equivalent, while luteolin-7-O-rutinoside and pinoresinol-4-O-β-D-glucoside were quantified using luteolin-7-O-glucoside calibration curve.

2.3. Human study design

The study protocol was approved by King's College London Research Ethics Committee (reference number: HR-18/19–12367) and it was conducted according to the guidelines laid down in the Declaration of Helsinki. The study was also registered in the National Institutes of Health ClinicalTrials.gov (NCT04095949). Eight healthy participants, five females and three males, mean age 26.4 ± 2.8 years and mean body mass index 22.8 ± 2.5 kg/m^2, were recruited. Participants were non-pregnant or lactating women, non-smoker, whose alcohol intake was < 80 g/day, who were not taking any chronic medication, any antibiotic treatment during the four months prior to the study or any dietary supplements. All participants gave their informed consent prior to participation.

This study was conducted at King's College London in the Metabolic Research Unit of the Department of Nutritional Sciences. Participants were asked to follow a low-(poly)phenol diet 24 h prior to the study day, as well as during the 24 h of the study day. Indications were given to avoid the consumption of (poly)phenol-rich foods including fruits, vegetables, cocoa products, chocolate, coffee, tea and nuts, as well as alcoholic and soft drinks. To monitor compliance, participants completed two 24-h dietary recalls (one 24 h before the study day and another one during the study day). On the morning of the study day, after 12 h fasted, participants consumed five *sous-vide* cooked artichokes (approx. 200 g), which represent a normal serving. Blood samples were taken at baseline (0 h) and at 0.5, 1, 2, 4, 6, and 24 h after artichoke consumption in 10-mL Vacutainer™ tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. To obtain plasma samples, the blood tubes were centrifuged at 1800×*g* for 15 min at 4 °C. Afterwards, plasma aliquots were stored at −80 °C until analysis. Urine samples were collected at baseline (spot urine) and at the intervals 0–2, 2–4, 4–8, and 8–24 h after artichoke intake. Urine samples were collected separately at each interval time in 2 L collection containers and the volume was measured before storing aliquots at −80 °C until analysis.

2.4. UHPLC-MS/MS analysis of biological samples

Plasma and urine samples were defrosted and micro-elution solid phase extraction using Oasis HLB 96-well plates (Waters, Eschborn, Germany) was performed, followed by analysis using ultra-high performance liquid chromatography coupled to a Triple Quadrupole Mass Spectrometer (LCMS8060, SHIMADZU, Kyoto, Japan), according to a validated method for the identification and quantification of 119 and 110 (poly)phenols with their respective authentic standards in plasma and urine respectively (Domínguez-Fernández, Xu, et al., 2021). For quantification purposes, two sets of calibration curve were included during the analysis of urine and plasma samples.

2.5. Statistical analysis

The results are presented as mean values ± standard deviation (SD) for (poly)phenols in *sous-vide* cooked artichokes, and as mean values ± standard error of the mean (SEM) for (poly)phenol metabolites in urine and plasma samples. PKsolver add-on program was used to perform pharmacokinetic analysis in Microsoft Excel (Zhang, Huo, Zhou, & Xie, 2010). GraphPad Prism (version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com) was used in urine and plasma samples to compare the mean differences at the defined time points. One-way repeated measures analysis of variance (ANOVA) was performed on urine samples, while for plasma samples a mixed model was applied due to missing values from one of the participants at 6 h. This mixed model uses a compound symmetry covariance matrix, it is fitted using Restricted Maximum Likelihood (REML) and can be interpreted as repeated-measures ANOVA. A Tukey test was applied as *a posteriori* test with a level of significance of 95%. Differences were considered significant at $p < 0.05$. Correlation analysis and comparison of 3'-methoxy-4'-hydroxycinnamic acid / 3'-hydroxy-4'-methoxycinnamic acid (ferulic/isoferulic acid) and 3-methoxy-4-hydroxybenzoic acid / 3-hydroxy-4-methoxybenzoic acid (vanillic/isovanillic acid) ratios between participants were performed using "R" version 3.6.2 (https://www.r-project.org/). For statistical analysis of 3-methoxy-4-hydroxybenzoic acid / 3-hydroxy-4-methoxybenzoic acid ratio, a Welch *t*-test was performed to compare the two groups as they were normally distributed and had different variances (no homoscedasticity).

3. Results and discussion

3.1. (Poly)phenolic composition of sous-vide artichoke

Volunteers consumed a 200 g portion of *sous-vide* artichokes equivalent to a typical meal. The *sous-vide* artichokes were analysed and a total of 31 (poly)phenolic compounds were identified and quantified as shown in Table 1. Total (poly)phenolic compounds accounted for 2568 ± 351 mg per 200 g which corresponds to 5727 ± 719 μmol. Approximately 95.6% were hydroxycinnamic acid derivatives, mainly CQAs (94.5%) while the remaining 4.4 % were mainly apigenin derivatives (2.5%) and luteolin derivatives (1.5%). CQAs are ubiquitous phenolic compounds present in many fruits and vegetables, such as berries, apples, potatoes, and aubergines among others (Li et al., 2020). However, only few foods, such as coffee, yerba maté, and artichoke, contain CQAs as the main phenolic group. Among them, the principal dietary source of CQAs is coffee. Although artichokes are not consumed so frequently, the contents reported per typical dietary serving of coffee (24–423 mg) (Clifford et al., 2017) are lower than in artichokes. (Poly)phenolic content quantified in edible parts of globe artichokes range from 536 to 1576 mg per 200 g of fresh weight (Azzini et al., 2007; D'Antuono et al., 2015). A recent review reported that volunteer studies had investigated acyl-quinic acid doses up to 5.5 mmol and a similar high dose was used in this study (5.4 mmol) but because of the higher diCQA contents this study used 8.62 mmol caffeic acid equivalents compared with a maximum of 4.38 mmol for studies with coffee. Compared with similar recent volunteer studies using coffee and maté, where diCQA accounted for 56 μmol and 213 μmol (8.4% and 24% of the total acyl-quinic acids), respectively, this study supplied 2.2 mmol diCQA, 54% of the total acyl-quinic acids, and this is the only modern volunteer study to investigate a test meal with significant 1-acyl-quinic acids content (1.95 mmol, 36% of the total acyl-quinic acids). Artichoke contains few 3'-methylated (poly)phenols, only 3 μmol of 3'-methoxy-4'-hydroxy-cinnamic acid (0.5% of the total (poly)phenols), much smaller than the FQA content of coffee (5%) and maté (8.4%), making the *sous-vide* artichoke a better dietary component with which to investigate the extent and regiochemistry of human COMT metabolism (Clifford et al., 2020).

Table 1
Concentration of the main (poly)phenolic compounds in *sous-vide* artichokes. Data are expressed as μmol/200 g of cooked artichokes (mean ± SD, n = 3).

	μmol/200 g
Cinnamic acids	
Dihydroxycinnamic acids	
3',4'-Dihydroxycinnamic acid	47.4 ± 0.8
1-O-Caffeoylquinic acid	9.0 ± 1.9
3-O-Caffeoylquinic acid	335.2 ± 30.3
4-O-Caffeoylquinic acid	453.2 ± 46.0
5-O-Caffeoylquinic acid	1,407.6 ± 155.3
1,3-O-Dicaffeoylquinic acid	897.1 ± 222.8
1,5-O-Dicaffeoylquinic acid	878.0 ± 189.9
3,5-O-Dicaffeoylquinic acid	389.4 ± 43.9
3,4-O-Dicaffeoylquinic acid	492.4 ± 49.6
4,5-O-Dicaffeoylquinic acid	383.7 ± 105.5
1,4-O-Dicaffeoylquinic acid	161.4 ± 13.6
Dicaffeoylquinic acid-glucoside	4.2 ± 0.2
Methoxycinnamic acids	
4'-Hydroxy-3'-methoxycinnamic acid	3.0 ± 0.5
Other cinnamic acids	
4'-Hydroxycinnamic acid	0.3 ± 0.0
p-Coumaroylquinic acid	12.5 ± 0.5
Hydroxybenzoic acids	–
3,4-Dihydroxybenzoic acid	1.7 ± 0.0
Flavonoids	–
Apigenin and derivatives	
Apigenin	2.6 ± 0.3
Apigenin 7-O-glucoside	35.1 ± 3.1
Apigenin 7-O-glucuronide	101.8 ± 11.3
Apigenin 7-O-rutinoside	3.5 ± 0.1
Luteolin and derivatives	
Luteolin	2.1 ± 0.1
Luteolin 7-O-glucoside	19.9 ± 1.3
Luteolin 7-O-glucuronide	54.3 ± 4.6
Luteolin 7-O-rutinoside	5.3 ± 0.2
Other flavonoids	
Quercetin	0.2 ± 0.1
Isorhamnetin	0.2 ± 0.0
Kaempferol-7-O-glucoside	15.2 ± 0.6
Naringenin-7-O-glucoside	1.8 ± 0.0
Vicenin-2	1.1 ± 0.0
Hesperidin	7.4 ± 2.6
Lignans	
Pinoresinol 4-O-β-D-glucoside	0.6 ± 0.0
Total phenolic compounds	5,727 ± 719

3.2. Artichoke (poly)phenols and metabolites in urine and plasma by UHPLC-MS/MS

After consumption of 200 g of *sous-vide* artichokes a validated, targeted UHPLC-MS/MS method developed using 119 authentic compounds (Domínguez-Fernández, Xu, et al., 2021) was used to identify and quantify up to 76 (poly)phenols in urine and 70 in plasma. Table S2 lists all quantified compounds with their common name and the recently recommended name for standardisation (Kay et al., 2020). 3-CQA and 5-FQA, plus some acyl-quinic acid metabolites often quantified after coffee consumption (caffeoylquinic acid phase II conjugates, feruloyl-glycine and isoferuloyl-glycine) were not included in the validated method and, therefore, were not targeted in plasma and urine samples in the

present study.

