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Effects of carrot and tomato juice consumption on faecal markers relevant to colon carcinogenesis in humans

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High intakes of carotenoid-rich fruits and vegetables are associated with a reduced risk of various cancers including colon cancer. A human intervention study with carrot and tomato juice should show whether a diet rich in carotenoids, especially high in β-carotene and lycopene, can modify luminal processes relevant to colon carcinogenesis. In a randomised cross-over trial, twenty-two healthy young men on a low-carotenoid diet consumed 330 ml tomato or carrot juice per d for 2 weeks. Intervention periods were preceded by 2-week depletion phases. At the end of each study period, faeces of twelve volunteers were collected for chemical analyses and use in cell-culture systems. Consumption of carrot juice led to a marked increase of β-carotene and α-carotene in faeces and faecal water, as did lycopene after consumption of tomato juice. In the succeeding depletion phases, carotenoid contents in faeces and faecal water returned to their initial values. Faecal water showed high dose-dependent cytotoxic and anti-proliferative effects on colon adenocarcinoma cells (HT29). These effects were not markedly changed by carrot and tomato juice consumption. Neither bile acid concentrations nor activities of the bacterial enzymes β-glucosidase and β-glucuronidase in faecal water changed after carrot and tomato juice consumption. Faecal water pH decreased only after carrot juice consumption. SCFA were probably not responsible for this effect, as SCFA concentrations and profiles did not change significantly. In summary, in the present study, 2-week interventions with carotenoid-rich juices led only to minor changes in investigated luminal biomarkers relevant to colon carcinogenesis.

Carotenoids: Tomatoes: Carrots: Juice: Faecal water

Colorectal cancer is one of the most common causes of cancer death in the Western world. Most of the colon tumours are sporadic and develop somatically in epithelial cells[1]. Apart from genetic factors, nutritional factors can markedly affect tumour development. While a high intake of red meat and animal fat is associated with an increased colon cancer risk, epidemiological studies often observed an inverse correlation between a high intake of fruit and vegetables and the incidence of colorectal cancer[2–4]. Protective effects of fruits and vegetables are attributed to ingredients, such as fibres, vitamins and carotenoids. To date, most of the experimental studies about carotenoids investigated the effects of β-carotene, which often showed anticarcinogenic properties in the colon[5–7]. A number of *in vitro* studies showed cytotoxic and anti-proliferative effects of β-carotene on different colon cancer cell lines[8–10]. However, human intervention and epidemiological studies could not clearly verify protective effects of β-carotene against colon cancer[11–15]. Other carotenoids such as lycopene and lutein have become of more interest in recent research. Epidemiological studies have suggested a decreased colorectal cancer risk with higher tomato consumption[16], and according to some *in vitro* studies, lycopene can also inhibit proliferation of human cancer cells[17–19].

The human colon is continuously exposed to a complex mixture of gut luminal compounds of dietary origin or digestive and microbial processes[20]. These compounds can contribute to colon tumour development by damaging the mucosa, consequently leading to an increased colonic crypt cell proliferation[21]. The analysis of faeces and faecal water therefore represents a useful, non-invasive possibility to assess protective and risk factors of colorectal carcinogenesis. In dietary intervention studies, the cytotoxicity of human faecal water is often used as a biological marker for colon cancer risk[22–24]. Most of these studies focused on bile and fatty acid concentrations in faecal water or its lipid extracts due to the role of fat as a potential tumour promoter in colon carcinogenesis.

Abbreviations: EC_{25}, effective concentration at 25 % reduction of cell viability; IC_{50}, half-maximal growth inhibitory concentration; MTT, 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide.

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To our knowledge, no data exist so far about modulations in the composition of faeces and faecal water due to changes in carotenoid intake.

The aim of the present study was to investigate whether a diet rich in carotenoids, especially high in β-carotene and lycopene, can modify processes relevant to colon carcinogenesis in the gastrointestinal lumen. Therefore, several faecal markers were used in the present study.

