Colonic availability of apple polyphenols – A study in ileostomy subjects

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Nutrition is thought to play an essential role in the pathogenesis of inflammatory and malignant gastrointestinal diseases. It is well known that plant ingredients such as polyphenols and flavonoids show anticarcinogenic effects both *in vitro* and in animal experiments, and may thus reduce the risk of colorectal cancer in man. The aim of the study was to determine the amount of polyphenols reaching the colon after oral intake of apple juice. After consumption of a polyphenol-free diet 11 healthy ileostomy volunteers drank 1 L of a polyphenol-rich cloudy apple juice. Ileostomy effluent was collected immediately before and 1, 2, 4, 6, and 8 h after consumption of apple juice. A broad spectrum of polyphenols was identified using HPLC-diode array detection (HPLC-DAD) as well as HPLC-ESI-MS/MS; quantitation was performed with HPLC-DAD. Most of the orally administered apple polyphenols were absorbed from or metabolized in the small intestine. Between 0 and 33% of the oral dose was recovered in the ileostomy bags with a maximum of excretion after 2 h. Phloretin glucuronide as product of polyphenol metabolism was detected in the ileostomy effluent. The present results show that most of the apple juice polyphenols are absorbed in the small intestine. Minor amounts of unmetabolized polyphenols are recovered in the ileostomy effluent, which would reach the colon under physiologic circumstances. These data have to be considered when polyphenols are used in model systems to show preventive effects in colorectal carcinogenesis.

Keywords: Apple juice / ESI / Flavonoids / HPLC / Ileostomy / Polyphenols

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1 Introduction

Fruits and vegetables such as apples, berries, and onions, beverages like red wine, coffee, green and black tea as well as cocoa and chocolate are the most important sources of polyphenols in the Western diet [1]. The daily polyphenol intake is about 1 g, estimated to be 2/3 flavonoids and 1/3 phenolic acids [2]. Today, dietary polyphenols receive considerable interest for their presumed role in the prevention of various degenerative diseases such as cancer [3–5]. They seem to play an important role as antioxidants and show protective effects in cardiovascular diseases and cancer [6–10].

Apples, *Malus domestica* (Rosaceae), are an important source for polyphenols [1]. As shown by Kahle *et al.* and other groups in apple fruits as well as apple juices, several classes of phenolic antioxidants are present such as flavonol monomers (mainly (–)-epicatechin) and oligomers (mainly procyanidin B_2) [2, 11, 12]. The polyphenol profiles of apples and apple juices are determined by chlorogenic acid (5-caffeoylquinic acid) besides smaller quantities of other hydroxycinnamic acid conjugates. Dihydrochalcones like phloridzin (phloretin 2′-*O*-glucoside) and phloretin 2′-*O*-xyloglucoside but no phloretin were measured in apple juices investigated. Minor amounts of quercetin glycosides were observed as well as anthocyanins such as cyanidin 3-*O*-galactoside in the skin of certain red apple varieties [13].

Recent studies using apple juices show protective effects on low-density lipoprotein oxidation [14]. DuPont *et al.* [15] have shown the increase of phenolic plasma concentrations after the consumption of cider. Plasma concentrations after juice consumption play an important role in the evaluation of biological effects of apple polyphenols.

Dose-dependent antiproliferative activity on colon as well as liver cancer cell has been observed using extracts derived from fresh apples [16]. In fact, the amount of polyphenols derived from apple juice is important for the ability to achieve protective effects in the colon. The question how much polyphenols reach the colon after the consumption of

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apple juice is still unsolved. For this reason, in our study 1 L of cloudy apple juice was consumed by subjects with a terminal ileostomy; the polyphenol content was quantitated in the ileostomy effluent.

2 Materials and methods

2.1 Subjects

The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Wuerzburg. After giving informed written consent, 11 healthy subjects with an end ileostomy participated in the trial (3 males, 8 females, and mean age of 40.8 years, range 23–50 years). They had undergone colectomy with terminal ileostomy between 2 months and 13.5 years (mean 4.4 years) prior to the study; the underlying conditions were Crohn's disease ($n = 7$), ulcerative colitis ($n = 2$), or familial adenomatous polyposis ($n = 2$). Patients who had been resected for colonic Crohn's disease had no evidence for small intestinal involvement. Concerning the type of surgery, only those patients were recruited into the study in whom no ileal resections had been performed.