Although there have been numerous human intervention studies of acyl-quinic acid bioavailability and metabolism (for review see (Clifford et al., 2020)), only two have used authentic standards for identification and quantification of every metabolite reported. The first one reported a total of 10 (Wong, Meinl, Glatt, Barron, Stalmach, Steiling, & Williamson, 2010) and the second one 56 compounds (Mills et al., 2017), and both of these used coffee as the acyl-quinic acid source.

Although participants were asked to follow a low-(poly)phenol diet 24 h prior to the consumption of artichokes, some compounds were detected in the basal samples of both urine and plasma (Table S3). The presence of (poly)phenolic compounds in basal samples is a common issue in bioavailability studies and may have several causes. For example, many (poly)phenolic metabolites have been reported to persist in the circulatory system in excess of 48 h (Del Rio et al., 2013), and it is almost impossible to completely eliminate (poly)phenol intake from the diet, because (poly)phenols are widespread in the plant kingdom and therefore present in almost all plant-based foods and food products. The situation is further complicated because phenylalanine and tyrosine metabolism overlaps with non-nutrient (poly)phenol metabolism. Metabolites of isoflavones, flavanones, flavonols, lignans and stilbenes, (poly)phenols not found in artichokes, and therefore derived from the background diet, are not discussed further in this manuscript, but their data for plasma and urine can be consulted in Table S4 and S5, respectively. Thus, out of 70 and 76 compounds quantified in plasma and urine, respectively, only 60 are included in Table 2 and 64 in Table 3 as feasible metabolites from artichoke. These (poly)phenolic metabolites belong to eight subgroups: (1) acyl-quinic acids and metabolites (2) C_6-C_3 unsaturated (cinnamic acids), (3) C_6-C_1 (benzoic acids), (4) C_6–C_2 (phenylacetic acids), (5) C_6-C_3 (propanoic acids), (6) C_6–C_1–N (hippuric acids), (7) C_6-C_0 (benzene diols and triols), (8) C_6-C_1 (benzaldehydes).

The same metabolites were found in urine and plasma except for cinnamic acid-4'-glucuronide, free 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid and 3-(2,3-dihydroxyphenyl)propanoic acid, which were found only in urine. Nine compounds were not quantified in every participant's plasma samples (Table 2). In urine, all compounds were quantified in every participant (n = 8), with the exception of 2'-hydroxycinnamic acid, 4-methyl-1,2-dihydroxybenzene and 4-methyl-2-hydroxybenzene-1-sulfate (Table 3). Phase-II metabolites were the main compounds, accounting for 38 in plasma and 39 in urine. These phase-II metabolites comprised sulfate (7), glucuronide (5 in urine and 4 in plasma), methyl (8), and glycine conjugates (4), as well as some methylated metabolites further conjugated with sulfate (11) or glucuronide (4).

3.3. Kinetics of artichoke (poly)phenols and metabolites

Food matrix can affect phytochemical and, specifically, hydroxycinnamic acid bioavailability (Bento-Silva et al., 2020). Although the complex metabolic pathway of acyl-quinic acids has been previously described, principally after coffee and maté consumption (Clifford et al., 2020) where they are consumed in solution, it has not been investigated fully for artichoke acyl-quinic acids where they are consumed in a solid matrix. Table 2 shows the kinetics of (poly)phenol metabolites in plasma at seven time points (0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 24 h), and expressed as Area Under the Curve (AUC, nM*h), peak plasma concentration (C_{max}, nM) and the time to reach C_{max} (T_{max}, h). Table 3 shows the excretion kinetics in urine expressed as nanomole per hour (nmol/h), for the four intervals studied (0–2 h, 2–4 h, 4–8 h and 8–24 h), as well as total excretion (μmol/24 h). Additionally, the individual kinetics of each compound in plasma and urine are presented graphically in **Figs. S1** and **S2**, respectively. Note that the SEM which sometimes is large relative to the mean values, indicate substantial person-to-person variation, and this is highlighted in Table 2 and Table 3 by presenting for each metabolite the range between minimum value and maximum value and for urine also the quotient of the maximum and minimum 24 h excretion value.

When analysing the complete set of kinetic curves (**Figs. S1** and **S2**), some compounds did not clearly increase during the 24 h after artichoke consumption. It can be suggested that they come from the background diet of the participants, which is supported by their presence at baseline after washout. These compounds marked with an asterisk in Table 2 and Table 3 included (1) benzene diols and triols (C_6-C_0) and (2) benzaldehydes (C_6-C_1), as well as some hydroxy- and methoxycinnamic acids, benzoic acids, phenylacetic acids, hydroxyphenylpropanoic acids and hippuric acids. We acknowledge that these compounds could, at least in part, come from the gut microbial metabolism of artichoke (poly)phenols. However, the absence in the present study of clear-cut increases in plasma and/or urine after the consumption of almost 6 mmol (poly)phenols, indicates that these are generated from other dietary food sources, with at most a small contribution from the *sous-vide* artichoke consumed. Henceforward, this study focusses on the remaining 35 quantified metabolites — see Figs. 1–3 and Table 2 and Table 3.

Based on the data obtained for these 35 compounds, the plasma appearance and clearance of (poly)phenol artichoke metabolites up to 24 h post-intake is represented by groups in Fig. 1, along with a condensed metabolic pathway. The complete proposed metabolic pathway of artichoke (poly)phenolic compounds, with the chemical structures of the individual metabolites, is presented in Fig. 2.

Two unmetabolized acyl-quinic acids (4-CQA and 5-CQA) appeared rapidly in plasma, the concentration increasing for at least 2 h, before falling some 50% by four hours post-consumption. It has been demonstrated that 4-CQA is absorbed by a saturable facilitated transport mechanism whereas 5-CQA is absorbed by passive paracellular diffusion, as reviewed by Clifford et al. (Clifford et al., 2017). Unmetabolized 4-CQA was detected in plasma and it has not previously been observed in plasma so far as we are aware (Clifford et al., 2020).

A recent review of acyl-quinic acid pharmacokinetics examined 70 data sets and concluded that the mean T_{max} for CQA was in the range 30 to 80 min (Clifford et al., 2020) among which coffee consistently produced T_{max} values in the range 30 to 60 min (Erk et al., 2012; Stalmach, Williamson, & Crozier, 2014). The doubling of T_{max} in this study compared with coffee indicates clearly that the artichoke matrix slowed absorption. COMT 3'-methylation produced 3-FQA and 4-FQA. Again, the T_{max} was no sooner than two hours, but the subsequent decline was a little slower, particularly for 4-FQA, which took four hours to decline by just over 50%, mainly due to one volunteer whose maximum was not before 24 h. The plasma and urine kinetic curves of this particular volunteer also stand out from the other volunteers in other compounds (**Figs. S3** and **S4**, respectively).

The plasma AUC for 3-FQA, which was not present preformed in artichoke (227 nM*h), is approximately half that for 5-CQA (441 nM*h), which suggests that 3-CQA has been rapidly and extensively 3'-methylated by COMT. The AUC value for 4-FQA (75 nM*h) is approximately half that for 4-CQA (151 nM*h) suggesting that 4-CQA is also easily 3'-methylated. Such methylation activity would be masked when beverages such as coffee and maté are consumed because of pre-existing FQA in the beverage.

Free 3',4'-dihydroxycinnamic acid was not detected in artichoke but low concentrations appeared rapidly in plasma (T_{max} 1–6 h) consistent with hydrolysis by chlorogenate esterase in the stomach or upper GIT, and consistent with little or no transport to the serosal side by Caco-2 cells or cultured gastric epithelial cells, as reviewed by Clifford et al. (Clifford et al., 2020).

However, much greater C_{max} concentrations with similar T_{max} values were recorded for the associated phase II conjugates, especially 4'-hydroxycinnamic acid-3'-sulfate (>1,000-fold), 3'-methoxycinnamic acid-4'-sulfate (>300-fold), 3'-methoxy-4'-hydroxycinnamic acid (>300-fold), 3'-methoxycinnamic acid-4'-glucuronide (>100-fold), and 3'-hydroxy-4'-methoxycinnamic acid (*ca* 50-fold) and provide clear evidence for the extensive and rapid acyl-quinic acid absorption, hydrolysis and phase II metabolism in the enterocyte and liver.

Table 2
Kinetics of (poly)phenol metabolites identified in plasma after artichoke consumption. Data are expressed as mean (minimum value - maximum value).

