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Effects of consumption of Brussels sprouts on intestinal and lymphocytic glutathione S-transferases in humans

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A high intake of glucosinolate-containing cruciferous vegetables, such as Brussels sprouts (*Brassica oleraceae*), has been linked to a decreased cancer risk, but the underlying mechanism is still unclear. The aim of this study was to reveal possible modulating effects of consumption of Brussels sprouts on duodenal, rectal and lymphocytic (i) glutathione S-transferase (GST) enzyme activity, (ii) GST isozyme levels and (iii) glutathione (GSH) content. Ten healthy non-smoking volunteers were randomly assigned to two groups in a cross-over design. Five persons started on a glucosinolate-free diet (control period), while the other five consumed 300 g/day cooked Brussels sprouts, at the expense of 300 g glucosinolate-free vegetables (sprouts period). After 7 days the regimen was changed for a further week. At the end of both periods blood samples and duodenal and rectal biopsies were taken. Mean GST activity showed marked differences between duodenal, rectal and lymphocytic cytosols (737 ± 54, 321 ± 29 and 154 ± 14 nmol/min/mg protein respectively), but was uninfluenced by the dietary regimen. Isozyme distribution varied greatly between the tissues. In duodenum GST-α, -π and -μ isozymes were expressed in considerable amounts (8441 ± 1365, 3002 ± 223 and 536 ± 248 ng/mg protein respectively). Rectal biopsies also contained above three GST classes, but here GST-π was the most pronounced expressed isozyme (2849 ± 246) followed by GST-μ (495 ± 242), while GST-α was only present in minor quantities (149 ± 31). In lymphocytes only GST-π (755 ± 96) and GST-μ (83 ± 54) could be detected. As a result of the dietary regimen rectal GST-α and -π levels were slightly increased at the end of the sprouts period, by 30 and 15% respectively. GSH contents were uninfluenced by the dietary regimen. In conclusion, consumption of glucosinolate-containing Brussels sprouts for 1 week results in increased rectal GST-α and -π isozyme levels. We hypothesize that these enhanced detoxification enzyme levels may partly explain the epidemiological association between a high intake of glucosinolates (cruciferous vegetables) and a decreased risk of colorectal cancer.

Introduction

Gastrointestinal tumors in general and colorectal cancer in particular are among the most common malignancies in Western society. Despite recent advances in diagnosis and therapy, the survival rates are still low (1). For this reason, research on prevention of gastrointestinal carcinogenesis is of the utmost importance. Epidemiological studies have shown that life-style factors, e.g. dietary habits, may largely determine each individual's tumor risk. Diets rich in cruciferous vegetables, such as Brussels sprouts (*Brassica oleraceae*), are associated with a lower risk (2). The anticarcinogenic properties of cruciferous vegetables have been mainly attributed to the degradation products of glucosinolates, e.g. (iso)thiocyanates and indoles (3). A possible mechanism of action of these constituents may be the induction of gastrointestinal detoxification enzymes (4–9). Important drug metabolizing or detoxification enzymes are glutathione S-transferases (GST*; EC 2.5.1.18), a family of isozymes divided into three main classes, α, π and μ (10–12). Recently a fourth class θ has been described in human and rat liver (13). GSTs catalyze the reaction of a wide variety of electrophiles with glutathione (GSH). Since most of the reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, GSTs may contribute considerably to carcinogen inactivation (12,14). Enhancement of this detoxifying enzyme system could potentially increase the capacity to withstand the burden of toxicants and (pre)carcinogens we are exposed to every day and may thus reduce the risk of developing cancer (15,16).

The aim of the present study was to reveal whether the GST detoxification system in humans could be induced by consumption of glucosinolate-containing Brussels sprouts.

Materials and methods

Outline of the study

Ten healthy non-smoking volunteers (five males and five females, mean age 24 ± 1 years, Quetelet index 22.0 ± 0.8 kg/m^2) were randomly assigned to a cross-over design experimental protocol. Five volunteers (three females and two males) started on a glucosinolate-free diet (control period), while the other five consumed 300 g/day cooked Brussels sprouts, at the expense of 300 g glucosinolate-free vegetables (sprouts period). After 7 days the dietary regimen was reversed for a further week. Volunteers were asked to keep to their habitual diet but to refrain from other glucosinolate-containing foods during the experiment. For their convenience a dietary exclusion list was provided. Furthermore, subjects were instructed not to consume more than one alcohol-containing drink per day and to abstain from any medication during the study.