Volunteers, materials and methods

Volunteers and study design

After screening history and medical examination, twenty-two non-smoking, non-supplement-taking, healthy men (age 28·7 ± 5·9 years; BMI 23·1 ± 2·0 kg/m^2) were selected for the intervention trial. The study protocol was approved by the medical ethical committee of the Landesärztekammer Baden-Württemberg (Germany). All participants gave their consent in writing, complied with the study protocol and completed both dietary interventions.

The study was designed as a randomised cross-over trial, consisting of two 14 d interventions with tomato juice or carrot juice (each 330 ml/d; Schoenenberger, Magstadt, Germany). The tomato juice (330 ml) provided 37·0 mg lycopene and 1·6 mg β-carotene; 330 ml of the carrot juice contained 27·1 mg β-carotene and 13·1 mg α-carotene. Intervention periods were preceded by wash-out phases on a low-carotenoid diet for 2 weeks. After the second intervention period, a third 2-week wash-out period succeeded, resulting in a study period of 10 weeks. In an earlier study, we observed that a 2-week intervention with similar amounts of carrot and tomato juices led to significant increases in plasma concentrations of β-carotene and lycopene, respectively[25]. Volunteers were told to consume the juices with main meals. Their daily diet was not restricted, but the volunteers were instructed to avoid fruit and vegetables high in carotenoids throughout the whole study (for detailed instructions, see Müller *et al.* [25]). During the last 4 d of every study phase, the volunteers received an energy- and macronutrient-balanced diet (51 ± 2 % energy as carbohydrates, 34 ± 2 % energy as fat, 15 ± 1 % energy as protein).

Collection of stool samples was restricted to twelve volunteers (six volunteers per intervention group). Complete 48 h stool samples were collected during the last 2 d of every 2-week period and directly frozen in a freezing toilet at −40°C. Samples were stored at −80°C until faecal analyses and the preparation of faecal water, respectively. Every volunteer had at least one defecation during the 2 d sampling period. The stool weight of the collected faecal samples (48 h stool) was 462 ± 206 g.

Materials

Unless otherwise described, all chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). Dulbecco's modified Eagle's medium, penicillin and streptomycin, fetal calf serum and phosphate-buffered saline were obtained from Life Technologies (Eggenstein, Germany). Organic solvents were purchased from Merck (Darmstadt, Germany). Carotenoids for HPLC standard solutions were obtained from Carl Roth (Karlsruhe, Germany).

Cell culture

The human colon adenocarcinoma cell line HT29 (passages 11–28) was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were routinely cultivated in 80 cm^2 cell-culture flasks from Nunc (Roskilde, Denmark) in Dulbecco's modified Eagle's medium (with 4·5 g glucose/l) containing 10 % (v/v) fetal calf serum, penicillin G (50 units/ml) and streptomycin (50 μg/ml). Cells were maintained in a humidified atmosphere with 5 % CO_2 at 37°C. Cell-culture medium was replaced three times per week.

Human faecal water preparation

The frozen faeces samples were thawed and homogenised at 4°C (with Stomacher® 400; Stomacher® Laboratory Systems, Northampton, UK). The faecal water fraction (the aqueous phase of human faeces) was prepared by ultracentrifugation of a sample of about 100 g homogenised stool at 60·000 ***g*** for 2 h at 4°C (ultracentrifuge Optima™ XL-100K; Beckmann Coulter, Krefeld, Germany). The supernatant fraction was collected and stored at −80°C.

To ensure sterile conditions for cytotoxicity and proliferation assays, faecal water was sterile filtered (stepwise filtration through 5, 0·65 and 0·22 μm filters, respectively; Millipore GmbH, Eschborn, Germany). Sterile filtered samples were additionally used for the analysis of bile acids and SCFA. Non-filtered faecal water samples served for the determination of carotenoids, pH and bacterial enzyme activities.

Extraction and analysis of carotenoids

Samples of homogenised faecal samples (10 ± 0·1 g, stabilised with 0·22 % butylhydroxytoluene in phosphate-buffered saline) were thawed, diluted with distilled water, and mixed thoroughly. A sample was used for carotenoid extraction with diethyl ether. The organic phase was dried under a stream of N_2 gas and dissolved in 100 μl HPLC mobile phase A (methanol–acetonitrile–2-propanol, 44:52:2 (by vol.), 1 % acetic acid).