	AUC (nM*h)	C_{max} (nM)	T_{max} (h)	n
Acyl-quinic acids				
5-O-Caffeoylquinic acid	414 (238–755)	78 (59–153)	2 (1–2)	8
3-O-Feruloylquinic acid	227 (89–359)	41 (26–75)	2 (1–2)	8
4-O-Feruloylquinic acid	75 (16–68)	4 (3–7)	2 (2–24)	8
4-O-Caffeoylquinic acid	151 (67–245)	25 (11–40)	2 (1–2)	8
Cinnamic acids (C_6-C_3 unsaturated)				
3',4'-Dihydroxycinnamic acids				
4'-Hydroxycinnamic acid-3′-sulfate	23,671 (13,151–46,386)	2,803 (1,468–4,549)	1 (1–6)	8
3'-Hydroxycinnamic acid-4′-sulfate	904 (372–2,013)	67 (38–171)	1 (1–6)	8
3',4'-Dihydroxycinnamic acid	37 (4–107)	2 (1–10)	6 (1–6)	8
3'-Hydroxycinnamic acid-4′-glucuronide	239 (97–384)	14 (12–32)	2 (1–6)	8
4'-Hydroxycinnamic acid-3′-glucuronide	92 (6–231)	6 (2–20)	6 (1–6)	8
4'-Hydroxy-3'-methoxycinnamic acids				
3'-Methoxycinnamic acid-4'-sulfate	10,180 (3,053–35,672)	704 (205–3,167)	6 (1–6)	8
4'-Hydroxy-3'-methoxycinnamic acid	10,297 (1,455–32,064)	651 (127–2,971)	6 (1–6)	8
3'-Methoxycinnamic acid-4'-glucuronide	3,670 (1,171–12,578)	269 (87–1,105)	6 (1–6)	8
3'-Hydroxy-4'-methoxycinnamic acids				
3'-Hydroxy-4'-methoxycinnamic acid	2,210 (1,072–3,107)	119 (85–226)	1 (1–6)	8
4'-Methoxycinnamic acid-3'-glucuronide	830 (342–1,427)	51 (41–114)	6 (1–6)	8
4'-Methoxycinnamic acid-3'-sulfate	310 (124–481)	19 (12–42)	1 (1–6)	8
Hydroxycinnamic acids				
Cinnamic acid-4'-sulfate	54 (24–67)	15 (9–19)	2 (1–2)	8
4'-Hydroxycinnamic acid*	40 (21–65)	2 (1–4)	4 (2–6)	8
2'-Hydroxycinnamic acid*	22 (0–22)	1 (0–2)	6 (0–6)	6
4'-Hydroxy-3',5'-dimethoxycinnamic acids				
4'-Hydroxy-3',5'-dimethoxycinnamic acid*	45 (26–90)	3 (2–6)	1 (1–6)	8
Benzoic acids (C_6-C_1)				
Benzoic acid	4,858 (3,834–5,886)	356 (236–572)	0 (0–4)	8
Hydroxybenzoic acids				
3-Hydroxybenzoic acid	1,787 (192–4,067)	94 (34–222)	1 (0–24)	8
2-Hydroxybenzoic acid *	5,840 (1,557–10,303)	308 (75–732)	24 (0–24)	8
4-Hydroxybenzoic acid *	554 (308–960)	33 (18–82)	0 (0–24)	8
3,4-Dihydroxybenzoic acids				
3-Hydroxybenzoic acid-4-sulfate	470 (201–898)	31 (22–76)	1 (1–6)	8
4-Hydroxybenzoic acid-3-sulfate	1,265 (444–2,592)	77 (36–224)	6 (1–6)	8
Other dihydroxybenzoic acids				
2,4-Dihydroxybenzoic acid *	1,202 (155–2,540)	86 (15–229)	1 (0–24)	8
2,5-Dihydroxybenzoic acid *	4,457 (0–19,293)	342 (0–2,103)	2 (0–24)	7
2,6-Dihydroxybenzoic acid *	3,425 (700–8,449)	220 (38–648)	0 (0–24)	8
3,5-Dihydroxybenzoic acid *	712 (72–623)	42 (28–86)	1 (0–4)	8
Methoxybenzoic acids				
3-Methoxybenzoic acid-4-sulfate	705 (67–1,529)	45 (7–123)	6 (1–6)	8
4-Methoxybenzoic acid-3-sulfate	1,235 (565–2,725)	76 (46–196)	6 (2–6)	8
4-Hydroxy-3,5-dimethoxybenzoic acid*	91 (9–98)	5 (3–9)	0 (1–24)	8
3-Hydroxy-4-methoxybenzoic acid-5-sulfate*	145 (0–188)	13 (0–28)	1 (0–24)	7
Phenylacetic acids (C_6-C_2)				
3′-methyoxyphenylacetic acid-4′-sulfate	91 (19–185)	6 (1–13)	6 (0–24)	8
3',4'-Dihydroxyphenylacetic acid*	364 (153–677)	25 (13–64)	1 (0–24)	8
Propanoic acids (C_6-C_3)				
3-(3',4'-Dihydroxyphenyl)propanoic acids				
3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate	19,225 (24–40,098)	1,330 (2–3,987)	6 (4–24)	8
3-(3',4'-Dihydroxyphenyl)propanoic acid	322 (77–523)	24 (8–43)	6 (4–6)	8
3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	348 (60–532)	24 (6–41)	6 (1–24)	8
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acids				
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	11,748 (190–19,587)	862 (73–1,959)	6 (4–24)	8
3-(3'-Methoxyphenyl)propanoic acid-4'-glucuronide	3,840 (492–8,366)	258 (24–772)	6 (4–24)	8
3-(3'-Methoxyphenyl)propanoic acid-4'-sulfate*	1,738 (551–3,232)	114 (28–281)	6 (6–24)	8
3-(3'-Hydroxy-4'-methoxyphenyl)propanoic acids				
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	997 (175–2,341)	63 (10–198)	6 (6–24)	8
3-(4'-Methoxyphenyl)propanoic acid-3'-sulfate*	864 (432–1,601)	46 (20–108)	6 (0–24)	8
Hydroxyphenylpropanoic acids				
3-(3'-Hydroxyphenyl)propanoic acid	73,905 (9,924–150,035)	5,970 (994–16,333)	24 (6–24)	8
2-(4'-Hydroxyphenoxy)propanoic acid*	1,243 (0–1,745)	94 (0–145)	6 (0–6)	6
3-(2'-Hydroxyphenyl)propanoic acid*	202 (0–400)	11 (0–23)	24 (0–24)	6
3-(3',5'-Dihydroxyphenyl)propanoic acid*	320 (77–823)	19 (11–59)	2 (0–24)	8
2-Hydroxy-3-(4'-hydroxyphenyl)propanoic acid*	522,253 (393,616–609,967)	23,038 (20,511–28,568)	24 (6–24)	8
Hippuric acids (C_6–C_1–N)				
3'-Hydroxyhippuric acid	62,165 (5,628–109,273)	5,470 (544–9,081)	24 (6–24)	8
Hippuric acid	197,765 (24,873–478,045)	12,310 (1,913–25,349)	24 (0–24)	8
2'-Hydroxyhippuric acid *	380 (130–912)	23 (10–75)	24 (0–24)	8
4'-Hydroxyhippuric acid *	2,036 (1,300–3,413)	106 (65–186)	6 (6–24)	8

(*continued on next page*)

Table 2 (*continued*)

	AUC (nM*h)	C_{max} (nM)	T_{max} (h)	n
Benzene diols and triols (C_6-C_0)				
4-Methyl-1,2-dihydroxybenzene*	2,442 (0–2,552)	193 (0–305)	0 (0–6)	4
4-Methyl-2-hydroxybenzene-1-sulfate*	14,577 (0–21,197)	1,715 (0–2,739)	0 (0–6)	4
2-Hydroxybenzene-1-glucuronide*	251 (0–306)	12 (0–30)	24 (0–24)	7
Pyrogallol-2'-sulfate*	1,915 (214–8,385)	113 (15–745)	24 (1–24)	8
1-Methylpyrogallol-sulfate*	2,096 (478–7,650)	124 (25–784)	24 (1–24)	8
2-Methylpyrogallol-1-sulfate*	2,873 (699–5,531)	149 (37–351)	0 (0–6)	8
Benzaldehydes (C_6-C_1)				
4-Hydroxybenzaldehyde *	1,958 (1,083–4,138)	123 (55–311)	6 (6–24)	8
4-Hydroxy-3-methoxybenzaldehyde*	155 (78–205)	8 (8–15)	1 (1–6)	8

AUC: Area Under the Curve; C_{max}: maximum concentration in plasma; T_{max}: time to reach the C_{max}; * not included in metabolic pathways proposal and bioavailability evaluation.

Because few studies have used authentic standards for quantification, it is difficult to make valid comparisons of the relative yields of particular metabolites observed in this study, with most of those previously reported. Nevertheless, the metabolites dominating the urine profile in this study generally correspond to those previously reported, but there are several noteworthy exceptions. Previous studies have reported that 3'-hydroxy-4'-methoxycinnamic acid is excreted primarily as the 3'-glucuronide (Clifford et al., 2020) and studies with authentic standards have confirmed this at 74–81% (Mills et al., 2017) and 92% (Wong et al., 2010) but in this study the free acid (53%) was dominant, accompanied by a significant amount of the 3'-sulfate (40%).

As in this study, Mills et al. (2017) and Wong et al. (2010) both reported that the dominant un-methylated metabolite of 3',4'-dihydroxycinnamic acid was the 3'-sulfate (81–94%), but this study with artichoke found that after hydrogenation of the cinnamic acid the 3'-glucuronide dominated (66%) whereas Mills et al. (2017) and Wong et al. (2010) reported that again the 3'-sulfate dominated (61–98%). This variation might not simply be a matrix effect but also result from person-to-person variation, e.g. UGT1A9 polymorphisms are well known, as reviewed by Clifford et al. (Clifford et al., 2020).

Cinnamic acids can be hydrogenated in the liver, but there is little evidence for this because the associated phenylpropanoic acids are barely detectable in plasma before two hours, and only begin to increase rapidly beyond four hours post-consumption. Accordingly, 3-(3',4'-dihydroxyphenyl)propanoic acid is predominantly formed from unabsorbed 3',4'-dihydroxycinnamic acid by the colonic gut microbiota, and absorbed by passive transcellular diffusion (Poquet, Clifford, & Williamson, 2008). Subsequent metabolism in the enterocytes and hepatocytes generates methylated, glucuronidated and sulfated conjugates among which 3-(4'-hydroxyphenyl)propanoic acid-3'-glucuronide (25 ± 6.2 μmol), 3-(4'-methoxyphenyl)propanoic acid-3'-glucuronide (27 ± 29 μmol), 3-(3'-methoxy-4'-hydroxyphenyl)propanoic acid (38 ± 33 μmol) and 3-(3'-methoxyphenyl)propanoic acid-4'-sulfate (49 ± 37 μmol) are the dominant forms excreted over 24 h.

The excretion of 3'-methoxy-4'-hydroxycinnamic acid, 3-(3'-methoxy-4'-hydroxyphenyl)propanoic acid and their phase II conjugates observed in this study cannot be directly compared with the data presented by Mills et al. (2017) or Wong et al. (2010) because coffee contains significant preformed FQA and this prevents the determination of the extent of 3'-methylation of 3',4'-dihydroxycinnamic acid and 3',4'-dihydroxyphenyl)propanoic acid. In contrast, artichoke with a negligible content of 3'-methylated (poly)phenols is an ideal test meal with which to assess the relative 3'- and 4'-methylation of 3',4'-dihydroxy-substituted substrates, a topic on which the literature lacks consensus (Clifford et al., 2020).

