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ORIGINAL CONTRIBUTION

Apple juice intervention modulates expression of ARE-dependent genes in rat colon and liver

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Abstract
Background The risk of cancer and other degenerative diseases is inversely correlated with consumption of fruits and vegetables. This beneficial effect is mainly attributed to secondary plant constituents such as polyphenols, supposed to play a major role in protection against ROS (reactive oxygen species)-associated toxicity.
Aim of the study To elucidate the potential of differently manufactured apple juices (clear AJ/cloudy AJ/smoothie, in comparison with a polyphenol-free control juice) to modulate expression of ARE-dependent genes.
Methods In male Sprague–Dawley rats ($n = 8$/group; 10d juice intervention, 4d wash-out; 4 treatment cycles), expression of target genes (superoxide dismutase, SOD1/SOD2; glutathione peroxidase, GPX1/GPX2; γ-glutamyl-cysteine ligase, GCLC/GCLM; glutathione reductase, GSR; catalase, CAT; NAD(P)H:quinone oxidoreductase-1, NQO1 and transcription factor erythroid-derived 2-like-2, Nrf2) was quantified with duplex RT-PCR, using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control.
Results In colon and liver of rats consuming polyphenol-free control juice, rather similar basic expressions were observed (relative GAPDH ratios ranging from 2 to 0.7 and 2.5–0.3, respectively). In the distal colon, apple juice intervention slightly but significantly induced most genes (e.g. GPX2, GSR, CAT, Nrf2; $p < 0.001$), whereas in the liver only GPX1 and NQO1 mRNA were up-regulated; other hepatic target genes were not affected or down-regulated (SOD1, SOD2, GCLC/M, GSR), concomitant with the absence of Nrf2 induction. Induction of antioxidant gene expression differed with juice type (cloudy AJ > clear AJ ~ smoothie).
Conclusion Taken together, the results underline the potential of polyphenol-rich apple juice to increase the expression of ARE-dependent antioxidant genes.

Keywords Antioxidant gene expression · Antioxidant response element · Apple juice intervention · Polyphenols · Rat colon and liver

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Abbreviations

AJ	Apple juice
ARE	Antioxidant response element
BSA	Bovine serum albumin
CAT	Catalase
DMSO	Dimethyl sulfoxide
DEPC	Diethyl pyrocarbonate
DMH	Dimethyl hydrazine
FCR	Folin-Ciocalteu Reaction
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCLC	Glutamate-cysteine ligase (catalytic subunit)
GCLM	Glutamate-cysteine ligase (modifier subunit)
GPX	Glutathione peroxidase
GPX1	Glutathione peroxidase-1 (cytosolic)
GPX2	Glutathione peroxidase (gastrointestinal)
GSR	Glutathione reductase
HPLC-PDA	High performance liquid chromatography-photodiode array detector
NQO1	NAD(P)H quinone oxidoreductase 1
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
ROS	Reactive oxygen species
rIOD	Relative integrated optical density
RT-PCR	Reverse transcription polymerase chain reaction
SOD	Superoxide dismutase
SOD1	Superoxide dismutase 1 (cytosolic)
SOD2	Superoxide dismutase 2
TEAC	Trolox equivalent antioxidant capacity
TRIR	Total RNA isolation reagent

Introduction

Apples and apple juice (AJ) represent a major source of fruit intake in the Western diet. Epidemiological studies have linked the consumption of apples with reduced risk of various cancers (including colorectal cancer), cardiovascular disease, diabetes and other reactive oxygen species (ROS)-related diseases [19]. Furthermore, results from a birth cohort study suggested an association between maternal apple intake and reduced risk of developing childhood asthma and allergic disease in 5-year-old children [41]. Antioxidant effects from apple juice consumption have been reported in an intervention with healthy men and women, resulting in decreased oxidation of low-density lipoprotein in plasma, which might be associated with a reduced risk of coronary artery disease [17]. These beneficial effects of apple juice are largely attributed to polyphenols, representing a major class of bioactive apple ingredients [7, 14]. Apple polyphenols (which mainly occur in peel, core and pulp) inhibit inflammatory gene expression and are potent antioxidants in vitro [6, 20, 32]. In addition to direct scavenging of free radicals and chelating of transition-metal cations, polyphenols can protect against ROS-mediated damage by elevating cellular antioxidant defense [29]. Rats, orally treated with polyphenols, showed increased hepatic mRNA expression of superoxide dismutase (Cu/Zn-SOD), glutathione peroxidase (GPX) and catalase (CAT), indicating that these antioxidant enzymes are regulated on a transcriptional level [42]. Expression of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and NAD(P)H quinone oxidoreductase 1 (NQO1), is mediated by the nuclear transcription factor (erythroid-derived 2)-like 2 (Nrf2), a key factor regulating genes, that encode antioxidant and detoxifying enzymes [22]. Results obtained from Nrf2 and kelch-like ECH-associated protein 1 (Keap1)-deficient mice support the emerging role of Nrf2 in protecting against diseases of the liver and gastrointestinal tract [2]. In an intervention study with ileostomy patients, ileostomy samples collected after consumption of cloudy AJ showed elevated gene expression of the peroxide-detoxifying enzyme glutathione S-transferase theta 2 (GSTT2) in HT-29 cells [37].

