RESEARCH ARTICLE

Gastrointestinal stability and bioavailability of (poly)phenolic compounds following ingestion of Concord grape juice by humans

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The in vitro gastrointestinal stability of (poly)phenolic compounds in Concord grape juice was compared with recoveries in ileal fluid after the ingestion of the juice by ileostomists. Recoveries in ileal fluid indicated that 67% of hydroxycinnamate tartarate esters, and smaller percentages of the intake of other (poly)phenolic compounds, pass from the small intestine to the colon. The juice was also ingested by healthy subjects with an intact functioning colon. Peak plasma concentrations (C_{max}) ranged from 1.0 nmol/L for petunidin-3-*O*-glucoside to 355 nmol/L for dihydrocoumaric acid. Urinary excretion, as an indicator of bioavailability, varied from 0.26% for total anthocyanins to 24% for metabolites of hydroxycinnamate tartarate esters. The C_{max} times of the anthocyanins indicated that their low level absorption occurred in the small intestine in contrast to hydroxycinnamate metabolites which were absorbed in both the small and the large intestine where the colonic microflora appeared responsible for hydrogenation of the hydroxycinnamate side chain. The bioavailability of the complex mixture of (poly)phenolic compounds in Concord grape juice, was very similar to that observed in previous studies when compounds were either fed individually or as major components in products containing a restricted spectrum of (poly)phenolic compounds.

Keywords:
Concord grape juice / Gastrointestinal stability / Plasma pharmacokinetics / (Poly)phenol bioavailability / Urinary excretion

1 Introduction

Concord grapes are produced by *Vitis labrusca* and the juice made from these grapes contains a wide variety of tartarate esters of hydroxycinnamates, procyanidins, anthocyanins and other flavonoids [1]. Grapes and grape products have been tested in in vitro test systems, as well as with animal models and in dietary interventions with humans [2–7] where they have been associated with anti-carcinogenic effects and improved cardiovascular and cognitive function [2, 5]. These effects have been attributed to (poly)phenolic compounds which can regulate gene expression and have antioxidant, anticarcinogenic, anti-inflammatory and anti-proliferative properties [8–11].

After drinking the juice, a series of in vivo biotransformations occur that can affect the bioactivity of the ingested (poly)phenolic compounds. It is, therefore, important to understand these processes and obtain information on the fate of the (poly)phenolic compounds as they pass through the body. Previous studies have shown that gastrointestinal stability and bioavailability depends on the type of compounds ingested [12, 13]. In general, the apparent bioavailability of these compounds, which the body treats as xenobiotics, is limited as measured by low peak plasma concentrations and low urinary excretion of the dose ingested [14]. However, it can be increased substantially when catabolites formed by the colonic microflora are taken into account [15]. There is growing evidence pointing to the importance of colonic metabolism of polyphenolic compounds in increasing their bioavailability and producing metabolites with enhanced bioactivity [16, 17].

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Abbreviations: COMT, catechol-O-methyltransferase; **EST**, esterase; **GT**, UDP-glucuronosyltransferase; **n.d.**, not detected; **n.q.**, not quantifiable; **RA**, reductase; **ST**, sulphonyl-O-transferase

This study investigated Concord grape juice (poly)phenolic compounds in an in vitro model of gastrointestinal digestion and assessed their in vivo recovery in ileal fluid after ingestion by volunteers with an ileostomy. In addition, the juice was consumed by healthy subjects with an intact functioning colon after which 0–24 h plasma profiles and urinary excretion of (poly)phenolic compounds and their metabolites were monitored.

2 Material and methods

2.1 Chemicals

Concord grape juice (100%) was supplied by Welch Foods, Inc. (Concord, MA). Procyanidins B_1 and B_2, *p*-coumaric acid, ethyl gallate, 3-(3′-hydroxyphenyl)propionic acid, 3-(4′-hydroxyphenyl)propionic acid and quercetin-3-*O*-glucoside were purchased from Fluka (Sigma-Aldrich Co. Ltd., Dorset, UK). Ferulic acid, (–)-gallocatechin, (–)-epicatechin, (+)-catechin and *trans*-resveratrol were obtained from Sigma-Aldrich Co. Ltd. (–)-Epigallocatechin and (–)-epicatechin-3-*O*-gallate were purchased from Apin Chemicals Ltd. Caffeic acid, malvidin-3,5-*O*-diglucoside were supplied by AASC Ltd. (Southampton, UK). Quercetin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside and myricetin were purchased from Extrasynthese (Lyon, France) while delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside were obtained from PhytoLab Gmbh & Co. KG (Germany). Cyanidin-3-*O*-sambubioside-5-*O*-glucoside was purchased from Polyphenols (Sandnes, Norway). Standards used for quantification had a purity of 99%. Feruloylglycine was a gift from Professor Takao Yokota, Teikyo University, Utsunomiya, Japan. Sodium diethyldithiocarbamate, sodium carbonate, pepsin from porcine gastric mucosa (EC 232–629-3), a porcine bile extract (EC 232–369-0) and pancreatin from hog pancreas (EC 232–468-9) were purchased from Sigma-Aldrich Co. Ltd. Formic acid and HCl were purchased from Fisher Scientific Ltd. (Loughborough, Leicestershire, UK) and methanol and acetonitrile from Rathburn Chemicals (Walkerburn, Scotland).

2.2 Feeding studies

The study protocol was approved by the University of Glasgow Medical Faculty Ethics Committee (FM 00207 and FM 05308). Three male and five female volunteers with an intact colon (20–40 years of age and a body mass index [BMI] range of 20.7–26.1 kg/m^2) participated in the study. They were non-smokers, under no medication, not taking any vitamins or other supplements and generally healthy. Prior to starting the study, volunteers followed a diet low in (poly)phenolic compounds for 2 days. This consisted in avoiding intake of fruits, vegetables, tea, coffee, wine, chocolate, whole grain and whole meal food items. On the morning of the study, volunteers came to the trial unit in a fasted state and refrained from eating for the first 3 h after acute intake of 350 mL of 100% Concord grape juice. Water was allowed to be consumed ad libitum. Blood was sampled at baseline, after 0.5, 1, 2, 3, 4, 6, 8 and 24 h through IV canulation and urine was collected at 0–2, 2–8 and 8–24 h. Lunch was provided and consisted of a selection of white rolls, ham, margarine, cheese and ready salted crisps.

With a similar feeding protocol, two male and two female volunteers with an ileostomy (44–57 years of age, a BMI range of 22.9–27.2 kg/m^2, who had had an ileostomy for 10.8 ± 2.9 years) also consumed 350 mL of juice. Ileal fluid was collected over a 24 h period and aliquots were acidified with 50% formic acid (pH 2) and stored at −80°C prior to analysis.

2.3 In vitro digestion model

The in vitro stability of Concord grape juice (poly)phenolics was assessed based on the method of Bermúdez-Soto et al. [18]. The juice was acidified to pH 2 with HCl and 5 mL aliquots added to 1.43 mL of simulated gastric juice (pH 2) containing 0.32% (w/v) of pepsin (USA Pharmacopoeia, 1990) in falcon tubes which were flushed with nitrogen to remove excess oxygen, sealed and incubated in a shaking water bath in darkness for 1 h at 37°C to simulate gastric conditions. At the end of the incubation period, aliquots were centrifuged and 20 μL samples of supernatant analysed by high-performance liquid chromatography-mass spectrometry (HPLC-MS). A solution of sodium carbonate (0.5 N) containing 1% (w/v) of pancreatin was added to the remainder of the incubate to simulate intestinal conditions (pH 7) and 950 μL of bile in sodium carbonate (1% w/v) was added (final volume of 14.2 mL). Samples were further flushed with nitrogen, and incubated in darkness in a shaking water bath for 2, 4 and 6 h at 37°C. At the end of each incubation period 350 μL of concentrated HCl was added to the samples to achieve pH 2, prior to centrifugation and analysis by HPLC-MS.

