DOI 10.1002/mnfr.200500211 Mol. Nutr. Food Res. 2006, *50*, 418–423

Studies on apple and blueberry fruit constituents: Do the polyphenols reach the colon after ingestion?

Kathrin Kahle[1], Michael Kraus[1], Wolfgang Scheppach[2], Matthias Ackermann[1], Friederike Ridder[1] and Elke Richling[1]

[1] Department of Food Chemistry, University of Wuerzburg, Am Hubland, Wuerzburg, Germany
[2] Department of Medicine II, Division of Gastroenterology, University of Wuerzburg, Wuerzburg, Germany

The aim of our studies was to determine the amount of polyphenols reaching the colon after oral intake of apple juice and blueberries. After a polyphenol-free diet healthy ileostomy volunteers consumed a polyphenol-rich cloudy apple juice while others consumed anthocyanin-rich blueberries. Ileostomy effluent was collected and polyphenols were identified using HPLC-DAD as well as HPLC-ESI-MS/MS; quantification 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. A higher amount of the blueberry anthocyanins under study (up to 85%, depending on the sugar moiety) were determined in the ileostomy bags and therefore would reach the colon under physiological circumstances. Such structure-related availability has to be considered when polyphenols are used in model systems to study potential preventive effects in colorectal diseases.

Keywords: Anthocyan / Apple / Blueberry / Flavonoids / Metabolism

Received: October 19, 2005; revised: January 9, 2006; accepted: January 11, 2006

1 Introduction

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 anti-carcinogenic effects both *in vitro* and in animal experiments and may thus reduce the risk of colorectal cancer in man [1]. Some risk factors for colon cancer establishment could be inflammatory bowel diseases (IBD) [2]. In Germany up to 300 000 persons suffer from IBD such as Crohn's disease (CD) and ulcerative colitis (UC) [3]. Mostly young people develop this chronic inflammation accompanied by diarrhea, pain, and, sometimes in the long term, carcinogenesis [2, 4]. It is a long-lasting aim to identify the factors causing this illness and develop efficient strategies for effective treatment or to gain information in the prevention of IBD.

Nowadays, the identification of chemopreventive food ingredients is one of the worldwide targets. Various research groups have investigated the effects of food ingredients on the colonic mucosa and other targets related to colonic diseases. The modulation of detoxification enzymes as well as signal transduction pathways involved in carcinogenesis have been investigated in the presence of polyphenols [5–8]. Antiinflammatory and antioxidative properties of polyphenols were observed in other studies [9, 10]. Additionally, oxidative DNA damages were reduced *in vitro* by apple ingredients [11]. Recently, *in vivo* studies showed a reduction of aberrant crypts in rats when fed with polyphenol-rich cloudy apple juices [12]. Especially anthocyanins showed antiproliverative and antioxidative as well as "cytoprotective" effects in colon cell line Caco-2 [13, 14].

In fact, the amount of polyphenols derived from apple juice, other fruits, and vegetables is important for the ability to achieve protective effects in the colon. However, information is rather scarce to date about the colonic availability of food-derived polyphenols [15–17]. The aim of our studies was to determine the amount of polyphenols reaching the colon after oral intake of apple juice as well as blueberry fruits. After polyphenol-free diet healthy ileostomy volunteers consumed a polyphenol-rich cloudy apple juice or anthocyanin-rich blueberry fruits; the ileostomy effluent was collected after consumption. Polyphenols were identified using HPLC-DAD as well as HPLC-ESI-MS/MS; quantification was performed with HPLC-DAD.

Correspondence: Dr. Elke Richling, Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany
E-mail: richling@pzlc.uni-wuerzburg.de
Fax: +49 931-888-5484

Abbreviations: IBD, inflammatory bowel diseases

2 Materials and methods

2.1 Chemicals

All chemicals and solvents were of analytical grade. Solvents were redistilled before use. ACN (Lichrosolv®) was purchased at Merck (Darmstadt, Germany); formic acid was from Fluka (Deisenhofen, Germany), and 3,4,5-trihydroxycinnamic acid (internal standard) was distributed by Aldrich (Steinheim, Germany). Methanol (HPLC grade) and hydrochloric acid were products from Fisher Scientific (Schwerte, Germany).