2.2 Study design

Volunteers avoided food containing polyphenols the day before the study. After a 10 h overnight fast they consumed 1 L of cloudy apple juice within 15 min. The subjects remained fasted for another 4 h and were then served a light meal which did not contain polyphenols. The ileostomy bags were collected immediately before and 1, 2, 4, 6, and 8 h after the start of the apple juice intake. The contents of the ileostomy bags were immediately frozen at $-24^{\circ}C$ and stored for 3 wk until extraction and analysis.

2.3 Chemicals

All chemicals and solvents were of analytical grade. Solvents were redistilled before use. ACN (Lichrosolv®) was from Merck (Darmstadt, Germany); formic acid was purchased from Fluka (Deisenhofen, Germany). Chlorogenic acid (5-caffeoylquinic acid) and caffeic acid were purchased from Roth (Karlsruhe, Germany). Phloretin (2′,4′,6′,4-tetrahydroxydihydrochalcone), quercetin 3-*O*-glucoside (isoquercitrin), quercetin 3-*O*-galactoside (hyperoside) as well as (+)-catechin and (−)-epicatechin were products from Roth. Phloridzin (2′-*O*-glucosyl-4′,6′,4-trihydroxydihydrochalcone), quercetin (3,5,7,3′,4′-pentahydroxyflavone), quercetin 3-*O*-rutinoside (rutin), and quercetin 3-*O*-rhamnoside (quercitrin) were obtained from Sigma (Steinheim, Germany). 4-*p*-Coumaroylquinic acid, quercetin 3-*O*-xyloside (reynoutrine), quercetin 3-*O*-arabinoside (avicularin), and phloretin 2′-*O*-xyloglucoside as well as procyanidin B_1 and procyanidin B_2 were kindly provided by Professor Dr. H. Becker (Saarbrücken, Germany) and Professor Dr. P. Winterhalter (Braunschweig, Germany), respectively. The internal standard (IS) 3,4,5-trihydroxycinnamic acid was purchased from Aldrich (Steinheim, Germany).

Eleven bottles of commercially available cloudy apple juice (valid through 25-05-2005) were purchased at a local grocery store. Juice samples were shaken and aliquots of 1 mL were submitted to polyphenol quantitation. The resolving amounts of 1 L were immediately used for the study.

2.4 HPLC-DAD analysis

The HPLC system used was a Hewlett-Packard 1100 HPLC gradient pump and a Hewlett-Packard 1100 photodiode array detector (Waldbronn, Germany), equipped with a Wisp 710b autosampler (Waters, Eschborn, Germany). Data acquisition and evaluation were performed with a Hewlett-Packard ChemStation software. A Hypersil™ Gold C_{18} column, 100×4.6 mm, with 3 µm particle size (Thermo, Runcorn, UK), was used. The mobile phase consisted of aqueous 0.1% v/v formic acid (A) and ACN (B). The gradient applied was 1–99% B in 40 min at a flow rate of 1 mL/min, and 25 µL injection volumes were used. The peaks were identified by comparison of retention time and UV spectra (200–600 nm) with authentic references. Dihydrochalcones, catechins, and procyanidins were determined at 280 nm, hydroxycinnamic acid derivatives at 320 nm, and flavonols at 360 nm. Cyanidin 3-*O*-galactoside was detected at 520 nm.

2.5 HPLC-MS/MS analysis

HPLC-ESI-MS/MS was performed with a TSQ 7000 tandem mass spectrometer system equipped with an ESI interface (Finnigan MAT, Bremen, Germany) and an Applied biosystems 140b pump (BAI, Bensheim, Germany). Data acquisition and evaluation were conducted on a DEC 5000/33 (Digital Equipment, Unterföhring, Germany) using Finnigan MAT ICIS 8.1 software. HPLC chromatographic separations were carried out on a Hypersil Gold C_{18} column, 100×2.1 mm, with 3 µm particle size (Thermo). The mobile phase consisted of aqueous 0.1% formic acid (A) v/v and ACN (B). The gradient applied was 5–99% B in 40 min at a flow rate of 0.2 mL/min, and 10 µL injection volume. The analysis was performed in the negative ionization mode. The spray capillary voltage was set to 3.2 kV, and the temperature of the heated capillary was $200^{\circ}C$. Nitrogen served as both sheath (70 psi) and auxiliary gas (10 units). The mass spectrometer was operated in the full-

scan mode, *m/z* 150–700, with a total scan duration of 1.0 s. MS/MS experiments were performed at a collision energy of 20–40 eV, with argon (2.0 mTorr) serving as collision gas. The obtained molecular ion peaks and mass spectra were compared to those of references measured before [11].