Carotenoids were determined by reverse-phase HPLC according to a recently described method[10].

Cytotoxicity and proliferation assays

Influences of faecal water on cell viability and proliferation were determined via the 3-[4,5-dimethyl-thiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. MTT is reduced by viable, metabolically active cells to a blue-coloured formazan dye. According to the manufacturer's protocol, cells were incubated with MTT solution (11 % (v/v) in cell-culture medium) for 1 h. After cell lysis (buffer with 10 % sodium dodecyl sulfate), the absorbance of the formed formazan was measured at 560 nm (reference wavelength 690 nm) using a microplate reader (SpectraFluor Plus; Tecan Deutschland GmbH, Crailsheim, Germany).

For cytotoxicity assays, HT29 cells were seeded into ninety-six-well cell-culture plates (60·000 cells/well) and grown until they reached confluence. Subsequently, cells were incubated

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for 24 h with faecal water samples used in the following concentrations: 10, 5, 2·5, 2 and 1 % (v/v) in cell-culture medium (see Fig. 2 (A)).

For proliferation assays, cells were seeded into ninety-six-well cell-culture plates (5000/well) and incubated with cell-culture medium overnight to allow cells to attach to the plate before treatment. After removing the medium, cells were incubated with faecal water in a concentration of 10, 5, 2, 1 and 0·5 % in cell-culture medium. Cell proliferation was measured after 1, 2 and 5 d of treatment using the MTT test as described earlier. A typical dose-dependent effect of a volunteer's faecal water samples on cell proliferation is shown in Fig. 2 (B).

Extraction, derivatisation and measurement of bile acids

Conjugated bile acids in faecal water samples (100 μl) were hydrolysed for 2 h at 80°C in 1 ml 1 M-alkaline methanol (80 %). After addition of 100 μl HCl (32 %, v/v) and evaporation to dryness under a stream of N_2 gas, the samples were dissolved in 5 ml 0·1 M-phosphate buffer (pH 7·0). The samples were purified using Oasis HLB-cartridges (30 mg; Waters, Milford, MA, USA) that were preconditioned with 2 ml methanol and 5 ml phosphate buffer. After washing with 2 ml buffer, bile acids were eluted with 4 ml methanol. Then 150 μl 1·8 mM-KOH-methanol were added to the dried eluates and samples were evaporated again at 80°C under a stream of N_2 gas and stored overnight at −20°C before derivatisation.

The derivatisation procedure and HPLC analysis of bile acids were performed as reported by Guldutuna *et al.* with some modifications[26]. The prepared samples were incubated with 50 μl dicyclohexyl-18-crown-6-ether (5 mmol/l dissolved in acetonitrile) and 50 μl of the fluorescent substance 4-bromomethyl-7-methoxycoumarin (5 mM in acetonitrile) for 1 h at 37°C. After cooling down for 10 min on ice and centrifugation at 800 ***g*** for 3 min, 20 μl of the samples were injected.

Separation was carried out with a water–acetonitrile (60:40, v/v) mobile phase A and an acetonitrile–methanol–butanol (47:23:30, by vol.) mobile phase B at a constant flow rate of 1 ml/min using a C_{18} column (5 μm; 250 × 4·6 mm; Vydac, Hesperia, CA, USA). Gradient elution was started with 80 % A and was performed by changing the mobile phase B to 100 % (0–5 min 20 % B, 5–15 min to 25 % B, 15–25 min to 30 % B, 25–30 min 30 % B, 30–35 min to 50 % B, 35–36 min 50 % B, 36–40 min to 60 % B, 40–41 min to 70 % B, 41–45 min to 80 % B, 45–55 min to 100 % B). Bile acids were determined with a fluorescence detector (Shimadzu model RF-535, Shimadzu, Duisburg, Germany; excitation at 340 nm, emission at 410 nm) and a chromatointegrator (Merck-Hitachi model D-2500; Merck-Hitachi Ltd., Tokyo, Japan). Peaks of cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, and ursodeoxycholic acid were identified based on relative retention times and by comparison with those of pure standard mixtures.