2.4 Extraction and analysis of juice, urine and plasma

Extraction and analysis of Concord grape juice, plasma and urine samples from healthy volunteers were carried out as described by Stalmach et al. [1]. Anthocyanins, metabolites and derivatives in plasma and urine samples were quantified against standards of delphinidin-3-*O*-glucoside (delphinidin derivatives), cyanidin-3-*O*-glucoside (cyanidin derivatives), petunidin-3-*O*-glucoside (petunidin derivatives), peonidin-3-*O*-glucoside (peonidin derivatives) and malvidin-3-*O*-glucoside (malvidin derivatives), based on the $[M\text{-}H]^+$ in selected reaction monitoring (SRM). Calibration curves ranged between 0.5 and 50 ng with linear coefficients of 0.99. Tartarate esters of hydroxycinnamates were quantified

with a photo diode array (PDA) detector at 325 nm in caffeic acid (*trans*-caftaric acid), *p*-coumaric acid (*trans*-coutaric acid) and ferulic acid (*trans*-fertaric acid) equivalents. Calibration curves ranged between 5 and 500 ng with linear coefficients of 0.99. Sulphated metabolites in plasma and urine samples were based on the calibration curves of available standards of the corresponding aglycone dihydrocaffeic acid, dihydroferulic acid, caffeic acid, ferulic acid, *p*-coumaric acid, *m*-dihydrocoumaric acid (aka 3-(3′-hydroxyphenyl)propionic acid) based on the $[M\text{-}H]^-$ in selected ion monitoring (SIM). Calibration curves ranged between 0.5 and 25 ng (plasma) with minimum linear coefficients of 0.98 and between 1 and 100 ng (urine) with linear coefficients of 0.99. Methylated and sulphated (epi)catechin and (epi)gallocatechin in urine and plasma were quantified against standards of (–)-epicatechin and (–)epigallocatechin, respectively, based on the $[M\text{-}H]^-$ in SIM. Calibration curves ranged between 0.5 and 250 ng with minimum linear coefficients of 0.97. A standard of myricetin was used to quantify a urinary myricetin metabolite based on the $[M\text{-}H]^-$ in SIM with calibration curves ranging between 0.5 and 250 ng and linear coefficients of 1.00.

2.5 Extraction of ileal fluid

Extraction of 0–24-h ileal effluent were carried out as described elsewhere [19]. Briefly, aliquots of ileal fluid were defrosted at room temperature prior to being extracted. Ethyl gallate was used as an internal standard. Triplicate, two-step extractions were carried out using acidified methanol containing 20 mmol/L of sodium diethyldithiocarbamate. After centrifugation, supernatants were then pooled and reduced to dryness before being resuspended in 2 mL of HPLC mobile phase containing 10% methanol. Once resuspended, the ileal fluid extracts were centrifuged at 16 100 × *g* for 30 min at 4°C, in a 0.2 μm Micro-Spin™ Eppendorf filter (Alltech Associates Applied Sciences Ltd., Lancashire, UK) after which 50 μL aliquots of the filtrate were analysed by HPLC-PDA-MS^n. Recoveries of the internal standard ranged between 69 and 74% ($n = 4$).

2.6 HPLC-PDA-MS^n analyses

Quantitative analysis of Concord grape juice (poly)phenolic compounds and metabolites in body fluids used a Surveyor HPLC with a PDA detector and an LCQ Duo ion trap mass spectrometer fitted with an electrospray interface (ESI) (Thermo Fisher Scientific, San Jose, CA). Samples were chromatographed at 40°C using 4 μm C_{18} Synergi 250 × 4.6 mm id. reversed phase column (Phenomenex, Macclesfield, UK) using an autosampler maintained at 4°C. The mobile phase was pumped at a flow rate of 1 mL/min. The column eluate initially passed through the PDA detector and was then split, with 0.3 mL/min directed to the mass spectrometer with ESI operating in full scan positive (detection of anthocyanins) or negative ionisation mode (100–1000 *m/z*), data-dependant MS^2 for other compounds. Details of the chromatography and mass spectrometry conditions are described elsewhere [1].

2.7 Data analyses

HPLC-MS data were processed using Xcalibur QualBrowser version 2.0.7 software (Thermo Fisher Scientific, Inc., 2007). Statistical analyses were performed using Minitab 13 (Minitab Ltd., Coventry, UK). Differences between concentrations recovered at baseline after 1 and 2 h treatment with simulated gastrointestinal juices were assessed using a non-parametric Kruskal–Wallis test. Differences between peak plasma concentrations and time to reach peak concentrations between anthocyanins glucosides and glucuronides were assessed using a non-parametric Wilcoxon matched pairs sign test.

3 Results

3.1 (Poly)phenolic content of Concord grape juice

Detailed HPLC–PDA–MS^2 identifications of (poly)phenolic compounds in 100% Concord grape juice are provided elsewhere [1]. A 350 mL serving contained 528 ± 11 μmol of a mixture of (poly)phenolics. This comprised 238 ± 6 μmol of a diverse spectrum of anthocyanins in the form of cyanidin-, delphinidin-, peonidin- petunidin- and malvidin-*O*-glycosides, 158 ± 5 μmol of free hydroxycinnamate and tartarate esters, 53 ± 2 μmol procyanidins, and smaller quantities of monomeric flavan-3-ols, flavonols, *trans*-resveratrol and gallic acid. Table 1 lists the 60 individual compounds that were identified and quantified.

3.2 In vitro gastrointestinal stability

The in vitro gastrointestinal stability of (poly)phenolic compounds in the Concord grape juice was assessed by incubation with artificial gastric and pancreatic juices. The data obtained are summarised in Figure 1. Recoveries after a 1-h incubation with simulated gastric juice at pH 2.0 ranged from 57% for the procyanidins to 88% for the anthocyanins, with recoveries for gallic acid, monomeric flavan-3-ols, flavonols and hydroxycinnamate tartarate esters of 58, 64, 77 and 82%, respectively. Following the 1-h incubation under gastric conditions, samples were transferred to the in vitro pancreatic digestion model which were analysed after a further 1, 3 and 5 h corresponding to overall incubation periods of 2, 4 and 6 h. After 2 h, recoveries ranged from 12% (total monomeric flavan-3-ols) to 84% (total hydroxycinnamate tartarate esters) and thereafter, up to 6 h, degradation continued at a substantially reduced rate (Fig. 1).

Table 1. Quantification of (poly)phenols in a 350 mL serving of 100% Concord grape juice

	μmol/350 mL ± SE
Anthocyanins	
—Delphinidin-3-*O*-glucoside	58 ± 1
—Delphinidin-3,5-*O*-diglucoside	6.8 ± 0.2
—Delphinidin-3-*O*-(6″-*O*-acetyl)glucoside	7.4 ± 0.1
—Delphinidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside	5.8 ± 0.1
—Delphinidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucosyl-5-*O*-glucoside	18 ± 2
—Cyanidin-3-*O*-glucoside	25.0 ± 0.0
—Cyanidin-3,5-*O*-diglucoside	5.0 ± 0.1
—Cyanidin-3-*O*-(6″-*O*-acetyl)glucoside	6.4 ± 0.6
—Cyanidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside	8.4 ± 0.6
—Cyanidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucosyl-5-*O*-glucoside	7.5 ± 0.9
—Malvidin-3-*O*-glucoside	8.1 ± 0.0
—Malvidin-3,5-*O*-diglucoside	7.3 ± 0.2
—Malvidin-3-*O*-(6″-*O*-acetyl)glucoside	2.2 ± 0.1
—Malvidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside	3.5 ± 0.3
—Malvidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucosyl-5-*O*-glucoside	9.0 ± 0.0
—Petunidin-3-*O*-glucoside	22 ± 1
—Petunidin-3,5-*O*-diglucoside	4.5 ± 0.2
—Petunidin-3-*O*-(6″-*O*-acetyl)glucoside	4.1 ± 0.1
—Petunidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside	3.0 ± 0.1
—Petunidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucosyl-5-*O*-glucoside	10 ± 1
—Peonidin-3-*O*-glucoside	6.8 ± 0.1
—Peonidin-3,5-*O*-diglucoside	4.6 ± 0.1
—Peonidin-3-*O*-(6″-*O*-acetyl)glucoside	1.4 ± 0.1
—Peonidin-3-*O*-(6″-*O*-p-coumaroyl)glucoside	1.4 ± 0.1
—Peonidin-3-O-(6″-*O*-p-coumaroyl)glucosyl-5-*O*-glucoside	2.5 ± 0.1
Total anthocyanins	*238 ± 6*
Flavan-3-ols	
—Gallocatechin	2.8 ± 0.0
—Epigallocatechin	1.1 ± 0.1
—Catechin	7.1 ± 0.2
—Epicatechin	22 ± 2
—Epicatechin-3-*O*-gallate	0.8 ± 0.0
—Procyanidin dimers, trimers, tetramers and hexamers	53 ± 2
Total flavan-3-ols	*87 ± 1*
Flavonols and flavones	
—Myricetin-*O*-glycoside	6.4 ± 0.1
—Myricetin-*O*-glucuronide	0.4 ± 0.0
—Quercetin-3-*O*-glucoside	7.8 ± 0.1
—Quercetin-3-*O*-glucuronide	10.5 ± 0.2
—Quercetin-3-*O*-galactoside	0.3 ± 0.1
—Kaempferol-3-*O*-glucoside	0.6 ± 0.0
—Kaempferol-*O*-galactoside	0.1 ± 0.0
—Isorhamnetin-3-*O*-glucoside	0.2 ± 0.0
—Laricitrin-*O*-glycoside	0.2 ± 0.0
—Luteolin-*O*-glycoside	0.1 ± 0.0
Total flavonols and flavones	*27 ± 1*
Hydroxycinnamates and tartarate esters	
—Caffeic acid	0.7 ± 0.1
—*p*-Coumaric acid	1.9 ± 0.1
—Ferulic acid	0.4 ± 0.0
—*trans*-Caftaric acid	133 ± 4
—*trans*-Coutaric acid	19 ± 1
—*trans*-Fertaric acid	4.4 ± 0.2
Total hydroxycinnamates	*158 ± 5*
Hydroxybenzoic acid	
—Gallic acid	18 ± 1
Stillbenes	
—*trans*-Resveratrol	0.5 ± 0.0
Total (poly)phenolic compounds	***528 ± 11***