2.6 Preparation of ileostomy fluids

The frozen ileostomy fluids were freeze-dried and homogenized. Aliquots (range 0.5–2 mg) were extracted three times with 20 mL of methanol. The solvent was evaporated at 40°C, and the extract was dissolved in 2 mL of methanol.

For recovery determination of sample preparation, references were added to a polyphenol-free ileostomy content. Sample preparations were performed as described above. Recoveries ranged between 98 and 102%.

2.7 Quantitation of apple juice and ileostomy fluids

Aliquots from a stock solution of chlorogenic acid, caffeic acid, 4-*p*-coumaroylquinic acid, phloretin 2′-*O*-xyloglucoside, phloridzin, phloretin, procyanidin B_1, procyanidin B_2, (+)-catechin, (−)-epicatechin, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinoside, quercetin 3-*O*-rhamnoside, quercetin, and quercetin 3-*O*-rutinoside (100 mg/L each) in methanol were diluted and 3,4,5-trihydroxycinnamic acid as IS (50 mg/L) was added.

Calibration curves (at the appropriate wavelengths according to the absorption maximum of the compounds) were used for quantitation. Polyphenols were quantitated by means of calibration curves (peak area divided by IS area *vs.* quotient of polyphenol and the IS concentration). Linearity was shown for 0.4–600 mg/L; LOQs ranged from 0.4 to 0.9 mg/L, LODs from 0.2 to 0.4 mg/L with an S/N of 3:1 [17], respectively. All experiments were performed in triplicate. Compounds were identified by comparison of retention time, UV spectra, and MS as well as MS/MS information using reference compounds as shown before [11].

No references were given for 3- and 5-*p*-coumaroylquinic acid, 4-caffeoylquinic acid (=kryptochlorogenic acid), and phloretin glucuronide. These compounds were identified using HPLC-MS/MS and quantitated as 4-*p*-coumaroylquinic acid, chlorogenic acid, and phloretin 2′-*O*-glucoside by HPLC-DAD, respectively.

Prior to HPLC-DAD and HPLC-MS/MS analyses apple juices and ileostomy fluid extracts were filtered using a Millipore membrane (0.45 μm; Roth), IS was added, and the samples were analyzed.

2.8 Statistics

Values are mean ± SD of triplicates. Differences were considered not significant at p values of >0.05 using one-way ANOVA and the Tukey test.

3 Results

3.1 Identification and quantitation of polyphenols in cloudy apple juice

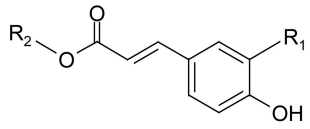
The main classes of polyphenols found in the cloudy apple juice under study were hydroxycinnamic acids (*i.e.*, chlorogenic acid, 4-*p*-coumaroylquinic acid), dihydrochalcones (phloretin glycosides), monomeric and oligomeric flavan-3-ols (catechins and procyanidins), and flavonols (quercetin glycosides). Structures are given in Fig. 1. Anthocyanins (cyanidin glycosides) are mentioned as constituent of apple skin in literature [18].

The polyphenol content of the cloudy apple juice used for the study (n = 11) is presented in Table 1. Juices were of identical production dates, and so far, the polyphenol contents did not vary significantly. Highest amounts were

Table 1. Polyphenol content (mg/L) of cloudy apple juice used in the ileostomy study determined by HPLC-DAD[a] (for details see Section 2)