2.2 Subjects

After giving informed written consent, healthy subjects with an end ileostomy participated in the trial. Patients had no evidence for small intestinal involvement. Concerning the type of operation, only those patients were recruited into the study in whom no ileal resections had been performed. The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Wuerzburg, Germany.

2.3 Blueberries

Lowbush blueberries, *Vaccinium augustifolium* (origin: Germany), were mixed and a portion size of 300 g was used in the dietary intervention study, whereas two aliquots of 5 g were separated for anthocyanin quantification.

2.4 Study design

The day prior the intervention study patients had to maintain a polyphenol-free diet. After a 10 h overnight fast 300 g minced lowbush blueberries with 100 g skim milk yoghurt (0.1% fat) were consumed within 15 min. The volunteers remained fasted for another 4 h and were then served a polyphenol-free light meal. The ileostomy bags were collected immediately before and 0.5, 1, 2, 4, 5, 6, and 8 h after the start of the blueberry intake. Ileostomy bags were immediately frozen at −24°C and stored until extraction and analysis.

2.5 Preparation of ileostomy fluids

Frozen ileostomy fluids were freeze-dried using a Christ Alpha 1–4 apparatus (Osterode, Germany) and carefully homogenized. Aliquots (0.2 g) were extracted ten times with 2.0 mL of methanol containing 500 mmol HCl, using sonication (Elma Transsonic 460, Singen, Germany) for 10 min and centrifugation at 6000 rpm for 6 min (Hettrich EBA-12, Tuttlingen, Germany). The solvent was evaporated at 35°C, and the extract was dissolved in 5 mL of aqueous 10% formic acid containing 400 µL of methanol. For recovery determination of sample preparation references were added to a polyphenol-free ileostomy fluid. Recoveries ranged between 85 and 99% (n = 3).

2.6 Quantification of anthocyanins in blueberries and ileostomy fluids

Fourteen blueberry anthocyanins were isolated from blueberry fruits by preparative techniques (data not shown). Identification of these references, delphinidin 3-*O*-galactoside (dp-3–gal), delphinidin 3-*O*-glucoside (dp-3-glc), delphinidin 3–*O*-arabinoside (dp-3-ara), cyanidin 3-*O*-galactoside (cy-3-gal), cyanidin 3-*O*-glucoside (cy-3-glc), cyanidin 3-*O*-arabinoside (cy-3-ara), petunidin 3-*O*-galactoside (pt-3-gal), petunidin 3-*O*-glucoside (pt-3-glc), petunidin 3-*O*-arabinoside (pt-3-ara), peonidin 3-*O*-galactoside (pn-3-gal), peonidin 3-*O*-glucoside (pn-3-glc), malvidin 3-*O*-galactoside (mv-3-gal), malvidin 3-*O*-glucoside (mv-3-glc), and malvidin 3-*O*-arabinoside (mv-3-ara) was confirmed by UV-VIS spectra and MS/MS measurement including information from literature [18–21]. Aliquots from stock solutions (between 42.2 and 79.8 mg/L) of anthocyanin references in aqueous 10% formic acid were diluted (range from 0.7 to 40 mg/L) and 3,4,5-trihydroxycinnamic acid as internal standard (50 mg/L) was added (v/v = 1/1). Anthocyanins were quantified by means of calibration curves (peak area divided by internal standard area *vs.* quotient of anthocyanin and the internal standard concentration). Linearity was given for 0.7–40 mg/L; limit of quantification was 0.35 mg/L and limit of determination was 0.2 mg/L, with an S/N of 3 : 1. All experiments were performed in triplicate. Compounds were identified by comparison of retention time, UV spectra (200–600 nm), and MS as well as MS/MS information using reference compounds. For pn-3-gal quantification was performed using pn-3-glc by HPLC-DAD.

