DOI 10.1002/mnfr.200700406 Mol. Nutr. Food Res. 2008, *52*, 330 – 341

Research Article

Consumption of Brussels sprouts protects peripheral human lymphocytes against 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and oxidative DNA-damage: results of a controlled human intervention trial

Christine Hoelzl[1], Hansruedi Glatt[2], Walter Meinl[2], Gerhard Sontag[3], Gerald Haidinger[1], Michael Kundi[4], Tatjana Simic[5], Asima Chakraborty[1], Julia Bichler[1], Franziska Ferk[1], Karel Angelis[6], Armen Nersesyan[1] and Siegfried Knasmüller[1]

[1] Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Vienna, Austria
[2] Department of Toxicology, German Institute of Human Nutrition (DifE), Potsdam-Rehbrücke, Nuthetal, Germany
[3] Department of Analytical and Food Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
[4] Institute of Hygiene and Medical Microbiology, Medical University of Vienna, Vienna, Austria
[5] Institute of Biochemistry, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[6] Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic

To find out if the cancer protective effects of Brussels sprouts seen in epidemiological studies are due to protection against DNA-damage, an intervention trial was conducted in which the impact of vegetable consumption on DNA-stability was monitored in lymphocytes with the comet assay. After consumption of the sprouts (300 g/p/d, n = 8), a reduction of DNA-migration (97%) induced by the heterocyclic aromatic amine 2-amino-1-methyl-6-phenyl-imidazo-[4,5-*b*]pyridine (PhIP) was observed whereas no effect was seen with 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-2). This effect protection may be due to inhibition of sulfotransferase 1A1, which plays a key role in the activation of PhIP. In addition, a decrease of the endogenous formation of oxidized bases was observed and DNA-damage caused by hydrogen peroxide was significantly (39%) lower after the intervention. These effects could not be explained by induction of antioxidant enzymes glutathione peroxidase and superoxide dismutase, but *in vitro* experiments indicate that sprouts contain compounds, which act as direct scavengers of reactive oxygen species. Serum vitamin C levels were increased by 37% after sprout consumption but no correlations were seen between prevention of DNA-damage and individual alterations of the vitamin levels. Our study shows for the first time that sprout consumption leads to inhibition of sulfotransferases in humans and to protection against PhIP and oxidative DNA-damage.

Keywords: Antioxidant / Comet assay / Cruciferous vegetables / Heterocyclic aromatic amines / Sulfotransferase

Received: February 23, 2007; revised: October 5, 2007; accepted: October 15, 2007

1 Introduction

Epidemiological studies indicate that consumption of Brassica vegetables is inversely related to the incidence of various forms of cancer [1]. In case-control studies, decreased risks for lung, stomach and colon cancers were observed and in cohort studies inverse associations were found (for reviews see [1, 2]). The strongest evidence for protective effects comes from studies in which the relation between the colon and colorectal tumors and consumption of cruci-

Correspondence: Dr. Siegfried Knasmüller, Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria
E-mail: siegfried.knasmueller@meduniwien.ac.at
Fax: +43-1-4277-9651

Abbreviations: Endo III, endonuclease, **FPG**, formamido-pyrimidine – DNA-glycosylase; **GPX**, glutathione peroxidase; **HAs**, heterocyclic aromatic amines; **PhIP,** 2-amino-1-methyl-6-phenyl-imidazo-[4,5-*b*]pyridine**; ROS**, reactive oxygen species; **SCGE**, single-cell gel electrophoresis; **SOD**, superoxide dismutase; **SULT**, sulfotransferase; **Trp-P-2,** 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole

ferous vegetables was investigated [3, 4]. The assumption that Brassicas may reduce colon cancer risks is further supported by investigations, which indicate that these vegetables inhibit induction of DNA-damage and preneoplastic lesions in rodents by heterocyclic aromatic amines (HAs); also in experiments with human cells protective effects towards these carcinogens were observed [5 – 8]. HAs are formed during cooking of meat [9] and are considered to be involved in the etiology of colon cancer [10].

Aim of our study was to elucidate whether Brussels sprouts protect humans against DNA-damage caused by HAs. Therefore, we developed a protocol for single-cell gel electrophoresis (SCGE) assays that allows studying HA-induced DNA-damage in lymphocytes before and after consumption of the vegetables. Over the last years, strong efforts have been made to identify HA-protective constituents and more than 600 individual compounds and dietary mixtures have been tested [11, 12]. However, data from human studies are scarce. It is not possible to monitor effects of HAs in man with conventional methods (*e. g.* in micronucleus assays or chromosomal aberration tests) due to the low levels of HAs in the diet and the only approaches that have been used are chemical analyses of metabolites in urine [13] and urinary bacterial mutagenicity assays [11], which provide only indirect evidence.

SCGE experiments are based on the measurement of DNA-migration in an electric field and have been used in many investigations to study prevention of formation of single- and double-strand breaks by dietary factors [14, 15]. In addition, protocols were developed for SCGE studies that enable monitoring the endogenous formation of oxidized bases with enzymes [16] and changes of the sensitivity towards DNA-damage induced by reactive oxygen species (ROS) [17]. Recently, Anderson and co-workers [18, 19] showed that HAs cause DNA-damage in human lymphocytes and conducted *in vitro* experiments in which they showed that certain dietary constituents protect the cells against amine-induced DNA-damage [19].

We used in the present study Brussels sprouts since we found earlier that they are more protective towards formation of HA-induced preneoplastic lesions in rats than other vegetables [20] and prevent induction of DNA-damage by the amines in human-derived liver cells [21]. The two HAs we used in the present study were 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) and 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP). Both compounds are carcinogenic in rodents and are contained in fried meats [22, 23]. PhIP is the most abundant HA in hamburgers and fried chicken [22, 24, 25]. Trp-P-2 is found less frequently but its genotoxic activity in liver cells is higher than that of other HAs [26] and it is also a more potent rodent carcinogen than other amines [27]. Both compounds are activated via hydroxylation by CYP1A isozymes [28], in the case of PhIP additional conjugation reactions take place that are catalyzed by sulfotransferases (SULTs). Therefore, we measured in the present study also the impact of sprout consumption on the activities of these enzymes.

Since it is assumed that also oxidative damage is involved in human cancer [29, 30], we monitored in the same trial also the formation of single- and double-strand breaks as well as DNA-damage due to formation of oxidized bases and alterations of the sensitivity of the lymphocytes towards ROS-induced DNA-migration. In order to provide mechanistic explanations, we studied the impact of sprout consumption on the activities of antioxidant enzymes, namely glutathione peroxidase (GPX) and superoxide dismutase (SOD) and on vitamin C levels in the plasma and investigated the scavenging effects of sprout juice in *in vitro* experiments.

2 Materials and methods

2.1 Chemicals

Trp-P-2 (CAS No. 62450-07-1) and PhIP (CAS No. 105650-23-5) were purchased from the Nard Institute (Nishinagasu Amagasaki, Japan), DMSO was from Merck (Darmstadt, Germany). RPMI 1640 medium, trypan blue, ethidium bromide, Histopaque-1077, nicotinamide adenine dinucleotide phosphate (NADPH), pentachlorphenol, 4-nitrophenol, vanillin and barium acetate were from Sigma-Aldrich (St. Louis, MO). Low and normal melting agarose (LMA, NMA) were from Invitrogen Life Technologies (Paisley, Scotland). [^{35}S]-3′-Phosphoadenosine-5′-phosphosulfate (PAPS) was from Perkin Elmer Life Sciences (Boston, MA). Endonuclease III (Endo III) and formamidopyrimidine – DNA-glycosylase (FPG) were produced as described by Boiteux *et al.* [31] and Rogers and Weiss [32].

2.2 Intervention studies

The design of the two intervention trials, which we carried out is shown in Fig. 1. The Austrian Ethical Commission approved the study and informed consent was obtained from all participants. Eight healthy, non-smoking volunteers (four males and four females) were included in the first experiment in which we monitored DNA stability parameters as well as GPX, SOD and vitamin C levels in plasma.