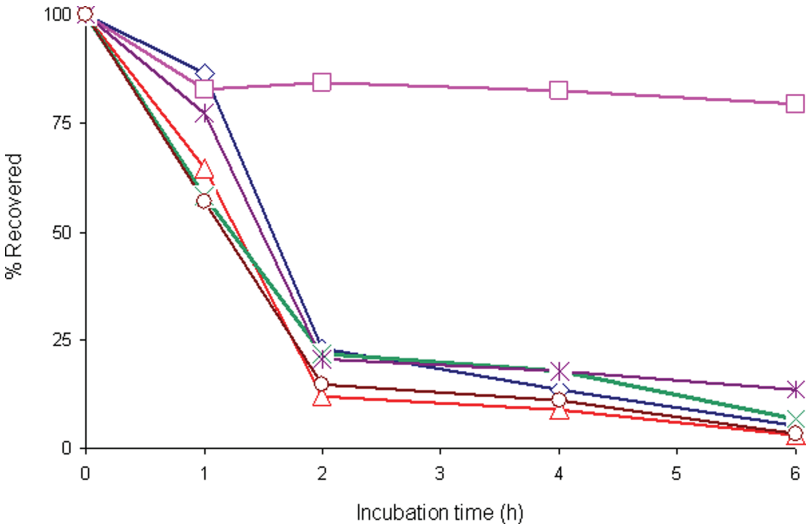

Figure 1. Recovery of the various groups of (poly)phenolics contained in Concord grape juice, expressed as a percentage of the initial amount incubated in simulated gastric juice at pH 2 (1 h) and simulated duodenal juice at pH 7 (2, 4, 6 h). ◇, Anthocyanidins; □, hydroxycinnamic acid tartarate esters; △, monomeric flavan-3-ols; ×, gallic acid; ⋆, flavonol glycosides; ◯, procyanidins.

More details of degradation in the initial 1 and 2 h incubation periods are presented in Table 2 which shows that after 2 h of in vitro gastrointestinal digestion there was an overall recovery of 40% of juice (poly)phenolics.

3.3 (Poly)phenolic compounds recovered in ileal fluid

The recovery of compounds in 0–24 h ileal fluid collected from ileostomy volunteers following acute intake of Concord grape juice provided insights into the combined in vivo effects of gastrointestinal stability and upper gastrointestinal absorption. Of the total 528 ± 11 μmol of (poly)phenolic compounds ingested 209 ± 26 μmol were recovered in ileal effluent, representing 40% of intake. This comprised 25 anthocyanins (47 ± 9 μmol), three hydroxycinnamate tartarate esters (104 ± 9 μmol), procyanidins (3.8 ± 1.1 μmol), flavonols (5.3 ± 3.2 μmol) and gallic acid (8.5 ± 1.7 μmol). Also present were four monomeric flavan-3-ols (2.3 ± 0.9 μmol) and 38 ± 13 μmol of (epi)catechin metabolites in the form of four (epi)catechin-*O*-sulphates (31 ± 10 μmol) and five methyl-*O*-(epi)catechin-*O*-sulphates (7.6 ± 3.1 μmol). When these metabolites are taken into account, the total (epi)catechin monomers in ileal fluid, from an intake of 33.4 μmol (Table 1), was 40.5 μmol which is a 121 ± 42% recovery.

Thus, the overall recovery of parent compounds and their metabolites in ileal fluid was 209 ± 26 μmol which is 40 ± 5% of intake. More details of the recoveries of the different types of compounds in ileal fluid and after a 2-h in vitro simulated gastrointestinal digestion are presented in Table 2. Although the two systems are far from identical, in general overall recoveries were broadly similar with a relatively high stability of the hydroxycinnamate tartarate esters compared to other components.

3.4 Bioavailability of Concord grape (poly)phenolic compounds in healthy volunteers

3.4.1 Anthocyanins

A total of 10 anthocyanins were identified in plasma and urine following ingestion of Concord grape juice by eight healthy volunteers with a functioning colon. These were 3-*O*-glucosides and *O*-glucuronides of delphinidin, petunidin, cyanidin, peonidin and malvidin (Table 3). In plasma, only the 3-*O*-glucosides and *O*-glucuronides of delphinidin and petunidin were detected albeit in very low but none-theless quantifiable amounts. Delphinidin-3-*O*-glucoside was present in samples from seven volunteers, petunidin-3-*O*-glucoside in five while delphinidin-*O*-glucuronide and petunidin-*O*-glucuronide were detected in plasma from all eight subjects. Peak plasma concentrations (C_{max}) ranged from 1.0 to 2.0 nmol/L, between 1.3 and 3.3 h (T_{max}) after ingestion. Plasma pharmacokinetic profiles of anthocyanins are presented in Figure 2. Although the C_{max} times of the glucuronidated metabolites appeared to be at later time points compared to the glucosides (1.3–1.4 h vs. 2.6–3.3 h), only delphinidin-3-*O*-glucuronide had a C_{max} that was significantly later than that of its 3-*O*-glucoside (3.3 ± 0.3 vs. 1.4 ± 0.4 h). Both petunidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside appeared to have a biphasic absorption profile (Fig. 2) which could be an indirect consequence of the extremely low concentrations of these compounds in plasma.

Urinary excretion of the anthocyanins ranged from 5.7 nmol for peonidin-3-*O*-glucoside to 368 nmol petunidin-*O*-glucuronide and total 0–24 h anthocyanin excretion amounted to 612 nmol which is equivalent to 0.26% intake (Table 3). Excretion expressed as a percentage of the amounts of different types of anthocyanins ingested ranged from 0.03% (delphinidin-*O*-glucuronide) to 0.85% (petunidin-*O*-glucuronide).

Table 2. Concentrations of various (poly)phenolic compounds in 100% Concord grape juice obtained following in vitro incubation with simulated gastric and pancreatic juices ($n = 2$) and percentages recovered in 0–24 h ileal fluid following ingestion of 350 mL of juice by ileostomy volunteers ($n = 4$)[a)]

	Initial concentration (μmol/L)	Gastric digestion (1 h) (μmol/L)	Pancreatic digestion (2 h) (μmol/L)	Recovery in vitro (%)	Recovery in ileal fluid (%)
Anthocyanin glycosides					
—Delphinidin	273 ± 6	221 ± 2	15 ± 1	5.5 ± 1	13 ± 2
—Cyanidin	149 ± 6	131 ± 2	55 ± 2	37 ± 1	17 ± 4
—Petunidin	124 ± 5	111 ± 1	17 ± 0	14 ± 1	27 ± 6
—Peonidin	48 ± 1	46 ± 1	23 ± 1	48 ± 2	23 ± 4
—Malvidin	86 ± 1	89 ± 2	49 ± 2	57 ± 1	34 ± 5
Total anthocyanins	*680 ± 17*	*598 ± 8*	*160 ± 5*	*24 ± 1*	*20 ± 4*
Hydroxycinnamates					
—*trans*-Caftaric acid	379 ± 12	306 ± 2	317 ± 4	84 ± 1	63 ± 6
—*trans*-Coutaric acid	53 ± 2	50 ± 1	48 ± 2	91 ± 4	88 ± 7
—*trans*-Fertaric acid	12 ± 1	9.2 ± 0.5	8.5 ± 0.9	71 ± 7	93 ± 9
Total tartarate esters	*444 ± 15*	*365 ± 1*	*373 ± 7*	*84 ± 2*	*67 ± 6*
Monomeric flavan-3-ols					
—Gallocatechin	8.0 ± 0.1	4.5 ± 0.0	0.2 ± 0.0	2.5 ± 0.1	2.9 ± 1.1
—Epigallocatechin	3.2 ± 0.4	2.7 ± 0.1	n.d.	n.d.	n.d
—Catechin	20 ± 1	16 ± 2	4.5 ± 0.1	23 ± 1	2.5 ± 0.9
—Epicatechin	62 ± 5	36 ± 2	6.6 ± 0.1	11 ± 0	8.8 ± 4.2
—Epicatechin-3-*O*-gallate	2.2 ± 0.1	1.5 ± 0.0	0.3 ± 0.0	14 ± 1	20 ± 3
—Sulphated and methylated (epi)catechins	n.d.	n.d.	n.d.	n.d.	133 ± 45
Total monomers	*95 ± 6*	*62 ± 4*	*12 ± 1*	*12 ± 1*	*121 ± 42*
Procyanidins					
Total procyanidins	*152 ± 5*	*87 ± 11*	*22 ± 0.1*	*15 ± 1*	*27 ± 8*[b)]
Flavonols					
Total flavonol glycosides	*76 ± 1*	*59 ± 1*	*16 ± 1*	*21 ± 1*	*38 ± 17*
Hydroxybenzoic acid					
—Gallic acid	*51 ± 3*	*30 ± 1*	*11 ± 1*	*22 ± 1*	*48 ± 10*
Total (poly)phenolics	***1498 ± 31***	***1200 ± 14***	***594 ± 13***	***40 ± 1***	***40 ± 5***