Puree addition to cloudy AJ (generating pulp-enriched apple juice, 'apple smoothie') was found to further increase the polyphenol concentration by 100% on average [40]. The polyphenol concentration of AJs strongly depends on cultivar (cider > table apples) and production technology (cloudy AJ > clear AJ) [40]. In intervention studies with dimethyl hydrazine (DMH)-treated rats, cloudy AJ showed a stronger reduction in number and overall mean size of aberrant crypt foci in distal colon than clear AJ, suggesting a preventive capacity against these preneoplastic lesions and thus against development of colon cancer. Both AJs, however, did not induce significant changes in expression of inflammatory (cyclooxygenase: COX-1, COX-2) and glutathione-associated genes (GST-P, GST-M; γ-glutamylcysteine synthetase) in the animals (treated with or without DMH) [4, 5].

The aim of the present study was to elucidate whether polyphenol-rich AJs modulate antioxidant gene expression in rats and to which extent antioxidant defense is influenced by juice type and polyphenol composition. We investigated the expression of selected Nrf2-dependent genes (Fig. 1) in rats after intervention with clear AJ, cloudy AJ and apple smoothie, in comparison with a polyphenol-free control juice. Additionally, we studied the gene expression of Nrf2, since this transcription factor is postulated to auto-regulate its own expression through an ARE-like promoter element, resulting in elevated nuclear accumulation of Nrf2 protein [23].

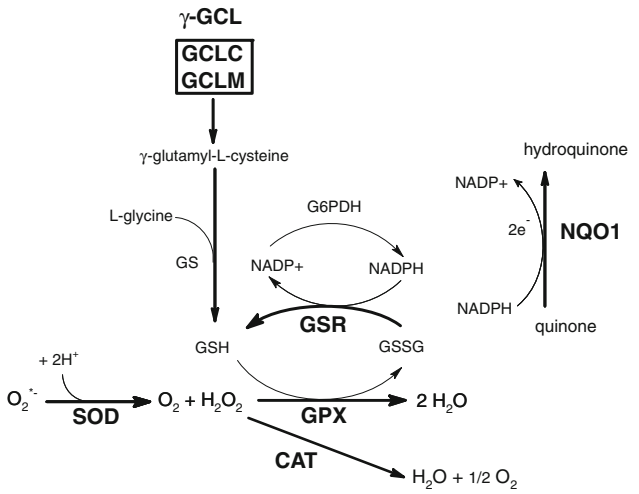

Fig. 1 Antioxidant defense system in mammalian cells. CAT, catalase; GCLC, glutamate-cysteine ligase (catalytic subunit); GCLM, glutamate-cysteine ligase (modifier subunit); GPX, glutathione peroxidase-1; GS, glutathione synthetase; G6PDH, glucose-6-phosphate dehydrogenase; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; SOD, superoxide dismutase