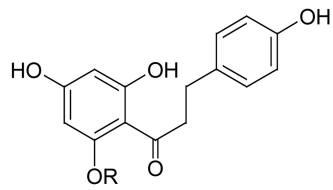
	mg/L
Kryptochlorogenic acid (4-caffeoylquinic acid)	14.1 ± 2.0
Chlorogenic acid (5-caffeoylquinic acid)	112.8 ± 3.5
Caffeic acid	5.3 ± 1.7
3-*p*-coumaroylquinic acid	7.1 ± 0.4
4-*p*-coumaroylquinic acid	18.4 ± 0.3
5-*p*-coumaroylquinic acid	4.2 ± 0.1
Σ hydroxycinnamic acids	161.9
Phloretin 2′-*O*-xyloglucoside	36.9 ± 1.8
Phloridzin (phloretin 2′-*O*-glucoside)	7.1 ± 0.7
Phloretin	ND[b]
Σ dihydrochalcone derivatives	44.0
Procyanidin B_1	5.3 ± 1.6
Procyanidin B_2	9.9 ± 0.3
(+)-Catechin	3.0 ± 0.5
(−)-Epicatechin	15.0 ± 2.1
Σ flavan-3-ols	33.2
Quercetin 3-*O*-glucoside	1.8 ± 0.2
Quercetin 3-*O*-galactoside	1.5 ± 0.3
Quercetin 3-*O*-xyloside	3.9 ± 0.1
Quercetin 3-*O*-arabinoside	0.9 ± 0.1
Quercetin 3-*O*-rhamnoside	2.7 ± 0.3
Quercetin	ND
Quercetin 3-*O*-rutinoside	ND
Σ flavonols	10.8
Total polyphenol amount	249.9

a) Mean ± SD; n = 11.
b) ND, not detectable.



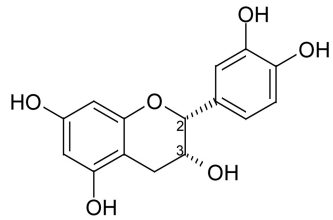
Hydroxycinnamic acids:

	R_1	R_2
caffeic acid	OH	H
caffeoylquinic acids	OH	quinic acid
coumaroylquinic acids	H	quinic acid



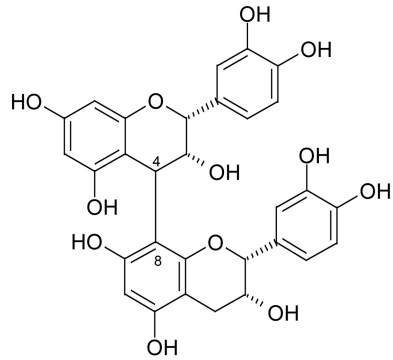
dihydrochalcones:

	R
phloretin	H
phloridzin	Glc
phloretin 2′-*O*-xyloglucoside	Xyl-Glc



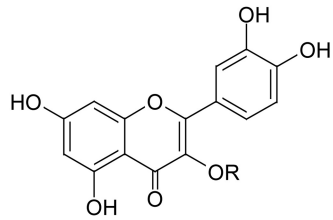
monomeric flavan-3-ols:

	2, 3
(+)-catechin	trans
(-)-epicatechin	cis



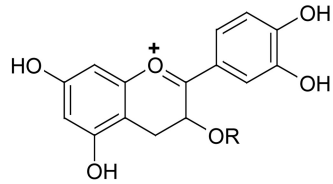
dimeric flavan-3-ols:

	monomers
procyanidin B_1	epicatechin-(4-8)-catechin
procyanidin B_2	epicatechin-(4-8)-epicatechin



flavonols:

	R
quercetin	H
quercetin-glycosides	glycosyl



anthocyanins:

	R
cyanidin	H
cyanidin-glycosides	glycosyl

Figure 1. Chemical structures of the major groups of apple polyphenols. Glc, glucose; Xyl, xylose.

determined for the group of hydroxycinnamic acids (average 161.9 mg/L), with chlorogenic acid as the dominating constituent (average 112.8 mg/L). The juices under study showed lower contents of dihydrochalcones (average 44 mg/L) and flavan-3-ols (33.2 mg/L). Quercetin and its derivatives were found to be minor constituents in the range from <0.2 to 3.9 mg/L in the juices. The free aglycones quercetin and phloretin as well as quercetin 3-*O*-rutinoside