It is known that age, body mass index (BMI) [33], intake of supplements [14], cigarette smoking [34] and dietary habits [35] may affect the results of SCGE trials [36]. Therefore, these parameters were recorded and attempts were made to exclude confounding factors. The average age of the probands was 33 ± 7, the BMI was on average 21.3 ± 1.5 for females and 22.5 ± 1.5 for males, all of them were non-smokers, none of them consumed supplements 2 months before and during the intervention and all of them were non-vegetarians. Five days before ("run-in" phase) and during the intervention, the participants consumed a controlled diet, refrained from intake of >200 g/d of citrus

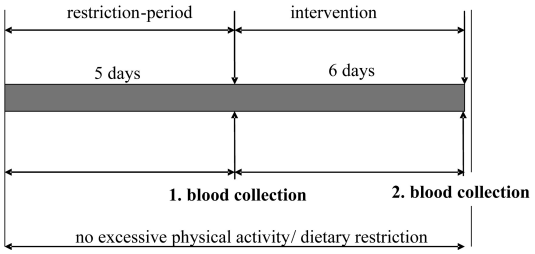


Figure 1. Design of the intervention study.

fruits, fruit juices, onions and whole-meal products and did not consume cruciferous vegetables. The participants were also asked not to consume >200 mL wine, beer or coffee per day and to avoid exhausting physical exercises, which may cause comet formation [37]. During the intervention, the participants consumed one portion (300 g) of Brussels sprouts/person/day over a period of 6 days. Deep-frozen sprouts were steamed for 10 min in a microwave (8 min, 950 W, Micromat 175Z, AEG Elektrolux, Stockholm, Sweden) at lunchtime and were consumed in two portions (150 g each), one at 12 noon and the second at 6 p.m. Blood was collected after the run-in phase on the sixth intervention day at 9 a.m. (3 h before consumption of the sprouts) and 15 h after the consumption of the last portion on the 12th day. The overall glucosinolate content (38 μmol/g) as well as individual glucosinolates of the sprouts cultivar used in the present study were characterized by chemical analysis (for details see Kassie *et al*. [6]). At the beginning and at the end of the study, 10 ml blood was collected in heparinized tubes (BN Vacutainer Systems, Plymouth, UK). Lymphocytes were isolated using Histopaque-1077 (density 1.077 g/L) according to Duthie *et al*. [38]. After the first centrifugation (10 min, 4°C, 650 × *g*), plasma was collected and stored at −80°C. Fresh lymphocytes were resuspended in PBS and used for SCGE experiments. Cells for enzyme measurements were stored at −80°C. For the biochemical assays, lymphocytes were lysed by three freeze-thaw cycles.

The impact of Brussels sprouts consumption on the expression and activities of SULT1A1 and 1A3 was investigated in a second independent experiment with identical design and the same individuals as in the first trial (except one) participated in this study.

2.3 SCGE with lymphocytes

The experiments were conducted according to the guidelines of Tice *et al*. [39]. To define optimal exposure concentrations for Trp-P-2 and PhIP, they were dissolved in DMSO and the cells exposed (37°C) to different concentrations for 30 min. All experiments were carried out with three cultures per experimental point and in each acute toxic effects were monitored with trypan blue, which measures the integrity of the cell membranes [40]. Only cultures with survival rates were ≥80% were analyzed for comet formation (50 cells/slide). To compare comet formation before and after the vegetable consumption, the cells were either analyzed without pretreatment under standard conditions (20 min electrophoresis, 25 V, 300 mA), or the nucleotides were treated with FPG for 30 min or with Endo III for 45 min according to the protocol described by Collins and co-workers [16, 41]. In parallel, experiments with the enzyme buffers were carried out which were composed as described by Collins *et al*. [16]. DNA-damage attributable to formation of oxidized bases purines or pyrimidines was determined by calculating the differences of the values obtained in presence and absence of the enzymes. To study alterations of the ROS sensitivity, lymphocytes (0.1×10^6 per experimental point) were exposed on ice to H_2O_2 (100 μM, 5 min), or to Trp-P-2 (0.2 mM) or PhIP (0.7 mM) at 37°C for 30 min.

In additional *in vitro* experiments, cells from one donor were treated with fresh sprout juice (5–80 μL) and H_2O_2 (50 μM) for 5 min. The juice was prepared after short cooking (10 min) of the vegetables with a blender, subsequently it was centrifuged twice (20 min at 3000 × *g* and 10 min at 10 000 × *g*) and filter sterilized (Schleicher and Schuell, 0.2 μm).

After the treatment, the cells were washed with PBS (pH 7.4), mixed with 80 μLl of LMA (0.5%) and transferred to agarose-coated slides. From each donor, three cultures were made in parallel and only cultures with a viability ≥80% were analyzed for comet formation. The slides were immersed in lysis solution (1% Triton X, 10% DMSO, 2.5 M NaCl, 10 mM Tris, 100 mM Na_2EDTA, pH 10.0) at 4°C for at least 1 h. After electrophoresis, the DNA was stained with 40 μL ethidium bromide (20 μg/mL) and the tail lengths (TL) and tail moments (TM) measured with an image analysis system [42].

2.4 Measurement of antioxidant enzymes

SOD activity was determined in cell lysates with the RANSOD test kit (Randox Labs., Ard-more, UK) [43], which is based on the use of xanthine and xanthine oxidase to generate O^{2-} radicals. The activity was measured spectrophotometrically by determination of the degree of inhibition of O_2 induced formation of a red formazan dye (λ = 505 nm). GPX was measured by the coupled assay procedure of Gunzler *et al*. [44]. All measurements were carried out in triplicate.

2.5 Determination of SULT1A1 and SULT1A3

The activities of SULT1A1 and SULT1A3 in the lymphocyte homogenates were measured by a modified radioenzymatic method according to Foldes and Meek [45] using characteristic substrates for SULT1A1 (4-nitrophenol) and SULT1A3 (vanillin). Briefly, 40 μg (SULT1A1) or 20 μg

(SULT1A3) of total protein were incubated in a final volume of 150 µL adjusted with ice-cold buffer (150 mM potassium chloride, 10 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) in presence of 50 pmol $PAP^{35}S$ (20 µCi) and 4 µM 4-nitrophenol (SULT1A1) or 33 µM vanillin (SULT1A3) at 37°C for 10 min. All measurements were made in duplicate. For each sample, background values were determined in tubes containing all components but the substrate. After incubation, unreacted PAPS was precipitated with 200 µL of each, 0.1 M barium acetate, 0.1 M barium hydroxide and 0.1 M zinc sulfate. Subsequently, 500-µL aliquots of the supernatants containing the sulfo-conjugate were analyzed in a Beckman LS6500 scintillation counter (Beckman Coulter, Fullerton, USA). Activities are expressed as pmol sulfo-conjugate formed per minute and milligram protein.

2.6 Immunodetection of SULT1A1 and SULT1A3

SULT1A1 and SULT1A3 were measured using Western blots [46]. The protein concentrations in the lymphocyte homogenates were determined with the BCA Protein Assay Kit (Pierce, Rockford, IL). Proteins (separated by SDS-PAGE according to Laemmli 1970 [47]) were transferred to a nitrocellulose membrane (Hybond™, ECL™, Amersham Biosciences, Freiburg, Germany). After blotting, the membrane was incubated for 60 min with primary antisera (diluted 1:10 000) raised in sheep against human SULT1A3 and 1A1 (both antibodies were kindly provided by M. W. H. Coughtrie, University of Dundee, UK) [48], followed by incubation with a second, peroxidase-conjugated antibody (Sigma-Aldrich. Munich, Germany). Subsequently, the membrane was treated with Western Lightning™ Chemiluminescence Reagent Plus (Perkin Elmer Life Sciences, Boston, MA) and analyzed with a luminescent image analyzer LAS-1000 (Raytest, Straubenhardt, Germany). The band areas and intensities between the samples were compared by use of the AIDA software package (Raytest, Urdorf, Switzerland).

2.7 Determination of vitamin C levels in plasma

Plasma vitamin C levels were determined by means of HPLC using a coulometric electrode array detector (ESA Coulochem Electrode Array System, Genova, Italy) and a reversed phase analytical column (Spherisorb ODS2, 5 µm, 250 × 4 mm, Hewlett Packard, Munich, Germany). As liquid phase, a 200-mM aqueous solution of KH_2PO_4 (pH 3.0) was applied as described by Rose *et al*. [49].