Measurement of short-chain fatty acids

SCFA were analysed by GC (HP5890II with automatic injector HP7673 II; Hewlett-Packard, Böblingen, Germany). Samples were deproteinated with 10 % formic acid. After high-speed centrifugation, the SCFA acetate, propionate and butyrate were separated isothermally on chromosorb WAW (80/100 mesh) with 20 % neopentenylglucosinolate and 2 % phosphoric acid. Operation conditions were: injection temperature 130°C, carrier flow of N_2 gas 25 ml/min, column oven temperature 130°C, temperature of the flame ionisation detector 250°C.

Activities of the bacterial enzymes β-glucosidase and β-glucuronidase

Bacterial enzyme acitivities were assayed in duplicate by a method of Hylla *et al.*[27] with some modifications. The β-glucosidase reaction was run at 37°C in a total volume of 1 ml consisting of 100 μl 1:10 diluted faecal water, 100 μl 0·01 M-*p*-nitrophenyl glucoside and 800 μl actetate buffer (0·1 mol/l; pH 4·5). The reaction was stopped after 0, 15, 30, 45, 60 and 75 min of incubation by adding 500 μl of a 1:1 mixture of glycine buffer (0·3 mol/l; pH 10·4) and distilled water. The *p*-nitrophenyl released was measured photometrically at 400 nm.

β-Glucuronidase was determined under similar conditions by using 100 μl 0·01 M-phenolphthalein-β-D-glucuronide, 400 μl 1:5 diluted faecal water and 400 μl acetate buffer. The release of phenolphthalein was measured at 552 nm. The enzyme activities were calculated within the linear reaction range by using standard curves for the corresponding substrates. They are expressed as mg liberated substrate per h and per ml faecal water.

Statistical methods

In order to simplify the comparison of dose-dependent cytotoxic or anti-proliferative effects of faecal water preparations from intervention periods and the preceding depletion phases, we calculated non-linear regression curves as represented in Fig. 2 using SigmaPlot version 4.01 from SPSS Science Software (Erkrath, Germany). According to the measurement range of cell assays, we used calculated effective concentrations at 25 % reduction of cell viability (EC_{25}) or half-maximal growth inhibitory concentrations (IC_{50}) from non-linear regression for the following statistical analyses.

All measured parameters were first tested for potential intervention, period or carry-over effects via cross-over design analyses (various *t* tests) using NCSS 97 (NCSS Systems, Kaysville, UT, USA). If period or carry-over effects could not be observed, data of both subject groups of the cross-over intervention trial (group 1, tomato then carrot juice, group 2, carrot then tomato juice) were merged. Paired Student's *t* tests served for analysis of intervention effects from carrot and tomato juice consumption (before *v.* after each juice consumption). These statistical calculations and correlations between different parameters were performed with SigmaStat® 3.0 (SPSS Science Software). Significance level was set at $P \leq 0{\cdot}05$. If the measured data were not normally distributed, equivalent non-parametric statistical tests were used.

As faecal material was limited, unfortunately we could not perform all analyses with every sample. Therefore, the number of investigated volunteer samples is always shown subsequently.

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Inter-assay CV for the analytical carotenoid, bile acid and SCFA measurements described earlier were below 10 %.

Results

Carrot juice supplementation (330 ml/d) for 2 weeks significantly increased faecal contents of β- and α-carotene (Fig. 1). Lycopene amounts were elevated after tomato juice (330 ml/d) consumption. After the following wash-out period, concentrations of these carotenoids in faeces decreased to initial values (Fig. 1).

Carotenoid concentrations in non-filtered faecal water – analysed from 500 μl faecal water samples of four volunteers – were also elevated after consumption of both juices. For example, β-carotene concentration was increased from 0·2(SD 0·2) μmol/l before to 49·2(SD 16·6)μmol/l after carrot juice consumption. Lycopene concentrations averaged 0·7(SD 1·5) μmol/l before and reached 83·1(SD 50·0) μmol/l after tomato juice consumption. Carotenoid concentrations in the sterile filtered faecal water samples were below the detection limit.