Materials and methods

Standard solutions and reagents

Total RNA Kit, PerfectBlue Gelsystem Maxi M and peqGold Universal-Agarose were obtained from Peqlab Biotechnologie GmbH (Erlangen, Germany) and total RNA Isolation Reagent (TRIR) from ABgene (Hamburg, Germany). DNA Primers were purchased from Eurofins MWG Operon (Ebersberg, Germany) and stored as 100 pM/μL stock solutions at −20 °C. Dimethyl sulfoxide (DMSO), bovine serum albumin (BSA) and glycerol were purchased from Sigma–Aldrich Chemical (Taufkirchen, Germany). AmpliTaq Gold™ DNA polymerase, Ambion RNALater Solution and High Capacity cDNA Reverse Transcription Kit were obtained from Applied Biosystems (Darmstadt, Germany) and UltraPure™ diethyl pyrocarbonate (DEPC)-Treated Water from Invitrogen (Karlsruhe, Germany). Molecular weight marker (100 bp) was provided by MBI Fermentas (St. Leon-Rot, Germany). All other chemicals were of reagent or molecular biology grade.

Production and analysis of apple juices

Clear and cloudy AJ was obtained from the same raw material, a mixture of cider apple varieties (Bohnapfel, 10%; Winterrambour, 10%; Maunzen, 45%) and table apples (35%) at the Geisenheim Research Center, as described in [39]. Briefly, the fruits were crushed, extracted in a horizontal press and the resulting juice was centrifuged, pasteurized and submitted to further treatment. Subsequently, the cloudy AJ was hot-filled into 0.75-L glass bottles. Clear AJ was manufactured from cloudy AJ by pectinase treatment and subsequent cross-flow filtration. Smoothie was manufactured from the variety Boskoop by blending of cloudy juice (produced as described above) with 40% apple puree [40]. The latter was obtained from the mash of Boskoop apples by enzymatic treatment (maceration enzyme and pectinase-free amylase), heating and subsequent passing through a finisher [40]. Polyphenol-free control juice was obtained from clear AJ by removing the polyphenols with SP70 adsorber resin treatment. The resulting colorless eluate contained the sugars, organic acids and minerals, whereas polyphenols were retained on the resin column. Bottled apple juices were stored at 4 °C until use.

Clear AJ, cloudy AJ and control juice were analyzed directly after membrane filtration (0.45 μm) for basic analytical juice parameters, total phenolics (Folin-Ciocalteu reaction, FCR; expressed as (+)-catechin equivalents), concentration of polyphenolic constituents (HPLC/photodiode array detector, PDA) and trolox equivalent antioxidant capacity (TEAC) as described in [30, 33, 40]. Basic juice parameters, total phenols and TEAC value of smoothie were determined in the supernatant after centrifugation; polyphenolic constituents were analyzed from methanolic extracts of freeze dried samples (HPLC/PDA) [40].

Animal treatment and tissue sampling

Male Sprague–Dawley rats ($n = 32$; 125–150 g b.w., CD; Charles River, Sulzfeld, Germany) were housed at room temperature with a 12-h light–dark cycle and were given free access to tap water. The animals received a total pathogen-free standard diet (Altromin 1314, Altromin Spezialfutter GmbH & Co. KG, Germany) throughout the whole study. After adaptation for 1 week, the animals were randomly divided into four groups of eight rats each, which received clear AJ, cloudy AJ, smoothie or polyphenol-free control juice instead of drinking water for ten consecutive days (juice uptake ad libitum). In a subsequent 4-day period, the juice was replaced by water (uptake ad libitum). This experimental design was repeated three times. Body weight of the animals was measured weekly. After a total of 56 days of treatment, the animals were sacrificed; caudate liver lobe and colon were excised. Liver specimens (20–25 mg) were immediately transferred into 5 mL RNAlater, and distal colon sections were flushed with PBS prior to snap freezing in liquid nitrogen and storage at −80 °C until use. The experimental protocol, as well as care and treatment of the animals, was approved by the ethics committee responsible for the administrative district of Kaiserslautern in accordance with the European Community Guiding Principles for the care and use of animals.