For sample preparation, 250 µL serum was defrosted on ice in the dark. Subsequently, an equal amount of 10% phosphoric acid was added. After centrifugation (4°C, 3 min, 15 000 × *g*), the supernatant was transferred into an ultracentrifugation tube (Ultrafree1-Centrifugal Filter Units, NMWL 5000, Millipore, Bedford, MA), covered with nitrogen and subjected to an additional centrifugation step (30 min, 5000 × *g*, 4°C). Subsequently, the filtrates were transferred into autosampler glass vials and the vitamin C contents measured with gallic acid as an internal standard.

2.8 Statistics

Differences in 90th percentile tail lengths were tested by ANOVA. For *in vitro* experiments, Dunnett's tests were performed to compare the different test conditions with the control condition in case of a significant main effect ($p < 0.05$) of experimental conditions. For analysis of experiments in human volunteers, two-factor ANOVA were carried out with before/after vegetable consumption as the experimental factor and subjects as a random factor. In all tests, two-sided *p*-values below 0.05 were considered significant. Prior assessment of statistical power revealed that applying a within subject design with eight volunteers and a targeted 30% reduction of DNA migration three replicates lead to a power above 95% and a significance level of 5%.

3 Results

3.1 *In vitro* experiments with HAs

To define optimal exposure concentrations of the amines, *in vitro* experiments with different concentrations of Trp-P-2 and PhIP were carried out. Representative results of these experiments are shown in Figs. 2a and b. With Trp-P-2, a concentration-dependent induction of DNA-migration was seen (Fig. 2a). The effects were significant at exposure concentrations ≥ 0.3 mM; under the same conditions also moderate cytotoxicity was observed. Also with PhIP, induction of DNA-migration was observed (Fig. 2b), but a significant effect was detected only at the highest concentration (0.7 mM) and the acute toxicity was less pronounced than that seen with Trp-P-2.

3.2 Impact of sprout consumption on HA-induced DNA-migration

The impact of sprouts consumption on HA-induced DNA-damage is shown in Fig. 3. No significant alteration of Trp-P-2-induced damage was seen after the intervention (Fig. 3a), whereas the effects of PhIP were completely alleviated (Fig. 3b). The results found in the different individuals are depicted in Fig. 3c and it can be seen that protective effects were observed in all participants.

3.3 Effect of Brussels sprouts consumption on protein levels and activities of SULT1A1 and 1A3

We showed earlier that SULTs play a critical role in the bioactivation of PhIP [50, 51]. Therefore, we decided to repeat the intervention study and to examine SULT expression

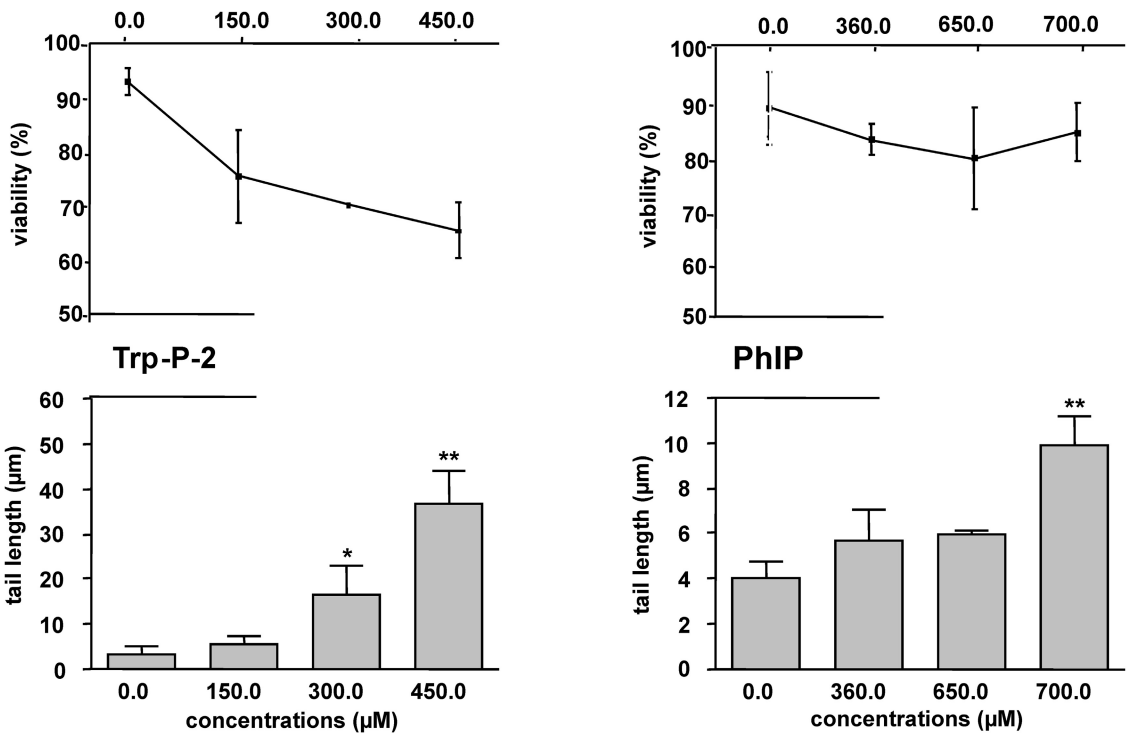


Figure 2. Induction of DNA migration and of acute toxic effects in peripheral human lymphocytes by Trp-P-2 (a) and PhIP (b). Each experimental point represents the means ± SD of results obtained with three cultures. Asterisks indicate statistical significance (* *p*-value < 0.05, ** *p*-value < 0.01).

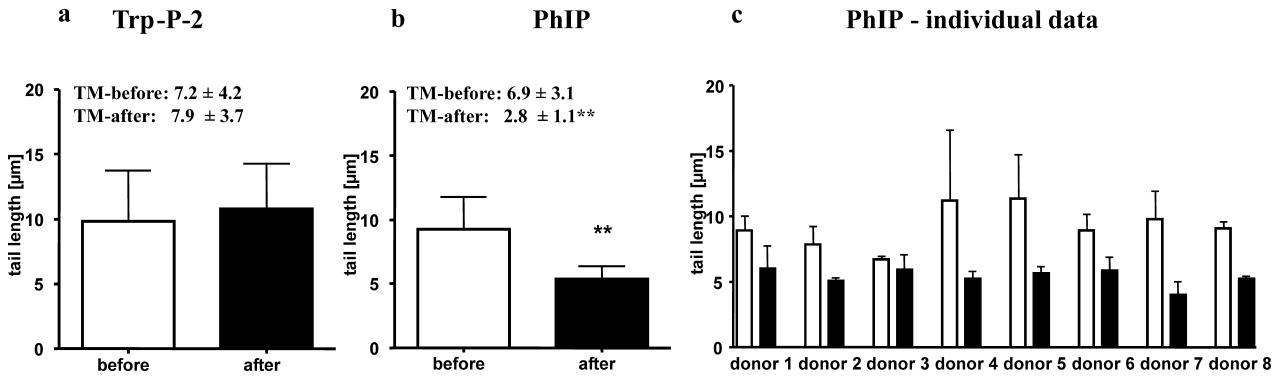


Figure 3. Prevention of HA-induced DNA damage in lymphocytes by consumption of Brussels sprouts (300 g/p/d, 6 days, $n = 8$). The cells were collected before and after consumption of the vegetables and analyzed for DNA migration after treatment with Trp-P-2 (a) or PhIP (3b) for 30 min. In (c) the results obtained with PhIP with the individual participants are depicted. With the solvent control (DMSO) the average tail lengths were 6.2 ± 0.9 before and 6.8 ± 1.7 after the intervention. From each individual, three cultures were made in parallel. TM, tail moment. Asterisks indicate statistical significance (* *p*-value < 0.05, ** *p*-value < 0.01).

before and after consumption of the vegetables in lymphocytes of the same subjects that had participated in the first experiment. SULT1A1 and 1A3 were the only SULT forms detected in the lymphocytes using an antiserum directed against SULT1A forms. SULT1B1 could not be detected although a highly sensitive antiserum was used. The effects of sprout consumption are shown in Figs. 4a – c. The protein levels and the activities of both isozymes were significantly reduced after the consumption. Although the levels of these enzymes varied substantially between the different individuals, a decrease was observed in each participant and a reduction in the enzyme activities was seen in all subjects except in one. According to information collected after the trial, these participants consumed all servings and had no digestive problems during the trial, but it cannot be excluded that other (yet unknown) dietary factors may have