Whereas stool weight did not change during the whole study, water content of faecal samples was significantly reduced by 4 % after carrot juice consumption in the first intervention period (Table 1). Additionally, a mean reduction of 0·38 in faecal water pH was observed after carrot juice consumption.

In cell-culture experiments, viability of confluent HT29 cells was always markedly reduced in a concentration-dependent manner after 24 h treatment with faecal water (Fig. 2 (A)). For comparison of cytotoxic effects from the different faecal water samples, we used calculated concentrations causing 25 % reduction in cell viability (EC_{25} values; Table 2). Carrot juice consumption did not cause a change in faecal water cytotoxicity. In contrast, EC_{25} values were increased on average by 1·1 % after tomato juice consumption. However, this reduction in cytotoxicity seemed to be negligible, as it was in the intra-individual range of the test system.

A strong dose-dependent inhibition of cell growth was observed after 5 d treatment of proliferating HT29 cells (Fig. 2 (B)). This incubation time was used for calculation of half-maximal inhibitory concentrations (IC_{50} values; Table 2). Neither tomato nor carrot juice consumption affected the anti-proliferative properties of faecal water.

Further markers in faecal water that could not markedly be modified by juice consumption were the activities of the two bacterial enzymes β-glucosidase and β-glucuronidase (Table 2), as well as the concentrations of bile acids and SCFA (Table 3).

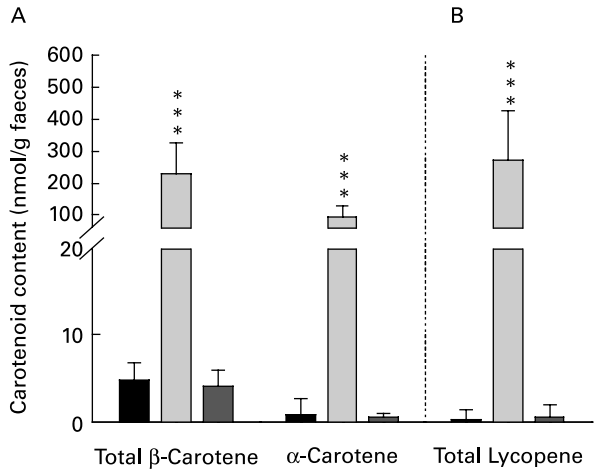

Fig. 1. Faecal content of carotenoids after 2-week periods of depletion preceding (■) and succeeding (□) carrot (A) and tomato (B) juice intervention (■). Carotenoids in faeces were determined including all-*trans* and *cis* configurations. No differences between the two intervention groups of the cross-over trial were observed in any intervention period ($P \geq 0{\cdot}37$), indicating that the sequence of juice consumption had no effect on carotenoid excretion. Therefore, data of faecal carotenoid amounts of both intervention groups were merged. Values are means from all volunteers (*n* 12 per study phase), with standard deviations represented by vertical bars. *** Mean value was significantly different from those preceding and succeeding the juice intervention ($P \leq 0{\cdot}001$).

Discussion

Several epidemiological studies have provided compelling evidence that a high intake of vegetables and fruits rich in carotenoids decreases the risk for many types of cancer including colorectal cancer[2,4,16,28]. Franceschi *et al.*[28] proposed a reduced risk of colorectal cancer of more than 20 % after one additional daily serving of most vegetables including carrots and tomatoes. Cancer-related molecular mechanisms of carotenoids such as cytotoxic, anti-proliferative and apoptosis-inducing effects have been identified *in vitro*[8–10,17]. Whether carotenoids can modulate these mechanisms *in vivo*, especially in the presence of other bioactive compounds within a complex food matrix, is not yet clear.