Prior HPLC-DAD and HPLC-MS/MS analyses ileostomy fluid extracts were centrifugated, internal standard was added, and the samples were analyzed.

2.7 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 Zorbax™ SB-C8 column, 150 mm × 2.1 mm, with 3.5 µm particle size

(Agilent Technologies, Waldbronn, Germany), was used. The mobile phase consisted of aqueous 10% formic acid (A) and ACN (B) v/v. The gradient applied was 2% B for 0–3 min, from 2 to 5% B for 3–17 min, from 5 to 6% B for 17–28 min, from 6 to 15% B for 28–35 min, from 15 to 30% B for 35–45 min, from 30 to 99% B for 45–50 min, and 99% B for 50–55 min at a flow rate of 0.35 mL/min, and 20 µL injection volumes were used. Anthocyanins were determined at 520 nm and the internal standard 3,4,5-trimethoxycinnamic acid at 280 nm.

2.8 HPLC-DAD-MS/MS analysis

HPLC-DAD-ESI-MS/MS was performed with a TSQ 7000 tandem mass spectrometer system equipped with an ESI interface (Finnigan MAT, Bremen, Germany) and a Hewlett-Packard 1100 HPLC gradient pump and a Hewlett-Packard 1100 photodiode array detector (Waldbronn, Germany), equipped with a Wisp 710b autosampler (Waters). MS-data acquisition and evaluation were conducted on a DEC 5000/33 (Digital Equipment, Unterföhring, Germany) using Finnigan MAT ICIS 8.1 software. DAD-Data acquisition and evaluation were performed with a Hewlett-Packard Chemstation software. HPLC chromatographic separations were carried out on a Zorbax™ SB-C8 column, 150 mm × 2.1 mm, with 3.5 µm particle size (Agilent Technologies). The mobile phase consisted of aqueous 10% formic acid (A) and ACN (B) v/v. The gradient applied was 2% B for 0–3 min, from 2 to 5% B for 3–17 min, from 5 to 6% B for 17–28 min, from 6 to 15% B for 28–35 min, from 15 to 30% B for 35–45 min, from 30 to 99% B for 45–50 min, and 99% B for 50–55 min at a flow rate of 0.35 mL/min, and 20 µL injection volumes were used. The analysis was performed in the positive ionization mode. The spray capillary voltage was set to 3.2 kV, and the temperature of the heated capillary was 200°C. Nitrogen served both as sheath (70 psi) and auxiliary gas (10 units). The mass spectrometer was operated in the full-scan mode, *m*/*z* 150–1000, with a total scan duration of 1.0 s. MS/MS experiments were performed at a collision energy of 20 eV, with argon (2.0 mTorr) serving as collision gas. The obtained molecular ion peaks and mass spectra were compared to those of the references measured before.

2.9 Apple polyphenols

Data on ileostomy study performance and quantification of apple polyphenols in ileostomy bags were published previously [22, 23].

3 Results

Colonic availability of orally consumed polyphenols or their metabolites was studied in subjects who have undergone colectomy with terminal ileostomy. In the present studies, the volunteers consumed 1 L of a cloudy apple juice containing 249.9 mg/L of polyphenols [22] or 300 g of blueberries with a total anthocyanin amount of 7834 mg/kg (data not shown). All polyphenols determined in the ileostomy fluids passed the small intestine completely 6–8 h after the consumption of apple juice or blueberries (see Table 1) [23]. In both studies, maximum of excretion was observed at 2 h.