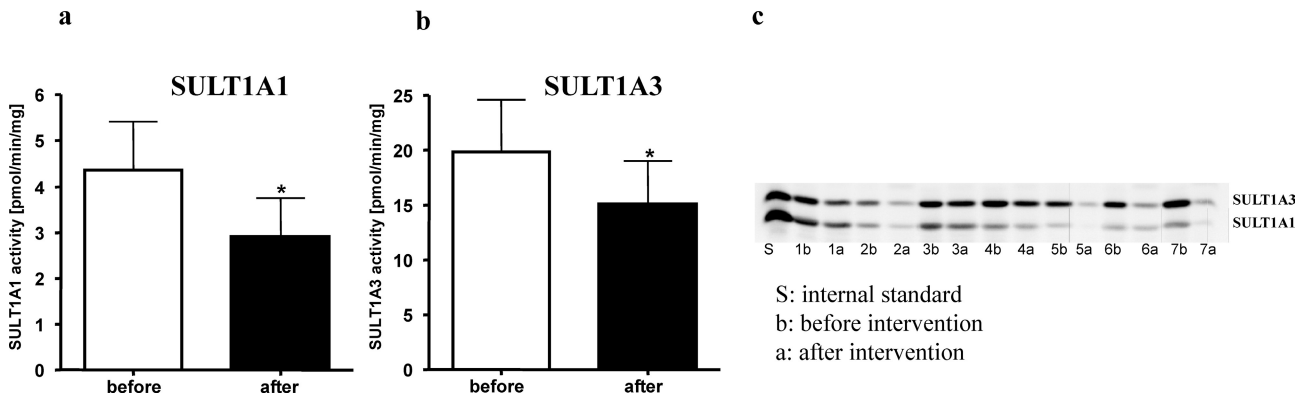


Figure 4. Impact of sprouts consumption on the expression and on the activities of SULT1A1 (a) and 1A3 (b) in homogenates of lymphocytes. The results of Western blot analyses are depicted in (c). White bars: means ± SD before consumption of sprouts, black bars: means ± SD after consumption of sprouts. Asterisks indicate statistical significance (*p-value < 0.05).

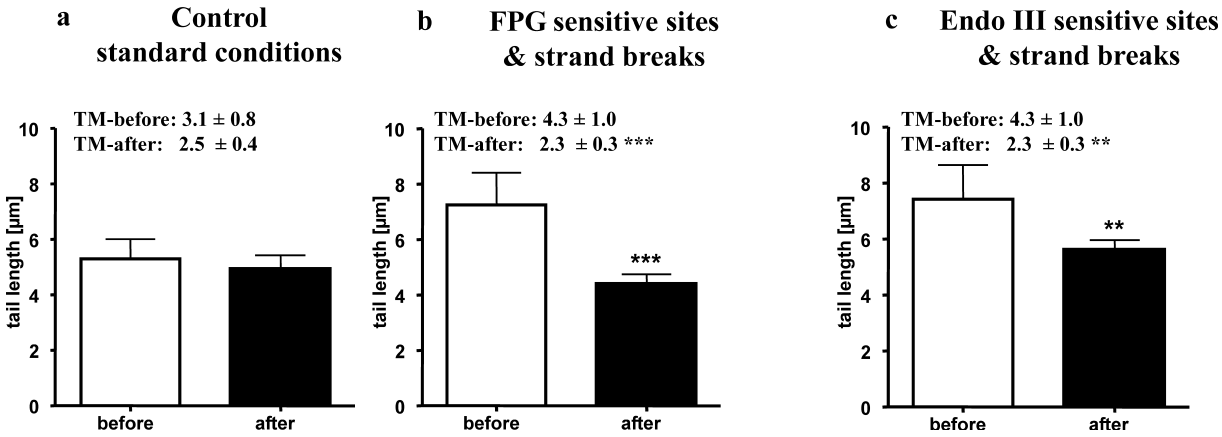


Figure 5. Prevention of oxidative DNA-damage in lymphocytes by Brussels sprouts (300 g/p/d, 6 days, n = 8). Before and after consumption of the vegetables, lymphocytes were collected and analyzed for DNA damage either under standard conditions (20-min electrophoresis, 25 V (a) or after exposure of the nucleotides to FPG (b) or Endo III (c). Values obtained with enzyme buffers were 5.4 ± 0.4 before and 4.5 ± 0.4 after intervention for Endo III and 5.3 ± 0.4 before and 4.5 ± 0.5 after the intervention period for FPG, respectively. TM, tail moment. Asterisks indicate statistical significance (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.0001).

caused an inhibition of SULT activity when the intervention started.

3.4 Effect of sprout consumption on endogenous and ROS-induced DNA-migration

When the SCGE experiments were carried out under standard conditions (20-min electrophoresis, 25 V) only weak DNA-migration was observed and no significant difference was seen before and after consumption of the vegetables (Fig. 5a). This condition reflects DNA-migration due to formation of single- and double-strand breaks as well as apurinic sites [39]. The extent of DNA-migration due to formation of oxidized DNA bases was calculated by subtracting the tail lengths measured after treatment of the nucleotides with the DNA lesion-specific enzymes (see Figs. 5b and c) from values obtained with the corresponding buffers, which were 5.4 ± 0.4 before the study and 4.5 ± 0.4 for Endo III and 5.3 ± 0.4 before and 4.5 ± after the intervention period for FPG. After consumption of the vegetables, no migration attributable to formation of oxidized purines was detectable (FPG-sensitive lesions) and DNA-migration due to oxidized purines was reduced by 45%.

Also in experiments with H_2O_2, which reflect the sensitivity of the cells towards exogenous DNA-damage caused by ROS ($OH^{\bullet}$, O^{2-}), a strong overall protective effect was observed, *i.e.* DNA-migration was reduced by 39% after consumption of the sprouts (Fig. 6a) and it can be seen in Fig. 6b that a decrease of DNA-migration was found in all participants.

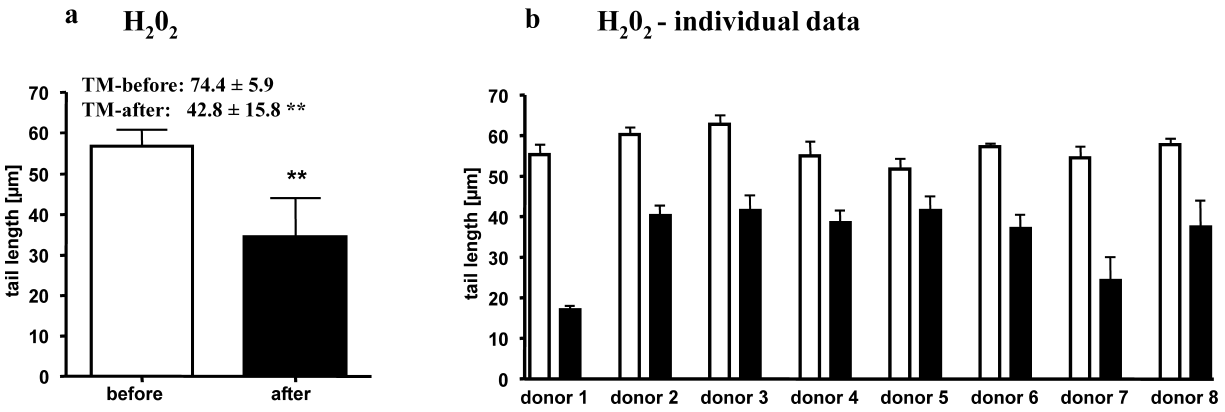
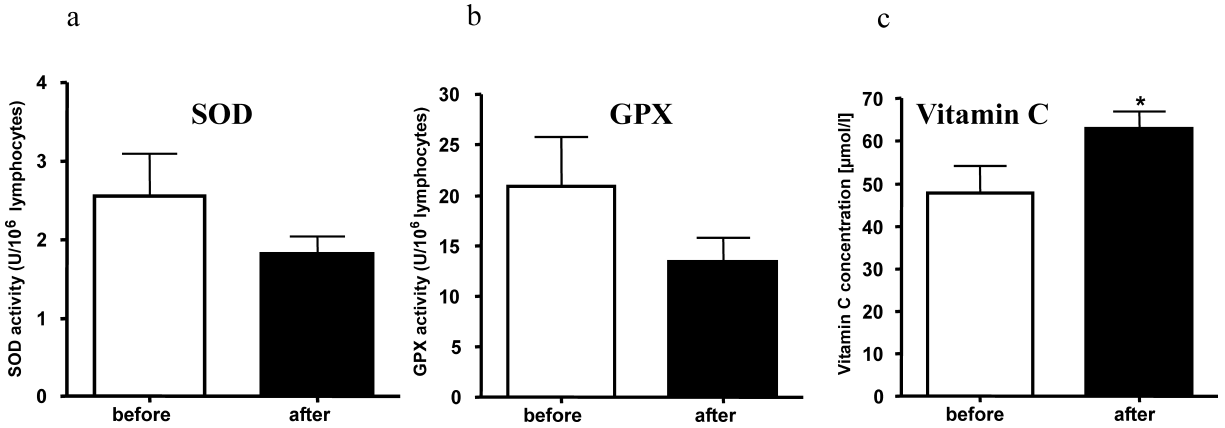