All standardized dinners were prepared and consumed at the University Hospital St Radboud. The Brussels sprouts used in this study were from the same batch as used previously by Bogaards *et al.* (17). One kilogram of Brussels sprouts contained the following glucosinolates: sinigrin 2.15 mmol, glucobrassicin 0.38 mmol, neoglucobrassicin 0.04 mmol, progoitrin 0.62 mmol and gluconapin 0.19 mmol.

Daily intakes of total energy and of the macronutrients fat, protein and carbohydrate were calculated from dietary diaries kept for 3 days in both periods, using the 1989/1990 release of the Netherlands nutrient data bank. At the end of both periods, biopsies were taken by endoscopy (i) from the descending part of the duodenum, 10 cm from the pyloris, and (ii) from the rectum, 10 cm from the anus, without previous laxation. In addition, blood samples were collected by venepuncture into EDTA-containing tubes. Lymphocytes were isolated using Histopaque-1077, according to the manufacturer's instructions (Sigma Diagnostics, St Louis, MO) and plasma samples were prepared by centrifugation at 2500 *g* for 10 min. All material was immediately frozen in liquid nitrogen and stored at –80°C. For preparation of cytosolic

*Abbreviations: GST, glutathione S-transferase; GSH, reduced glutathione.

fractions, biopsies were quickly thawed using cold running water and homogenized with buffer A (0.25 M saccharose, 20 mM Tris, 1 mM dithiothreithol, pH 7.4) using disposable polypropylene pestles and microtubes (Kontes, Van Oortmerssen, Rijswijk, The Netherlands). The homogenate was centrifuged at 150 000 *g* at 4°C for 1 h. Lymphocytes were slowly thawed and buffer A was added for homogenization as mentioned above. The homogenate was centrifuged at 12 000 *g* (4°C) for 30 min. Aliquots of post-centrifugation supernatant, representing the cytosolic fraction, were frozen in liquid nitrogen and stored (–80°C) until assayed.

This study was approved by the local Medical Ethical Review Committee and informed consent was obtained from the participants prior to the start of the experiment.

Assays

Plasma thiocyanate concentration was determined according to Pettigrew and Fell (18). Protein concentration was assayed in triplicate by the method of Lowry *et al.* (19), using bovine serum albumin as the standard. Total GST enzyme activity with 1-chloro-2,4-dinitrobenzene as substrate was determined in triplicate according to Habig *et al.* (20). Cytosolic samples were subjected to SDS–PAGE (11% w/v acrylamide) and Western blotting (21). The Western blots were incubated with monoclonal antibodies against human GST class-α, -μ and -π as described previously (21,22). Class-α antibodies react against GST A1-1, GST A1-2 and GST A2-2, class-μ antibodies recognize GST M1a-1a, GST M1a-1b and GST M1b-1b and class-π antibodies are directed against GST P1-1. Cross-reactivity of the above-mentioned monoclonal antibodies with recently discovered (i.e. GST M2-M5) or yet unknown isoforms cannot be excluded. Specific binding of the monoclonal antibodies was detected with 4-chloro-1-naphthol after incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulins as second antibody (Dakopatts, Glostrup, Denmark). Staining on the immunoblots was quantified using a laser densitometer (Ultrascan; LKB, Bromma, Sweden). Known amounts of purified GST-α, -μ and -π were run in parallel with the experimental samples and served as standards for calculation of the absolute amounts of these enzymes (7,21,22). Glutathione was quantified by high performance liquid chromatography after reaction with monobromobimane as described previously (7).

Materials

1-Chloro-2,4-dinitrobenzene, bovine serum albumin, glutathione and dithiothreithol were purchased from the Sigma Chemical Co. (St Louis, MO) and monobromobimane from Calbiochem Co.

Statistical analysis

All values given are means ± SEM. Student's paired *t*-test was used to assess statistical significance between the control and sprouts periods.

Results

Total daily intakes of energy and of macronutrients and plasma thiocyanate concentrations in the control and sprouts periods are given in Table I. There was no statistical difference between the periods for total daily caloric intake and for the intake of the macronutrients protein, fat and carbohydrate. However, the dietary regimen resulted in a significantly higher (1.8 times) plasma thiocyanate concentration in the sprouts period as compared with the control period.

