Eur J Nutr (2008) 47:226–234
DOI 10.1007/s00394-008-0726-7

ORIGINAL CONTRIBUTION

Intervention with cloudy apple juice results in altered biological activities of ileostomy samples collected from individual volunteers

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Received: 31 March 2008
Accepted: 3 July 2008
Published online: 17 July 2008

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Abstract *Background* Apple juice is considered to be an important component of the healthy diet, with anticancer activities in colon cancer animal models and key ingredients have numerous chemoprotective activities in human colon cells in vitro. *Aim of the study* Since only little is known on comparable activities in the human colon in vivo, here a pilot study was performed to assess related mechanisms caused by ileostomy samples from volunteers that had consumed apple juice. *Methods* Ileostomy samples were collected after intervention (0–8 h) with cloudy apple juice (1 l). They were characterized analytically for major apple polyphenols and biologically in HT29 colon cells for their potential to cause genotoxic damage, protect from the genotoxic insult by hydrogen peroxide (H_2O_2) and modulate the expression of GSTT2, an enzyme related to antioxidative defence against different peroxides. *Results* The analytical determination of polyphenols in the ileostomy samples revealed that the majority of the compounds were recovered in the samples collected 2 h after intervention. The comparison of genotoxic effects of samples before intervention and 2 h after intervention revealed a considerable variation of genotoxic response, but there was a trend for reduced genotoxicity in three of eight persons (P) after intervention. Samples collected at 2 h protected HT29 cells from genotoxic damage by H_2O_2 (for 4 of 8 persons), resulted in an increased GSTT2 expression (for 2 of 6 persons) and of GSTT2 promotor activity (2 of 6 persons). *Conclusions* The intervention with apple juice results in bioavailable concentrations of related polyphenols in the gut lumen, which could contribute to reduced genotoxicity, enhanced antigenotoxicity and favorable modulation of GSTT2 gene expression in some individuals. The pilot study for the first time used this combination of faecal biomarkers which in larger cohorts may either reveal overall significant alterations of chemoprotection or may be used to identify individuals which could particularly benefit from a personalized nutrition.

Key words ileostomy – colon cancer chemoprevention – comet assay – gene expression – HT29 cells

EJN 726

Introduction

Population-based studies, including case control and cohort studies, have indicated that eating sufficient portions of fruit and vegetables may reduce the risk of developing cancer, especially cancers of the digestive tract [25]. Therefore, this dietary habit, together with other lifestyle factors, such as increasing the intensity and duration of physical activity, or reducing consumption of meat especially red meat, has been recommended by health agencies for a better protection against the aforementioned types of cancer [6]. Still, the link between dietary factors and cancer protection is difficult to establish, and the protective role of fruits and vegetables is somewhat controversial [11, 23]. It is therefore, important to continue exploring possible interactions between dietary and potential cancer risk factors, and to appropriately stratify epidemiological studies [23]. Furthermore, possible protective effects should be substantiated with more detailed studies of the mechanisms of protective dietary components, combined with measuring effects in human intervention studies [5].

Apple juice is an important component of fruit intake in Europe. Recent animal experiments have shown a protective activity of cloudy apple juice with respect to carcinogenesis in the distal colon of rats induced by 1,2-dimethylhydrazine (DMH) treatment [3]. The underlying mechanisms are not yet understood and warrant further investigation. Polyphenols extracted from apple juice contain a number of different flavonoids with known antioxidative effects [4]. Apple polyphenol extracts, as well as some of their major ingredients like chlorogenic acid, phloretin and quercetin were shown to inhibit the growth of the colon tumor-derived cell line HT29 [27], which may partially be related to inhibition of different growth factor signalling events [2, 15]. In HT29 cells treated with the apple polyphenol extract, a modulation of gene expression patterns occurred, including an upregulation of several genes involved in drug detoxification, notably *GSTP1* and *GSTT2* [27]. Induction of *GSTT2* could also be recapitulated using a corresponding reporter construct (K. Palige et al., unpublished data). In an LT97 human colon adenoma cell line also *GSTT2* was transcriptionally elevated, in addition to other target genes [26]. *GSTT2* has peroxidase activity towards *t*-butyl hydroperoxide, cumene hydroperoxide (Cum-OOH), and substrates also include the physiologically available arachidonic acid peroxides, such as 5-hydroperoxyeicosatetraenoic acid (5-HpETE) and arachidonic acid 15-hydro peroxide which potentially damage DNA and may be deactivated by the enzyme's peroxidase activity [10, 12, 24]. HT29 and LT97 cells, which were pretreated with the apple polyphenol extract, were better able to cope with oxidative stress caused by peroxides which may be deactivated by GSTT2-2 peroxidase activity [16].