Figure 6. Impact of Brussels sprouts consumption on H_2O_2-induced DNA migration in peripheral lymphocytes. White bars: DNA migration before vegetable consumption, black bars: DNA migration after vegetable consumption. TM, tail moment. Asterisks indicate statistical significance (**p-value < 0.01).



Figures 7. Impact of sprouts consumption on the activities of SOD (a) and GPX (b) and on the vitamin C concentrations in the plasma (c). White bars: means ± SD before consumption of sprouts, black bars: means ± SD after consumption of sprouts. Asterisks indicate statistical significance (*p-value < 0.05).

3.5 Effect of sprout consumption on the activities of antioxidant enzymes

The activities of SOD and GPX in plasma before and after sprouts consumption are depicted in Figs. 7a and b; it can be seen that no significant alterations were found at the end of the trial.

3.6 Effect of Brussels sprouts consumption on vitamin C concentration

The effects of vegetable consumption on plasma vitamin C levels are shown in Fig. 7c. The average concentration was increased by 37% after the intervention. However, correlation analyses failed to find significant relations between the reduction of the different parameters of oxidative damage (*i. e.* ROS sensitivity and formation of oxidized bases) and alterations of the vitamin concentrations in the different individuals.

3.7 Effect of Brussels sprouts juice on ROS-induced DNA-damage in vitro

The results of experiments that were conducted to study ROS scavenging effects of sprout juice are shown in Figs. 8a and b. Exposure of lymphocytes to different amounts of juice caused no alterations of the pattern of DNA-migration (Fig. 7a), but a strong protective effect towards H_2O_2-induced damage was observed under identical conditions (Fig. 8b).

4 Discussion

The results of the present study show that the SCGE technique can be used in human intervention trials to detect protective effects of dietary factors towards HA-induced DNA-damage. At present, evidence for potential protective effects towards HAs in humans is restricted to results gener-

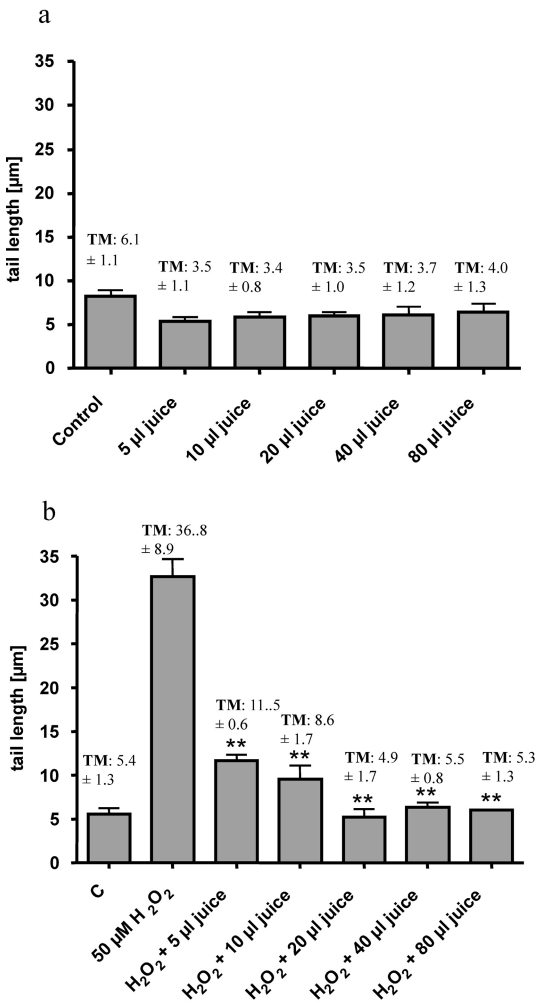


Figure 8. Effect of Brussels sprout juice on ROS-induced DNA damage in lymphocytes *in vitro*. The cells were treated with different amounts of sprout juice (5–80 µL) in absence or presence of H_2O_2 (50 µM). TM, tail moment. Asterisks indicate statistical significance (**p-value < 0.01).

ated with indirect methods such as chemical analyses of urinary excretion of HA metabolites [11] and urinary bacterial mutagenicity tests [13, 52, 53] and we anticipate that the approach described in this article will be useful for future investigations.

One of the disadvantages of our *ex vivo* experiments is due to the fact that relatively high concentrations of HAs are required as the activities of cytochrome enzymes which catalyze the activation of HAs are low in lymphocytes [54]. However, as mentioned above Anderson and co-workers [19] carried out *in vitro* SCGE experiments with lymphocytes and high doses of different HAs and identified a number of protective dietary components, for example quercetin, silymarin and myricetin. These compounds were also found to reduce induction of preneoplastic lesions and/or DNA-damage in inner organs of laboratory rodents and in some cases suppress chemical carcinogenesis [55–58] indicating that comet experiments with peripheral blood cells reflect indeed protective effects.

As described in Section 3, consumption of Brussels sprouts lead to pronounced protection towards PhIP-induced DNA-migration (Figs. 3b and c) whereas no effect towards Trp-P-2 was seen. This can be at least partly explained by the fact that the levels and activities of SULT1A1, which plays a key role in the activation of PhIP but not of Trp-P-2 [50, 51], were significantly lower after the intervention (Figs. 4a–c). We found in earlier experiments with V79 cells expressing human SULT1A1 that PhIP but not Trp-P-2 is activated by this isozyme to DNA-reactive metabolites (Muckel and Glatt, unpublished). When lymphocytes were simultaneously treated with pentachlorphenol, a known inhibitor of SULTs *in vitro* [59, 60] and Trp-P-2 or PhIP, pronounced inhibition of DNA-migration caused by the pyridine was observed whereas the DNA-damaging potential of the tryptophan pyrolysate was not affected [61]. These observations show that SULT plays a crucial role in the activation of PhIP and provide a plausible explanation for protective effects, which were seen towards PhIP in the present study.

SULT1A1 is not only involved in the activation of PhIP but also catalyses the formation of mutagenic metabolites from other carcinogens, such as aminocarbolines some homocyclic aromatic amines [62], nitroarenes [63], polycyclic aromatic hydrocarbons [64], secondary nitro alkanes [65] and Maillard products [51, 66–68] and it is conceivable that the inhibition of this isozyme by Brussels sprouts leads to protection against DNA-damage caused by these compounds in humans. To our knowledge, the present study is the first, which shows that a dietary factor causes inhibition of SULT in humans. Attempts to find out which constituents of Brussels sprouts account for the inhibition and to clarify in animal experiments if the activities of SULT are also reduced in inner organs are under way.