As shown in Table 1, after the consumption of 1 L of cloudy apple juice only up to 33% of the ingested polyphenols

Table 1. Relative amounts of polyphenols consumed by 1 L of cloudy apple juice in relation to the polyphenol amounts recovered in ileostomy fluids (µmol)

	Apple juice	0 h	1 h	2 h	4 h	6 h	8 h	Total excretion, (µmol)	Total excretion, (%)
4-Caffeoylquinic acid	39.8 ± 5.6	ND	2.8 ± 1.6	4.2 ± 1.8	2.0 ± 1.4	0.28 ± 0.23	0.03 ± 0.08	9.31	23
5-Caffeoylquinic acid	318.4 ± 9.9	0.33 ± 1.1	7.9 ± 2.9	16.9 ± 2.9	6.8 ± 3.0	1.1 ± 1.1	0.20 ± 0.28	33.23	10
Caffeic acid	29.4 ± 9.4	ND	ND	ND	ND	ND	ND	ND	0
3-*p*-Coumaroylquinic acid	21.0 ± 1.2	ND	1.2 ± 0.38	3.6 ± 0.67	1.5 ± 1.0	0.38 ± 0.17	0.09 ± 0.15	6.77	33
4-*p*-Coumaroylquinic acid	54.4 ± 0.89	ND	2.4 ± 0.38	3.6 ± 0.56	3.0 ± 0.62	1.2 ± 0.21	0.09 ± 0.15	10.29	18
5-*p*-Coumaroylquinic acid	12.4 ± 0.30	ND	0.29 ± 0.03	0.38 ± 0.21	0.47 ± 0.15	0.21 ± 0.15	0.06 ± 0.11	1.41	11
Phloretin 2′-*O*-xyloglucoside	64.9 ± 3.2	ND	3.0 ± 0.46	6.2 ± 1.2	3.5 ± 1.0	0.41 ± 0.18	0.04 ± 0.07	13.15	20
Phloretin 2′-*O*-glucoside	16.3 ± 1.6	ND	ND	ND	ND	ND	ND	ND	0
Phloretin-glucuronide	ND	ND	0.95 ± 0.19	2.5 ± 0.59	2.0 ± 0.51	0.33 ± 0.11	0.02 ± 0.07	5.80	
Phloretin	ND	ND	0.15 ± 0.18	0.73 ± 0.36	0.69 ± 0.32	0.15 ± 0.18	0.07 ± 0.15	1.79	
Procyanidin B_1	9.2 ± 2.8	ND	ND	ND	ND	ND	ND	ND	0
Procyanidin B_2	17.1 ± 0.52	ND	ND	ND	ND	ND	ND	ND	0
(+)-Catechin	10.3 ± 1.7	ND	ND	ND	ND	ND	ND	ND	0
(−)-Epicatechin	51.7 ± 7.2	ND	2.1 ± 0.62	3.8 ± 0.62	2.8 ± 0.90	0.44 ± 0.49	0.07 ± 0.14	9.21	17
Quercetin 3-*O*-glucoside	3.9 ± 0.43	ND	ND	ND	ND	ND	ND	ND	0
Quercetin 3-*O*-galactoside	3.2 ± 0.65	ND	ND	ND	ND	ND	ND	ND	0
Quercetin 3-*O*-xyloside	9.0 ± 0.24	ND	ND	ND	ND	ND	ND	ND	0
Quercetin 3-*O*-arabinoside	2.1 ± 0.24	ND	0.04 ± 0.07	0.05 ± 0.07	0.04 ± 0.07	ND	ND	0.13	6
Quercetin 3-*O*-rhamnoside	6.0 ± 0.67	ND	0.25 ± 0.16	0.20 ± 0.13	0.13 ± 0.13	0.05 ± 0.09	ND	0.63	10
Quercetin	ND	ND	ND	ND	ND	ND	0.03 ± 0.10	0.03	
Total polyphenol amount	669.1	0.33	21.08	42.16	22.93	4.55	0.70	91.75	14

Mean ± SD; $n = 11$.
ND = not detectable (LOD see [23]).