Table II shows duodenal, rectal and lymphocytic GST activities, GST isozyme levels and GSH contents at the end of the control period, as well as after consumption of Brussels sprouts. Mean total GST activity at the end of the control period showed marked differences between duodenal, rectal and lymphocytic cytosols (737 ± 54, 321 ± 29 and 154 ± 14 nmol/min/mg protein respectively). Overall GST activity at these sites was not influenced by the dietary regimen. Distribution of the isozymes varied greatly between the tissues. In duodenum GST-α, -π and -μ isozymes were expressed in considerable amounts (8441 ± 1365, 3002 ± 223 and 536 ± 248 ng/mg protein respectively). Rectal biopsies also contained all three GST classes, but here GST-π was the most pronounced isozyme (2849 ± 246) and GST-α was only present in minor quantities (149 ± 31). In lymphocytes only GST-π (755 ± 96) and GST-μ (83 ± 54) were detected. As a result of the dietary regimen rectal GST-α and -π levels were slightly increased at the end of the sprouts period, by 30 and 15% respectively. Duodenal and rectal GSH levels were similar and ~4-fold higher than found in lymphocytes, but GSH concentrations were uninfluenced by the dietary regimen.

Table I. Effects of dietary regimen on total daily energy intake, macronutrient balance and plasma thiocyanate concentration in healthy non-smoking volunteers

Period	Total daily energy (kCal/day)	Protein (% total energy)	Fat (% total energy)	Carbohydrate (% total energy)	SCN^- (μg/ml)
Control	2403 ± 143	15 ± 1	32 ± 1	52 ± 1	2.98 ± 0.34
Sprouts	2409 ± 189	16 ± 1	33 ± 1	50 ± 2	5.21 ± 0.25[a]

Daily intake of total energy (kCal/day), protein, fat and carbohydrate (expressed as percentage of total energy intake) and plasma thiocyanate concentration (μg SCN^-/ml plasma) were determined as described in Materials and methods. Values given are means ± SEM. Student's paired *t*-test was used to assess statistical significance between the control and sprouts periods: [a]$P < 0.005$.

Table II. Effects of consumption of glucosinolate-containing Brussels sprouts on duodenal, rectal and lymphocytic glutathione S-transferase activity, isozyme levels and glutathione concentration in healthy non-smoking volunteers

Parameter	Period	Duodenum	Rectum	Lymphocyte
GST activity	Control	737 ± 54	321 ± 29	154 ± 14
	Sprouts	760 ± 52	306 ± 24	165 ± 20
GST-α	Control	8441 ± 1365	149 ± 31	ND
	Sprouts	8136 ± 1068	193 ± 37[b]	ND
GST-π	Control	3002 ± 223	2849 ± 246	755 ± 96
	Sprouts	2889 ± 229	3261 ± 201[a]	664 ± 59
GST-μ	Control	536 ± 248	495 ± 242	83 ± 54
	Sprouts	482 ± 177	530 ± 229	267 ± 156
GSH	Control	46 ± 2	44 ± 1	11 ± 1
	Sprouts	50 ± 2	42 ± 3	13 ± 2

ND, not detectable. Detection limit of the assay 50 ng/mg. Glutathione S-transferase activity (nmol/min/mg protein), glutathione S-transferase-α, -π and -μ levels (ng/mg protein) and glutathione concentration (nmol/mg protein) were determined as described in Materials and methods. Values given are means ± SEM. Student's paired *t*-test was used to assess statistical significance of differences between the control and sprouts periods: [a]$P < 0.050$ and [b]$P < 0.005$.

Discussion

It is well accepted that dietary habits affect the development of human cancer in general and gastrointestinal tumors in particular (2). For instance, high caloric, fat and alcohol intake are regarded as risk factors (23). Furthermore, the human diet contains a large number of well-known (pre)carcinogens that may be of relevance in this respect (15,16,24). However, apart from carcinogens our diet also contains anticarcinogenic compounds, i.e. minor non-nutritive constituents of vegetables and fruits which are correlated with decreased cancer risk (6,25,26). From animal studies it has been suggested that these compounds may, at least in part, be effective by virtue of enhancing detoxification systems (6,7,22,26). These detoxification enzymes, such as GSTs can reduce carcinogenicity by conjugation reactions with GSH, which is an efficient biochemical mechanism that transforms the carcinogen to a compound with lower biological activity and increased excretability (12,14).