RNA extraction and RT-PCR

Reverse transcription (RT)-PCR of the RNA expressed from the genes for SOD (SOD1, SOD2), GPX (GPX1, GPX2), γ-GCL (GCLC, catalytic subunit; GCLM, modulatory subunit), GSR, CAT and NQO1 was performed using tissue samples from colon and liver. Liver tissue specimens were homogenized (500 µL TRK lysis buffer; 5000 rpm, two cycles, Precellys 24 homogenizer) [25]; total RNA was isolated (peqGOLD Total RNA Kit) and checked. Total RNA from colon tissue was isolated with total RNA Isolation Reagent (TRIR, ABgene®), according to the acid guanidinium thiocyanate–phenol–chloroform method [9]. RNA purity and integrity were assured by optical density (260/280 ratio >1.9; Nanodrop 1000, Peqlab Biotechnologie GmbH, Erlangen, Germany) and by visual assessment of 28S and 18S rRNA gel images (2:1 brightness, [15]), respectively. Subsequently, all samples were snap frozen and stored at −80 °C until use. Reverse transcription was performed with 2 µg of total RNA, 5 mM $MgCl_2$, 1× RT-Buffer, 1 mM dNTPs, 25 U MuLV reverse transcriptase and 2.5 µM random hexamers in a final reaction volume of 25 µL. The use of random hexamers allows a more accurate representation of the whole mRNA sequence, by avoiding formation of secondary structures. Reactions were carried out in a thermocycler (PTC-100, MJ-Research/Bio-Rad) starting with an initial step of 10 min at 25 °C, followed by reverse transcription for 120 min at 37 °C and a subsequent 10-min enzyme denaturation step at 95 °C. Primers (rat mRNA sequences: NCBI UNIGENE database) were designed with Primer Express V. 2.0 Software (Applied Biosystems), analyzed with OligoCalc software [21] and selected according to the following criteria: length between 19 and 21 bases, optimal 21 bases; T_m between 51 and 60 °C, optimal T_m at 52–54 °C; length of amplification product between 290 and 576 bp, so as not to overlap with the 890-bp GAPDH amplification product; G + C content, >47%; repetitive sequences, absent; repetitive bases, stretches of >5 identical bases (such as poly Ts) were normally avoided. Specificity of primers was assured by submitting their sequences to Primer-BLAST search against user-selected database, accessible at the National Center for Biotechnology Information [3]. All primer sequences are added in Table 1 in the Online Supporting Material. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous reference gene [8]. Annealing temperatures were calculated according to Marmur and Doty [26], and duplex RT-PCR (target/reference gene) was performed using AmpliTaq Gold™ DNA polymerase (Applied Biosystems). The reaction mix (40 µL) consisted of 3 µL (10 pmol/µL) of each forward primer and reverse primer, 2 µL (2 µg) cDNA, 20 µL (1U) DNA polymerase and 6 µL water. Cycling was performed under following conditions: initial enzyme activation (95 °C for 10 min), subsequent amplification (a total of 30 cycles; denaturation: 95 °C for 30 s; annealing: 52/54 °C for 30 s and extension: 72 °C for 45 s) and a final 10-min extension at 72 °C. Under these conditions, mRNA amplification remained in the exponential phase, and competition of primer pairs was excluded. To assure the absence of genomic DNA in the samples, a negative control (total RNA as template) and a no template control (NTC) were included in each PCR experiment. All colon or liver cDNA samples ($n = 32$; target and control gene) were simultaneously amplified and electrophoresed (1.3% agarose gel in 1× TAE buffer, 85 Volt DC, 90 min). A 100-bp DNA ladder was run on every gel. Ethidium bromide-stained DNA bands were visualized under UV-transillumination (Eagle Eye II, Stratagene), and net band integrated optical density (IOD) of target gene and GAPDH was quantified with ImageJ Software (Version 1.41o) [1]. Areas of user-defined selections were used to calculate relative integrated optical density (relative IOD, rIOD: target gene/GAPDH ratio) [36]. For all studied genes, primer efficiency was assured to be greater than 90% by serial dilution experiments (R^2: 0.936–0.995), except for SOD1 (R^2: 0.823). Variation coefficients (CV) were calculated for independently replicated samples (inter-assay CV in colon: GPX2, 5.4; Nrf2, 9.3) and for PCR runs of all target genes (intra-assay CV: 1.7–9.7%, colon; CV: 3.7–16.8%, liver). Because the coefficient of variation in replicate PCRs was typically 10–20% in the linear range, the precision of PCR was sufficient to measure fourfold differences in template concentration, which is in accordance with the results of Halford et al. 1999 [16].

Statistical analysis

Results from liver and colon specimen of eight rats per juice group are expressed as boxplots (basic expression, rIOD) and mean and SD (difference between control and apple juice groups. Anderson–Darling test was used for analysis of normal distribution. rIOD values of colon/liver samples and of control/apple juice groups were analyzed for significant difference with unpaired, one-sided Mann–Whitney U-test (data not normally distributed): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (significantly different). Statistics were performed with R-Project software.