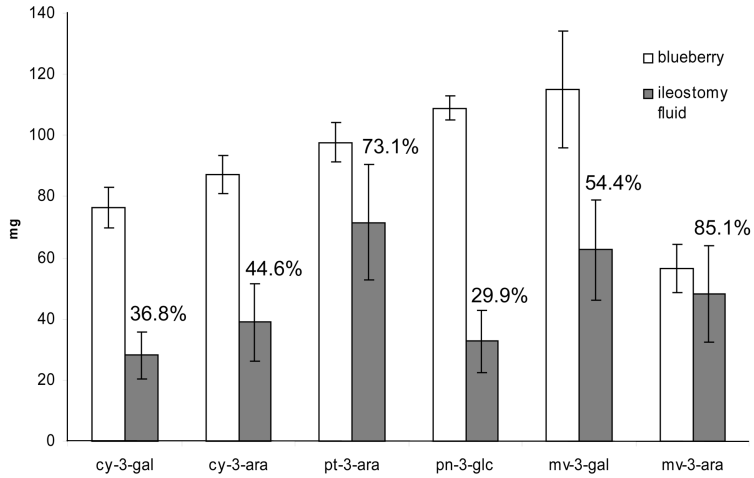

Figure 1. Comparison of the anthocyanins delphinidin 3-*O*-galactoside (dp-3-gal), delphinidin 3-*O*-glucoside (dp-3-glc), delphinidin 3-*O*-arabinoside (dp-3-ara), cyanidin 3-*O*-glucoside (cy-3-glc), petunidin 3-*O*-glucoside (pt-3-glc), petunidin 3-*O*-galactoside (pt-3-gal), and malvidin 3-*O*-glucoside (mv-3-glc) determined in wild blueberries *vs.* anthocyanin contents (mg) detected in the ileostomy fluids of patients ($n = 5$) under study. Recovery in percentage; data are expressed as mean ± SD.

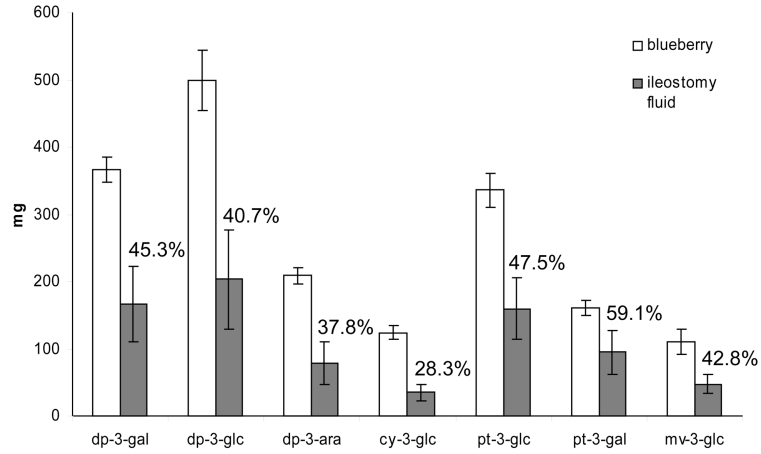

Figure 2. Comparison of the anthocyanins cyanidin 3-*O*-galactoside (cy-3-gal), cyanidin 3-*O*-arabinoside (cy-3-ara), petunidin 3-*O*-arabinoside (pt-3-ara), peonidin 3-*O*-galactoside (pn-3-gal), peonidin 3-*O*-glucoside (pn-3-glc), malvidin 3-*O*-galactoside (mv-3-gal), and malvidin 3-*O*-arabinoside (mv-3-ara) determined in wild blueberries *vs.* anthocyanin contents (mg) determined in the ileostomy fluids of patients ($n = 5$) under study. Recovery in percentage; data are expressed as mean ± SD.