A number of animal studies showed that several other dietary components, for example conjugated linoleic acid [69], coffee-specific diterpenoids [70, 71] and isothiocyanates (breakdown products of glucosinolates contained in cruciferous vegetables) [72], protect against PhIP-induced DNA-damage and induction of preneoplastic lesions [11, 12, 71] However, for all these compounds other mechanisms of protection, *i. e*. induction of phase II enzymes such as glucuronosyl-transferase and glutathione-S-transferase [72] as well as alterations of the activities of activating enzymes (CYP1A, N-acetyltransferases) were postulated [71, 73]. Walters *et al*. [74] reported recently the results of a human study in which they found increased excretion of the detoxification product N^2-hydroxy-N^2-PhIP-glucuronide after continuous consumption of Brussels sprouts. This interesting finding is not in contrast to our assumption that inhibition of SULT1A1 accounts for the protective effects towards PhIP as it is conceivable that inhibition of activa-

tion of PhIP by SULTs may lead to increased formation of detoxification products.

Consumption of Brussels sprouts was also found to cause strong protection towards oxidative DNA-damage. The sensitivity of the cells towards ROS (H_2O_2) induced DNA-damage was reduced by 39% (Figs. 6a and b), endogenous formation of oxidized purines was completely inhibited and DNA-damage attributable to oxidized pyrimidines was reduced by 45% (Figs. 5b and c). These effects could be not explained by induction of the activities of the antioxidant enzymes GPX and SOD (Figs. 7a and b) and the results of our *in vitro* experiments (Fig. 8b) are in agreement with the findings of Zhu *et al*. [75] and suggest that sprout juice contains constituents that are highly protective against ROS. Since the effects were seen after short treatment of the cells, *i. e*. under conditions under which enzyme activities are not induced, it is likely that these compounds act as direct scavengers.

Brussels sprouts contain relatively high amounts of vitamin C [76], therefore, we hypothesized that this vitamin may be responsible for the protective effects seen in the intervention trial. Although the serum concentrations were significantly increased at the end of the intervention (Fig. 6c), statistical analyses failed to find correlations between prevention of oxidative DNA-damage and changes in the vitamin concentrations at the individual level. In earlier studies, significant reduction of oxidative DNA-damage in lymphocytes was only observed after consecutive consumption of high doses of the vitamin C (1000 mg/person/day) [77], whereas supplementation with lower amounts was not effective [78 – 80]. In *in vitro* experiments concerning the antioxidative properties of Brussels sprout extracts, it was found that the most effective fraction coeluted with sinigrin, a glucosinolate that is contained in Brussels sprouts, furthermore the authors stressed that the pattern of activity of the extract was different from that seen with vitamin C [75]. These findings indicate that compounds other than vitamin C account for the antioxidant effects [81].

Our observation of a reduction of oxidative DNA-damage after consumption of Brussels sprouts is in agreement with the findings of Verhagen *et al*. [82, 83], who showed that uptake of the vegetables reduces the levels of 8-oxo-dG in the urine of humans. Also in experiments with rats, a significant reduction of excretion the oxidized base was seen and this effect was paralleled by decreased formation of oxidized guanine in the kidney [84]. In this context, it is notable that Gedik *et al*. [85] showed that urinary excretion of 8-oxo-dG in humans correlates with FPG-specific DNA-migration in lymphocytes.

Also in human intervention studies with other vegetables and fruits, for example with tomato puree (25 g/person/14 days), kiwi juice (a single dose of 500 mL) and a mix of germinated sprouts, protection towards oxidative DNA-damage was observed in SCGE assays [86, 87]. On the contrary, no effects were seen in a trial, in which a relatively large amount of a mixed vegetable-fruit concentrate (500 g/person/day) was consumed by the participants [88]. Also, continuous uptake of 600 g of fresh fruits and vegetables/person/day had no impact on the parameters monitored in the present study and on 8-oxo-dG excretion in the urine [89]. These negative findings are probably due to the fact that only specific plant foods are protective against oxidative DNA-damage.

Our study involved only few participants and in order to further substantiate our findings, further trials with a cross over design, additional endpoints and a higher number of probands are planned. As described above (Section 2.8) the calculation of the statistical power of our experiments support the assumption of a DNA protective effects of sprouts consumption against PhIP- and ROS-induced DNA-damage and our findings provide plausible explanation of the cancer protective effects seen in animal experiments [90] and in epidemiological studies [1, 91].

The authors have declared no conflict of interest.

5 References

[1] Steinmetz, K. A., Potter, J. D., Vegetables, fruit, and cancer prevention: a review, *J. Am. Diet Assoc*. 1996, *96*, 1027 – 1039.

[2] Van Poppel, G., Verhoeven, D. T., Verhagen, H., Goldbohm, R. A., *Brassica* vegetables and cancer prevention. Epidemiology and mechanisms, *Adv. Exp. Med. Biol*. 1999, *472*, 159 – 168.

[3] Lee, H. P., Gourley, L., Duffy, S. W., Esteve, J., *et al*., Colorectal cancer and diet in an Asian population – a case-control study among Singapore Chinese, *Int. J. Cancer* 1989, *43*, 1007 – 1016.

[4] Seow, A., Yuan, J. M., Sun, C. L., Van Den Berg, D., *et al.*, Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study, *Carcinogenesis* 2002, *23*, 2055 – 2061.

[5] Schwab, C., Kassie, F., Qin, H.-M., Sanyal, R., *et al.,* Development of test systems for the detection of compounds which prevent the genotoxic effects of heterocyclic aromatic amines: preliminary results with constituents of cruciferous vegetables and other dietary constituents, *J. Environ. Pathol. Toxicol. Oncol*. 1999, *18*, 109 – 188.

[6] Kassie, F., Uhl, M., Rabot, S., Grasl-Kraupp, B., *et al.*, Chemoprevention of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen, *Carcinogenesis* 2003, *24*, 255 – 261.

[7] Kassie, F., Rabot, S., Uhl, M., Huber, W., *et al.*, Chemoprotective effects of garden cress (Lepidium sativum) and its constituents towards 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions, *Carcinogenesis* 2002, *23*, 1155 – 1161.

[8] Huber, W. W., McDaniel, L. P., Kaderlik, K. R., Teitel, C. H., *et al.,* Chemoprotection against the formation of colon DNA adducts from the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat, *Mutat. Res*. 1997, *376*, 115 – 122.

[9] Jagerstad, M., Skog, K., Formation of meat mutagens, *Adv. Exp. Med. Biol*. 1991, *289*, 83 – 105.

[10] Augustsson, K., Skog, K., Jagerstad, M., Dickman, P. W., Steineck, G., Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study, *Lancet* 1999, *353*, 703 – 707.

[11] Schwab, C. E., Huber, W. W., Parzefall, W., Hietsch, G., *et al.,* Search for compounds that inhibit the genotoxic and carcinogenic effects of heterocyclic aromatic amines, *Crit .Rev. Toxicol*. 2000, *30*, 1 – 69.

[12] Dashwood, R. H., Modulation of heterocyclic amine-induced mutagenicity and carcinogenicity: an ‚A-to-Z' guide to chemopreventive agents, promoters, and transgenic models, *Mutat. Res*. 2002, *511*, 89 – 112.

[13] Frandsen, H., Frederiksen, H., Alexander, J., 2-Amino-1-methyl-6-(5-hydroxy-)phenylimidazo[4,5-b]pyridine (5-OH-PhIP), a biomarker for the genotoxic dose of the heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), *Food Chem. Toxicol*. 2002, *40*, 1125 – 1130.

[14] Moller, P., Loft, S., Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair, *Mutat. Res*. 2004, *551*, 79 – 89.

[15] Moller, P., Loft, S., Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies, *Am. J. Clin. Nutr*. 2002, *76*, 303 – 310.

[16] Collins, A. R., Duthie, S. J., Dobson, V. L., Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA, *Carcinogenesis* 1993, *14*, 1733 – 1735.

[17] Duthie, S. J., Collins, A. R., Duthie, G. G., Dobson, V. L., Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidized pyrimidines) in human lymphocytes, *Mutat. Res*. 1997, *393*, 223 – 231.

[18] Anderson, D., Dobrzynska, M. M., Basaran, N., Basaran, A., Yu, T. W., Flavonoids modulate comet assay responses to food mutagens in human lymphocytes and sperm, *Mutat. Res*. 1998, *402*, 269 – 277.