To link the above-described findings better to the in vivo situation, it was the objective of this study to determine whether apple juice intervention in humans could affect genotoxin levels in the gut lumen. Furthermore, the capacity of those apple juice components which passed the small intestine for modulation of *GSTT2* expression and for prevention of oxidative genotoxic stress was studied in HT29 cells using ileostomy samples from volunteers who had consumed cloudy apple juice [14]. The samples were collected at different time points after intervention and were characterized analytically and biologically for various parameters associated with chemoprotection.

Materials and methods

Design and procedure of intervention study

A detailed description of the ileostomy study, and of the preparation of ileostomy bags have been reported previously [14]. Quantification of polyphenols in the apple juices under study and the ileostomy samples were performed using HPLC-DAD and HPLC-MS/MS methods as described earlier [13, 14].

The study was approved by the Ethics Commission of the University of Wuerzburg. The 11 volunteers gave their informed consent to participate in the study and agreed to avoid all foods that contain polyphenols the day before the study started. After an overnight fasting period, all volunteers drank 1 l of cloudy apple juice within 15 min. A light meal which did not contain polyphenols was served 4 h later. The ileostomy bag was removed before (control value) and 1, 2, 4 and 6 h after the start of the apple juice intake (persons 1 and 2). When we recognized, that after 6 h, polyphenols had not completely passed the small intestine, samples were taken from the remaining persons at 8 h (persons 3–11) as well. All ileostomy samples were immediately frozen at −20°C for storage, freeze-dried and homogenized. For cell culture experiments, ileostomy samples were thawed and reconstituted with phosphate buffered saline (PBS). The samples were then stored at −80°C.

Cell line and cell culture

HT29 colon carcinoma cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Life Technology, USA)

supplemented with 10% foetal calf serum (FCS) and antibiotics [1% penicillin/streptomycin (v/v); Roche Molecular Biochemicals, Meylan, France] according to our laboratory standard culture conditions. Confluent cultures were passaged 3 or 4 days after trypsinization. HT29 cells were maintained under sterile conditions at 37°C in a 95% humidified incubator (5% CO_2). Every batch of HT29 cells was routinely checked for mycoplasma contaminations using highly sensitive PCR analysis (Minerva Biolabs GmbH, Germany). In the experiments described here, cell passages 30–41 were used.

Detection of genotoxicity and antigenotoxicity

DNA damage was measured using single cell micro gel electrophoresis (Comet assay). The alkaline version of this assay was performed to detect single strand breaks and alkali-labile sites, such as apyrimidinic and apurinic (AP)-sites that are formed when bases are lost. HT29 cells were incubated with the ileostomy samples (5%, v/v) for 24 h. H_2O_2 (obtained as a 30% aqueous solution from Merck, Darmstadt, Germany) was used as the genotoxic reference compound (positive control) and to induce DNA damage (challenge) after preincubation of the cells with the ileostomy samples to measure their antigenotoxic capacity. The cell suspensions were treated with H_2O_2 at 37°C for 5 min. After this, the suspensions were diluted by adding DMEM and were centrifuged (2,000*g*, 5 min) to recover the cells. The cell pellets were resuspended in DMEM and stored on ice.

Finally, cell suspensions were centrifuged (2,000*g*, 5 min), and cells were embedded into agarose on microscopical slides, and the Comet assay was performed according to our published procedure [7]. The extent of DNA migration was determined for 150 DNA spots per treatment using the image analysing system of Perceptive Instruments (Suffolk, UK, http://www.perceptive.co.uk). The intensity of fluorescence in the comet tail, expressed as "tail intensity", was the evaluation criterion presented in the table and graphs. Each experiment was performed independently at least three times.

GSTT2: gene expression with real-time PCR

HT29 cells were seeded into 6-well culture dishes (Falcon, UK) at 0.4×10^6 cells/well and cultured for 48 h. Ileostomy samples were added at a concentration of 5% (v/v) and incubation was done for 24 h. RNA was isolated using RNeasy mini plus kit (QIAGEN, Hilden, Germany). Total RNA was checked for purity and stability by gel electrophoresis. First-strand cDNA was synthesized using the SuperScript II reverse transcriptase (Invitrogen GmbH, Karlsruhe, Germany) with 3 μg of DNA free total RNA according to the manufacturer's protocol. Real-time PCR was performed on the iCycler iQ® instrument (Bio-Rad GmbH München, Germany) with the iQ™ SYBR® Green Supermix (Bio-Rad GmbH München, Germany). PCR was performed with a set of specific targets (*GSTT2*) and reference (*GAPDH*) gene primers as described before [22]. Average threshold cycle (Ct) values were used to determine the relative differences between control and treated groups and the REST® tool program [21] was utilized for the data normalization.