reached the ileostomy bags and, under physiological circumstances, would reach the colon. In total, 14% (91.8 µmol) of the consumed polyphenols determined in apple juice (669.1 µmol) were recovered in the ileostomy bags within 8 h. In contrast to the polyphenols determined in cloudy apple juice, no caffeic acid and the dimeric procyanidins B_1 and B_2 as well as (+)-catechin, phloretin 2′-*O*-glucoside (phloridzin), quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, and quercetin 3-*O*-xyloside were detectable in the ileostomy fluid within 8 h after apple juice intake. Except for phloretin, no phase II metabolites, such as quercetin glucuronide or sulfate were identified in the ileostomy fluids using HPLC-ESI-MS/MS analysis. Although not present in the juice, the phloretin aglycon was determined in the ileostomy extract with a maximum of excretion between 2 and 4 h. Its conjugated form, phloretin glucuronide occurred with a maximum at 2 h, indicating that phloridzin was hydrolyzed before absorption [23].

In contrast, 28 – 85% of the ingested anthocyanins from blueberries were found in the ileostomy fluids (Figs. 1 and 2). For petunidin 3-*O*-galactoside (pt-3-gal) and petunidin 3-*O*-glucoside (pt-3-glc) 59.1 and 47.5% of the consumed dose excreted into the ileostomy bags, whereas 45.3 and 40.7% of delphinidin 3-*O*-galactoside (dp-3-gal) and delphinidin 3-*O*-glucoside (dp-3-glc) were excreted. Cyanidin 3-*O*-glucoside (cy-3-glc), and delphinidin 3-*O*-arabinoside (dp-3-ara) were metabolized and/or absorbed intensively (recovery was 28.3 and 37.8%), whereas for malvidin 3-*O*-glucoside (mv-3-glc) (42.8%) metabolization took place to a lesser extent.

The arabinose conjugated anthocyanins such as malvidin 3-*O*-arabinoside (mv-3-ara) and petunidin 3-*O*-arabinoside (pt-3-ara) were determined in the ileostomy bag with recovery rates of 85.1 and 73.1%, whereas for peonidin 3-*O*-glucoside (pn-3-glc) (recovery 29.9%) and cyanidin 3-*O*-galactoside (cy-3-gal) (36.8%) degradation or absorption took place to a higher amount. Recoveries of malvidin 3-*O*-galactoside (mv-3-gal) and cyanidin 3-*O*-arabinoside (cy-3-ara) were determined with 54.4 and 44.2% of the consumed dose showing lower absorption or degradation ability.

4 Discussion

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. In contrast, most of the blueberry anthocyanins (up to 85% depending on the sugar moiety or degree of methoxylation) were determined in the ileostomy bags and therefore would reach the colon under physiological circumstances.

In general, differences in the absorption in the small intestine or conjugation *via* the human metabolism of the ingested phenolic substances were observed depending on the nature of compounds and sugar conjugation.

Up to now only limited information has been published about the colonic availability of food-derived polyphenols. Some groups have used animal models to investigate the amount of polyphenols reaching the colon [24], whereas others have performed ileostomy studies to determine the amount of quercetin, quercetin conjugates, or chlorogenic acid after the consumption of polyphenol-rich food [15–17]. Additionally, other groups have investigated colonic anthocyanin metabolism *in vitro* [25, 26].

Our studies show that absorption kinetics and bioavailability are probably governed by the type of β-D-glycosidically linked sugar moiety bound to the anthocyanidin aglycon and the consumed amount thereof. Considering the recoveries of the malvidin, petunidin, and cyanidin glycosides in the ileostomy fluids, their metabolism seems to be dependent of the sugar moiety bound to the anthocyanidin aglycon. Glucosides are degraded or absorbed to a higher extent than galactosides, whereas arabinosides were recovered in highest amounts in the ileostomy fluids. Accordingly, quercetin 3-*O*-rhamnoside and quercetin 3-*O*-arabinoside occurred in the ileostomy fluids, but quercetin 3-*O*-glucoside was not detectable in the ileostomy fluid after apple juice consumption. These results are in accordance with the findings on phloretin 2′-*O*-glucoside and phloretin 2′-*O*-xyloglucoside metabolism in our previous study using apple juice [23]. No phloretin 2′-*O*-glucoside, but the aglycon phloretin and phloretin glucuronide – no phloretin sulphate – were detected. These findings are in good agreement with the literature where these metabolites were observed in animal models [27, 28].