[19] Anderson, D., Basaran, N., Dobrzynska, M. M., Basaran, A. A., Yu, T. W., Modulating effects of flavonoids on food mutagens in human blood and sperm samples in the comet assay, *Teratog. Carcinog. Mutagen* 1997, *17*, 45 – 58.

[20] Kassie, F., Parzefall, W., Musk, S., Johnson, I., *et al.*, Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells, *Chem. Biol. Interact*. 1996, *102*, 1 – 16.

[21] Laky, B., Knasmuller, S., Gminski, R., Mersch-Sundermann, V. *et al.,* Protective effects of Brussels sprouts towards B[a]P-induced DNA damage: a model study with the single-cell gel electrophoresis (SCGE)/Hep G2 assay, *Food Chem. Toxicol*. 2002, *40*, 1077 – 1083.

[22] W.H.O. IARC Heterocyclic aromatic amines, in: W.H.O. International Agency for Research on Cancer (Ed.), *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some natural occuring substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mykotoxines*, IARC, Lyon 1993, pp. 229 – 242.

[23] W.H.O. IARC Trp-P-2 and its acetate, in: W.H.O. International Agency for Research on Cancer (Ed.), *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans:Some natural occuring substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mykotoxines*, IARC, Lyon 1983, pp. 255 – 263.

[24] W.H.O. IARC Heterocyclic aromatic amines, in: W.H.O. International Agency for Research on Cancer (Ed.), *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some natural occuring substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mykotoxines*, IARC, Lyon 1993, pp. 29.

[25] Ito, N., Hasegawa, R., Sano, M., Tamano, S., *et al.,* A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), *Carcinogenesis* 1991, *12*, 1503 – 1506.

[26] Knasmuller, S., Parzefall, W., Sanyal, R., Ecker, S., *et al.,* Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens, *Mutat. Res*. 1998, *402*, 185 – 202.

[27] Overvik, E., Gustafsson, J. A., Cooked-food mutagens: current knowledge of formation and biological significance, *Mutagenesis* 1990, *5*, 437 – 446.

[28] Jagerstad, M., Skog, K., Genotoxicity of heat-processed foods, *Mutat. Res*. 2005, *574*, 156 – 172.

[29] Ames, B. N., Shigenaga, M. K., Gold, L. S., DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis, *Environ. Health Perspect*. 1993, *101 (Suppl. 5)*, 35 – 44.

[30] Hussain, S. P., Hofseth, L. J., Harris, C. C., Radical causes of cancer, *Nat. Rev. Cancer* 2003, *3*, 276 – 285.

[31] Boiteux, S., O'Connor, T. R., Laval, J., Formamidopyrimidine-DNA glycosylase of *Escherichia coli*, cloning and sequencing of the fpg structural gene and overproduction of the protein, *EMBO J*. 1987, *6*, 3177 – 3183.

[32] Rogers, S. G., Weiss, B., Exonuclease III of *Escherichia coli* K-12, an AP endonuclease, *Methods Enzymol*. 1980, *65*, 201 – 211.

[33] Demirbag, R., Yilmaz, R., Gur, M., Celik, H., *et al.*, DNA damage in metabolic syndrome and its association with antioxidative and oxidative measurements, *Int. J. Clin. Pract*. 2006, *60*, 1187 – 1193.

[34] Park, E., Kang, M. H., Smoking and high plasma triglyceride levels as risk factors for oxidative DNA damage in the Korean population, *Ann. Nutr. Metab.* 2004, *48*, 36 – 42.

[35] Kazimirova, A., Barancokova, M., Volkovova, K., Staruchova, M., *et al.,* Does a vegetarian diet influence genomic stability? *Eur. J. Nutr*. 2004, *43*, 32 – 38.

[36] Hoelzl, C., Bichler, J., Ferk, F., Simic, T., *et al.,* Methods for the detection of antioxidants which prevent age related diseases: a critical review with particular emphasis on human intervention studies, *J. Physiol. Pharmacol*. 2005, *56 (Suppl. 2)*, 49 – 64.

[37] Hartmann, A., Plappert, U., Raddatz, K., Grunert-Fuchs, M., Speit, G., Does physical activity induce DNA damage? *Mutagenesis* 1994, *9*, 269 – 272.

[38] Duthie, S. J., Ross, M., Collins, A. R., The influence of smoking and diet on the hypoxanthine phosphoribosyltransferase (hprt) mutant frequency in circulating T lymphocytes from a normal human population, *Mutat. Res*. 1995, *331*, 55 – 64.

[39] Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., *et al.,* Single-cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing, *Environ. Mol. Mutagen.* 2000, *35*, 206 – 221.

[40] Lindl, T., Bauer, J., *Zell- und Gewebekultur*, Fischer Stuttgart, Jena, New York 1994.

[41] Collins, A. R., Dobson, V. L., Dusinska, M., Kennedy, G., Stetina, R., The comet assay: what can it really tell us? *Mutat. Res*. 1997, *375*, 183 – 193.

[42] Helma, C., Uhl, M., A public domain image-analysis program for the single-cell gel-electrophoresis (comet) assay, *Mutat. Res*. 2000, *466*, 9 – 15.

[43] Misra, H. P., Fridovich, I., The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *J. Biol. Chem*. 1972, *247*, 3170 – 3175.

[44] Gunzler, W. A., Kremers, H., Flohe, L., An improved coupled test procedure for glutathione peroxidase (EC 1-11-1-9) in blood, *Z. Klin. Chem. Klin. Biochem*. 1974, *12*, 444 – 448.

[45] Foldes, A., Meek, J. L., Rat brain phenolsulfotransferase: partial purification and some properties, *Biochim. Biophys. Acta* 1973, *327*, 365 – 374.

[46] Tovey, E. R., Baldo, B. A., Comparison of semidry and conventional tankbuffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes, *Electrophoresis* 1987, *8*, 384 – 387.

[47] Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* 1970, *227*, 680 – 685.

[48] Richard, K., Hume, R., Kaptein, E., Stanley, E. L., *et al.,* Sulfation of thyroid hormone and dopamine during human development: ontogeny of phenol sulfotransferases and arylsulfatase in liver, lung, and brain, *J. Clin. Endocrinol. Metab.* 2001, *86*, 2734 – 2742.

[49] Rose, R. C., Bode, A. M., Analysis of water-soluble antioxidants by high-pressure liquid chromatography, *Biochem. J.* 1995, *306*, 101 – 105.

[50] Muckel, E., Frandsen, H., Glatt, H. R., Heterologous expression of human N-acetyltransferases 1 and 2 and sulfotransferase 1A1 in Salmonella typhimurium for mutagenicity testing of heterocyclic amines, *Food Chem. Toxicol.* 2002, *40*, 1063 – 1068.

[51] Glatt, H. R., Activation and inactivation of carcinogens by human sulfotransferases. In: Pacifici, G. M., Coughtrie, M. W. H. (Eds.), *Human sulfotransferases*, Taylor & Francis, London 2005, pp. 281 – 306.

[52] Felton, J. S., Knize, M. G., Bennett, L. M., Malfatti, M. A., *et al.,* Impact of environmental exposures on the mutagenicity/carcinogenicity of heterocyclic amines, *Toxicology* 2004, *198*, 135 – 145.

[53] Steinkellner, H., Rabot, S., Freywald, C., Nobis, E., *et al.,* Effects of cruciferous vegetables and their constituents on drug metabolizing enzymes involved in the bioactivation of DNA-reactive dietary carcinogens, *Mutat. Res.* 2001, *480 – 481*, 285 – 297.

[54] Raucy, J. L., Ingelman-Sundberg, M., Carpenter, S., Rannug, A., *et al.,* Drug metabolizing enzymes in lymphocytes, *J. Biochem. Mol. Toxicol.* 1999, *13*, 223 – 226.

[55] Deep, G., Agarwal, R., Chemopreventive efficacy of silymarin in skin and prostate cancer, *Integr. Cancer Ther.* 2007, *6*, 130 – 145.

[56] Ren, W., Qiao, Z., Wang, H., Zhu, L., Zhang, L., Flavonoids: promising anticancer agents, *Med. Res. Rev.* 2003, *23*, 519 – 534.

[57] Sanchez-Perez, Y., Carrasco-Legleu, C., Garcia-Cuellar, C., Perez-Carreon, J., *et al.,* Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis, *Cancer Lett.* 2005, *217*, 25 – 32.