In the present study, 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid and 3-hydroxy-4-methoxybenzoic acid were found as sulfates in plasma and urine, but only 3-methoxy-4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid were found free, and only in urine. It is noticeable that while beyond eight hours the rate of excretion of the methyl and sulfate conjugated derivatives declines, it increases for free 3,4-dihydroxybenzoic acid. These benzoic acids are usually considered to be flavonoid metabolites (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014) and are rarely reported at more than trace levels in human intervention studies with a flavonoid-free acyl-quinic acid source (Clifford et al., 2020; Rodriguez-Mateos et al., 2014). Accordingly, the 30.9 μmol of 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid and 3-hydroxy-4-methoxybenzoic acid, collectively, could be derived by gut microbiota catabolism of the 82 μmol of luteolin glycosides consumed, however, β-oxidation and COMT methylation of 3',4'-dihydroxycinnamic acid, whether free or released from CQA, must have occurred because these benzoic acids increased significantly in plasma ($p < 0.05$) within two hours of consumption (Fig. 1), and the *sous-vide* artichoke supplied only 1.7 μmol of pre-existing 3,4-dihydroxybenzoic acid, while 4.2 μmol were excreted during the first four hours after the intervention. Even if the total 4.2 μmol came from hepatic β-oxidation of 3',4'-dihydroxycinnamic acid, 4'-hydroxy-3'-methoxycinnamic acids and 3'-hydroxy-4'-methoxycinnamic acids, this would be a minor pathway after the consumption of 8.7 mmol total caffeic acid equivalents (*ca* 0.04% yield).

Greater plasma concentrations at six hours compared with two hours, and non-zero concentrations at 24 h, indicate that gut microbiota metabolism also has a role in their production, either through catabolism of luteolin, or by hydrolysis of unabsorbed CQA and diCQA providing both quinic acid and 3',4'-dihydroxycinnamic acid. Both hydrolysis products may yield 3,4-dihydroxybenzoic acid, quinic acid via gut microbiota dehydroxylation and dehydrogenation (Pinta et al., 2018), and 3',4'-dihydroxycinnamic acid via hepatic β-oxidation. 3,4-dihydroxybenzoic acid is a substrate for COMT-3- and to a lesser extent also 4-methylation, as reviewed by Clifford et al. (Clifford et al., 2020). It is clear from the 24-hour excretion data that the acyl-quinic acids can only be minor sources because even if all 30.6 μmol of these 3,4-di-substituted benzoic acids were so generated, this was achieved after the consumption of 8.7 mmol total caffeic acid and 5.4 mmol quinic acid (*ca* 0.2% yield).

3',4'-Dihydroxyphenylacetic acid and 3'-methoxyphenylacetic acid-4'-sulfate showed plasma profiles similar to 3-(3',4'-dihydroxyphenyl) propanoic and 3-(3'-methoxy-4'-hydroxyphenyl)propanoic acid, not peaking before 6 h post-consumption and with lower but non-zero concentrations at 24 h indicative of colonic microbial origin (Fig. 1).

Plasma concentrations of 3-hydroxybenzoic acid at 6 and 24-hours post-consumption were little changed, whereas 3'-hydroxyhippuric acid and 3-(3'-hydroxyphenyl)propanoic acid were >3-fold higher at 24 h. These were major metabolites quantified in both plasma (AUC = 62165 nM*h and 73905 nM*h; C_{max} = 5470 nM and 5970 nM, respectively) and urine (max. excretion rate = 6585 nmol/h and 24732 nmol/h, respectively) and it is clear from the plasma profiles that excretion would continue beyond 24 h. Their formation is initiated by the 4'-dehydroxylation of unabsorbed 3',4'-dihydroxyphenyl-substituted metabolites by the gut microbiota, generating 3-(3'-hydroxyphenyl)propanoic acid, followed by β-oxidation to 3-hydroxybenzoic acid and conjugation with glycine in the liver. Consistent with the results of this

Table 3
Urinary excretion (0–24 h) of (poly)phenol metabolites after consumption of artichokes containing 5,727 μmol of (poly)phenols. Data is expressed as mean values ± SEM.

Excretion rate of artichoke (poly)phenolic metabolites in urine. Total excretion in 24 h includes the range of values among participants: minimum value (min)-maximum value (max) and the quotient.

	n	0–2 h (nmol/h)	2–4 h (nmol/h)	4–8 h (nmol/h)	8–24 h (nmol/h)	Total excretion 24 h (μmol)		
						Mean	Min.-max.	Quotient
Acyl-quinic acids								
5-O-Caffeoylquinic acid	8	432 ± 85	389 ± 78	120 ± 29	5 ± 1	2.2 ± 1.1	1.2–4.9	4.08
3-O-Feruloylquinic acid	8	393 ± 46	374 ± 52	119 ± 16	8 ± 2	2.1 ± 0.7	1.2–3.1	2.58
4-O-Feruloylquinic acid	8	111 ± 10	152 ± 20	64 ± 9	4 ± 1	0.8 ± 0.3	0.4–1.2	3.00
4-O-Caffeoylquinic acid	8	41 ± 11	49 ± 13	19 ± 6	1 ± 0	0.3 ± 0.2	0.1–0.8	8.00
Cinnamic acids (C_6-C_3 unsaturated)								
3′,4′-Dihydroxycinnamic acids								
4'-Hydroxycinnamic acid-3′-sulfate	8	5,062 ± 983	4,465 ± 723	3,380 ± 626	415 ± 79	39 ± 17	18–64	3.55
3'-Hydroxycinnamic acid-4′-sulfate	8	823 ± 167	922 ± 180	619 ± 160	35 ± 9	6.5 ± 3.3	2.5–11	4.40
3',4'-Dihydroxycinnamic acid	8	22 ± 6	18 ± 6	78 ± 36	46 ± 22	1.1 ± 1.4	0.3–4.6	15.33
3'-Hydroxycinnamic acid-4′-glucuronide	8	49 ± 7	62 ± 10	58 ± 7	15 ± 3	0.7 ± 0.2	0.4–1.0	2.50
4'-Hydroxycinnamic acid-3′-glucuronide	8	55 ± 9	77 ± 15	69 ± 15	11 ± 2	0.7 ± 0.3	0.2–1.2	6.00
4′-Hydroxy-3′-methoxycinnamic acids								
3'-Methoxycinnamic acid-4'-sulfate	8	3,033 ± 726	2,528 ± 642	3,186 ± 899	1,081 ± 186	41 ± 23	26–105	3.96
4'-Hydroxy-3'-methoxycinnamic acid	8	4,227 ± 946	3,517 ± 829	4,100 ± 999	1,636 ± 252	58 ± 28	16–77	4.81
3'-Methoxycinnamic acid-4'-glucuronide	8	1,470 ± 163	1,917 ± 321	2,224 ± 407	894 ± 195	30 ± 12	14–48	3.43
3′-Hydroxy-4′-methoxycinnamic acids								
3'-Hydroxy-4'-methoxycinnamic acid	8	710 ± 106	437 ± 108	797 ± 115	279 ± 53	10 ± 2	7.2–14	1.94
4'-Methoxycinnamic acid-3'-glucuronide	8	111 ± 13	130 ± 20	102 ± 20	26 ± 3	1.3 ± 0.4	4.6–16	3.48
4'-Methoxycinnamic acid-3'-sulfate	8	320 ± 33	433 ± 93	435 ± 79	273 ± 76	7.6 ± 3.7	0.9–1.9	2.11
Hydroxycinnamic acids								
Cinnamic acid-4'-sulfate	8	97 ± 14	110 ± 13	40 ± 4	19 ± 14	0.9 ± 0.7	0.5–2.6	5.20
4'-Hydroxycinnamic acid*	8	8 ± 1	9 ± 1	18 ± 6	11 ± 5	0.3 ± 0.2	0.1–0.8	8.00
Cinnamic acid-4'-glucuronide*	8	3 ± 1	3 ± 1	3 ± 0	8 ± 7	0.1 ± 0.2	0.02–0.5	25.00
2'-Hydroxycinnamic acid*	6	5 ± 2	4 ± 2	3 ± 1	2 ± 1	0.0 ± 0.1	0.002–0.1	50.00
4′-Hydroxy-3′,5′-dimethoxycinnamic acids								
4'-Hydroxy-3',5'-dimethoxycinnamic acid*	8	50 ± 10	19 ± 4	38 ± 12	23 ± 20	0.7 ± 0.9	0.1–2.7	2.70
Benzoic acids (C_6-C_1)								
Benzoic acid	8	154 ± 57	61 ± 16	283 ± 69	496 ± 214	9.5 ± 10	1.7–29	17.06
Hydroxybenzoic acids								
3-Hydroxybenzoic acid	8	50 ± 10	46 ± 22	111 ± 10	281 ± 128	5.1 ± 5.8	1.5–19	12.67
2-Hydroxybenzoic acid*	8	91 ± 27	56 ± 18	64 ± 14	17 ± 7	0.8 ± 0.6	0.2–2.0	10.00
4-Hydroxybenzoic acid*	8	2,633 ± 115	1,929 ± 152	2,706 ± 552	1,192 ± 257	39 ± 16	24–73	3.04
3,4-Dihydroxybenzoic acids								
3,4-Dihydroxybenzoic acid	8	233 ± 53	232 ± 70	600 ± 122	737 ± 186	15 ± 10	6.0–33	5.50
4-Hydroxybenzoic acid-3-sulfate	8	253 ± 47	281 ± 60	225 ± 77	45 ± 23	2.7 ± 2.0	0.9–6.8	7.55
3-Hydroxybenzoic acid-4-sulfate	8	175 ± 43	281 ± 71	266 ± 66	52 ± 23	2.8 ± 2.1	1.2–7.3	6.08
Other dihydroxybenzoic acids								
2,4-Dihydroxybenzoic acid*	8	140 ± 58	115 ± 40	105 ± 34	42 ± 15	1.4 ± 1.5	0.2–4.6	23.00
2,5-Dihydroxybenzoic acid*	8	1,103 ± 768	972 ± 688	631 ± 385	797 ± 426	19 ± 32	1.3–97	74.62
2,6-Dihydroxybenzoic acid*	8	87 ± 31	71 ± 22	58 ± 18	26 ± 7	1.0 ± 0.8	0.2–2.7	13.50
3,5-Dihydroxybenzoic acid*	8	202 ± 69	98 ± 25	133 ± 33	106 ± 31	2.6 ± 1.8	0.4–5.0	12.50
Methoxybenzoic acids								
4-Hydroxy-3-methoxybenzoic acid	8	230 ± 67	116 ± 33	386 ± 103	230 ± 69	5.8 ± 4.2	1.2–12	10.00
3-Methoxybenzoic acid-4-sulfate	8	153 ± 44	121 ± 26	226 ± 52	104 ± 23	3.1 ± 1.8	0.7–5.4	7.71
4-Methoxybenzoic acid-3-sulfate	8	40 ± 14	84 ± 24	113 ± 27	32 ± 5	1.2 ± 0.6	0.7–2.6	3.71
4-Hydroxy-3,5-dimethoxybenzoic acid*	8	18 ± 4	12 ± 2	15 ± 2	12 ± 6	0.3 ± 0.3	0.1–1.0	10.00
3-Hydroxy-4-methoxybenzoic acid-5-sulfate*	8	39 ± 12	21 ± 3	23 ± 4	14 ± 7	0.4 ± 0.4	0.1–1.4	14.00
Phenylacetic acids (C_6-C_2)								
3′-Methyoxyphenylacetic acid-4′-sulfate	8	11 ± 2	6 ± 1	19 ± 6	8 ± 1	0.2 ± 0.1	0.04–0.4	10.00
3',4'-Dihydroxyphenylacetic acid*	8	450 ± 72	365 ± 49	441 ± 41	320 ± 62	8.5 ± 3.7	5.2–16	3.08
Propanoic acids (C_6-C_3)								
3-(3′,4′-Dihydroxyphenyl)propanoic acids								
3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate	8	17 ± 5	25 ± 9	328 ± 54	440 ± 58	8.4 ± 3.2	18–37	2.06
3-(3',4'-Dihydroxyphenyl)propanoic acid	8	16 ± 9	14 ± 4	136 ± 19	233 ± 31	4.3 ± 1.6	4.1–12	2.93
3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	8	42 ± 25	43 ± 15	1,149 ± 276	1,259 ± 90	25 ± 6.2	2.1–6.5	3.09
3-(4′-Hydroxy-3′-methoxyphenyl)propanoic acids								
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	8	89 ± 23	87 ± 29	2,154 ± 491	1,827 ± 647	38 ± 33	9–108	12.00
3-(3'-Methoxyphenyl)propanoic acid-4'-glucuronide	8	20 ± 4	17 ± 5	149 ± 58	193 ± 59	3.8 ± 3.3	18–131	7.28
3-(3'-Methoxyphenyl)propanoic acid-4'-sulfate	8	73 ± 35	75 ± 37	1,237 ± 425	2,713 ± 707	49 ± 37	1.7–11	6.47
3-(3′-Hydroxy-4′-methoxyphenyl)propanoic acids								