Results

Juice composition and antioxidant capacity

Basic analytical juice parameters, summarized in Table 1, were in the usual range of apple juices [40]. The pattern of individual polyphenols (Table 2), determined by HPLC–PDA,

Table 1 Basic analytical juice parameters and total phenols

Parameter	[unit]	Juice			
		Control Juice	Clear AJ	Cloudy AJ	Smoothie [40]
Relative density	20/20	1.04918	1.05001	1.05055	1.0566
Brix	°	11.78	12.05	12.21	13.52
Conductivity [20 °C]	$S \times m^{-1}$	2,160	2,250	2,210	2,560
Sorbitol	g/L	4.6	4.6	4.7	6.2
pH-value		3.43	3.13	3.13	3.1
Total titratable acid[a]	g/L	7.09	7.34	7.44	10.6
Extract	g/L	127.7	129.8	131.4	147.8
Glucose	g/L	23.7	19.6	18.9	30.4
Fructose	g/L	71.4	68.2	69.2	69.6
Sucrose	g/L	16.9	20.2	21.7	23.3
Ash	g/L	n.a.	n.a.	n.a.	2.88
Potassium	mg/L	1,146	1,114	1,145	1,366
Magnesium	mg/L	48	49	51	53
Calcium	mg/L	49	43	49	47
Sodium	mg/L	7	9	8	10.4
Sugar-free extract	g/L	15.7	21.8	21.6	18.3
Σ Sugars	g/L	112	108	109.8	123.3
Total phenols (FCR)[b]	mg/L	n.d.	1,027	1,343	1,339

n.d. not detectable, *n.a.* not analyzed

[a] expressed as malic acid at pH 8.1

[b] folin reaction; expressed as (+)-catechin equivalents

was almost similar in the filtrate of clear and cloudy AJ, with chlorogenic acid (5-caffeoylquinic acid) as major compound, followed by procyanidin B2, 4-coumaroylquinic acid and epicatechin. The smoothie exhibited much higher concentrations of chlorogenic acid, phloretin-2′-xyloglucoside, phloridzin and quercetin glycosides. Procyanidins (B2, C1) and coumaroylquinic acids, however, were found in lower concentrations. The sum of individual polyphenols in smoothie exceeded the respective values of clear and cloudy AJ by 22 and 26%. In the control juice, the adsorber resin treatment resulted in complete elimination of phenolic compounds (ascertained by both, HPLC–PDA and FCR), whereas other juice parameters remained largely unchanged (Table 1).

Basic expression of antioxidant genes in colon and liver

Colon and liver of rats consuming polyphenol-free control juice showed distinct expression of most target genes (Fig. 2), with high homogeneity between the eight animals studied. In the colon, highest mRNA levels were observed for SOD1 and GPX1 (rIOD > 1.5), whereas expression of most target genes (including the gastrointestinal GPX2) was at best slightly higher compared to the endogenous control GAPDH (rIOD 1–1.5). In the liver, basic rIOD of most genes was lower or equal to the colon, except for SOD1 and CAT, which exhibited higher values.

Modulation of gene expression by AJs

Consumption of polyphenol-rich AJs resulted in juice-specific changes (higher or lower gene expression, compared to treatment with polyphenol-free juice, Fig. 3). Even though differences to the control were relatively small, they were still significant due to the high expression homogeneity within each group.

In colon, most target genes were up-regulated by apple juice intervention: A highly significant induction ($p < 0.001$) occurred for CAT (all AJs), GPX2 (clear AJ, cloudy AJ), GSR (clear AJ, cloudy AJ) and the transcription factor Nrf2 (cloudy AJ, smoothie). SOD1, GCLC/M and NQO1 were less elevated, and other genes were unchanged (clear AJ: GCLM, Nrf2; cloudy AJ: GPX1, NQO1, SOD1; smoothie: SOD1, GPX1, NQO1, GCLC, GPX2) or down-regulated (clear AJ: SOD2, GPX1, NQO1; cloudy AJ: SOD2, GCLC). In the liver, only GPX1 and NQO1 mRNA were up-regulated; other hepatic target genes were not affected or down-regulated.