Reporter gene assay for GSTT2 promotor activity

Promoter constructs of human *GSTT2*, driving expression of Firefly luciferase in the vector pGL3 were kindly provided by Dr. Paul R. Buckland (Cardiff, UK) [9]. Among the polymorphic variants, the most prevalent A form was used. 2.5×10^6 HT29 cells were seeded in a 6-cm dish. The next day, the cells were transfected with 0.8 μg *GSTT2* in pGL3 (or empty pGL3 for control) and 0.04 μg of the Renilla luciferase expressing construct pRL-TK (Promega GmbH, Mannheim, Germany) using lipofectamine 2000 (Invitrogen, Karlsruhe, Germany) according to the recommendations of the manufacturer. A ratio of lipofectamine (μl) /DNA (μg) of 6:1 was used. After 24 h, the cells were trypsinized and seeded into 96-well plates (2×10^4 cells per well). The next day, ileostomy samples and corresponding controls were added, and incubation continued for 24 h. Thereafter, the cells were washed with PBS and processed for measurement of luciferase activities with the Dual Glo™ assay kit (Promega GmbH, Mannheim, Germany) according to the method recommended by the manufacturer. Luminiscence was read in a LumiStar Galaxy reader (BMG Labtechnologies, Durham, NC, USA).

Statistical analysis

Results represent the mean value ± SD of triplicate and independently repeated experiments unless stated otherwise. Data were analysed using the one- and two-way ANOVA with Bonferroni's post test in order to determine the presence of statistically significant differences.

Results

The kinetics of total polyphenol excretion (flavonoids and phenolic acids such as chlorogenic acid) was

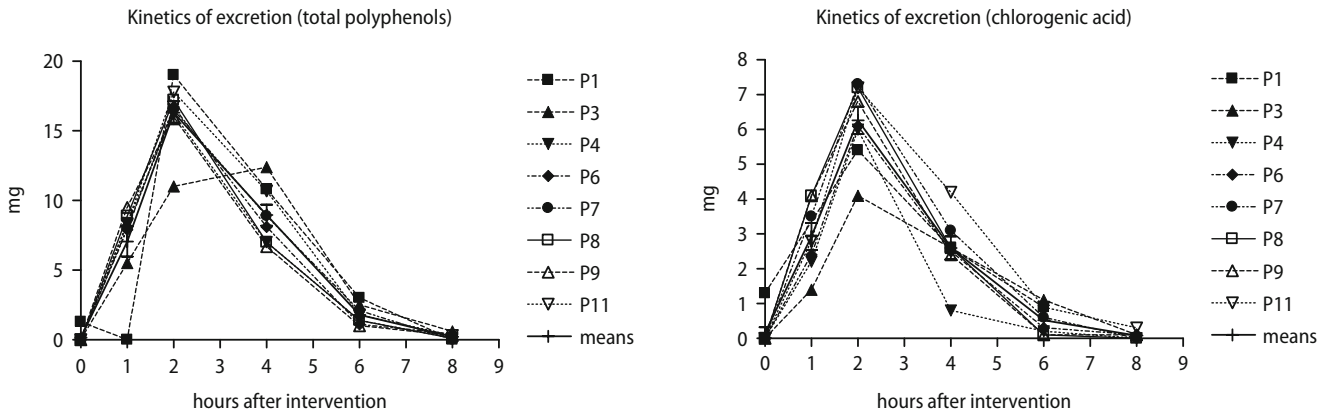

Fig. 1 Total polyphenol and chlorogenic acid excretion. Kinetics of excretion of total polyphenols and of chlorogenic acid in ileostomy samples (n = 8) obtained before (0 h) and after (1–8 h) intervention with apple juice

determined following ingestion of cloudy apple juice. From Fig. 1, it is apparent that the majority of the compounds were recovered in the samples collected 2 h after the intervention. In average, a total of 16.0 ± 3.5 mg polyphenols were retrieved from the samples of 11 subjects. The average volume of the samples was 360 ± 143 ml. Chlorogenic acid concentrations were also at their maximum in the 2 h samples and the absolute mean values of the 11 subjects were 6.0 ± 1.0 mg. Interestingly, person number 3 seemed to have a delayed response, in that the total polyphenol concentrations were higher in the 4 h sample than in the 2 h sample.