a) Data expressed as mean values ± SE.
b) Recovered as dimeric procyanidins.
n.d., not detected.

3.4.2 Flavonols, flavones and flavan-3-ols

The juice contained low levels of nine flavonol glycosides and one flavone glycoside (Table 1), and trace quantities of metabolites of these compounds appeared transiently in plasma of a small number of volunteers. Only a myricetin-*O*-glucoside-*O*-sulphate appeared in urine in quantifiable amounts equivalent to 0.5% of myricetin intake.

Although flavan-3-ol monomers were present in the juice in slightly higher levels than the flavonols, and it is known that they are absorbed in the proximal gastrointestinal tract [15,19,21], their metabolites too did not accumulate in plasma in quantifiable amounts. However, ten flavan-3-ol metabolites were detected in urine with the amounts excreted over the 24-h collection period ranging from 45 ± 14 nmol for an *O*-methyl-(epi)catechin-*O*-glucuronide to 2301 ± 225 nmol for two (epi)catechin-*O*-sulphate isomers (Table 4). Total flavan-3-ol metabolites excretion was 4476 ± 308 nmol corresponding to 13.7% of intake. Recovery of (epi)catchin metabolites at 15.1% was substantially higher than that of (epi)gallocatechins at 3.5% as noted previously in other studies [19–21]. No procyanidins were detected in either plasma or urine of any of the eight volunteers.

3.4.3 Hydroxycinnamate tartarate esters

Nine compounds derived from the hydroxycinnamate tartarate esters, *trans*-caftaric acid, *trans*-coutaric and *trans*-fertaric acid were quantified in plasma and 13 in urine. Caffeic acid-3′-*O*-sulphate, ferulic acid-4′-*O*-sulphate, dihydrocaffeic acid-3′-*O*-sulphate and *m*-dihydrocoumaric acid (aka 3-(3′-hydroxyphenyl)propionic acid) were detected in plasma from all eight volunteers, while six subjects accumulated ferulic acid, and five volunteers accumulated dihydroferulic acid-4′-*O*-sulphates, caffeic acid, *p*-coumaric acid, and a dihydrocoumaric acid-*O*-sulphate, tentatively identified as the 3′-*O*-sulphate. Plasma pharmacokinetic profiles of the hydroxycinnamate metabolites are presented in Figure 3. The data in Table 5 shows that C_{max} values ranged from 47 nmol/L

Table 3. Pharmacokinetic analysis of anthocyanins detected in plasma and excreted in 0–24 h urine of healthy volunteers following acute ingestion of 350 mL of 100% Concord grape juice[a)]

	Plasma				24-h Urinary excretion	
	C_{max} (nmol/L)	T_{max} (h)	*AUC* (nmol·h/L)	$T_{1/2}$ (h)	nmol	Percentage of intake[b)]
Delphinidin-3-*O*-glucoside	1.4 ± 0.3	1.4 ± 0.4	3.5 ± 0.5	0.96	36 ± 12	0.06 ± 0.02
Delphinidin-*O*-glucuronide	1.5 ± 0.4	3.3 ± 0.3	6.9 ± 2.2	1.0	32 ± 9	0.03 ± 0.01
Petunidin-3-*O*-glucoside	1.0 ± 0.5	1.3 ± 0.5	2.0 ± 0.6	n.q.	17 ± 6	0.08 ± 0.03
Petunidin-*O*-glucuronide	2.0 ± 0.5	2.6 ± 0.2	7.6 ± 2.9	1.4	368 ± 74	0.85 ± 0.17
Cyanidin-3-*O*-glucoside	n.q.	n.q.	n.q.	n.q.	15 ± 6	0.06 ± 0.03
Cyanidin-*O*-glucuronide	n.q.	n.q.	n.q.	n.q.	19 ± 11	0.04 ± 0.02
Peonidin-3-*O*-glucoside	n.q.	n.q.	n.q.	n.q.	5.7 ± 1.6	0.08 ± 0.02
Peonidin-*O*-glucuronide	n.q.	n.q.	n.q.	n.q.	63 ± 13	0.38 ± 0.08
Malvidin-3-*O*-glucoside	n.q.	n.q.	n.q.	n.q.	9.3 ± 2.2	0.11 ± 0.03
Malvidin-*O*-glucuronide	n.q.	n.q.	n.q.	n.q.	46 ± 13	0.15 ± 0.04
Total	-	-	-	-	*612*	*0.26 ± 0.05*

a) Data expressed as mean values ± SE.
b) Expressed as a percentage of intake relative to the individual anthocyanins ingested.
AUC, area under the plasma concentration curve; C_{max} peak plasma concentration; n.q., not quantifiable; T_{max}, time to reach C_{max}; $T_{1/2}$, elimination half-life.

(caffeic acid-3′-*O*-sulphate) to 178 nmol/L (caffeic acid) for metabolites with T_{max} values of <2 h after juice intake. The C_{max} of the hydrogenated metabolites, *m*-dihydrocoumaric acid, dihydrocaffeic acid-3′-*O*-sulphate, dihydroferulic acid-4′-*O*-sulphate and the putative *m*-dihydrocoumaric acid-3′-*O*-sulphate ranged from 27 to 355 nmol/L with T_{max} values of 3.9–6.0 h.

Information on urinary excretion of hydroxycinnamates and their metabolites is summarised in Table 6. As previously noted in a feeding study with coffee [22], low levels of certain hydroxycinnamates were detected at baseline. These were *m*-dihydrocoumaric acid, the putative dihydrocoumaric acid-3′-*O*-sulphate, caffeic acid-3′-*O*-sulphate, dihydrocaffeic acid-3′-*O*-sulphate, ferulic acid-4′-*O*-sulphate and isoferulic acid-3′-*O*-glucuronide which were detected in the urine of some, but not all, of the volunteers, in amounts ranging from 0.2 μmol to 9.9 μmol (Table 6). A total of 62 ± 7 μmol were excreted in the 24-h period after juice consumption, although 24 ± 4 μmol were excreted following a 36-h low-polyphenol diet (baseline). When adjusted for baseline excretion, the 0–24 h excretion of hydroxycinnamate-derived compounds was equivalent to 24 ± 3% of intake.

4 Discussion

This study investigated the bioavailability of (poly)phenolic compounds in Concord grape juice, which comprise a mixture of anthocyanins, flavan-3-ols, flavonol glycosides, flavone glycosides, hydroxycinnamates and other phenolic compounds (Table 1). This involved (i) monitoring the gastrointestinal stability of these compounds by incubation of the juice with simulated gastric and pancreatic juices, (ii) determining their recovery in ileal fluid following ingestion of the juice by volunteers with an ileostomy and (iii) by following plasma pharmacokinetics and urinary excretion of (poly)phenolic compounds after consumption of the juice by healthy volunteers with an intact functioning colon.

Incubation of the juice in the in vitro model simulating gastric and pancreatic digestion resulted an overall 40% recovery of (poly)phenolic compounds after 2 h (Table 2, Fig. 1). As previously reported, (poly)phenolic compounds are relatively stable under gastric conditions at pH 2 but can undergo major losses under simulated duodenal digestion at pH 7 [18,23,24]. Monomeric and dimeric flavan-3-ols were the most affected, which is in accordance with a recent study by Kahle et al. reporting an almost complete degradation of procyanidin B2 and epimerisation of (–)-epicatechin to (–)-catechin after a 2 h incubation in the gastric milieu [25].