No difference in juice uptake was observed between the four experimental groups (daily volumes ranging between

Table 2 Phenolic constituents and antioxidant capacity (TEAC) of clear AJ, cloudy AJ and smoothie

Phenolic constituents [mg/L][a]	Juice		
	Clear AJ	Cloudy AJ	Smoothie [40]
Procyanidin B1	5.7	8.5	9.3
(+) Catechin	8.7	6.9	15.9
Procyanidin B2	62.0	71.2	25.8
(−)-Epicatechin	57.2	36.3	34.9
Procyanidin C1	32.3	35.8	18.9
Σ Flavanols	165.9	158.7	104.8
Phloretin-2′-xyloglucoside	40.6	40.9	126.5
Phlorizin	11.6	9.0	84.5
Σ Dihydrochalcons	52.2	49.9	211.0
Chlorogenic acid	186.8	177.8	253.6
Cryptochlorogenic acid[b]	4.7	5.3	6.1
Caffeic acid	0.0	0.2	0.4
3-Cumaroylquinic acid	4.9	4.5	1.4
4-Cumaroylquinic acid	49.3	41.8	17.8
5-Cumaroylquinic acid	4.3	5.5	1.5
Σ Cinnamic acids	249.8	235	280.8
Quercetin-3-gal	1.5	1.3	3.6
Quercetin-3-glc	0.7	0.6	1.1
Quercetin-3-xyl	0.5	0.3	2.2
Quercetin-3-ara	0.8	0.4	3.5
Quercetin-3-rha	2.1	1.5	2.7
Σ Flavonols	5.6	4.1	13.1
Σ Polyphenols	473.5	447.7	609.7
TEAC (mmol/L Trolox)	8.5	9.8	10.8

[a] HPLC–PDA of methanolic extracts

[b] Calculated as chlorogenic acid

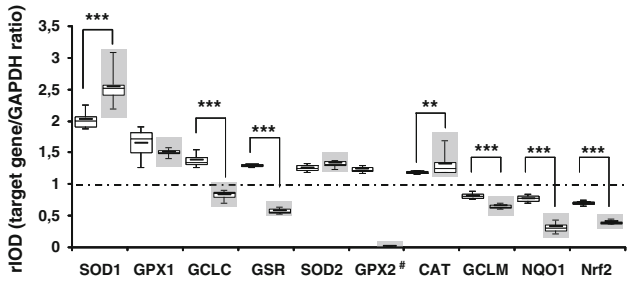

Fig. 2 Basic expression of antioxidant target genes in colon (left box, white background) and liver (right box, gray background) of rats ($n = 8$) after intervention with polyphenol-free control juice. Values represent relative integrated optical density (rIOD, normalized to endogenous reference gene GAPDH (IOD = 1), displayed as box-plots; minimum, 25th quartile, 50th (median), 75th quartile, maximum and mean (bold line); # trace (rIOD < 0.02). Data were analyzed for significant difference in rIOD of target gene in colon and liver: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (one sided Mann–Whitney U-test, unpaired, data not normally distributed)

25 and 35 mL/animal); similarly, weekly body weight gain did not show significant differences, indicating no preference for a specific juice.

Discussion

The high amounts of total titratable acid and total phenols in clear and even more distinctly in cloudy AJ reflect the major proportion of cider apples used. Correspondingly, concentration of titratable acid in smoothie, monovarietally produced from Boskoop apples, distinctly exceeded the respective values of clear and cloudy AJ [33, 40]. In cloudy AJ, concentration of total phenolics (determined by FCR) was slightly higher than in clear AJ and smoothie. It should, however, be considered that the amount of total phenols in the smoothie (determined in the supernatant after centrifugation) is probably underestimated, since particle-bound polyphenols in the pulp fraction were not covered. The high concentration of individual polyphenols in smoothie (determined in the methanolic extract; Table 2) might be attributed to the use of Boskoop apples as raw materials and to the addition of apple puree to the original cloudy juice [40]. The antioxidant capacity of the AJs (8.5–10.8 mmol/L Trolox) and total phenolics (1027–1339 mg/L) distinctly exceeded those of conventional apple juices (2.7–4.3 µmol/mL Trolox and 289.2–1020, respectively) [27, 33]. TEAC values of the clear and cloudy AJ were rather similar, reflecting the largely similar contents of polyphenols and oligomeric procyanidins. The TEAC value of smoothie was analyzed in the supernatant after centrifugation [40]; this might have caused some loss of antioxidant compounds bound to cloud particles, as already discussed for the polyphenol fraction. In the control juice, no TEAC activity was detected, because of the absence of polyphenols.

