Habitual consumption of fruits and vegetables: associations with human rectal glutathione S-transferase

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The glutathione (GSH)/glutathione S-transferase (GST) system is an important detoxification system in the gastrointestinal tract. A high activity of this system may benefit cancer prevention. The aim of the study was to assess whether habitual consumption of fruits and vegetables, especially citrus fruits and brassica and allium vegetables, is positively associated with parameters reflecting the activity of the GSH/GST enzyme system in human rectal mucosa. GST enzyme activity, GST isoenzyme levels of GST-alpha (A1-1, A1-2 and A2-2), -mu (M1-1) and -pi (P1-1), and GST levels were measured in rectal biopsies from 94 subjects. Diet, lifestyle, GSTM1 and GSTTI null polymorphisms were assessed. Mean GST enzyme activity was 237 nmol/min/mg protein (SD = 79). Consumption of citrus fruits was positively associated with GST enzyme activity [difference between high and low consumption: 28.9 (95% confidence interval (CI) = 9.3–48.6) nmol/min/mg protein], but was not associated with the other parameters. A positive association with brassica vegetables was found among carriers of the GSTM1-plus genotype [difference between high and low consumption: 22.6 (95% CI = 0.2–45.0) nmol/min/mg protein], but not among GSTM1-null individuals (−25.8 nmol/min/mg protein), 95% CI = −63.3–11.8). This is in line with a positive association between consumption of brassica vegetables and GSTM isoenzyme level [difference between high and low consumption: 67.5%, 95% CI = (68.8–162.7%)]. Consumption of allium vegetables was not associated with GST enzyme activity, but negatively with GSTP1-1 levels [difference between high and low consumption: −23.3%, 95% CI = (−35.5: −8.6)]. Associations were similar among those with the GSTTI-plus and GSTTI-null genotype. In conclusion, variations in habitual consumption of fruits, particularly citrus fruits, and of vegetables, in particular brassica vegetables, among those with the GSTM1-plus genotype, may contribute to variations in human rectal GST enzyme activity.

Introduction

Given their role in absorption, digestion and transport, the colon and rectum are constantly challenged by potentially harmful compounds, including mutagens and carcinogens. The large intestine possesses several defence mechanisms to counteract damage of the colorectal mucosa by such reactive compounds. These include the ability to up-regulate detoxification systems (1,2).

Essential is the glutathione (GSH)/glutathione S-transferase (GST) detoxification system, which comprises antioxidant reduced GSH and GSTs (EC 2.5.1.18); a family of phase II enzymes, which, in humans, consists of four main subgroups: alpha(α), mu(μ), pi(π) and theta(θ).

Because this system plays an important role in detoxification of a broad range of carcinogens (3), high GST enzyme activity has been suggested as being beneficial to cancer prevention (4). Considering the low GST enzyme activity in the colon and rectum compared with tissues in which cancer occurs less frequently, we hypothesized previously that GST enzyme activity might be critically low and related to high rates of carcinogenesis in this organ (5,6).

Colonic GST enzyme activity and GST protein levels vary considerably between individuals (7,8), which may be related to differential susceptibility to colorectal cancer. Individuals with homozygous deletions of GSTM1 or GSTTI (null genotype) do not have detectable GSTM1 or GSTTI enzyme activity, respectively, and were postulated to be at higher risk of colorectal cancer. However, no consistent associations of GSTM1 and GSTTI null polymorphisms with risk of colorectal cancer have been observed (9). Apart from inherited polymorphisms in GSTs, individuals may differ in GST enzyme activity due to differential exposure to bioactive compounds.

In vivo and in vitro studies have shown that a variety of dietary compounds or their metabolites can induce the GSH/GST detoxification system. These include glucosinolate metabolites and dithiolthiones present in brassica vegetables, diallyl sulfides present in allium vegetables, limonoids and flavonoids present in citrus fruits (3,10–17), and butyrate produced by colonic fermentation of fibre (8).

Evidence for induction of the human rectal GSH/GST detoxification system was found in a crossover study among 10 volunteers consuming 300 g/day of cooked Brussels sprouts during 7 days. This yielded 30 and 15% increases of GST-alpha and GSTP1-1 protein levels, respectively. However, no effect upon GST enzyme activity was found (11), and taking 3 g/day of broccoli supplements for 14 days also did not influence GST enzyme activity in lymphocytes or colon mucosa (10).