The samples before intervention (0 h) and after intervention (2 h) were compared for genotoxic activity in HT29 colon tumor cells. As shown in Fig. 2, there was a considerable variation of genotoxic response. In comparison to the medium control only the ileostomy sample of subject 1 (P1, 0 h) was significantly genotoxic ($P \leq 0.01$). But there was a trend of increased genotoxicity as compared with the medium control also in the 0 h samples of subjects 8 and 11. For subject 1, the sample collected 2 h after cloudy apple juice intervention was significantly ($P \leq 0.01$) less genotoxic than before the intervention. A trend in the same direction of reduced genotoxicity after the intervention (sample 2 h) was also observed for persons 8 and 11. There was no indication for an increased level of genotoxicity after intervention for samples of any of the volunteers. The statistical evaluation was also done with data from the whole group (2 h), which were compared with the control (0 h). This analysis yielded no significant differences (P = 0.0659) (data not shown).

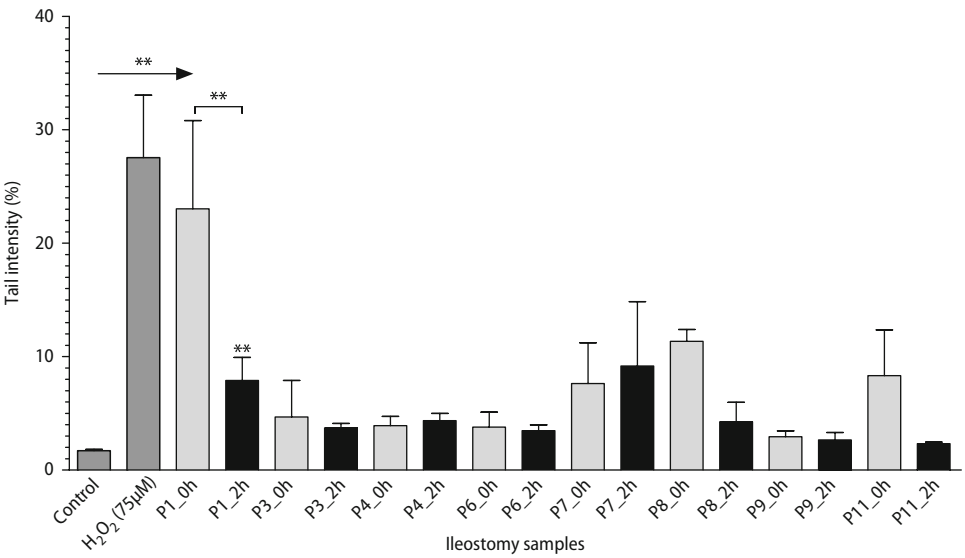

Fig. 2 Genotoxicity before and after intervention. Effects of apple juice intervention on genotoxic activities of ileostomy samples (5% v/v, n = 8) obtained before (0 h) and after 2 h intervention with apple juice. The determinations were performed with the Comet assay in HT29-treated cells. H_2O_2 was used as a positive control. Data are expressed as mean ± SD of three experiments. Two-way ANOVA with Bonferroni's post test was used to compare the statistical differences between 0 and 2 h ileostomy samples and one-way ANOVA with Bonferroni's post test was used to compare each subject to untreated control; $^{**}P \leq 0.01$

Table 1 Genotoxic effects of H_2O_2 in human colon cells (HT29) treated (24 h) with ileostomy samples (5%, v/v) obtained before and after intervention with apple juice [samples were from before (0 h) and 2 h after intervention]