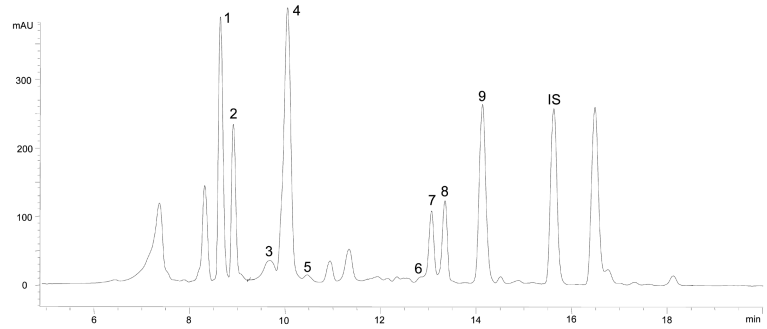


Figure 2. HPLC elution profile of an extract of ileostomy fluid 2 h after apple juice consumption detected at 320 nm. Unlabeled peaks are of intestinal constituents. 1, chlorogenic acid; 2, kryptochlorogenic acid; 3, 3-*p*-coumaroylquinic acid; 4, 4-*p*-coumaroylquinic acid; 5, 5-*p*-coumaroylquinic acid; 6, quercetin 3-*O*-arabinoside; 7, quercetin 3-*O*-rhamnoside; 8, phloretin 2′-xyloglucoside; 9, phloretin glucuronide. IS, internal standard (3,4,5-trimethoxycinnamic acid). For details see Section 2.

were not detectable. No cyanidin 3-galactoside was identified using HPLC-DAD at 520 nm. The data shown are in good agreement with the recently identified polyphenols in apple juices from different apple varieties and commercially available apple juices [11].

For identification and quantitation, HPLC-DAD and HPLC-ESI-MS/MS in the negative mode were used as described previously [11]. Structural elucidation was performed by comparing the spectroscopic data with those of references.

3.2 Identification and quantitation of polyphenols in ileostomy fluids

Eleven healthy ileostomy patients consumed 1 L of cloudy apple juice. Ileostomy bags were removed 0, 1, 2, 4, 6, and 8 h after juice consumption. Polyphenol amounts in the ileostomy fluids were determined using HPLC-DAD analysis directly after sample preparation. Dihydrochalcones, catechins, and procyanidins were determined at 280 nm, hydroxycinnamic acid derivatives at 320 nm, and flavonols at 360 nm. Reliable structural elucidation of all compounds under study was performed by means of retention time, UV maximum, and HPLC-ESI-MS/MS fragmentation pattern as shown previously [11]. An HPLC-DAD chromatogram of an ileostomy extract 2 h after the consumption of 1 L cloudy apple juice (λ = 320 nm) is shown in Fig. 2. Chlorogenic acid, kryptochlorogenic acid, 3-*p*-coumaroylquinic acid, 4-*p*-coumaroylquinic acid, 5-*p*-coumaroylquinic acid, quercetin 3-*O*-arabinoside, quercetin 3-*O*-rhamnoside, phloretin 2′-*O*-xyloglucoside, phloretin glucuronide, and 3,4,5-trimethoxycinnamic acid as IS was identified at 320 nm. Unlabeled peaks are of nonphenolic intestinal constituents.

Time courses of the apple juice polyphenols occurring in the ileostomy fluids of all 11 patients in a period of 8 h are shown in Figs. 3, 4. As shown in Fig. 3 maximum amounts of the hydroxycinnamic acids such as kryptochlorogenic acid, chlorogenic acid, and 3-, 4-, and 5-*p*-coumaroylquinic acid emerge in the ileostomy bag at 2 h. One patient has

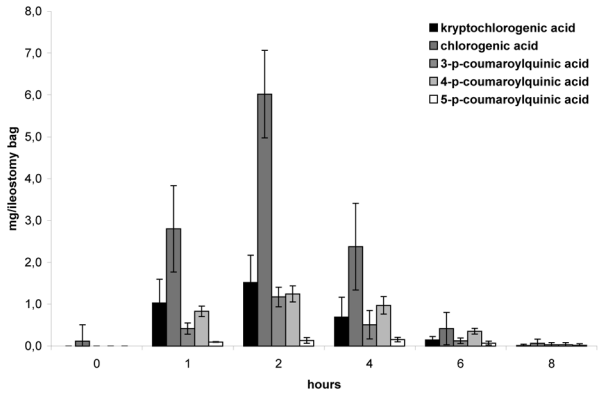


Figure 3. Time course of excretion of the hydroxycinnamic acid derivatives, kryptochlorogenic acid, chlorogenic acid, 3-, 4-, and 5-*p*-coumaroylquinic acid, in the ileostomy fluids of patients (n = 11) under study. Data are expressed as mean ± SD.