Nonetheless, indications of the up-regulation of GST enzyme activity by brassica and allium vegetables were found in blood plasma, urine and saliva (11–15). Moreover, duodenal GST-α and GST-π protein levels were higher among

Abbreviations: CDNB, 1-chloro-,2,4 dinitrobenzene; CI, confidence interval; HNPPC, hereditary non-polyposis colorectal cancer; GSH, reduced glutathione; GST, glutathione S-transferase; p25, 25th percentile; p75, 75th percentile.
subjects consuming vegetables at least four times a week, and antral GSTT1 protein levels were higher among subjects consuming fruits at least four times a week compared with those who consumed these products less frequently. However, no associations between frequency of consumption of fruits and vegetables and GST enzyme activity were found in these tissues (16).

Induction of the GSH/GST detoxification system by fruits and vegetables may partially account for the observed inverse associations between their consumption and risk of colorectal cancer (12,18). We investigated whether habitual consumption of fruits and vegetables, in particular of brassica and allium vegetables and citrus fruits, is positively associated with the following components of the rectal GSH/GST detoxification system: GST enzyme activity, GST-α, GSTM1-1 and GSTP1-1 isoenzyme and GSH levels.

Methods

This cross-sectional study comprises a sub-study nested in a case-control study on dietary factors, genetic susceptibility and somatic mutations in sporadic and hereditary colorectal adenomas (19). In addition to the data collected in the main study, rectal biopsies were taken from participants who enrolled in one of the participating hospitals.

Study population

Between December 1995 and February 1998, subjects undergoing colonoscopy or sigmoidoscopy in the outpatient clinic of the Department of Gastroenterology and Hepatology of University Medical Centre Nijmegen, The Netherlands, were recruited by their gastroenterologist. Eligible subjects were Dutch speaking Caucasians, between 18 and 75 years old at day of endoscopy and having no history of colorectal resection, polyposis coli, colorectal cancer or chronic inflammatory bowel disease. Subjects with and without adenomas, either with or without a family background of hereditary non-polyposis colorectal cancer (HNPPC) according to the Amsterdam I criteria (20) were included. All HNPPC family members were first-degree relatives of patients with colorectal or endometrial cancer, but were free of cancer themselves. Subjects who did not belong to a HNPPC family were excluded if diagnosed with colorectal adenoma >3 years prior to recruitment. The medical ethical committees of Wageningen University and University Medical Centre Nijmegen approved the study protocol.

Rectal biopsies were taken from 106 (82%) of the 130 eligible subjects. Twelve subjects did not return dietary and/or lifestyle questionnaires. Thus, the final study population consists of 94 subjects, including 44 members of 26 HNPPC families.

Data collection

After providing written informed consent, participants underwent endoscopy during which six biopsies from healthy mucosa were taken from rectal mucosa within 10 cm from the anal verge. Biopsy specimens were immediately frozen in liquid nitrogen and stored at −80°C. Additionally, 30 ml of EDTA blood was drawn and stored at −20°C.

Clinical information regarding the presence and characteristics of adenomas, HNPPC and indication for endoscopy was abstracted from medical records.

We requested that participants complete a questionnaire on lifestyle and socio-economic factors and a validated semi-quantitative food-frequency questionnaire (21). The questionnaire assesses consumption of fruits and vegetables with high reproducibility after a year (fruits, Spearman’s \( r = 0.61 \) and 0.77; vegetables, \( r = 0.76 \) and 0.65 for males and females, respectively), while relative validity versus the means of 12 24-h recalls was moderate (fruits, Spearman’s \( r = 0.56 \) and 0.38; vegetables, \( r = 0.56 \) and 0.31 for males and females, respectively) (21). Nutrient intake was calculated using the 1996 computerized version of the Dutch food composition table (22).

Laboratory assays

Rectal biopsies were processed into cytosolic fractions (11). Total GST enzyme activity was assayed by spectrophotometric determination of 1-chloro-2,4-dinitrobenzene (CDNB) conjugation with GST (23). Total intracellular reduced glutathione (GSH) level was quantified by high performance liquid chromatography after reaction with monobromobimane using a modification of the method of Fahey and Newton (24,25). GST-α (GSTA1-1, A1-2 and A2-2), GSTM1-1 and GSTP1-1 isoenzyme levels were determined by western blotting (11). GST enzyme activity and levels of isoenzymes and GSH were expressed per milligram of intracellular protein, as determined colorimetrically according to the method of Lowry et al. (26). Protein and enzyme activity measurements were done in duplicate. The within- and between-assay coefficients of variations were 10–15% for protein and 5–10% for GST enzyme activity measurements, respectively.