In the present study, HPLC analysis showed that consumption of carrot and tomato juices caused an increase in the amounts of β-carotene and lycopene in faeces and faecal water. Stepwise filtration showed that carotenoids in faecal water are present in structures with a diameter size ranging between 0·65 and 5·0 μm. HT29 cells can take up carotenoids from emulsions with particles sized between 0·3 and 1·0 μm[29]. In carrots and tomatoes, β-carotene and lycopene, respectively, occur as membrane-bound semi-crystalline structures derived from plastids[30]. The release of carotenoids from the food matrix is a key step in their bioavailability. Goni *et al.*[31] demonstrated by an *in vitro* gastrointestinal digestion model that carotenoids can be released from the food matrix by bacterial enzyme activity and assumed that β-carotene was potentially available for absorption in the colon, because it was not utilised as substrate during *in vitro* colonic fermentation. To our present knowledge, it is unknown in which form carotenoids (associated to indigestible food residues, as precipitated crystals, or incorporated into micelles) occur in faecal water and if they could at least in part be taken up by colonocytes.

Gut luminal compounds, especially free reactive and soluble substances, can contribute to colon tumour development[32]. In the present study, the investigations of filtered faecal water should show potential indirect effects of a carotenoid-rich diet on faecal water composition and its impact on the cell-culture model system HT29. Particularly, non-absorbed water-soluble compounds present in vegetable juices or produced by the intestinal microflora as a consequence of dietary changes could be of importance. HT29 treatment with non-filtered faecal water should provide additional

Table 1. General markers of faecal samples from the intervention trial
(Mean values and standard deviations and number of tested volunteers per intervention group)

Intervention...	Carrot juice				Tomato juice				
	Before consumption		After consumption		Before consumption		After consumption		
Marker	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*n*
Water content (%)	78	6	74*	4	73	5	78	4	6†
Faecal water pH	6·73	0·45	6·34*	0·42	6·76	0·47	6·67	0·41	12

* Mean value was significantly different from that before juice consumption ($P \leq 0{\cdot}05$).
† Carry-over effects ($P = 0{\cdot}4$) were observed during the statistical cross-over design analyses. Therefore, only the first 14 d treatments of the two volunteer groups were included in the analysis of intervention effects from carrot and tomato juice consumption.

information about direct carotenoid effects on the colonic epithelium in the presence of other luminal compounds.

In line with previous studies[33], filtered faecal water samples from our intervention trial showed pronounced cytotoxic effects on confluent HT29 cells. Cytotoxicity, however, was not modified by carrot or tomato juice consumption (Table 2) and it was independent from dilution effects (correlation with water amount in faeces: r −0·04; $P = 0{\cdot}77$). Non-filtered faecal water samples exceeded carotenoid levels that had cytotoxic potential in our previous study[10] (30 μmol/l) and showed stronger cytotoxic effects than filtered ones, but their cytotoxicity was not affected by carrot and tomato juice consumption.

Although we observed strong anti-proliferative effects of isolated β-carotene (IC_{50}: 11–16 μmol/l after 5 d treatment) in a previous study[10], we could not detect modifications in anti-proliferative effects of non-filtered faecal water after carrot and tomato juice consumption (data not shown). An explanation for the lack of effects of the juice interventions might be the high cytotoxic and anti-proliferative potential of faecal water. Even samples containing low or non-detectable amounts of carotenoids (for example, samples from washout phases or filtered samples) had to be diluted for cytotoxicity and proliferation measurements. For example, to reach IC_{50}, faecal water had at least to be diluted about 100-fold (see Table 2). Therefore, it was not possible to detect potential but low carotenoid effects in our test system. Other luminal compounds seem to be more active.

Bile acids are considered to contribute to colon carcinogenesis by disturbing the balance between proliferation, differentiation and apoptosis in colonic cells[33]. *In vitro*, lithocholic acid, chenodeoxycholic acid and deoxycholic acid showed cytotoxic effects on human colon tumour cell lines, including HT29[34]. In the present study, concentrations of bile acids in faecal water did not change significantly after carrot or tomato juice consumption (Table 3). Total concentration of bile acids measured in faecal water was with maximal 33·6 μmol/l considerably below effective IC_{50} bile acid concentrations for HT29 cells[34]. This does not indicate direct bile acid effects on faecal water cytotoxicity in our test system.