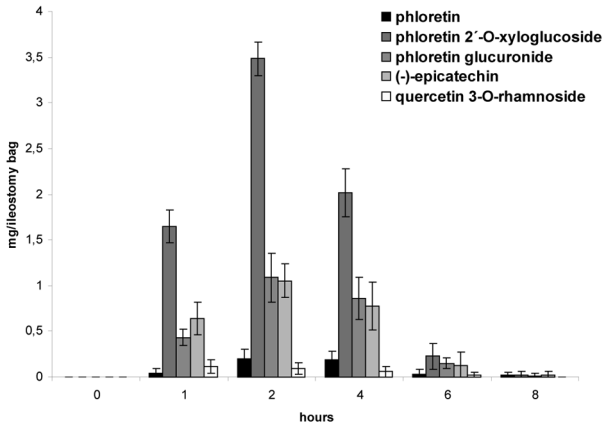


Figure 4. Time course of excretion of the dihydrochalcones phloretin, phloretin 2′-xyloglucoside, and phloretin glucuronide, and (−)-epicatechin and quercetin 3-*O*-rhamnoside in the ileostomy fluid of patients (n = 11) under study. Data are expressed as mean ± SD.

shown minor amounts of chlorogenic acid in the ileostomy effluent at the beginning of the study. Identically, for (−)-epicatechin and phloretin 2′-*O*-xyloglucoside maximum excretion was observed in the ileostomy bag after 2 h (see Fig. 4). Caffeic acid and the dimeric procyanidins B_1 and B_2

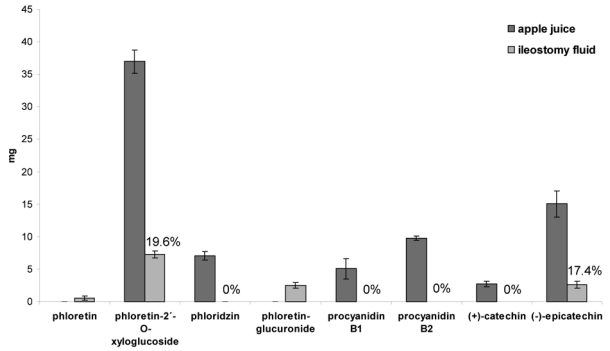


Figure 5. Comparison of the dihydrochalcones and flavan-3-ols determined in cloudy apple juice *versus* polyphenol contents detected in the ileostomy fluid of patients ($n = 11$) under study. Recovery is in percentage; data are expressed as mean ± SD.

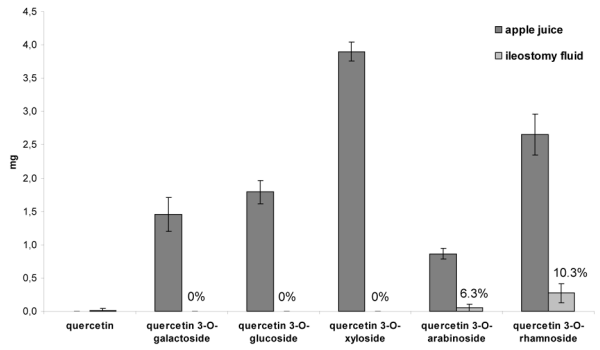


Figure 6. Flavonols determined in cloudy apple juice in comparison to the polyphenol contents detected in the ileostomy fluid of patients ($n = 11$) under study. Recovery is in percentage; data are expressed as mean ± SD.