In the literature, β-glucosidases, namely cytosolic β-glucosidase (CBG) and lactase phloridzin hydrolase (LPH), located at the brush border membrane and involved in the deglycosylation of polyphenols, have been reported to be present in the small intestine [1, 29, 30]. In our study, no free anthocyanin aglycons did occur in the ileostomy fluid. Up to now, it is still not clear if compounds have been deglycosylated prior to absorption, absorbed as glycosides, subjected to conjugation steps, or have been metabolized and need further investigations.

The polyphenols present in apple juice were recovered in the ileostomy fluids strongly depending on their chemical nature. For hydroxycinnamic acid esters, such as 3-, 4-, 5-*p*-coumaroylquinic acids, the colon availability seemed to depend on the position of esterification [23]. For the group of selected procyanidin dimers (procyanidin B_1 and B_2) a cleavage into monomers or absorption seems to occur in the intestinal metabolism. No procyanidin B_2 or B_2, but (−)-epicatechin was detected in the ileostomy fluid [23]. Deprez *et al.* [31] have reported the absorption of intact procyanidin dimers and trimers using colon carcinoma (Caco-2) cells whereas Spencer *et al.* [32] observed the degradation of procyanidins to (−)-epicatechin monomers and dimers in simulated gastric juice. In some other studies, the monomers (−)-epicatechin as well as (+)-catechin were *O*-methylated and glucuronidated in the small intestine [33]. We observed no free (+)-catechin neither *O*-methylated nor glucuronidated (+)-catechin and/or (−)-epicatechin in the ileostomy fluids using HPLC-ESI-MS/MS analysis.

Our results show that after apple juice or blueberry intake polyphenols reach the end of the small intestine unmetabolized in healthy ileostomy patients and therefore could contribute to prevent from colonic diseases. Some compounds occur in the conjugated form in the ileostomy fluids whereas others were absorbed. It is important to keep these figures in mind when studies with polyphenols *in vitro* are devised in order to avoid unphysiologically high concentrations.

The study was performed as a part of NutritionNet, supported by the German Federal Ministry of Education and Research (BMBF); project No. 01EA0102. We thank all volunteers for their participation. Sincerest thanks go to Antje Volk, Gerda Dusel, and Ines Holub (Division of Gastroenterology, University of Wuerzburg) for taking care of the participants and their support within the study performance, Professor Dr. Peter Schreier for many helpful hints and support, Henriette Zeßner and Professor Dr. Hans Becker (Saarbrücken), as well as Professor Dr. Peter Winterhalter (Braunschweig) for providing polyphenol references.

5 References

[1] Scalbert, A., Williamson, G., *J. Nutr.* 2000, *130*, 2073S–2085S.
[2] Eaden, J. A., Abrams, K. R., Mayberry, J. F., *Gut* 2001, *48*, 526–535.
[3] Logan, R. F., *Gut* 1998, *42*, 309–311.
[4] Fiocchi, C., *Chin. J. Dig. Dis*. 2005, *6*, 6–9.

[5] Harborne, J. B., Williams, C. A., *Phytochemistry* 2000, *55*, 481 – 504.

[6] Williams, R. J., Spencer, A., Rice-Evans, C., *Free Rad. Biol. Med*. 2004, *36*, 838 – 849.

[7] Marko, D., Puppel, N., Tjaden, Z., Jakobs, S., Pahlke, G., *Mol. Nutr. Food Res*. 2004, *48*, 318 – 325.

[8] Kern, M., Tjaden, Z., Ngiewih, Y., Puppel, N., *et al.*, *Mol. Nutr. Food Res*. 2005, *49*, 317 – 328.

[9] Rice-Evans, C., *Curr. Med. Chem*. 2001, *8*, 797 – 807.