Challenge for 5 min with H_2O_2 (μM)	Person 1					Person 8					Person 11				
	0 h		2 h		Difference	0 h		2 h		Difference	0 h		2 h		Difference
	Mean	SD	Mean	SD	0–2 h	Mean	SD	Mean	SD	0–2 h	Mean	SD	Mean	SD	0–2 h
Control	2.9	0.5	2.3	0.7	0.6	2.0	0.5	1.4	0.2	0.7	2.7	0.7	3.3	0.8	−0.5
4.7	5.7	2.0	3.9	1.2	1.8	2.2	0.9	1.2	0.5	0.9	2.0	0.5	3.0	0.5	−1.0
9.4	3.7	0.9	4.0	1.5	−0.3	2.4	0.2	2.1	0.9	0.4	4.2	1.7	3.2	1.1	0.9
18.8	4.4	2.4	4.1	2.0	0.3	7.8	4.6	3.1	0.4	4.7	*11.0	3.6	3.5	0.9	§§**7.5**
37.5	5.9	1.0	5.6	2.1	0.3	**11.8	2.3	2.7	1.1	§§§**9.1**	**13.4	0.9	***10.3	2.2	3.2
75.0	*9.8	1.8	***9.5	2.3	0.3	***21.4	4.1	***7.6	1.7	§§§**13.7**	***24.7	2.8	***18.3	1.8	§**6.4**
150.0	***28.3	5.0	***25.2	0.9	3.2	***41.6	4.2	***14.2	0.9	§§§**27.4**	***44.1	7.0	***30.8	2.3	§§§**13.3**

Challenge for 5 min with H_2O_2 (μM)	Person 4					Person 7					Person 9				
	0 h		2 h		Difference	0 h		2 h		Difference	0 h		2 h		Difference
	Mean	SD	Mean	SD	0–2 h	Mean	SD	Mean	SD	0–2 h	Mean	SD	Mean	SD	0–2 h
Control	3.4	1.1	3.3	0.9	0.1	1.4	0.5	4.7	1.7	−3.3	2.5	0.5	3.4	0.8	−0.9
4.7	4.3	1.0	3.8	1.0	0.5	5.0	1.8	4.3	2.1	0.7	*12.5	0.9	10.6	5.1	1.9
9.4	8.8	0.6	**14.4	2.2	−5.6	10.1	1.0	7.7	1.0	2.4	***16.8	2.3	*14.6	4.8	2.1
18.8	***20.1	3.8	***29.1	1.4	§§**−8.9**	6.3	0.3	*10.6	1.5	−4.4	***28.6	3.5	***22.2	2.5	6.4
37.5	***37.8	5.8	***42.0	6.1	−4.1	***21.5	8.9	***24.1	2.3	−2.6	***30.6	1.1	***40.2	5.9	§**−9.6**
75.0	***41.9	3.5	***35.2	1.2	6.7	***35.5	3.3	***51.1	2.6	§§§**−15.6**	***47.9	2.5	***34.7	4.4	§§§**13.2**
150.0	***39.9	0.5	***41.0	2.8	−1.0	***53.4	4.5	***56.8	3.0	−3.4	***45.8	5.8	***42.4	0.2	3.4

Shown are tail intensities (%) (means ± SD) of three independent Comet assay experiments
Values in bold print indicate significant ($^{§}P \leq 0.05$, $^{§§}P \leq 0.01$, $^{§§§}P \leq 0.001$) differences between the ileostomy samples obtained before (0 h) and after 2 h intervention
$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.01$ indicate significant differences to the untreated controls (cell culture medium with PBS) or H_2O_2 treatments

Table 1 compares the dose-related genotoxic effects of H_2O_2 in cells pretreated with medium or with ileostomy samples colleted before (0 h) or after invention (2 h) with apple juice. Two of the three antigenotoxic samples (P8, P11) also protected from H_2O_2-mediated DNA damage. In addition, the 2 h sample from person 9 (Table 1) and from person 3 (Fig. 3) significantly protected more from H_2O_2-