Our results on basic expression levels of GPX1 and GPX2 agree well with reported gene/GAPDH ratios in distal colon of male rats [12] and underline the high expression of the fairly ubiquitous GPX1 [11]. Significant tissue-specific differences were found particularly for GPX2 (distinctly expressed in colon, but not in liver), in line with previous reports [10].

The intervention with polyphenol-rich AJs resulted in significantly increased expression of most antioxidant genes in the colon. In a study with rats, given i.p. injections of DMH or NaCl, intervention with clear or cloudy apple juice did not significantly change transcript levels of glutathione-associated enzymes in epithelial cells of distal colon [4, 5]. The higher polyphenol concentration achieved for the juices in the present study might account for the more pronounced modulation of gene expression. Similar

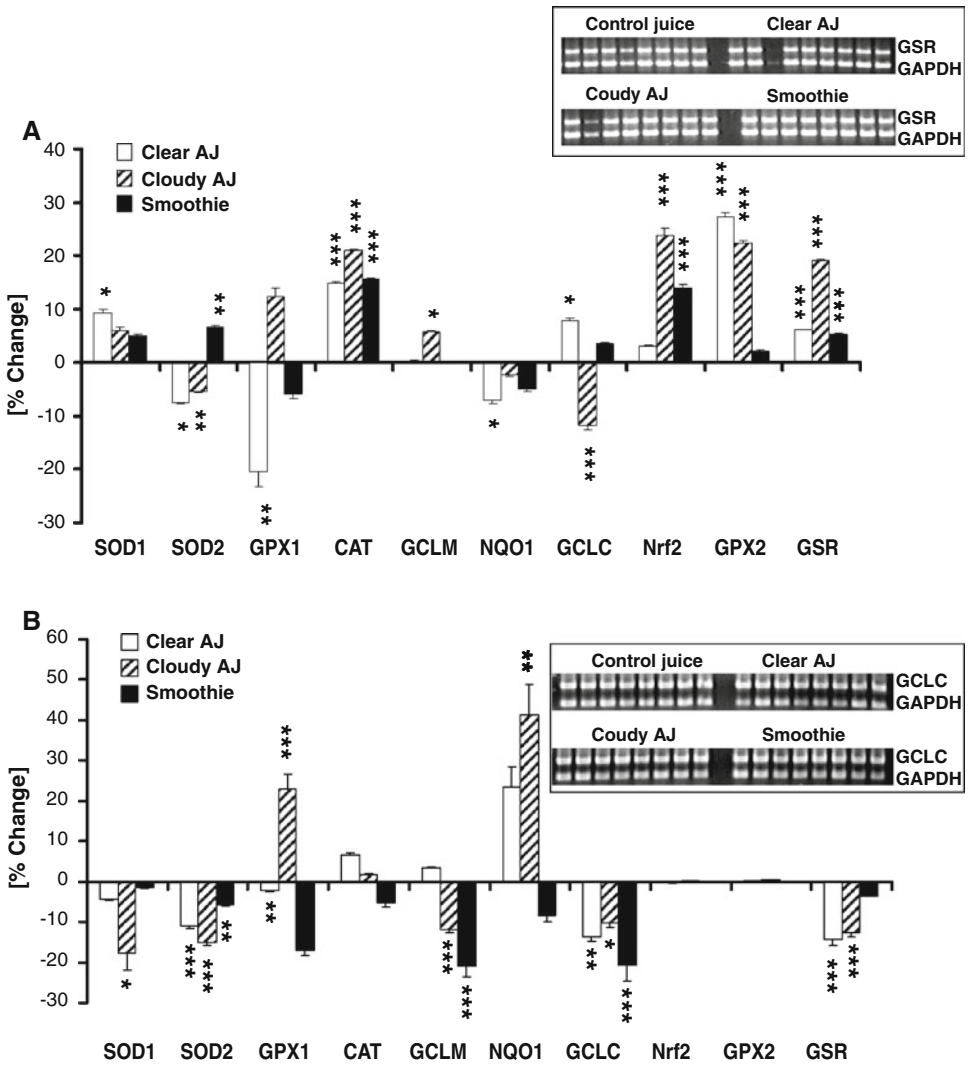

Fig. 3 Change in redox-sensitive gene expression in **a** colon and **b** liver of rats after intervention with clear AJ, cloudy AJ and smoothie, compared to (polyphenol-free) control juice: Data were calculated from relative integrated optical density (rIOD, normalized to the reference gene GAPDH: IOD = 1) and presented as percent difference to the respective mean basic levels (obtained from the polyphenol-free control juice group). Representative gel images for GSR expression in colon and GCLC expression in liver are displayed in the boxes. Values represent mean percent differences and SD from eight animals per juice group; significant difference to control juice group: $*p < 0.05$, $^{\#}p = 0.052$, $**p < 0.01$, $***p < 0.001$ (one-sided Mann–Whitney U-test, unpaired, data not normally distributed)

to our results, a higher preventive effectiveness has also been reported for intervention with cloudy when compared to clear apple juice in rats, by monitoring aberrant crypt foci and assessing cell proliferation and DNA damage [5]. In human cell lines, derived from colon carcinoma (HT-29) and adenoma (LT97), mixtures of apple polyphenols have been shown to up-regulate several glutathione S-transferase genes (including GSTT2 and GSTP1) and to inhibit cell growth [38].

In liver, most antioxidant genes were down-regulated (SOD1, SOD2, GCLC/M, GSR) or not affected by intervention with phenolic apple juices, concomitant with the absence of Nrf2 induction. The down-regulation of genes, involved in antioxidant defense, could be a consequence of cellular ROS scavenging by polyphenols leading to decreased oxidant status. GPX1 and NQO1 expression, however, was found significantly elevated by cloudy AJ. This might be ascribed to the fact that the transcription of ARE-driven genes is only in part regulated