The grape juice anthocyanins were stable under acidic conditions in the presence of pepsin, with an average recovery for the 25 anthocyanins of 88%, falling to 24% following simulated digestion with pancreatin and bile extract. Malvidin, peonidin and cyanidin glycosides, with respective recoveries of 57, 48 and 37%, were more stable than petunidin (14%) and delphinidin (5.5%) glycosides (Table 2). Increased recovery of petunidin, peonidin and malvidin derivatives were observed in the 24-h ileal effluent compared to delphinidin and cyanidin conjugates (data not presented). This is in keeping with a study in which ileostomists were fed bilberry, and malvidin and petunidin glycosides, with methoxy groups on the B ring, were recovered in ileal fluid in greater quantities than cyanidin and delphinidin glycosides, which have B-ring hydroxyl groups [26]. This highlights the increased stability/reduced absorption conferred by the *O*-methylation. Previous studies investigating the gastrointestinal stability of anthocyanins using in vitro and animal models have reported similar results with recoveries ranging from 8 and 57% [18,24,27,28]. Losses of anthocyanins are thought to result from their conversion into reversible pseudobases, quinoidal bases and chalcones

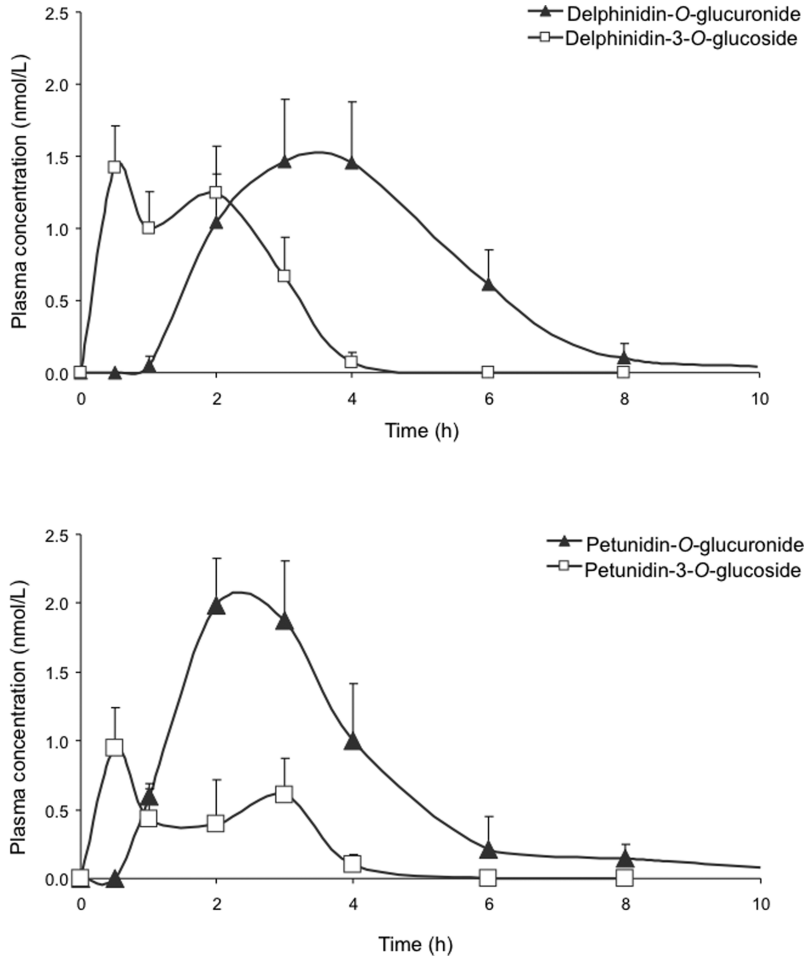

Figure 2. Pharmacokinetic profile of delphinidin-3-*O*-glucoside and delphinidin-*O*-glucuronide (top graph) and petunidin-3-*O*-glucoside and petunidin-*O*-glucuronide (bottom graph) in plasma of healthy volunteers ($n = 8$), 0–24 h following acute intake of 238 μmol of anthocyanins contained in a 350 mL-serving of Concord grape juice.

Table 4. Urinary excretion of flavan-3-ol metabolites following intake of 350 mL of 100% Concord juice by healthy volunteers ($n = 8$)[a)]

Flavan-3-ol metabolites *(no. isomers)*	Baseline	0–24 h	% Intake[b)]
(Epi)catechin-*O*-sulphate *(2)*	n.d.	2301 ± 225	
(Epi)catechin-*O*-glucuronide	n.d.	236 ± 21	
O-Methyl-(epi)catechin-*O*-sulphate *(5)*	n.d.	1757 ± 111	
O-Methyl-(epi)catechin-*O*-glucuronide	n.d.	45 ± 14	
Total (epi)catechin metabolite excretion		*4340 ± 175*	*15.1 ± 1.1*
O-Methyl-(epi)gallocatechin-*O*-sulphate	n.d.	137 ± 33	3.5 ± 0.8
Total flavan-3-ol excretion		*4476 ± 308*	*13.7 ± 0.9*

a) Data expressed in nmol as mean values ± SE ($n = 8$).
b) Expressed as a percentage of intake (28.7 μmol for epicatechin, 3.9 μmol for epigallocatechin).
n.d., not detected.

under neutral pH and alkaline conditions as well as hydrolysis to various phenolic acids, such as 3,4-dihydroxybenzoic acid (aka protocatechuic acid) [23,24], analyses of which were beyond the scope of the current study.

The hydroxycinnamate tartarate esters, of which *trans*-caftaric acid was the major component, were the most stable of the compounds investigated with a recovery of 84% after 2 h of duodenal digestion (Table 2, Fig. 1). The gastrointestinal stability of such compounds has not previously been investigated, although caffeoylquinic acids, quinic esters of hydroxycinnamates, have been reported to be stable when incubated with various gastrointestinal fluids [29–32]. However, after long incubation periods with simulated duodenal juice, isomerisation and hydrolysis of the quinic moiety has been observed [25]. Flavan-3-ol monomers and procyanidins were much less stable in vitro with recoveries of 12–13% after a 2-h incubation with most degradation occurring during pancreatic digestion (Table 2). Breakdown of procyanidins did not result in an increase in monomeric flavan-3-ols as previously reported [33]. In fact, the overall concentration