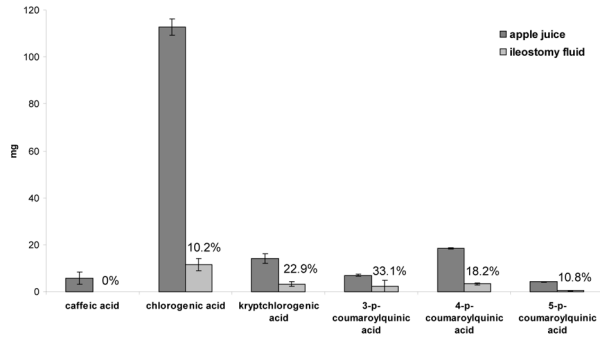


Figure 7. Comparison of the hydroxycinnamic acid contents determined in cloudy apple juice *versus* polyphenols detected in the ileostomy fluid of patients ($n = 11$) under study. Recovery is in percentage; data are expressed as mean ± SD.

as well as (+)-catechin and phloridzin were not detectable in the ileostomy fluid within 8 h after apple juice intake. All compounds determined in the ileostomy bags had passed the small intestine completely 8 h after the apple juice consumption. Although not present in the juice, the phloretin aglycon was determined in the ileostomy extract with a maximum excretion between 2 and 4 h; also, its conjugated form, phloretin glucuronide, occurs with a maximum at 2 h, indicating that phloridzin was hydrolyzed before absorption. In the group of flavonols, quercetin 3-*O*-rhamnoside and traces of quercetin 3-*O*-arabinoside (in 5 of 11 patients under study), but no quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, and quercetin 3-*O*-xyloside were recovered. No quercetin glucuronide or sulfate was identified in the ileostomy fluids using HPLC-ESI-MS/MS analysis. Only in one participant, free quercetin aglycon was found in the 8 h ileostomy fluid probably derived from lunch but not as a degradation product from the apple juice flavonol glycosides. One volunteer showed maximum polyphenol excretion at 4 h, whereas the other ten patients under study showed a maximum at 2 h.

Comparing the amounts of polyphenols consumed *via* cloudy apple juice with the amounts of polyphenols appearing in the ileostomy fluids the main differences are obvious. In general, 0–33.1% of the ingested substances from apple juice were found in the ileostomy fluids. These findings are presented in Figs. 5–7. For (−)-epicatechin and phloretin 2′-*O*-xyloglucoside 17.4 ± 4.2% and 19.6 ± 0.2% of the consumed dose were excreted into the ileostomy bags, whereas only 10.3 ± 5.0% and 6.3 ± 6.2% of the flavonols quercetin 3-*O*-rhamnoside and quercetin 3-*O*-arabinoside were excreted. Considering the recoveries of the caffeoylquinic acids (chlorogenic and kryptochlorogenic acid) and 3-, 4-, and 5-*p*-coumaroylquinic acid in the ileostomy fluids, their metabolism seems to be dependent on the position of esterification. Chlorogenic acid and 5-*p*-coumaroylquinic acid, bound at position 5, were metabolized intensively (recovery was 10.2 ± 2.5% and 10.8 ± 3.2%) whereas kryptochlorogenic acid (22.9 ± 6.4%) and 4-*p*-coumaroylquinic acid (18.2 ± 1.8%)-bound in position 4-metabolization took place to a lesser extent. Recovery of 3-*p*-coumaroylquinic acid was determined with 33.1 ± 2.7%, showing lowest absorption or metabolization ability. The dimeric procyanidins B_1 and B_2 as well as the monomeric (+)-catechin did not occurr in the ileostomy fluid. Therefore, measurable amounts of (−)-epicatechin derived either from the apple juice itself or as a part of the procyanidin cleavage were determined.

With our results it is still not clear if compounds have been absorbed or subjected to conjugation steps. Some unidentified peaks have occurred in the ileostomy fluids, which will be further investigated.

4 Discussion

Data in humans on the absorption from or biotransformation of polyphenols in the small intestine are limited. The question of colonic availability of orally consumed poly-

phenols or their metabolites can be studied in subjects who have undergone colectomy with terminal ileostomy. In the present study, 11 volunteers with ileostoma consumed 1 L of a cloudy apple juice containing 249.9 mg/L of polyphenols. Approximately 66.9–100% of the ingested phenolic substances were absorbed in the small intestine or conjugated *via* the human metabolism.