As a result of limited tissue availability, not all biopsy samples could be analysed completely; this resulted in 78 samples analysed for GST enzyme activity, 94 for GSH, 91 for GST-α and GSTP1-1 and 76 for GSTM1-1 isoenzyme levels.

DNA was extracted from 200 µl frozen whole blood (QIAamp blood kit, Qiagen Inc.), diluted to a concentration of ~20 ng/µl and stored at 4°C until use. GSTM1 and GSTTI genotypes were determined using a general multiplex polymerase chain reaction method followed by electrophoresis (27). A β-globin gene fragment of 350 bp was present as a positive control.

Data analysis

Because all biopsies in which GSTM1-1 protein was not detected were obtained from GSTM1-null genotype carriers, we excluded these observations in analyses regarding this isoenzyme. We log-transformed GST-α, GSTM1-1 and GSTP1-1 protein levels to yield approximately normally distributed variables. Nutrient, alcohol and fat intakes were adjusted, separately for men and women, for total energy intake using the residual method (28).

Spearman correlation coefficients were computed to quantify the strength of the association between GST enzymes, GSH and GST parameters. Linear mixed models were fitted to associate dietary, lifestyle and medical determinants with GST enzyme activity, GSH and log-transformed GSTM1-1 and GSTP1-1 levels. In these models, family membership was incorporated as random intercept to account for the presence of relatives in the study population, and HNPPC and presence of adenomas at index endoscopy were included as indicator variables to account for the study design. Because no null genotype carriers of the associated polymorphism has been identified in any of the genes encoding GST-α, we used Tobit regression (29), which explicitly allows for censored distributions of the dependent variable, to model this isoenzyme.

Two series of models were fitted: a first to investigate the association between lifestyle and medical factors, and a second to address the association between consumption of fruits and vegetables and GSH/GST parameters. In the latter, total energy intake was included to control for potential confounding and to reduce the impact of under- and over-reporting of intake (28). To compensate for potential confounding, the following factors that potentially relate to both consumption of fruits and vegetables and to GST enzyme activity were added separately and mutually to the model: current and past smoking, sex and use of NSAIDs or paracetamol as indicator variables, and as continuous variables: age (years), body mass index (kg/m²) consumption of coffee, tea, (red) meat, energy-adjusted (saturated) fat, alcohol, red wine in grams per day and vitamin E (equivalents/day). Current smoking was the only factor that changed the regression coefficient of interest with >5 U in most models. To enhance comparability, we adjusted all models for this factor. Additionally, regression equations regarding GST isoenzymes were adjusted for energy-adjusted fat.

Fruits and vegetables were treated as continuous variables, tertiles and quadratic terms. Because Akaike’s Information Criteria and likelihood ratio tests—for tertiles and quadratic terms, respectively—showed a similar fit to continuous terms, only models containing the latter are presented.

To evaluate hypothesized effect modification by GSTM1 and GSTTI genotype and explore effect modification by smoking, age, sex, HNPPC and adenoma status, interaction terms were included and tested by Wald tests. Subsequently, to evaluate whether the observed associations may be attributed to fractions related to dietary fibre, vitamin A, vitamin C, β-carotene and folate, we added these food components to the models. Finally, we checked whether influential outliers were present. This appeared not to be the case.

The tests of statistical significance were two-sided and considered to be significant at the level of 5%. The analyses were conducted using Statistical Analysis Software (SAS version 8.0, Sas Institute, Cary, NC).

Results

In Table I the characteristics of the entire study population are presented. The characteristics of subjects in whose rectal biopsies the GST enzyme activity, one or more of the isoenzymes and/or GSH could not be assessed, did not differ from those subjects in which these factors were assessed (results not presented). Figure 1 shows the distribution of GST enzyme activity, isoenzymes and GSH according to GSTM1 genotype.
As expected, GSTM1-1 protein could not be detected in any of the biopsies of GSTM1-null genotype carriers, whereas detectable levels were present in all GSTM1-plus genotype carriers. GST-α protein could not be detected in 51 (56.0%) of the 91 samples. Among the 76 subjects in whom GST-α, GSTM1-1 and GSTP1-1 levels could be assessed, GSTP1-1 attained highest levels in 71 (93.4%) subjects, while GSTM1-1 levels were highest in the remaining five subjects; GST-α was only present in minor quantities. No differences in GST enzyme activity, GSH and GST isoenzyme levels could be detected according to GSTM1 and GSTT1 genotype or their combination (results not presented).