A low faecal pH has been associated with a decreased incidence of colon cancer[35]. For instance, changes in the composition of the gut flora and the bacterial production of potentially cancer-protective SCFA are linked to this observation. We observed a marked decrease in faecal water pH

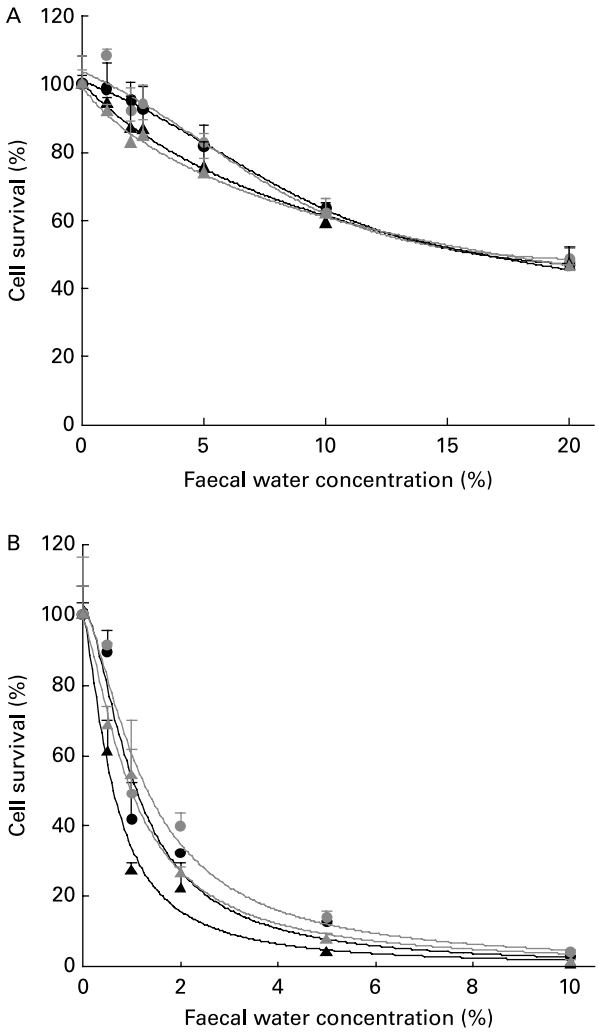

Fig. 2. Dose-dependent cytotoxic (A) and anti-proliferative (B) effects of faecal water samples (non-linear regression curves) from one volunteer on HT29 cells. (A) Cytotoxicity was assessed by the 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test after 24 h treatment. (B) Growth inhibition effects were measured via MTT after 5 d of treatment. Values are the means of one experiment with triplicate determination and are related to the untreated control, which was set at 100 %, with standard deviations represented by vertical bars. Samples are: (●), before carrot juice consumption; (●), after carrot juice consumption; (▲), before tomato juice consumption; (▲), after tomato juice consumption.

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Table 2. Biological effects of faecal water depending on the study phase
(Mean values and standard deviations and number of tested volunteers per intervention group)

Intervention...	Carrot juice				Tomato juice				
	Before consumption		After consumption		Before consumption		After consumption		
Marker	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*n*
Cytotoxicity: EC_{25} values (%)†	6·8	2·7	7·7	1·5	4·8	1·7	5·9*	1·1	6‡
Growth inhibition: IC_{50} values (%)§	0·8	0·2	0·9	0·3	1·1	0·5	1·0	0·6	7
Activity of bacterial β-glucosidase (mg substrate/h × ml)	1·0	0·8	1·2	0·6	1·0	0·4	1·3	0·7	6
Activity of bacterial β-glucuronidase (mg substrate/h × ml)	0·5	0·6	0·5	0·3	0·3	0·2	0·4	0·3	6

* Mean value was significantly different from that before juice consumption ($P \leq 0{\cdot}05$).
† EC_{25} values were calculated by non-linear regression and represent the effective concentrations of faecal water samples in cell-culture medium that lead to a 25 % reduction of HT29 cell viability compared with the untreated control after 24 h treatment.
‡ Period effects ($P = 0{\cdot}02$) were observed during the statistical cross-over design analyses. Therefore, only the first 14 d periods of the two intervention groups were included in the analysis of effects from carrot and tomato juice consumption.
§ IC_{50} values were calculated by non-linear regression and represent the inhibition concentrations of faecal water samples indicating a 50 % reduction of HT29 cell growth compared with the untreated control after 5 d treatment.

after carrot juice consumption. Whether this effect was due to an increased biochemical activity of the microflora or changes in the flora composition should be investigated in further studies. The intake of dietary fibre increased by an average of 7·9 and 8·6 % by carrot and tomato juice consumption, respectively (data not shown). Differences between the effects of carrot and tomato juice intervention could be caused by variations in the composition of dietary fibre or differences in the juice matrices which could influence the sites of colonic fermentation.