[58] Yanaida, Y., Kohno, H., Yoshida, K., Hirose, Y., *et al.,* Dietary silymarin suppresses 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in male F344 rats, *Carcinogenesis* 2002, *23*, 787 – 794.

[59] Visser, T. J., Kaptein, E., Glatt, H., Bartsch, I., *et al.,* Characterization of thyroid hormone sulfotransferases, *Chem. Biol. Interact.* 1998, *109*, 279 – 291.

[60] Daimon, H., Sawada, S., Asakura, S., Sagami, F., Inhibition of sulfotransferase affecting *in vivo* genotoxicity and DNA adducts induced by safrole in rat liver, *Teratog. Carcinog. Mutagen.* 1997, *17*, 327 – 337.

[61] Hölzl, C., Schutzeffekte von Kohlsprossen vor freien Sauerstoffradikalen und heterozyklischen Aminen, Nutritional Sciences, University of Vienna, Vienna 2004, Thesis, pp. 72.

[62] King, R. S., Teitel, C. H., Kadlubar, F. F., *In vitro* bioactivation of N-hydroxy-2-amino-alpha-carboline, *Carcinogenesis* 2000, *21*, 1347 – 1354.

[63] Boelsterli, U. A., Ho, H. K., Zhou, S., Leow, K. Y., Bioactivation and hepatotoxicity of nitroaromatic drugs, *Curr. Drug Metab.* 2006, *7,* 715 – 727.

[64] Hempel, N., Gamage, N., Martin, J. L., McManus, M. E., Human cytosolic sulfotransferase SULT1A1*, Int. J. Biochem. Cell. Biol.* 2007, *39*, 685 – 689.

[65] Ku, W. W., Bigger, A., Brambilla, G., Glatt, H., *et al.*, Strategy for genotoxicity testing – metabolic considerations, *Mutat. Res.* 2007, *627*, 59 – 77.

[66] Glatt, H., Sulfotransferases in the bioactivation of xenobiotics, *Chem. Biol. Interact*. 2000, *129*, 141 – 170.

[67] Glatt, H., Meinl, W., Use of genetically manipulated *Salmonella typhimurium* strains to evaluate the role of sulfotransferases and acetyltransferases in nitrofen mutagenicity, *Carcinogenesis* 2004, *25*, 779 – 786.

[68] Meinl, W., Meerman, J. H., Glatt, H., Differential activation of promutagens by alloenzymes of human sulfotransferase 1A2 expressed in *Salmonella typhimurium*, *Pharmacogenetics* 2002, *12*, 677 – 689.

[69] Futakuchi, M., Cheng, J. L., Hirose, M., Kimoto, N., *et al.,* Inhibition of conjugated fatty acids derived from safflower or perilla oil of induction and development of mammary tumors in rats induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), *Cancer Lett.* 2002, *178*, 131 – 139.

[70] Cavin, C., Holzhaeuser, D., Scharf, G., Constable, A., *et al.,* Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity, *Food Chem. Toxicol.* 2002, *40*, 1155 – 1163.

[71] Huber, W. W., Teitel, C. H., Coles, B. F., King, R. S. *et al.,* Potential chemoprotective effects of the coffee components kahweol and cafestol palmitates via modification of hepatic N-acetyltransferase and glutathione S-transferase activities, *Environ. Mol. Mutagen.* 2004, *44*, 265 – 276.

[72] Mori, Y., Koide, A., Tatematsu, K., Sugie, S., Mori, H., Effects of alpha-naphthyl isothiocyanate and a heterocyclic amine, PhIP, on cytochrome P-450, mutagenic activation of various carcinogens and glucuronidation in rat liver, *Mutagenesis* 2005, *20*, 15 – 22.

[73] Dashwood, R. H., Xu, M., The disposition and metabolism of 2-amino-3-methylimidazo-[4,5-f]quinoline in the F344 rat at high versus low doses of indole-3-carbinol, *Food Chem. Toxicol.* 2003, *41*, 1185 – 1192.

[74] Walters, D. G., Young, P. J., Agus, C., Knize, M. G., *et al.,* Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans, *Carcinogenesis* 2004, *25*, 1659 – 1669.

[75] Zhu, C., Poulsen, H. E., Loft, S., Inhibition of oxidative DNA damage *in vitro* by extracts of brussels sprouts, *Free Radic. Res.* 2000, *33*, 187 – 196.

[76] Podsedek, A., Sosnowska, D., Redzynia, M., Anders, B., Antioxidant capacity and content of *Brassica oleracea* dietary antioxidants, *Int. J. Food Sci. Technol.* 2006, *41(s1)*, 49 – 58.

[77] Panayiotidis, M., Collins, A. R., *Ex vivo* assessment of lymphocyte antioxidant status using the comet assay, *Free Radic. Res.* 1997, *27*, 533 – 537.

[78] Welch, R. W., Turley, E., Sweetman, S. F., Kennedy, G., *et al.,* Dietary antioxidant supplementation and DNA damage in smokers and nonsmokers, *Nutr. Cancer* 1999, *34*, 167 – 172.

[79] Anderson, D., Phillips, B. J., Yu, T. W., Edwards, A. J., *et al.,* The effects of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with "low" or "high" cholesterol levels, *Environ. Mol. Mutagen.* 1997, *30*, 161 – 174.

[80] Brennan, L. A., Morris, G. M., Wasson, G. R., Hannigan, B. M., Barnett, Y. A., The effect of vitamin C or vitamin E supplementation on basal and H2O2-induced DNA damage in human lymphocytes, *Br. J. Nutr.* 2000, *84*, 195 – 202.

[81] Williamson, G., Faulkner, K., Plumb, G. W., Glucosinolates and phenolics as antioxidants from plant foods, *Eur. J. Cancer Prev.* 1998, *7*, 17 – 21.

[82] Verhagen, H., Poulsen, H. E., Loft, S., van Poppel, G., *et al.*, Reduction of oxidative DNA-damage in humans by brussels sprouts, *Carcinogenesis* 1995, *16*, 969 – 970.

[83] Verhagen, H., de Vries, A., Nijhoff, W. A., Schouten, A., *et al.*, Effect of Brussels sprouts on oxidative DNA-damage in man, *Cancer Lett.* 1997, *114*, 127 – 130.

[84] Deng, X. S., Tuo, J., Poulsen, H. E., Loft, S., Prevention of oxidative DNA damage in rats by Brussels sprouts, *Free Radic. Res.* 1998, *28*, 323 – 333.

[85] Gedik, C. M., Boyle, S. P., Wood, S. G., Vaughan, N. J., Collins, A. R., Oxidative stress in humans: validation of biomarkers of DNA damage, *Carcinogenesis* 2002, *23*, 1441 – 1446.

[86] Porrini, M., Riso, P., Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption, *J. Nutr.* 2000, *130*, 189 – 192.

[87] Collins, B. H., Horska, A., Hotten, P. M., Riddoch, C., Collins, A. R., Kiwifruit protects against oxidative DNA damage in human cells and *in vitro*, *Nutr. Cancer* 2001, *39*, 148 – 153.

[88] van den Berg, R., van Vliet, T., Broekmans, W. M., Cnubben, N. H. *et al.,* A vegetable/fruit concentrate with high antioxidant capacity has no effect on biomarkers of antioxidant status in male smokers, *J. Nutr.* 2001, *131*, 1714 – 1722.

[89] Moller, P., Vogel, U., Pedersen, A., Dragsted, L. O., *et al.,* No effect of 600 grams fruit and vegetables per day on oxidative DNA damage and repair in healthy nonsmokers, *Cancer Epidemiol. Biomarkers Prev.* 2003, *12*, 1016 – 1022.

[90] Wiseman, A., Dietary anticancer isothiocyanates (ITC) in *Brassica* raise the reduced-glutathione barrier to DNA-damage in the colon, *Trends Food Sci. Technol.* 2005, *16*, 215 – 216.

[91] Verhoeven, D. T., Goldbohm, R. A., van Poppel, G., Verhagen, H., van den Brandt, P. A., Epidemiological studies on *Brassica* vegetables and cancer risk, *Cancer Epidemiol. Biomarkers Prev.* 1996, *5*, 733 – 748.