by Nrf2, and the ARE motif may also interact with other DNA-binding proteins (e.g. c-JUN: NQO1) [13, 18]. To the observed tissue-specific gene expression, differences in availability and metabolism of apple polyphenols in colon and liver are supposed to contribute [24, 31]. Moreover, the extent of polyphenol absorption in the small intestine, largely determined by chemical structure and nature/amount of metabolites formed by microflora, is a major influential factor [34, 35].

Taken together, all phenolic-rich apple juices modulated redox-sensitive gene expression. Although cloudy and clear AJ were made from the same raw material and the composition of the membrane filtrates was almost identical, cloudy AJ was a more potent inducer of redox-sensitive genes than clear AJ. This gives strong evidence that the cloud fraction contributes to the increased antioxidant potential of cloudy AJ. Lipids (49%), proteins (24%), polyphenols (18%, unidentified) and cell wall polysaccharides (7.4%) have been tentatively identified in the

cloud fraction (particle size: 1 to 5 μm) of a cloudy apple juice [5]. In addition to polyphenols, apple-derived dietary fiber, fermented by intestinal microflora to generate short-chain fatty acids (mainly butyrate), may contribute to the up-regulation of antioxidant gene expression, as described for glutathione S-transferases after incubation of tumor cell lines with butyrate [28]. Furthermore, it was discussed that cloud colloids in the juice might protect entrapped procyanidins from absorption in the small intestine, thus elevating their availability in the colon [4]. The authors proposed that cloud colloids in the juice might protect entrapped procyanidins from absorption in the small intestine, thus elevating their availability in the colon.

The lower induction of gene expression by the smoothie, compared to cloudy AJ, might be due to distinctly lower concentrations of specific polyphenols (procyanidins B2, C1; coumaroylquinic acids). Additional studies with differently composed juices/isolated phenolics are required to clarify the effect of individual phenolics on gene expression. Additionally, the increase in centrifugable solids by apple puree addition (from 0.1 up to 8–15%; [40]) might lead to increased binding of polyphenolic constituents, resulting in impaired availability in the gastrointestinal tract or delayed release.

Conclusions

The results of the intervention study in rats support the potential of polyphenol-rich apple juices to increase induction of redox-sensitive gene expression (colon > liver). The antioxidant potential of the juices varied depending on the juice type (cloudy AJ > clear AJ ∼ smoothie).

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