(*continued on next page*)

Table 3 (*continued*)

Excretion rate of artichoke (poly)phenolic metabolites in urine. Total excretion in 24 h includes the range of values among participants: minimum value (min)-maximum value (max) and the quotient.

	n	0–2 h (nmol/h)	2–4 h (nmol/h)	4–8 h (nmol/h)	8–24 h (nmol/h)	Total excretion 24 h (μmol)		
						Mean	Min.-max.	Quotient
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	8	63 ± 35	60 ± 31	810 ± 332	1,446 ± 568	27 ± 29	7.6–95.9	12.62
3-(4'-Methoxyphenyl)propanoic acid-3'-sulfate*	8	330 ± 133	212 ± 71	324 ± 74	409 ± 170	8.9 ± 8.3	2.9–28.7	3.90
Hydroxypropanoic acids								
3-(3'-Hydroxyphenyl)propanoic acid	8	213 ± 170	526 ± 469	1,011 ± 396	6,585 ± 3,549	110 ± 166	2.5–419	167.60
2-(4'-Hydroxyphenoxy)propanoic acid*	8	34 ± 14	65 ± 39	1,216 ± 205	1,586 ± 218	30 ± 12	14–43	3.07
3-(2'-Hydroxyphenyl)propanoic acid*	8	10 ± 1	8 ± 2	12 ± 2	8 ± 2	0.2 ± 0.1	0.1–0.4	4.00
3-(2',3'-Dihydroxyphenyl)propanoic acid*	8	4 ± 1	3 ± 1	16 ± 5	37 ± 13	0.7 ± 0.6	0.03–1.9	63.33
3-(3',5'-Dihydroxyphenyl)propanoic acid*	8	293 ± 145	277 ± 150	153 ± 55	157 ± 56	4.3 ± 4.7	1.2–15	12.50
2-Hydroxy-3-(4'-hydroxyphenyl)propanoic acid*	8	241 ± 54	166 ± 25	333 ± 100	231 ± 58	5.8 ± 3.9	1.8–12	6.67
Hippuric acids (C_6–C_1–N)								
3'-Hydroxyhippuric acid	8	3,656 ± 1,719	2,562 ± 1,162	7,791 ± 2,691	24,723 ± 5,898	439 ± 297	66–858	13.00
Hippuric acid	8	45,554 ± 18,622	26,701 ± 7,939	45,755 ± 15,041	51,400 ± 14,796	1,150 ± 970	185–3,162	17.09
2'-Hydroxyhippuric acid *	8	1,308 ± 253	1,325 ± 352	1,273 ± 246	1,357 ± 379	32 ± 21	6.3–71	11.27
4'-Hydroxyhippuric acid *	8	1,710 ± 372	1,340 ± 250	2,317 ± 289	1,420 ± 285	38 ± 16	24–66	2.75
Benzene diols and triols (C_6-C_0)								
4-methyl-1,2-dihydroxybenzene*	7	743 ± 292	373 ± 148	156 ± 48	130 ± 43	3.2 ± 3.8	<LOQ-11	>275.00
4-Methyl-2-hydroxybenzene-1-sulfate *	7	13,073 ± 6,439	7,174 ± 3,173	3,551 ± 1,011	2,955 ± 1,542	56 ± 88	<LOQ-257	>372.46
2-Hydroxybenzene-1-glucuronide*	8	28 ± 9	18 ± 5	47 ± 16	48 ± 17	1.0 ± 1.0	0.3–3.0	10.00
Pyrogallol-2'-sulfate*	8	178 ± 117	75 ± 29	93 ± 32	32 ± 13	1.4 ± 1.6	0.2–5.1	25.50
1-Methylpyrogallol-sulfate*	8	141 ± 53	70 ± 27	204 ± 96	73 ± 37	2.4 ± 2.6	0.4–7.9	19.75
2-Methylpyrogallol-1-sulfate*	8	13 ± 3	6 ± 2	12 ± 7	2 ± 0	0.1 ± 0.1	0.01–0.4	40.00
Benzaldehydes (C_6-C_1)								
4-Hydroxybenzaldehyde*	8	7 ± 2	7 ± 2	8 ± 3	5 ± 2	0.1 ± 0.1	0.03–0.3	10.00
4-Hydroxy-3-methoxybenzaldehyde*	8	12 ± 3	8 ± 2	9 ± 1	6 ± 1	0.2 ± 0.1	0.1–0.3	3.00

b) Total excretion of the main subclasses of artichoke (poly)phenolic metabolites 0–24 h.

	0–4 h		4–24 h		0–24 h	
	Excretion (μmol)	Recovery (%)	Excretion (μmol)	Recovery (%)	Excretion (μmol)	Recovery (%)
Acyl-quinic acids	3.9 ± 0.5	0.07	1.6 ± 0.2	0.03	5.5 ± 0.7	0.09
3',4'-Dihydroxycinnamic acids	23.1 ± 4.0	0.40	25.2 ± 4.1	0.44	48.3 ± 7.3	0.84
4'-Hydroxy-3'-methoxycinnamic acids	33.4 ± 6.6	0.58	95.8 ± 15.2	1.66	129.2 ± 20.3	2.24
3'-Hydroxy-4'-methoxycinnamic acids	4.3 ± 0.6	0.07	14.6 ± 1.9	0.25	18.9 ± 2.0	0.33
Cinnamic acid-4'-sulfate	0.4 ± 0.0	0.01	0.5 ± 0.2	0.01	0.9 ± 0.2	0.02
3-Hydroxybenzoic acid	0.2 ± 0.0	0.00	4.9 ± 2.1	0.09	5.1 ± 2.0	0.09
3,4-Dihydroxybenzoic acids	2.9 ± 0.6	0.05	17.7 ± 4.1	0.31	20.6 ± 4.6	0.36
Methoxybenzoic acids*[1]	1.3 ± 0.3	0.02	8.8 ± 1.8	0.15	10.1 ± 1.9	0.17
3′-methoxyphenylacetic acid-4'-sulfate	0.0 ± 0.0	0.00	0.2 ± 0.0	0.00	0.2 ± 0.0	0.00
(3',4'-Dihydroxyphenyl)propanoic acids	0.3 ± 0.1	0.00	37.4 ± 3.3	0.65	37.6 ± 3.3	0.65
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acids	0.7 ± 0.3	0.01	89.9 ± 24.9	1.56	90.6 ± 24.9	1.57
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	0.2 ± 0.1	0.00	26.4 ± 10.4	0.46	26.6 ± 10.4	0.46
3-(3'-Hydroxyphenyl)propanoic acid	0.8 ± 0.7	0.01	109.4 ± 58.3	1.89	110.2 ± 58.6	1.91
Benzoic acid	0.4 ± 0.1	0.01	9.1 ± 3.6	0.16	9.5 ± 3.6	0.16
Total excretion	**71.9 ± 11.5**	**1.25**	**441.3 ± 93.3**	**7.64**	**513 ± 92**	**8.89**

* not included in metabolic pathways proposal and bioavailability evaluation. *[1] Only including 3-hydroxy-4-methoxybenzoic acid, 3-methoxybenzoic acid-4-sulfate and 4-methoxybenzoic acid-3-sulfate.

volunteer study, 3-(3'-hydroxyphenyl)propanoic acid was the most abundant metabolite after *sous-vide* artichoke had been subject *in vitro* to a six-hour human gut microbiota incubation (Domínguez-Fernández, Ludwig, et al., 2021).