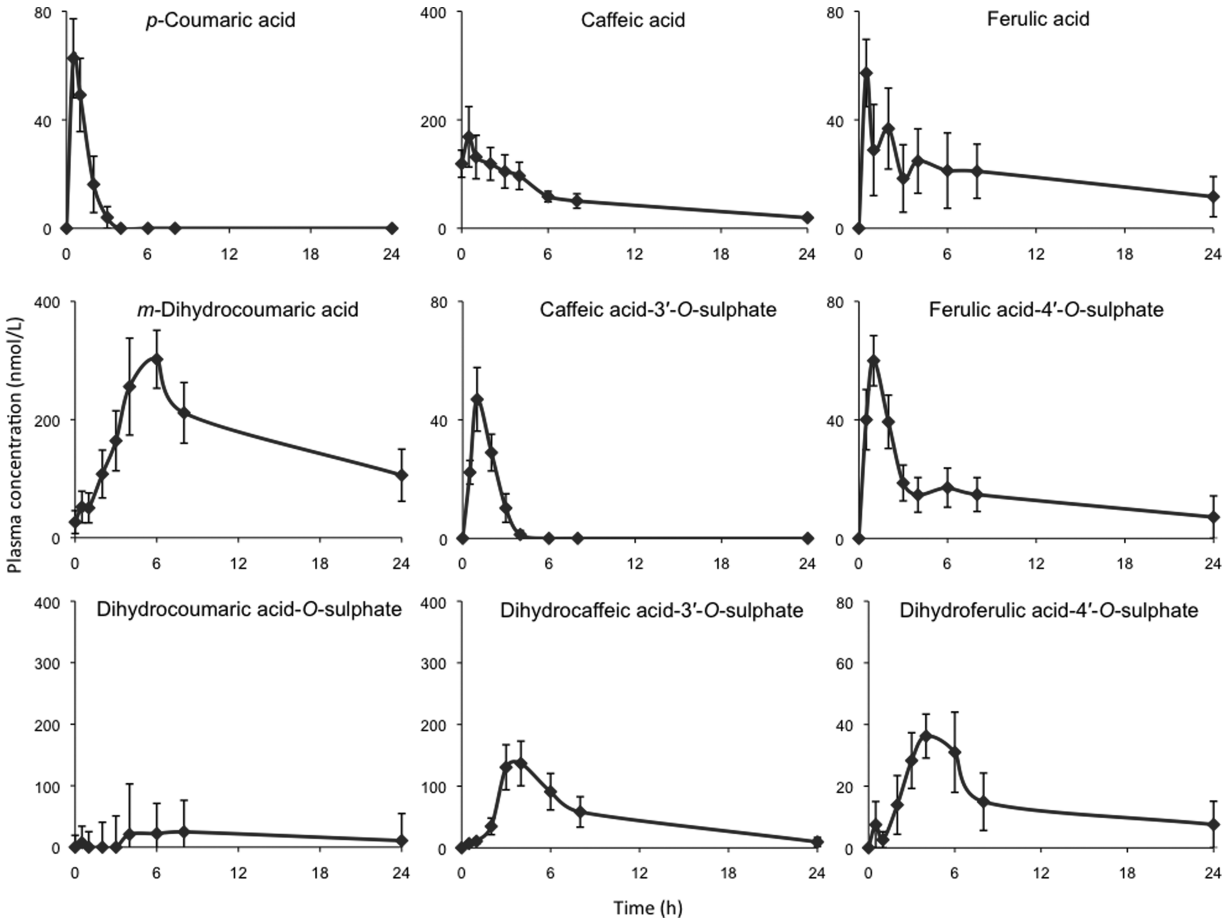

Figure 3. Pharmacokinetic profile of metabolites identified in plasma of healthy volunteers ($n = 8$), 0–24 h following intake of 155 μmol of tartarate esters of hydroxycinnamic acids contained in a single 350 mL-serving of Concord grape juice.

Table 5. Pharmacokinetic analysis of hydroxycinnamate metabolites detected in the plasma of healthy volunteers following acute ingestion of 350 mL of 100% Concord grape juice[a)]

	C_{max} (nmol/L)	T_{max} (h)	AUC (nmol·h/L)
p-Coumaric acid	64 ± 14	0.7 ± 0.1	88 ± 27
m-Dihydrocoumaric acid	355 ± 57	5.8 ± 0.5	4080 ± 968
Dihydrocoumaric acid-*O*-sulphate	27 ± 1	6.0 ± 2.0	388 ± 92
Caffeic acid	178 ± 53	0.5 ± 0.2	1306 ± 312
Caffeic acid-3′-*O*-sulphate	47 ± 11	1.0 ± 0.0	90 ± 23
Dihydrocaffeic acid-3′-*O*-sulphate	161 ± 37	3.9 ± 0.4	1164 ± 368
Dihydroferulic acid-4′-*O*-sulphate	42 ± 9	4.4 ± 0.7	359 ± 164
Ferulic acid	63 ± 13	1.8 ± 0.9	468 ± 215
Ferulic acid-4′-*O*-sulphate	63 ± 9	1.2 ± 0.2	370 ± 115

a) Data expressed as mean values ± SE ($n = 8$).
AUC, area under the plasma concentration curve; C_{max} peak plasma concentration; T_{max}, time to reach C_{max}.

of flavan-3-ol monomers following incubation with simulated gastric juice for 1 h declined by ∼35% despite previous studies reporting their stability at pH 2 [33, 34]. Such discrepancy may have resulted from the simulated gastric juice composition which in the current study, in contrast to previous in vitro experiments, contained pepsin that may have impacted on monomeric flavan-3-ol stability.

After acute ingestion of Concord grape juice by ileostomy volunteers, 40% of the intake of (poly)phenolic compounds were recovered in 24-h ileal effluent, comparable to the recovery following in vitro gastrointestinal incubations. The quantity of (poly)phenolic compounds detected in ileal fluid after supplementation of ileostomists gives an indication of the amounts that will pass from the small to the large

Table 6. Urinary excretion of hydroxycinnamate metabolites following acute intake of 350 mL of 100% Concord juice by healthy volunteers ($n = 8$)[a)]

	Baseline[b)]	0–24 h	
p-Coumaric acid	n.d.	0.5 ± 0.2	
Coumaric acid-*O*-sulphate	n.d.	1.6 ± 0.4	
m-Dihydrocoumaric acid	0.2 ± 0.2	3.0 ± 2.3	
Dihydrocoumaric acid-*O*-sulphate	2.1 ± 0.6	5.9 ± 0.8	
Coumaric and coutaric acid intake (%)			*42 ± 13%*[c)]
Caffeic acid-3′-*O*-sulphate	0.8 ± 0.2	4.3 ± 0.4	
Caffeic acid-4′-*O*-sulphate	n.d.	0.2 ± 0.0	
Dihydrocaffeic acid	n.d.	0.9 ± 0.3	
Dihydrocaffeic acid-3′-*O*-sulphate	3.7 ± 1.3	17.1 ± 3.3	
Caffeic and caftaric acid intake (%)			*14 ± 2%*[c)]
Ferulic acid-4′-*O*-sulphate	9.9 ± 2.4	15.8 ± 3.0	
Dihydroferulic acid-4′-*O*-sulphate	2.4 ± 0.8	2.7 ± 0.9	
Dihydroferulic acid	n.d.	1.9 ± 0.6	
Isoferulic acid-3′-*O*-sulphate	n.d.	0.1 ± 0.0	
Isoferulic acid-3′-*O*-glucuronide	0.5 ± 0.2	2.5 ± 0.3	
Feruloylglycine	4.3 ± 1.1	5.3 ± 1.1	
Ferulic acid and fertaric acid intake (%)			*217 ± 41%*[c)]
Total hydroxycinnamate excretion as a % of intake			**24 ± 3%**[c)]

a) Data expressed in μmol as mean values ± SE ($n = 8$).
b) Baseline values correspond to 24-h urinary excretion following a 2-day low (poly)phenolic diet.
c) Corrected for baseline excretion.

intestine in healthy subjects with a functioning colon. The presence of flavan-3-ols in ileal effluent has previously been reported following acute intake of green tea by ileostomists [19] where there was a 33% recovery compared to only 7.2% in the current investigation. This may be associated with the varying flavan-3-ol intakes, 634 μmol with green tea compared to 33 μmol with Concord grape juice. The presence of sulphated and methylated (epi)catechin accounting for 133 ± 45% of the amount of (epi)catechin ingested suggests that a small amount of the ingested procyanidins were hydrolysed into smaller units and subsequently absorbed, as noted in ileostomists following apple juice consumption [35], which subsequently underwent methylation and sulphation prior being effluxed back into the lumen, as observed with green tea flavan-3-ols [19]. Recoveries of anthocyanins and other (poly)phenolic compounds in ileal fluid after grape juice consumption were broadly in line with those observed with the in vitro digestion model with the hydroxycinnamates again being recovered in highest amounts relative to intake (Table 2).

After ingestion of Concord grape juice by healthy subjects, trace levels of anthocyanins were detected in plasma and urine. Urine contained 3-*O*-glucosides and *O*-glucuronides of delphinidin, cyanidin, petunidin, peonidin and malvidin, whereas only the petunidin and delphinidin conjugates accumulated in plasma in detectable quantities with C_{max} values of ≤2 nmol/L (Fig. 2, Table 3), seemingly as a consequence of absorption in the small intestine, as indicated by early T_{max} values in the range of 1.3–1.4 h for petunidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside. Arguably, the later T_{max} values of the petunidin and delphinidin glucuronides may be a consequence of post-absorption phase II metabolism converting the anthocyanin glucosides to glucuronides. Urinary excretion levels varied slightly depending upon the anthocyanidin moiety, but were overall low with 0.26% of intake excreted. The low C_{max} values and limited urinary excretion of anthocyanins are in keeping with data obtained in other studies [36–39].

The ratio of petunidin:delphinidin conjugate levels in plasma at C_{max} was 1.6 but this increased to 5.7 for 24-h urinary excretion. This implies that more petunidin, mainly in the glucuronidated form, was excreted in urine than was in the circulation compared to delphinidin. This could be a consequence of phase II 5′-methylation in the kidneys resulting in the conversion of delphinidin to petunidin derivatives, as previously reported in rats [40, 41]. Alternatively, it is possible that petunidin glycosides are turned over in the circulatory system at a faster rate than their delphinidin counterparts without this being reflected in increased plasma C_{max} levels.