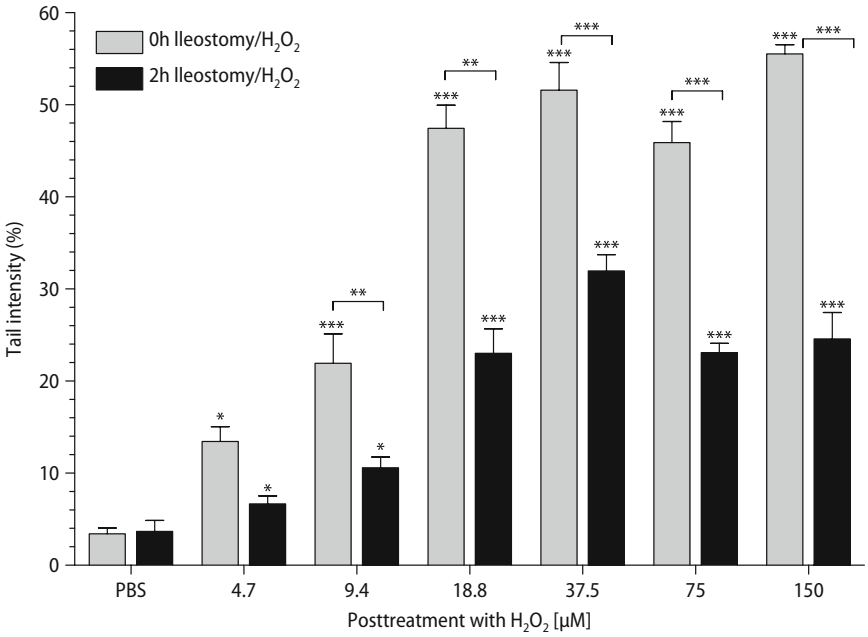

Fig. 3 Effects of ileostomy samples derived from subject 3 on DNA damage induced by H_2O_2 (37°C, 5 min) in HT29 cells. Cells were pretreated for 24 h with the ileostomy samples obtained before (0 h) and after 2 h intervention with apple juice. The figure shows tail intensities (%) (mean ± SD, $n = 3$). Statistical differences were determined by one-way ANOVA with Bonferroni's post test

mediated damage than did the corresponding 0 h samples from the same subjects. The results obtained with samples from person 3 were an interesting exception since the 2 h sample of this person was the most protective, without exhibiting a reduced baseline genotoxic potential and the data are therefore plotted in Fig. 3. Pretreatment of cells with three other 2-h samples (P1, P4, P7), did not result in reduced genotoxic effects by H_2O_2, compared to the corresponding samples collected at 0 h (Table 1).

Since apple extracts have been shown to increase expression of *GSTT2* [27], we next addressed the question of whether the ileostomy samples might also induce this enzyme that may deactivate other peroxides than H_2O_2. Figure 4 shows that the 2-h samples of P8 and P11 tended to enhance expression of the *GSTT2* mRNA in comparison with cells treated with the corresponding sample at 0 h, as determined by real-time PCR. The results did not reach statistical significance after three independent replications possibly on account of the experimental variability. The samples sizes were too limited in quantity and it was therefore not possible to perform additional determinations. Two-hour samples from P1, P4, P7 and P9 in contrast had no apparent capacity to elevate *GSTT2* mRNA levels. Samples of subject 3 could not be investigated also due to limited amounts available.

The samples were also investigated using a newly established reporter assay measuring *GSTT2* promoter activity. HT29 cells were transfected with a luciferase expression construct under control of the most prevalent form of a *GSTT2* promoter sequence [9]. Apple polyphenol extracts have the capacity to activate this reporter, with detectable induction upon treatment for 24 h and optimal induction at 48 h treatment (K. Palige et al., unpublished data). Cells were treated for 24 h with ileostomy samples taken at 0 and 2 h after the intervention with apple juice. The obtained reporter activity was compared. The 2-h sample of person 11 induced *GSTT2* promoter activities, relative to the cells treated with the 0 h sample, a very weak induction was detected with the sample from patient 1 (Fig. 5). No induction was seen with samples from persons 4, 7, 8, or 9. These data, although obtained under not optimal conditions for reporter stimulation, are in support of a regulatory activity of apple constituents which have passed the ileum with respect to *GSTT2* expression.

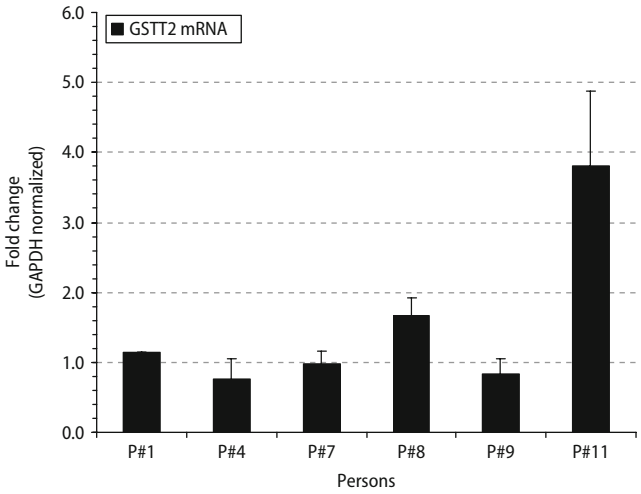

Fig. 4 GSTT2 mRNA induction in HT29 cells by ileostomy samples. Transcriptional expression of *GSTT2* gene was measured by SYBR green I real-time PCR in HT29 cells. Cells were treated for up to 24 h with ileostomy samples (5%, v/v) obtained before (0 h) and after intervention (2 h) with apple juice. The differences in the average threshold cycle (Ct) values were determined and normalized to the expression of *GAPDH* mRNA. The data reflect the effects of the 2 h samples against the 0 h samples (average of three separate experiments, mean ± SD)