Both metabolites have been quantified in plasma and urine after coffee and maté consumption (Gómez-Juaristi, Martínez-López, Sarria, Bravo, & Mateos, 2018; Gómez-Juaristi et al., 2018), but the yields of 3-(3'-hydroxyphenyl)propanoic acid and 3'-hydroxy-hippuric acid in urine over 24 h were both <0.1% of the total 3',4'-dihydroxycinnamic acid intake, in marked contrast to their prominence in the profile observed in this study

Benzoic and hippuric acids are always significant components of human urine and probably all dietary (poly)phenols which reach the colon are potential sources. Another potential source of particular relevance to this study is the quinic acid derived from the 5.4 mmol acyl-quinic acids consumed, which can be converted to benzoic acid by a combination of gut microbiota catabolism and hepatic dehydrogenation, as reviewed by Clifford et al. (Clifford et al., 2020). In this study it was noticeable that the excretion of both increased during the study, peaking in the 8–24-hour period consistent with a role for the gut microbiota.

Unexpectedly, 2-(4'-hydroxyphenoxy)propanoic acid was found in plasma samples from six participants and in urine samples from 8 participants. Additionally, the kinetics in plasma and urine (**Figs. S1** and **S2**, respectively) indicate that it might originate from the artichokes

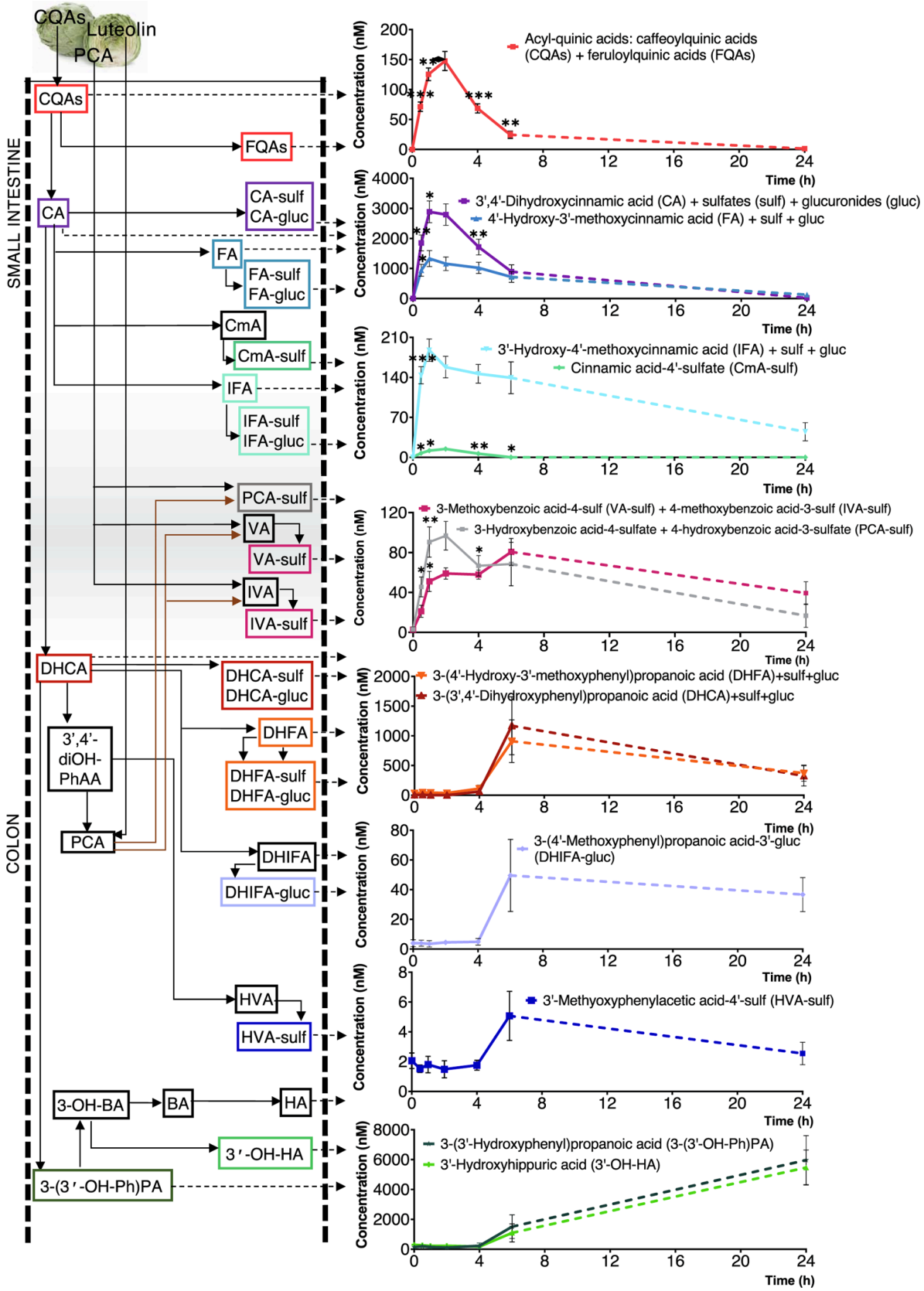

Fig. 1. Plasma profiles of artichoke (poly)phenol metabolites with a summary of the proposed metabolic pathway. BA: benzoic acid; HA: hippuric acid; PCA: 3,4-dihydroxybenzoic acid; 3',4'-diOH-PhAA: 3',4'-dihydroxyphenylacetic acid.