After drinking Concord grape juice, flavan-3-ol sulphate, methyl and glucuronide metabolites were detected in urine but not plasma. The overall 0–24 h excretion was equivalent to 15.1% of (epi)catechin intake and 3.5% of the ingested (epi)gallocatechin (Table 4). The high excretion of (epi)catechin metabolites, relative to intake, which is not accompanied by similarly high C_{max} values, is in keeping with data obtained in other investigations [19–21, 42], indicating that it is a general phenomenon and not one limited to high intakes of green tea flavan-3-ols. Similarly, the low level of excretion of (epi)gallocatechin metabolites compared to (epi)catechin derivatives has been noted previously in studies with green tea [19, 20] and a polyphenol-rich drink [21].

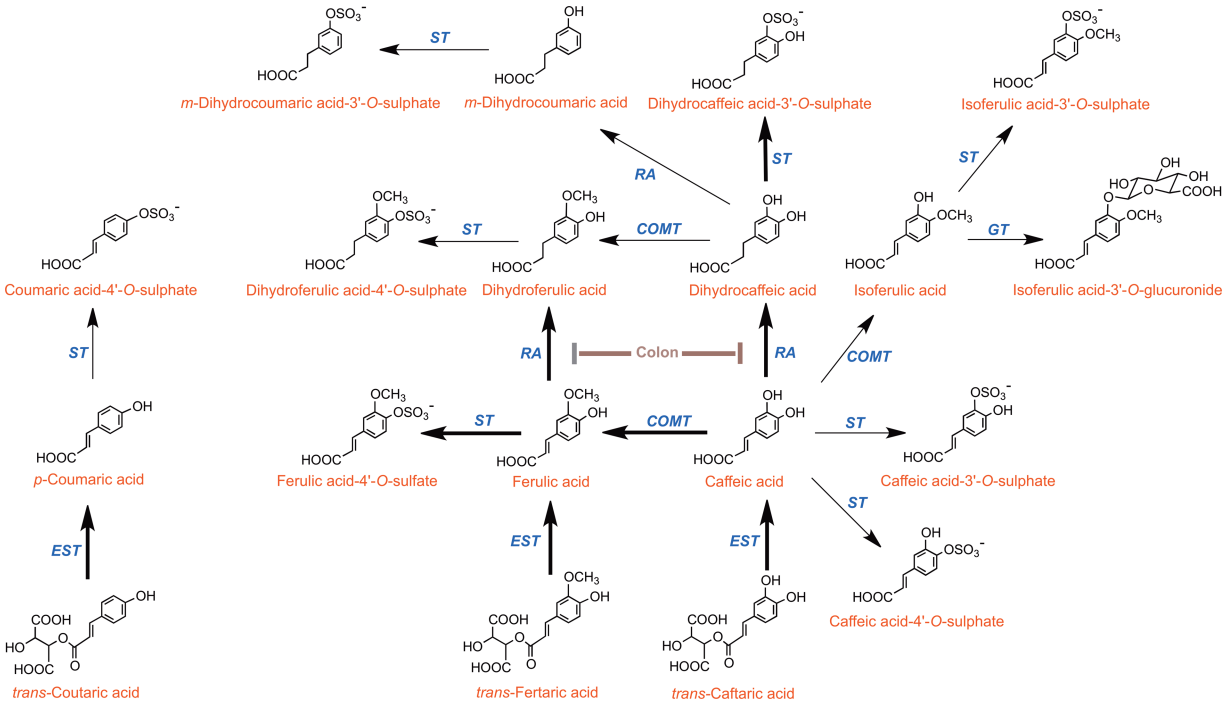

Figure 4. Proposed metabolism of hydroxycinnate tartarate esters following the ingestion of 350 mL of Concord grape juice by human volunteers. The identification of dihydrocoumaric acid-3′-*O*-sulphate is tentative with the location of the sulphate moiety based on the presence of *m*-dihydrocoumaric acid, (aka 3-(3′-hydroxyphenyl)propionic acid) in plasma and urine, and the subsequent sulphation of the 3′-hydroxyl group. Identification of the sulphates of caffeic acid, dihydrocaffeic acid, ferulic acid and dihydroferulic acid, and isoferulic acid-3′-*O*-glucuronide were based on MS–MS and co-chromatography with metabolites identified by HPLC-MS^2 in plasma and urine in an earlier feeding study with a caffeoylquinic acid-rich coffee [44]. COMT, catechol-*O*-methyltransferase; EST, esterase; GT, UDP-glucuronosyltransferase; RA, reductase; ST, sulphonyl-*O*-transferase. Bold arrows indicate major pathways. Steps initiated in the colon are indicated.

The hydroxycinnamate tartarate esters generated the highest in vitro and in vivo gastrointestinal tract recoveries with the ileal fluid data indicating that substantial amounts of *trans*-caftaric acid and *trans*-coutaric acid will pass from the small intestine to the colon (Table 2). After the juice was ingested by healthy subjects, nine hydroxycinnamate metabolites appeared in plasma with relatively high C_{max} values (Table 5). The appearance of *p*-coumaric acid, caffeic acid and ferulic acid in the circulatory system with T_{max} times of 0.7–1.8 h indicates that these compounds are being released in the proximal gastrointestinal tract by the action of esterases on coutaric acid, caftaric acid and fertaric acid. The notably short T_{max} of 0.5 h for caffeic acid and 0.7 h for *p*-coumaric acid suggests a rapid cleavage of the tartarate esters and an efficient absorption of the released hydroxycinnamates. As well as appearing in the circulatory system as free acids, caffeic acid and ferulic acid were also detected in plasma as sulphated metabolites. Other hydroxycinnamates, dihydrocoumaric acid, a dihydrocoumaric acid-*O*-sulphate along with sulphated metabolites of dihydrocaffeic acid and dihydroferulic acid, had later C_{max} values of 3.9–6.0 h indicative of events occurring in the large intestine, and in keeping with the passage of the three parent tartarate esters in the colon. The appearance of these metabolites suggests that hydrogenation of the hydroxycinnamate side chain takes place in the large but not the small intestine, as illustrated in Fig. 4, as consequence of the action of the colonic microflora. Alternatively, hydrogenation can also take place in the liver [43].

Urinary excretion of hydroxycinnamates consisted of a similar spectrum of metabolites to that found in plasma with the additional appearance of small amounts of sulphate and glucuronide conjugates of isoferulic acid (Table 5). The recovery of urinary coutaric acid metabolites was 42% of intake and that of caftaric acid 14%, while metabolites derived from fertaric acid were excreted in amounts equivalent to 217% of the amounts ingested. This could be a consequence of 3′-methylation of caffeic acid yielding ferulic acid. Proposed routes for the metabolism of the hydroxycinnamate tartarate esters are illustrated in Fig. 4. They are similar, but not identical, to pathways elucidated for the metabolism of caffeic acid and ferulic acid derived from caffeoyl- and feruloylquinic acids following the ingestion of coffee by human volunteers [22, 44]. In the pathway of tartarate esters metabolism in Fig. 4, free *p*-coumaric acid and a sulphated derivative, possibly *p*-coumaric acid-4′-*O*-sulphate, are involved in the metabolism of coutaric acid. Production of dihydrocoumaric acids (i.e., hydroxyphenylpropionic acids) have also been

linked with colonic microflora-mediated catabolism of procyanidins [45].

In conclusion, we note that the bioavailability of main components in the complex mixture of (poly)phenolic compounds in Concord grape juice, based on urinary excretion, was very similar to that observed in previous studies when the compounds were either fed individually or as major components in supplements containing a restricted spectrum of (poly)phenolic compounds. There is, therefore, as obtained in an earlier study with a (poly)phenol-rich drink [21], no evidence of major interactions impacting upon transport and absorption into the circulatory system that limit the bioavailability of dietary (poly)phenolics.

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5 References

[1] Stalmach, A., Edwards, C. A., Wightman, J. D., Crozier, A., Identification of (poly)phenolic compounds in Concord grape juice and their metabolites in human plasma and urine after juice consumption. *J. Agric. Food Chem.* 2011, *59*, 9512–9522.

[2] Balu, M., Sangeetha, P., Murali, G., Panneerselvam, C., Age-related oxidative protein damages in central nervous system of rats: modulatory role of grape seed extract. *Int. J. Dev. Neurosci.* 2005, *23*, 501–507.

[3] Castilla, P., Echarri, R., Davalos, A., Cerrato, F. et al., Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am. J. Clin. Nutr.* 2006, *84*, 252–262.

[4] Hudson, T. S., Hartle, D. K., Hursting, S. D., Nunez, N. P. et al., Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. *Cancer Res.* 2007, *67*, 8396–8405.

[5] Krikorian, R., Nash, T. A., Shidler, M. D., Shukitt-Hale, B. et al., Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *Br. J. Nutr.* 2010, *103*, 730–734.