In recent years, a number of studies with ileostomy patients have been performed to get a closer insight into the colonic bioavailability as well as absorption rates of food-derived flavonoids and aromatic acids. Hollman *et al.* [19] and Walle *et al.* [20] investigated the bioavailability of quercetin glycosides. Whereas Hollman found that about 52% of quercetin derived from a meal of cooked onions was absorbed in the small intestine, Walle and coworkers found absorption rates of up to 81%, suggesting hydrolyzation of the glycosides to quercetin in the small intestine by β-glucosidases prior to absorption. Our data support the observation that quercetin glycosides are extensively hydrolyzed even if no free quercetin, quercetin glucuronide or sulfate was detected. However, it is well known that absorption of quercetin is more rapid after intake of onions, rich in glucosides, than after intake of apples containing both glucosides and other glycosides [21]. Absorption kinetics and bioavailability are probably governed by the type of glycoside and the consumed amount thereof. In our study, a total of 10.8 mg quercetin glycosides were consumed at once whereas in the other studies amounts up to 100 mg were given. Especially, polyphenols linked to a rhamnose moiety have to reach the colon to be hydrolyzed by rhamnosidases of the microflora prior to absorption [22, 23]. The same probably applies to polyphenols linked to arabinose [12], which is in good agreement to our data where quercetin 3-*O*-rhamnoside and quercetin 3-*O*-arabinoside occurred in the ileostomy fluids whereas all other quercetin glycosides such as quercetin 3-*O*-glucoside were not detectable.

Olthof *et al.* [24] studied the absorption of chlorogenic and caffeic acid in ileostomy patients. In contrast to our data, volunteers consumed no food but high amounts of pure chlorogenic acid (1000 mg) and caffeic acid (500 mg). According to Olthof, 67% of chlorogenic and 5% of caffeic acid were recovered in the ileostomy fluids. In contrast, 10.2% of chlorogenic acid, 0% for caffeic acid, and 22.9% of kryptochlorogenic acid were determined in the ileostomy fluid extracts in our study. This could be explained by the lower consumption of chlorogenic acid from apple juice where absorption and/or hydrolization mechanisms could be different. To our knowledge, no quantitative data on the absorption of 3-, 4-, 5-*p*-coumaroylquinic acids are available in literature.

In a previous study, Crespy *et al.* [25] showed that phloretin and phloridzin were absorbed in the small intestine, using an *in situ* intestinal perfusion model. After perfusion of phloridzin, they found that the corresponding aglycone as well as conjugated derivatives (glucuronide and/or sulfate) appeared in the lumen; 80% of phloridzin was hydrolyzed. In a following study, the group of Crespy tested the bioavailability of phloretin and phloridzin in rats [26]. Phloretin was recovered in plasma in small amounts and no trace of intact phloridzin was detected, indicating that this glucoside must be hydrolyzed before absorption and metabolism. These results are in agreement with our data where no phloridzin but the aglycon phloretin and phloretin glucuronide were detected. In our study, using HPLC-ESI-MS/MS analysis no phloretin sulfate but phloretin glucuronide was detectable in the ileostomy extracts. We suppose the position of glucuronidation to be 2′ or 4′ as already discussed in literature [12]. In fact, the group of Crespy has used glucuronidase and sulfatase enzymes and cannot differentiate between bond glucuronides and sulfates. About the absorption and metabolism of phloretin 2′-*O*-xyloglucoside, no literature is available. In general, it is important that 19.6 ± 0.2% phloretin 2′-*O*-xyloglucoside but no phloridzin reach the end of the small intestine and so far the colon.

Procyanidins differ from most other plant polyphenols because of their polymeric nature and high molecular weight. On the basis of *in vitro* experiments it has been suggested that procyanidins from cocoa break down into monomeric units during their passage through the gastrointestinal tract [27–29]. In contrast, Rios *et al.* [30] have reported that most of the ingested procyanidins reach the small intestine intact. Our data support the observation that procyanidins were cleaved into monomers, because no procyanidin B_1 or B_2 was detected in the ileostomy fluid, but 17.4 ± 4.2% of the consumed (−)-epicatechin.

In another study, (−)-epicatechin as well as (+)-catechin were *O*-methylated and glucuronidated in the small intestine [31]. We observed no (+)-catechin as well as no *O*-methylated or glucuronidated (+)-catechin in the ileostomy fluids using HPLC-ESI-MS/MS analysis.

Our results show that after apple juice intake some of the polyphenols reach the end of the small intestine unmetabolized in healthy ileostomy patients. It is important to keep these figures in mind when studies with polyphenols *in vitro* are devised in order to avoid unphysiologically high concentrations.

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