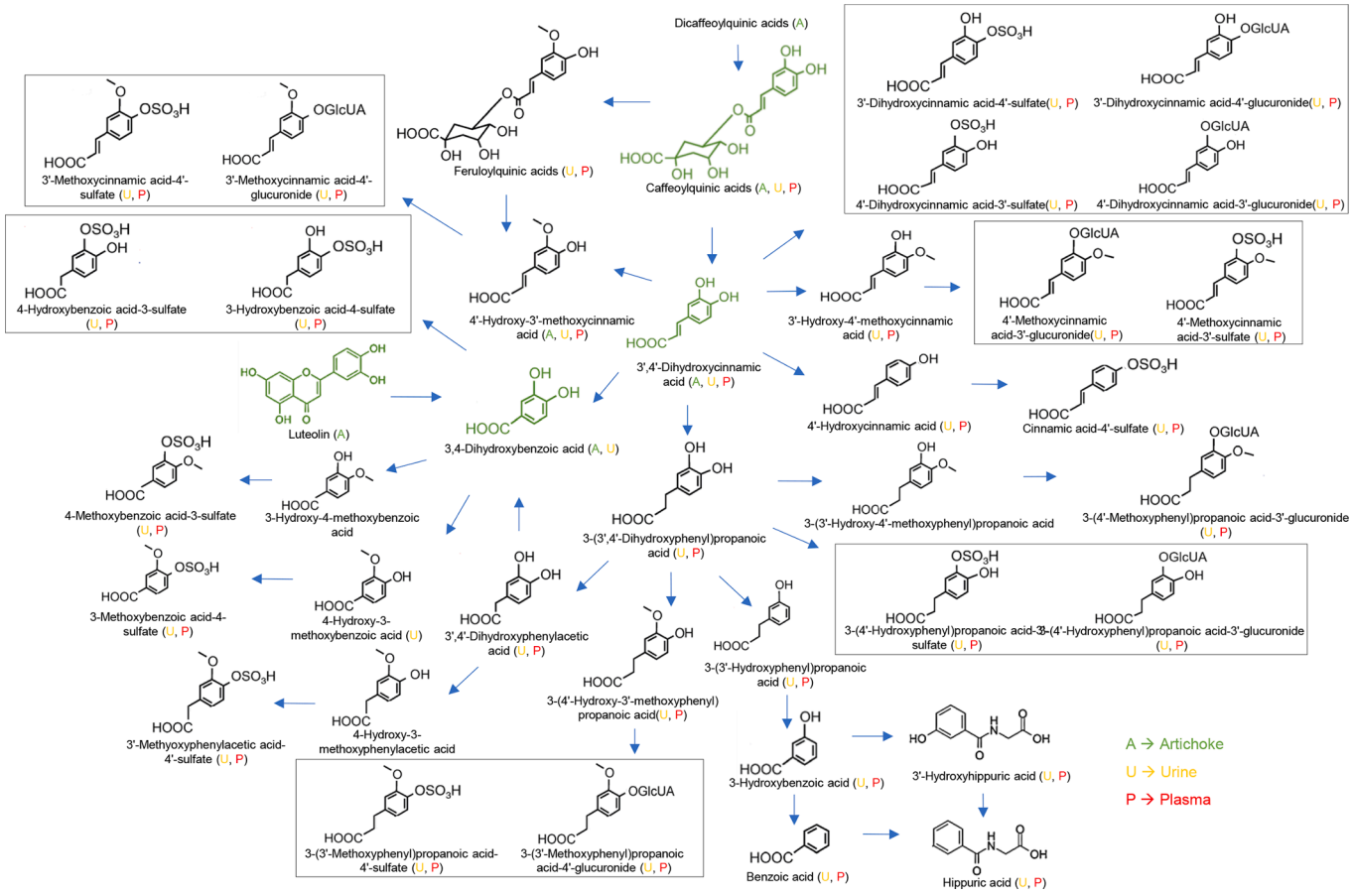

Fig. 2. Proposed metabolic pathway of (poly)phenolic metabolites after the intake of sous-vide cooking artichokes, accompanied with their chemical structures. Compounds detected in A (artichokes), U (urine) and P (plasma). Chemical structures in green are referred to compounds found in *sous-vide* artichokes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consumed. A progressively rising rate of excretion (Table 3) culminating in 1216 and 1586 nmol/h in the 4–8 and 8–24-hour periods, respectively, clearly points to a colonic origin. This compound has not been described before as a typical metabolite after consumption of artichokes or any other acyl-quinic acid-rich food, but it has been reported as the main metabolite of the herbicide fenoxaprop-P and its ethyl ester under anaerobic condition, (Anonymous, 2007) and such residues on the artichokes are almost certainly its origin. Recent data for toxicity of these herbicides in Zebra Fish considered 2-(4'-hydroxyphenoxy)propanoic acid the least toxic fenoxaprop-P metabolite (Xu, Jing, Zhai, & Li, 2020). Additionally, 2-(4'-hydroxyphenoxy)propanoic acid is approved to use in cosmetics (Draelos et al., 2013) and such products might have contributed to the zero-time excretion after wash-out. Therefore, while its presence in plasma and urine was unexpected, it does not seem to be a cause for concern.

3.4. (Poly)phenol intake and total urinary excretion

Total amounts of the artichoke metabolites excreted in urine in 24 h by groups and percentages relative to the 5.7 mmol of (poly)phenols consumed are summarised in Table 3. With the aim of distinguishing between an earlier absorption, probably in upper GIT, and a later absorption, further in the intestinal tract, the total excretion is also shown between 0 and 4 h and between 4 and 24 h

Because of overlap in the metabolism of dietary (poly)phenols and some nutrients, for example phenylalanine and tyrosine, hormones, for example adrenaline and noradrenaline, and some medications, for example aspirin (acetyl-salicylic acid), it is impossible in volunteer studies of this type to say with certainty that the totality of phenolic metabolites excreted in urine are derived from the test food consumed. Even if they are derived from artichoke, they are not necessarily derived from artichoke acyl-quinic acids and flavonoids, because of the possible contribution from phenylalanine and tyrosine. An estimate of yield in 24 h from the 5.7 mmol of artichoke (poly)phenols consumed which includes all the possible artichoke phenolic metabolites quantified in urine during this study (2.3 mmol) results in a total urinary recovery of *ca* 40%. However, this must be an over-estimate and it is not unreasonable to exclude at least the major metabolites that can be associated with origins other than the artichoke consumed, including compounds from Table 3 which did not show clear-cut kinetics, as well as 3'-hydroxyhippuric acid (439 ± 297 μmol) and hippuric acid (1150 ± 970 μmol), as it is well known that they have other origins (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014), as indicated by their basal levels in plasma and urine. Therefore, when excluding these compounds, the total urinary excretion decreases to 0.5 mmol (total recovery of 8.9%). However, we acknowledge that this will give rise to an under-estimate because some unquantifiable portion of these metabolites will have been produced from the acyl-quinic acids.

While this strategy might serve to set upper and lower limits to the true yield over 24 h (8.9–40%), the fact that the mean rate of excretion of certain colonic metabolites (e.g. 3-(4'-hydroxyphenyl)propanoic acid-3'-glucuronide, 3-(3'-methoxyphenyl)propanoic acid-4'-sulfate, 3-(3'-hydroxyphenyl)propanoic acid, 3'-hydroxy-hippuric acid and hippuric acid) was still rising in the 8–24-hour period suggests that some derived from artichoke, perhaps from substrates within the matrix, might not have been excreted until the next day, as seen in the wash-out data at zero-time. In this sense, for future studies on artichoke bioavailability, it would be interesting to collect urine samples for 48 h.

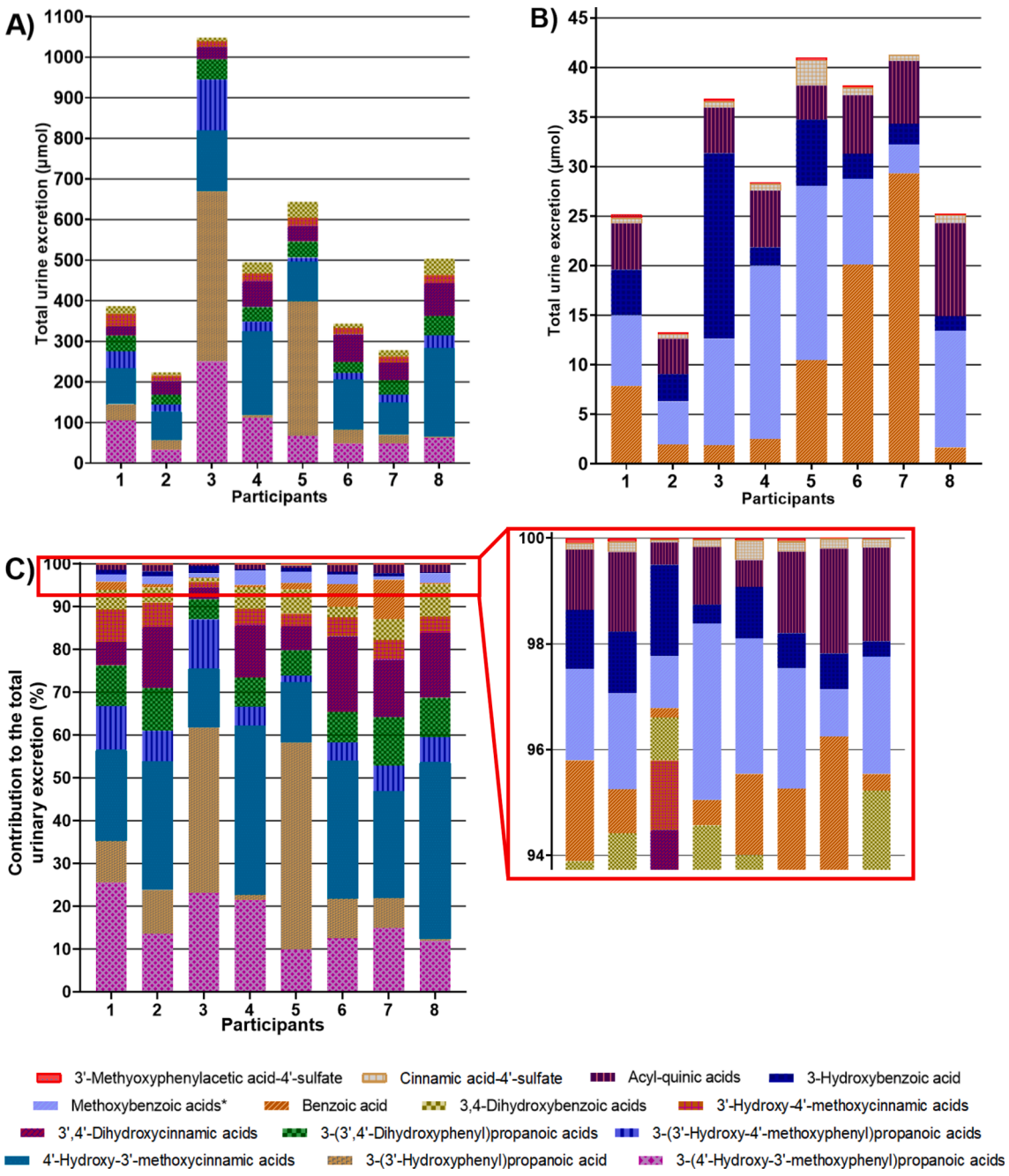

Fig. 3. A) Cumulative urinary excretion of the major groups of (poly)phenolic metabolites in 24 h. B) Relative contribution of each major group of (poly)phenolic metabolites to the total urinary excretion in 24 h. C) Cumulative urinary excretion of the (poly)phenolic metabolites in 24 h. For metabolites in each group see Table 2. *Only including 4-hydroxy-3-methoxybenzoic acid, 3-methoxybenzoic acid-4-sulfate and 4-methoxybenzoic acid-3-sulfate.

The monitoring in urine of metabolites which are unique to a particular class of substrate with no connection to endogenous / basal metabolism, such as the ellagitannin-derived urolithins, has demonstrated that such extended periods of excretion can occur (Truchado et al., 2012) especially when the relevant substrate is known to bind to proteins, potentially including the gut mucosa. Dicaffeoylquinic acids bind to proteins more strongly than caffeoylquinic acids, at least in model systems (Clifford et al., 2020), and the relatively high dose of dicaffeoylquinic acids used in this study (2.2 mmol diCQA, 54% of the total acyl-quinic acids) might be significant in this regard.

From the 0.5 mmol of artichoke phenolic metabolites excreted, the main compounds were: 4'-hydroxy-3'-methoxycinnamic acid derivatives (129 μmol), 3-(3-hydroxyphenyl)propanoic acid (110 μmol) and 3-(4'-hydroxy-3'-methoxyphenyl)propanoic acid derivatives (91 μmol). In agreement with their colonic origin, 3-(4'-hydroxy-3'-methoxyphenyl) propanoic acid and 3-(3'-hydroxyphenyl)propanoic acid were excreted almost exclusively after 4 h. In contrast, although 4'-hydroxy-3'-methoxycinnamic acid derivatives were excreted in higher amounts after 4 h, there was an earlier excretion also before 4 h. Similarly, 3',4'-dihydroxycinnamic acids and cinnamic acid-4'-sulfate were also excreted before and after 4 h after artichoke consumption. This is in accordance with the previously reported biphasic behaviour of some of these compounds, in particular 3'-methoxycinnamic acid-4'-sulfate, but also 3'-methoxycinnamic acid-4'-glucuronide and 4'-hydroxycinnamic acid-3′-sulfate (Clifford et al., 2020). Acyl-quinic acids were the only compounds which were mainly excreted before 4 h, in accordance with

their predominant absorption in the small intestine.