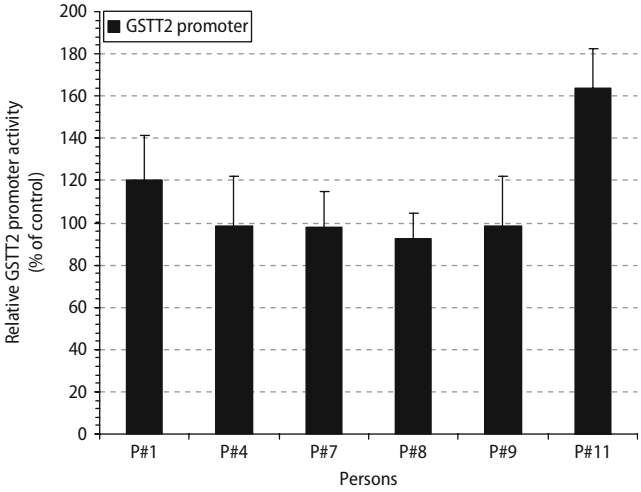

Fig. 5 Effects of ileostomy samples on *GSTT2* promotor activity. HT29 cells were transfected with a *GSTT2* promoter construct driving expression of Firefly luciferase and treated with the indicated ileostomy samples for 24 h. Thereafter, reporter activity was assayed and normalized to a cotransfected Renilla luciferase reporter. Shown are the changes of reporter activity in presence of the 2 h ileostomy samples of the indicated subjects in comparison with the 0 h (100%) samples

Discussion

This study deals with the investigation of possible chemoprotective effects in the gut lumen of humans resulting from apple juice consumption. In this context the reported pilot study with 11 ileostomy subjects is a first exploratory effort to assess whether different faecal biomarkers can be used to determine the reduction of specific risk parameters related to exposure. Foremost, the analytical determination of polyphenols in the ileostomy samples revealed that