[10] Gerhäuser, C., Klimo, K., Heiss, E., Neumann, I., *et al*., *Mutat. Res*. 2003, *523 – 524*, 163 – 172.

[11] Janzowski, C., Schäfer, S., Fritzen, S., Baum, M., *et al*., *Proc. Am. Ass. Cancer Res*. 2004, *45*, 731.

[12] Barth, S. W., Fähndrich, C., Bub, A., Dietrich, H., *et al.*, *Carcinogenesis* 2005, *26*, 1414 – 1421.

[13] Guerra, M. C., Galvano, F., Bonsi, L., Speroni, E., *et al*., *Br. J. Nutr*. 2005, *94*, 211 – 220.

[14] Yi, W., Fischer, J., Krewer, G., Akoh, C. C., *J. Agric. Food Chem*. 2005, *53*, 7320 – 7329.

[15] Hollman, P. C. H., De Vries, J. H. M., Van Leeuwen, S. D., Mengelers, M. J. B., Katan, M. B., *Am. J. Clin. Nutr*. 1995, *62*, 1276 – 1282.

[16] Walle, T., Otake, Y., Walle, K., Wilson, F. A., *J. Nutr*. 2000, *130*, 2658 – 2661.

[17] Olthof, M. R., Hollman, P. C. H., Katan, M. B., *J. Nutr*. 2001, *131*, 66 – 71.

[18] Prior, R. L., Lazarus, S. A., Cao, G., Muccitelli, H., Hammerstone, J. F., *J. Agric. Food Chem*. 2001, *49*, 1270 – 1276.

[19] Kähkönen, M. P., Heinämäki, J., Ollilainen, V., Heinonen, M., *J. Sci. Food Agric*. 2003, *83*, 1403 – 1411.

[20] Wu, X., Prior, R., *J. Agric. Food Chem*. 2005, *53*, 3101 – 3113.

[21] Goiffon, J.-P., Mouly, P. P., Gaydou, E. M., *Anal. Chim. Acta* 1999, *382*, 39 – 50.

[22] Kahle, K., Kraus, M., Richling, E., *Mol. Nutr. Food Res*. 2005, *49*, 797 – 806.

[23] Kahle, K., Kraus, M., Scheppach, W., Richling, E., *Mol. Nutr. Food Res*. 2005, *49*, 1143 – 1150.

[24] He, J., Magnuson, B. A., Giusti, M. M., *J. Agric. Food Chem*. 2005, *53*, 2859 – 2866.

[25] Keppler, K., Humpf, H.-U., *Bioorg. Med. Chem*. 2005, *13*, 5195 – 5205.

[26] Fleschhut, J., Kratzer, F., Rechkemmer, G., Kulling, S., *Eur. J. Nutr*. 2006, *45*, 7 – 18.

[27] Crespy, V., Morand, C., Besson, C., Manach, C., *et al*., *J. Nutr*. 2001, *131*, 2109 – 2114.

[28] Crespy, V., Aprikian, O., Morand, C., Besson, C. *et al*., *J. Nutr*. 2002, *132*, 3227 – 3230.

[29] Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., *Am. J. Clin. Nutr*. 2004, *79*, 727 – 747.

[30] Hollman, P. C. H., Katan, M. B., *Biomed. Pharmacother*. 1997, *51*, 305 – 310.

[31] Deprez, S., Mila, I., Huneau, J.-F., Tome, D., Scalbert, A., *Antioxid. Redox. Signal*. 2001, *3*, 957 – 967.

[32] Spencer, J. P. E., Chaudry, F., Pannala, A. S., Srai, S. K., *et al*., *Biochem. Biophys. Res. Commun*. 2000, *272,* 236 – 241.

[33] Kuhnle, G., Spencer, J. P. E., Schroeter, H., Shenoy, B., *et al*., *Biochem. Biophys. Res. Commun*. 2000, *277*, 507 – 512.