Total excretion of artichoke (poly)phenols in the 4–24 h period (441 μmol) was six times higher than excretion from 0 to 4 h (72 μmol), suggesting that high levels of artichoke (poly)phenols reached the colon where they are metabolised by the gut microbiota and further absorbed. These results are in accordance with a previous study where the 70% of the total acyl-quinic acids consumed were reported to reach the colon, as reviewed by Clifford et al. (Clifford et al., 2017).

It is very difficult to make meaningful comparisons regarding the relative yield of metabolites from different foods and beverages, not only for the reasons stated above, but also because different investigators reported different numbers of metabolites, and often used different standards for their quantification. Stalmach et al. (Stalmach et al., 2009) who quantified 17 metabolites using authentic standards recorded a 29.1% recovery after the consumption of coffee but they included several metabolites not quantified in the current study and excluding these their recovery falls to 23.3%. However, the current study quantified many metabolites not determined by Stalmach et al, and if the comparison is restricted to the 13 metabolites quantified in both studies the artichoke yielded only 4%, approximately one sixth the yield from coffee. The equivalent comparison with a coffee dose–response study (Stalmach et al., 2014) for 10 metabolites gives yields of 29.2%, 24.5% and 20.7% with increasing dose (412, 635 and 795 μmol), compared with 3.4% in the present study for a dose of 5,700 μmol. The artichoke matrix is probably a major factor in this lower yield. It could be hypothesised that the solid matrix delayed the absorption and further excretion of the artichoke metabolites and, as mentioned before, 48 h urine should be collected in future studies.

Despite the low percentage recovery, the greater content of (poly)phenols in a serving of *sous-vide* artichokes (5.7 mmol) led to an average excretion in 24 h of 534 μmol (poly)phenol metabolites, much higher than the 100–160 μmol reported after single servings of coffee containing 412–795 μmol (see Clifford et al., 2020 and references therein). Although, in part, this is because more metabolites were quantified in the artichoke study, and in part because artichoke supplies tyrosine and phenylalanine which coffee does not, it demonstrates clearly that artichoke is a significant dietary source of (poly)phenols. Additionally, this points out the importance of using reliable analytical methods with authentic standards, including as many metabolites as possible, for making comparisons in the metabolism of different food and beverages.

There is no entirely satisfactory method to assess whether a 200 g portion of *sous-vide* artichoke has more health-promoting potential than other sources of acyl-quinic acids such as apple, coffee or maté. The only acyl-quinic acid metabolite for which there is unequivocal evidence of modest health benefit is 3'-methoxycinnamic acid-4'-sulfate. It has been demonstrated that this metabolite produces vasorelaxation in isolated mouse saphenous and femoral arteries and aortae *in vitro* (Van Rymenant, Grootaert, et al., 2017; Van Rymenant, Van Camp, et al., 2017). In addition, its precursor, 5-caffeoylquinic acid, has been correlated with reduction in diastolic and systolic blood pressure in an acute clinical study with 23 volunteers (Mubarak et al., 2012), and beverages containing acyl-quinic acids have produced an acute improvement in flow mediated dilatation (Mills et al., 2017; Naylor et al., 2020), suggesting that those who regularly consume acyl-quinic acids frequently, might gain at least a modest health benefit long term.

3.5. Interindividual variability

The bioactivity of (poly)phenols consumed depends on their metabolites and the concentration they reach in the organism. However, high person-to-person variations are found in the metabolic profile of (poly)phenols. Therefore, for a better understanding of (poly)phenol beneficial effects, there is an increasing interest in understanding how interindividual variability affect their bioavailability and metabolism (Clifford et al., 2017).

Fig. 3 represents the total urinary excretion in 24 h of the eight participants by major (**A**) and minor (**B**) (poly)phenolic groups. Firstly, there are important differences in the total excretion of artichoke (poly)phenol metabolites among participants (245–1090 μmol) and, therefore, in their total urinary recovery (4.3–18.9%). The rest of the material might have been eliminated in the faeces or transformed into CO_2 as previously observed after isotopically labelled anthocyanins consumption (Czank, Cassidy, Zhang, Morrison, Preston, Kroon, & Kay, 2013). Further, the different compound groups did not contribute equally to this variability, and there is also a high variability among participants in the relative contribution of major and minor groups, as shown in Fig. 3C.

(Poly)phenolic compounds showing the highest variability were 3-(3'-hydroxyphenyl)propanoic acid (168-fold) and 3-(4'-methoxyphenyl) propanoic acid-3'-glucuronide (13-fold), among major groups; and 3-hydroxybenzoic acid (13-fold) among minor groups, having all of them a predominant microbial origin.

Interestingly, gut microbial metabolites showed higher interindividual variability among the (poly)phenols quantified. In agreement, gut microbiota composition has been pointed out as the main factor affecting the interindividual variability of the bioavailability and metabolism of acyl-quinic acids (Kerimi, Kraut, da Encarnacao, & Williamson, 2020) In this regard, it can be observed in Fig. 3A that participant 3 excreted by far the highest amount of (poly)phenols, mainly because of higher excretion of the typical gut microbiota derived 3-(3'-hydroxyphenyl)propanoic acid, 3-(3'-hydroxy-4'-methoxyphenyl) propanoic and 3-(3'-methoxy-4'-hydroxyphenyl)propanoic acid.

Additionally, other host-specific factors such as genetic factors or adaptation to the habitual diet are also responsible for some variability (Kerimi et al., 2020). In this sense, polymorphism affecting the methylation activity of hydroxycinnamic acids by COMT is a matter of great interest, especially in the present study due to the presence of very low levels of pre-existing ferulic acid in artichokes (Clifford et al., 2020). Thus, 3'-methylation of 3',4'-dihydroxycinnamic acid exceeded 4'-methylation in every participant; however, there were differences in the 4'-hydroxy-3'-methoxycinnamic acid / 3'-hydroxy-4'-methoxycinnamic acid ratio (2.79:1 to 10.93:1). In order to study tendencies in the ratio of participants, the values were arranged in increasing order and graphed in a scatter plot (**Fig. S5(A)**). The result suggested the presence of two groups statistically different ($p < 0.05$), one with lower ratio and another one with higher ratio, except for participant 6 whose value was in the middle. Similar approach was followed with 3-hydroxy-4-methoxybenzoic acid / 4-hydroxy-3-methoxybenzoic acid ratio. Once again, 3'-methylation of 3,4-dihydroxybenzoic acid was predominant over 4'-methylation in every participant but there were differences in the ratio (2.32:1 to 24.04:1). When plotting the values (**Fig. S5(B)**), however, participants with lower or higher 3'-hydroxy-4'-methoxycinnamic acid /4'-hydroxy-3'-methoxycinnamic ratio did not correspond with participants with lower or higher 3-hydroxy-4-methoxybenzoic acid / 4-hydroxy-3-methoxybenzoic acid ratio, respectively. Additionally, 3-hydroxy-4-methoxybenzoic acid / 4-hydroxy-3-methoxybenzoic acid ratio was continuous, so no groups could be compared. Although the mechanism involved in methylation might be the same for 3',4'-dihydroxycinnamic acid and 3,4-dihydroxybenzoic acid, it should be considered that the formation of 3,4-dihydroxybenzoic acid seems to involve principally colonic metabolism and therefore it is also subjected to gut microbiota variability. In order to be sure of these tendencies, however, more data from studies with larger sample size must be performed, if possible, having regard to genetic polymorphisms.

4. Conclusions

In the present study an extensive metabolic activity was observed after *sous-vide* artichoke consumption with 76 metabolites quantified using authentic standards in the plasma and urine of participants. The evaluation of their individual kinetic profiles led to consider 35 metabolites as clear artichoke (poly)phenol metabolites. According to the profiles they showed in plasma and urine, as well as their chemical

structures, a metabolic pathway was proposed. The metabolic pathway involves phase-II and microbial reactions which should be considered when investigating artichoke (poly)phenol potential health benefits. 3'-Methoxy-4'-hydroxycinnamic acid, 3'-methoxycinnamic acid-4'-sulfate, 4'-hydroxycinnamic acid-3'-sulfate, 3-(3'-methoxy-4'-hydroxyphenyl) propanoic acid, 3-(4'-methoxyphenyl)propanoic acid-3'-glucuronide, 3-(3'-hydroxyphenyl) propanoic acid and hippuric acids were the major compounds excreted. >80% of the artichoke (poly)phenol metabolites quantified in urine were excreted after 4 h post-consumption, suggesting that high levels of artichoke (poly)phenols reached the colon and were metabolised by the gut microbiota before absorption. Although the total recovery of metabolites calculated was lower than reported after consumption of other acyl-quinic acid-rich foods (e.g. after coffee consumption), the rising excretion rate of some colonic metabolites at 8–24 h suggests excretion beyond the 24 h recorded. The yield of individual metabolites excreted varied considerably between 1.94-fold for 3'-hydroxy-4'-methoxycinnamic acid, and as much as 168-fold for 3-(3'-hydroxyphenyl)propanoic acid, with higher variations found in general for gut microbial metabolites. Substantial interindividual variations were observed also in the balance between 3'- and 4'-methylation of 3',4'-dihydroxyphenyl-substituted substrates, and in the handling of 4-FQA.

CRediT authorship contribution statement

Maite Domínguez-Fernández: Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Paul Young Tie Yang:** Formal analysis, Visualization. **Iziar A. Ludwig:** Conceptualization, Project administration, Supervision, Writing - review & editing. **Michael N. Clifford:** Formal analysis, Writing - review & editing. **Concepción Cid:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing. **Ana Rodriguez-Mateos:** Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130620.

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