the majority of the apple phenolic compounds were recovered in the ileostomy samples collected 2 h after intervention, and chlorogenic acid was one of the predominant detected polyphenols. Such a compound could be responsible for reducing exposure to genotoxins and oxidants in the gut lumen, thus reducing the likelihood of damage to the DNA of colon cells, as has been demonstrated recently [8]. To analyse this we determined the genotoxicity of the ileostomy samples before and after intervention of eight volunteers, for whom samples were still available in sufficient quantities. The method was used according to procedures established to study genotoxic activities of faecal water. Faecal water genotoxicity is a biomarker method being developed to indicate whether dietary intervention with, e.g. pro- and prebiotics may result in a reduced exposure to genotoxic agents [7, 18]. In rats there seem to be a direct association between development of tumors and degree of faecal water genotoxicity [17]. The studies have, however, shown that there is a high inter- and intraindividual variability of faecal water genotoxicity, even in subjects consuming identical diets [20]. Nevertheless, the impact of dietary intervention still yields significant results when analysing each subject as his own control, as we have also seen in this study with ileostomy samples from three of the eight investigated subjects. In rats, there was a reduction of DNA damage in the colon cells of animals receiving cloudy apple juice [3]. Since apple juice contains polyphenols, typical antioxidants, we next addressed the question as to whether this reduction of genotoxicity is associated with protection against oxidants, like H_2O_2 that is also available in the colon [1]. Using a challenge assay, which is performed by first treating model cells with biological samples obtained before or after intervention and then challenging them with genotoxic agents, here it was found that several samples obtained 2 h after intervention indeed reduced the genotoxic response toward H_2O_2. This antigenotoxicity of the ileostomy samples could be due to a direct antioxidative effect by the polyphenols excreted in the 2-h samples. Among others, especially chlorogenic acid could be responsible for this effect, since it also reduced H_2O_2 genotoxicity in the challenge assay [8]. The comparison of genotoxic and antigenotoxic effects of samples before and 2 h after intervention revealed a considerable variation of responses and only two of the subjects showed significant differences for both endpoints. Self evidently the numbers of these subjects is too small to find significant associations, but the lack of association also could relate to completely different mechanisms. In the context of a reduced basal genotoxicity, apple ingredients may be scavenging or inactivating genotoxic and toxic components naturally available in the gut lumen. In the context of the challenge assay, antigenotoxicity could also mean that the apple phenols are enhancing stress response or antioxidant defence in HT29 cells, thus leading to an enhanced deactivation of H_2O_2. Enzymes involved could be catalase (CAT) and glutathione peroxidases (GPX) of which *GPX2* was shown to be transcriptionally activated by AE in LT97 colon adenoma cells using gene expression studies with custom made cDNA-microarray arrays [26]. Next to these target genes, a number of other genes which produce products related to defence against other factors of oxidative stress, such as superoxide dismutase 2 (*SOD2*), glutathione reductase (*GSR*), metallothionein 1X (*MT1X*), and glutathione *S*-transferase theta 2 (*GSTT2*) were modified according to the array analyses. Of particular interest in this context is *GSTT2*, the expression of which is altered by apple polyphenols not only in HT29 cells [27] but also in LT97 colon adenoma cells [26]. This is why we chose to investigate more closely, the effects of ileostomy samples on *GSTT2* expression, using two fully new biomarker approaches. One was to assess the transcriptional induction of *GSTT2* by the samples using real-time PCR and the other was to study effects on *GSTT2* promoter activity using a novel reporter gene assay. The main objective of the present study was to use these identified "apple-juice sensitive parameters", to compare the effects of ileostomy samples obtained before and after intervention with apple juice and to assess how this relates to the excreted apple products. For person 11 there was an increase of *GSTT2* promotor activity and also an increase of mRNA after incubation of HT29 cells with ileostomy samples in the 2-h sample in comparison to the control at 0 h. It is not known which compounds in the ileostomy sample are responsible for the observed *GSTT2* gene regulation. Chlorogenic acid may be a candidate, since it has been found to weakly induce the *GSTT2* promoter (K.Palige et al., unpublished data). It is efficiently excreted, and reaches the relatively highest levels in the ileostomy sample of person 11 at 2 h after intervention. However, the other ileostomy samples of this study containing nearly similar amounts of chlorogenic acid did not respond to these parameters. Thus the reduced genotoxicity after intervention, the responses in the challenge assay and for gene expression were highly different in different individuals whereas the bioavailability of apple components was surprisingly similar. This finding is of extreme importance and shows different degrees of pharmacological, genomic and dietary variability in humans [19]. Together all factors are responsible for differences in susceptibility and deserve more in depth investigation. In the long run it may be possible to identify different individuals who may more or less profit from the habit of con-

suming apple juice on the basis of their gut luminal contents and thus be used as a parameter to better define strategies of personalized nutrition.

Altogether, we may conclude that intervention with apple juice results in bioavailable concentrations of related polyphenols in the gut lumen. In some individuals these could contribute to reduced genotoxicity, enhanced antigenotoxicity and favorable modulation of *GSTT2* gene expression, together with other ingredients of the gut lumen content which are inter individually highly different Thus, the effects were not significant on a group level and the number of subjects that participated in the study as well as the amount of samples were both too small to show an intervention effect or to disprove the possibility that apple juice could lead to chemoprotection in the gut lumen. The results were obtained when healthy patients received single acute doses with relatively large volume of apple juice (1 l). In the future it would be of interest to have data on subacute doses applied over a longer time period with more realistic amounts of juice (200–300 ml). The pilot study, however, for the first time used this new combination of faecal biomarkers which in larger cohorts may either reveal significant alterations that contribute to reduced genotoxic exposure and thus to chemoprotection of colon cells. Alternatively these studies may give rise to the identification of particular individuals who may profit most from apple juice intervention. Taken together, it appears as if ileostomy samples, especially 2 h after intervention with cloudy apple juice, causes a number of biological effects related to chemoprotection, and that these effects have also been shown to be mediated by the apple extracts and/or individual phenolic components.

Acknowledgments The authors wish to acknowledge the excellent technical assistance of Mrs. Esther Woschee at the Institute for Nutrition, Friedrich-Schiller-University of Jena. *GSTT2* reporter assays were performed by Ms. Katja Palige, and Ms. Gabriele Schulz-Raffelt. We thank Antje Volk and Gerda Dusel for taking care of the study at the Division of Gastroenterology, University of Wuerzburg and we appreciate all volunteers for their participation. This project was supported by a grant from the Bundesministerium für Bildung und Forschung (*BMBF*, FKZ.01EA0103), Germany.

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