[6] Shanmuganayagam, D., Warner, T. F., Krueger, C. G., Reed, J. D. et al., Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis* 2007, *190*, 135–142.

[7] Singletary, K. W., Stansbury, M. J., Giusti, M., Van Breemen, R. B. et al., Inhibition of rat mammary tumorigenesis by concord grape juice constituents. *J. Agric. Food Chem.* 2003, *51*, 7280–7286.

[8] Ahmad, N., Adhami, V. M., Afaq, F., Feyes, D. K. et al., Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. *Clin. Cancer Res.* 2001, *7*, 1466–1473.

[9] Lazze, M. C., Savio, M., Pizzala, R., Cazzalini, O. et al., Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. *Carcinogenesis* 2004, *25*, 1427–1433.

[10] Terra, X., Montagut, G., Bustos, M., Llopiz, N. et al., Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. *J. Nutr. Biochem.* 2009, *20*, 210–218.

[11] van der Woude, H., Gliszczynska-Swiglo, A., Struijs, K., Smeets, A. et al., Biphasic modulation of cell proliferation by quercetin at concentrations physiologically relevant in humans. *Cancer Lett.* 2003, *200*, 41–47.

[12] Crozier, A., Jaganath, I. B., Clifford, M. N., Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* 2009, *26*, 1001–1043.

[13] Scholz, S., Williamson, G., Interactions affecting the bioavailability of dietary polyphenols in vivo. *Int. J. Vitam. Nutr. Res.* 2007, *77*, 224–235.

[14] Manach, C., Williamson, G., Morand, C., Scalbert, A. et al., Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* 2005, *81*, 230S–242S.

[15] Clifford, M. N., Crozier, A., in: Crozier, A., Ashihara, H., Tomas-Barbéran, F. (Eds.), *Teas, Cocoa and Coffee. Plant Secondary Metabolites and Health*, Wiley-Blackwell, Oxford 2011, pp. 45–98.

[16] Kemperman, R. A., Bolca, S., Roger, L. C., Vaughan, E. E., Novel approaches for analysing gut microbes and dietary polyphenols: challenges and opportunities. *Microbiology-Sgm* 2010, *156*, 3224–3231.

[17] Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S. et al., Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. *Mol. Nutr. Food Res.* 2011, *55*, S35–S43.

[18] Bermúdez-Soto, M. J., Tomás-Barberán, F. A., García-Conesa, M. T., Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to in vitro gastric and pancreatic digestion. *Food Chem.* 2007, *102*, 865–874.

[19] Stalmach, A., Mullen, W., Steiling, H., Williamson, G. et al., Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Mol. Nutr. Food Res.* 2010, *54*, 323–334.

[20] Auger, C., Mullen, W., Hara, Y., Crozier, A., Bioavailability of polyphenon E flavan-3-ols in humans with an ileostomy. *J. Nutr.* 2008, *138*, 1535S–1542S.

[21] Borges, G., Mullen, W., Mullan, A., Lean, M. E. J. et al., Bioavailability of multiple components following acute ingestion of a polyphenol-rich juice drink. *Mol. Nutr. Food Res.* 2010, *54*, S268–S277.

[22] Stalmach, A., Mullen, W., Barron, D., Uchida, K. et al., Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: identification of biomarkers of coffee consumption. *Drug Metab. Dispos.* 2009, *37*, 1749–1758.

[23] Fleschhut, J., Kratzer, F., Rechkemmer, G., Kulling, S. E., Stability and biotransformation of various dietary anthocyanins in vitro. *Eur. J. Nutr.* 2006, *45*, 7–18.

[24] Perez-Vicente, A., Gil-Izquierdo, A., Garcia-Viguera, C., In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. *J. Agric. Food Chem.* 2002, *50*, 2308–2312.

[25] Kahle, K., Kempf, M., Schreier, P., Scheppach, W. et al., *Eur. J. Nutr.* 2011, *50*, 507–522.

[26] Kraus, M., Kahle, K., Friedericke, R., Schantz, M. et al., in: Qian, M. C., Rimando, A. M. (Eds.), *Flavor and Health Benefits of Small Fruits*, American Chemical Society, Washington 2010, pp. 159–176.

[27] He, J., Wallace, T. C., Keatley, K. E., Failla, M. L. et al., Stability of black raspberry anthocyanins in the digestive tract lumen and transport efficiency into gastric and small intestinal tissues in the rat. *J. Agric. Food Chem.* 2009, *57*, 3141–3148.

[28] Wu, X. L., Pittman, H. E., Prior, R. L., Fate of anthocyanins and antioxidant capacity in contents of the gastrointestinal tract of weanling pigs following black raspberry consumption. *J. Agric. Food Chem.* 2006, *54*, 583–589.

[29] Farah, A., Guigon, F., Trugo, L. C., *The effect of human digestive fluids on chlorogenic acids isomers from coffee*. In the proceedings of 21st International Conference on Coffee Science, Montpellier, ASIC: Montpellier 2006, pp. 93–96.

[30] Olthof, M. R., Hollman, P. C., Katan, M. B., Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* 2001, *131*, 66–71.

[31] Rechner, A. R., Spencer, J. P. E., Kuhnle, G., Hahn, U. et al., Novel biomarkers of the metabolism of caffeic acid derivatives *in vivo*. *Free Radic. Biol. Med.* 2001, *30*, 1213–1222.

[32] Takenaka, M., Nagata, T., Yoshida, M., Stability and bioavailability of antioxidants in garland (*Chrysanthemum coronarium* L.). *Biosci. Biotechnol. Biochem.* 2000, *64*, 2689–2691.

[33] Spencer, J. P. E., Chaudry, F., Pannala, A. S., Srai, S. K. et al., Decomposition of cocoa procyanidins in the gastric milieu. *Biochem. Biophys. Res. Commun.* 2000, *272*, 236–241.

[34] Zhu, Q. Y., Holt, R. R., Lazarus, S. A., Ensunsa, J. L. et al., Stability of the flavan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *J. Agric. Food Chem.* 2002, *50*, 1700–1705.

[35] Kahle, K., Huemmer, W., Kempf, M., Scheppach, W. et al., Polyphenols are intensively metabolized in the human gastrointestinal tract after apple juice consumption. *J. Agric. Food Chem.* 2007, *55*, 10605–10614.

[36] Frank, T., Janssen, M., Netzel, M., Strass, G. et al., Pharmacokinetics of anthocyanidin-3-glycosides following consumption of *Hibiscus sabdariffa L. extract*. *J. Clin. Pharmacol.* 2005, *45*, 203–210.

[37] Garcia-Alonso, M., Minihane, A. M., Rimbach, G., Rivas-Gonzalo, J. C. et al., Red wine anthocyanins are rapidly absorbed in humans and affect monocyte chemoattractant protein 1 levels and antioxidant capacity of plasma. *J. Nutr. Biochem.* 2009, *20*, 521–529.

[38] Mertens-Talcott, S. U., Rios, J., Jilma-Stohlawetz, P., Pacheco-Palencia, L. A. et al., Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich acai juice and pulp (*Euterpe oleracea Mart.*) in human healthy volunteers. *J. Agric. Food Chem.* 2008, *56*, 7796–7802.

[39] Wiczkowski, W., Romaszko, E., Piskula, M. K., Bioavailability of cyanidin glycosides from natural chokeberry (*Aronia melanocarpa*) juice with dietary-relevant dose of anthocyanins in humans. *J. Agric. Food Chem.* 2010, *58*, 12130–12136.

[40] Talavera, S., Felgines, C., Texier, O., Besson, C. et al., Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *J. Agric. Food Chem.* 2005, *53*, 3902–3908.

[41] Tsuda, T., Horio, F., Osawa, T., Absorption and metabolism of cyanidin 3-*O*-β-D-glucoside in rats. *FEBS Lett.* 1999, *449*, 179–182.

[42] Stalmach, A., Troufflard, S., Serafini, M., Crozier, A., Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. *Mol. Nutr. Food Res.* 2009, *53*, S44–S53.

[43] Moridani, M. Y., Scobie, H., O'Brien, P. J., Metabolism of caffeic acid by isolated rat hepatocytes and subcellular fractions. *Toxicol. Lett.* 2002, *133*, 141–151.

[44] Stalmach, A., Steiling, H., Williamson, G., Crozier, A., Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Arch. Biochem. Biophys.* 2010, *501*, 98–105.

[45] Appeldoorn, M. M., Vincken, J. P., Gruppen, H., Hollman, P. C., Procyanidin dimers A1, A2, and B2 are absorbed without conjugation or methylation from the small intestine of rats. *J. Nutr.* 2009, *139*, 1469–1473.