The major colonic SCFA acetate, propionate and butyrate that play a central role in colon homeostasis[36] could not have been exclusively responsible for the observed decrease in faecal water pH. Their concentrations in faecal water did not change significantly after carrot juice consumption (and also not after tomato juice consumption). Perhaps SCFA contributed to an increased resorption of water in the distal colon and therefore to the observed decrease in water content of faecal samples after carrot juice consumption (Table 1).

Some bacterial enzymes of the gut flora including β-glucuronidase and β-glucosidase are associated with the generation of potentially carcinogenic metabolites in the colon[37]. In the present study, the activities of β-glucuronidase and β-glucosidase were in the range described in earlier studies[27,38], but were not significantly modified by carrot and tomato juice consumption (Table 2).

We cannot completely exclude the lack of effects due to the limited number of data. To increase the yield of faecal water in future studies, we recommend the modification of the faecal water preparation method, for example, dilution of faecal samples with buffer before centrifugation and use of filters with a larger diameter.

Although *in vitro* experiments with cancer cells have their limitations, they provide important information on molecular

Table 3. Exposition markers of faecal water depending on subject treatment
(Mean values and standard deviations of eight volunteers per intervention group)

Intervention...	Carrot juice				Tomato juice			
	Before consumption		After consumption		Before consumption		After consumption	
Compound	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bile acids (μmol/l)								
CA	1·8	1·0	1·2	0·7	1·8	1·4	2·2	1·9
CDCA	1·0	0·5	0·9	0·6	0·9	0·7	1·3	1·0
DCA	5·8	6·5	3·4	2·3	6·2	4·4	5·2	3·3
LCA	1·1	0·7	1·3	0·9	1·4	0·7	1·8	1·2
UCA	2·7	2·1	1·8	0·6	3·0	2·4	3·9	3·6
Total bile acids	12·4	8·9	8·6	3·3	13·3	7·0	14·5	8·7
SCFA (mmol/l)								
Acetate	75·5	20·4	76·8	22·6	68·8	21·8	70·1	23·3
Butyrate	27·1	11·3	28·5	11·8	23·5	11·3	23·3	8·9
Propionate	22·4	10·3	18·6	6·7	21·9	9·1	18·3*†	8·5
Total SCFA	124·9	34·5	123·9	36·5	114·2	35·0	111·7	31·1

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UCA, ursodeoxycholic acid.
* Mean value was significantly different from that before juice consumption ($P \leq 0{\cdot}05$).
† The reduction of propionate in faecal water after tomato juice consumption seemed to be negligible, as it was in the intra-individual concentration range of the depletion phases of the whole study.

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mechanisms. We selected HT29 cancer cells for our cell-culture test system, as permanent normal human colonic cell lines are not available at present. HT29 cells are well characterised, showed low variance in our earlier experiments and are often used for investigations about the influence of luminal contents on colon carcinogenesis[24,32,39]. Subsequently, conducting a human intervention study that includes the investigation of colonic biopsy material would be of great interest, even though taking biopsies represents an invasive method and also has methodological problems.

In summary, although consumption of both carotenoid-rich juices for 2 weeks increased the carotenoid level in faeces, no changes in the cytotoxic and anti-proliferative properties of faecal water, in the bile and SCFA concentrations, as well as bacterial enzyme activities in faecal water, were observed. This indicates that other anticarcinogenic mechanisms of carrots and tomatoes seem to be of greater importance. Further intervention studies focusing on other markers related to cancer prevention such as protection from DNA damage, induction of phase II enzymes and changes in inflammation markers are required.

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