Previously Brussels sprouts have been found to influence hepatic and intestinal GSTs in rodents (7,27–32). In humans evidence for induction of biotransformation enzymes by dietary

anticarcinogenic compounds is sparse. Pantuck *et al.* (33,34) noticed an increased plasma clearance of some drugs after consumption of cruciferous vegetables, indicating enhancement of hepatic and/or intestinal biotransformation activity. Hoensch *et al.* (35) measured a decreased monooxygenase activity in the small intestine in subjects on a semisynthetic diet deficient in vegetable compounds. To our knowledge no data on effects of consumption of Brussels sprouts on the gastrointestinal GST detoxification system in humans have been reported.

By exchanging glucosinolate-rich vegetables for glucosinolate-free vegetables in the human diet one could also introduce additional variations, e.g. such as higher or lower caloric intake or a shift in macronutrient balance, which could have a significant impact on carcinogenesis or biotransformation. However, in our study caloric intake and macronutrient balance in both periods were identical and only plasma thiocyanate concentration (a biological marker for glucosinolate exposure) was increased at the end of the sprouts period. Therefore, in our opinion the observed effects on GSTs are most likely the result of differences in glucosinolate exposure.

Although overall GST enzyme activity did not change after consumption of Brussels sprouts, a small but statistically significant increase in GST-α and -π levels was observed in rectal biopsies. Similar changes in GST isozyme levels without significant alteration in overall enzyme activity after feeding naturally occurring dietary anticarcinogens have been reported in rats (7,22).

Recently we found an inverse relationship between GST enzyme activity and tumor incidence for the organs of the human gastrointestinal tract (36). Tissues with high GST activity, such as liver and small intestine, had a low tumor incidence and vice versa. A similar relationship seems to exist for GST-α content; tissues with high GST-α content (liver and small intestine) have a low tumor risk, whereas tissues with a low GST-α content (colon/rectum, breast and lung) have a high tumor risk. In this respect we hypothesize that the relatively modest elevation in rectal GST-α level, in addition to the induction of GST-π, may be of great biological importance with reference to the very low basal GST-α levels found in the rectum.

Tissue sampling in chemopreventive studies by endoscopy is invasive, time consuming, costly and not without risk. Therefore, more convenient sampling procedures with a lower risk of complications, such as venous puncture, would be more favorable for evaluating response to dietary anticarcinogens. Recently Bogaards *et al.* (17) reported elevated plasma GST-α levels in healthy human volunteers on a Brussels sprouts diet, results which are in full agreement with our recently published data (37). It was hypothesized that these elevated plasma levels may be used as a biomarker, since they reflect normal cell turnover after selective induction of GST-α in tissues such as liver and intestine. However, our data indicate that both GST-α and GST-π were induced in the rectum, whereas only changes in plasma GST-α levels were found. From earlier studies it is known that normal human liver contains GST-α in very large quantities and only traces of GST-π (38,39). Therefore, it seems most reasonable to assume that elevated plasma GST-α levels without concomitant changes in GST-π levels reflect effects on hepatic and not on intestinal GSTs.

Szarka *et al.* (40) found a significant correlation between GST enzyme activity in lymphocytes and colorectal tissue in controls (Spearman's rank correlation coefficient $r_s = 0.81$, $P < 0.00001$). They suggested that lymphocytic GST activity could be used in future chemopreventive trials to monitor colorectal responsiveness to anticarcinogenic regimens. In our study lymphocytic and rectal GST activities are significantly correlated only in the control and not in the sprouts period ($r_s = 0.78$, $P = 0.008$ and $r_s = 0.56$, $P = 0.090$ respectively). Therefore, in our opinion plasma GST levels and lymphocytic GST activities seem less useful as biomarkers for the effects of anticarcinogens on intestinal GST activities and tissue samples are still needed in these kind of chemoprevention studies.

In conclusion, our data demonstrate that consumption of glucosinolate-containing Brussels sprouts for 1 week results in increased rectal GST-α and -π isozyme levels. We hypothesize that these induced detoxification enzyme levels may, at least in part, explain the epidemiological association between high intake of cruciferous vegetables (glucosinolates) and decreased risk of colorectal cancer.

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