No correlation was detected between GST isoenzymes, GSH and GST enzyme activity. However, after adjusting for HNPCC, adenoma status, smoking behaviour, sex, age, GSTM1 and GSTT1 genotype, a weak, negative correlation between GSH and GST enzyme activity appeared \((r = -0.24, P = 0.049)\).

Table II describes GST enzyme activity according to GSTM1 and GSTT1 genotype, lifestyle and medical factors. Smokers had a higher GST enzyme activity compared with never smokers; former smoking subjects tended to have a higher GST enzyme activity compared with those who had never smoked, although the difference was not significant. Strikingly, the presence of adenomas at index endoscopy was associated with a 44.9 nmol/min/mg protein lower GST enzyme activity. Restricting the analysis to subjects having rectal adenomas increased this estimate to 57.7 nmol/min/mg protein \([95\% \text{ confidence interval (CI)} = -6.1–121.6]\), although statistical significance was lost. GST enzyme activity was not associated with age, sex, HNPCC, GSTM1 or GSTT1 genotype and history of adenomas. None of the associations was modified by GSTM1 or GSTT1 genotype (results not shown).

Those belonging to a family with known HNPCC had 48.8% (95% CI = 20.0–67.3) lower GST-α and 98.9% (95% CI = 15.1–100.0) lower GSH protein levels compared with individuals who do not belong to a HNPCC family. These associations did not depend on GSTM1 and GSTT1 genotype. Current smokers had 103.2% (95% CI = 20.6–242.5) higher GST-α and 53.1% (95% CI = 9.6–113.7) higher GSTP1-1 protein levels compared with never smokers, while levels of current and ex-smokers did not differ. GSTP1-1 protein levels were lower for those with a previous diagnosis of colorectal adenomas compared with those without such a diagnosis \([-37.8\% (95\% \text{ CI} = -55.4–-13.3)\)], although no differences between those with and without current adenomas could be detected. For other isoenzymes and characteristics mentioned in Table II, no differences were found.

Associations of fruits and vegetables with GST enzyme activity, according to GSTM1 genotype, are presented in Table III. Consumption of fruits, in particular citrus fruits, was positively associated with GST enzyme activity. Total consumption of vegetables was positively associated with GST enzyme activity among GSTM1-plus carriers, but not GSTM1-null carriers. This tended to be true for all subgroups except allium, although the modification was only statistically significant for total vegetables and brassica vegetables. No association between juices and GST enzyme activity could be detected.

When brassica vegetables and citrus fruits were included together in the model, their parameter estimates remained essentially the same.

Consumption of citrus fruits, which was associated with total GST enzyme activity, was not associated with GST-α \([\text{difference between high and low consumption: } 9.7\% (95\% \text{ CI} = -10.7–34.7)]\), GSTM1-1 \([8.1\%, 95\% \text{ CI} = (-26.3–58.3)]\),

Table I. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men ((n = 45))</th>
<th>Women ((n = 49))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of study population</td>
<td></td>
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</tr>
<tr>
<td>Members of HNPCC families</td>
<td>18 (40.0%)</td>
<td>26 (53.1%)</td>
</tr>
<tr>
<td>Non-HNPCC subjects</td>
<td>27 (60.0%)</td>
<td>23 (46.9%)</td>
</tr>
<tr>
<td>Demographic and lifestyle factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>45.7 ± 13.3</td>
<td>47.9 ± 13.9</td>
</tr>
<tr>
<td>Body mass index (kg/m²), mean ± SD</td>
<td>25.8 ± 2.5</td>
<td>24.9 ± 3.6</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>14 (31.1%)</td>
<td>16 (33.3%)</td>
</tr>
<tr>
<td>Intake of paracetamol (tablets/year), median (p25, p75)</td>
<td>1.0 (0.5, 4.0)</td>
<td>2.6 (1.0, 6.0)</td>
</tr>
<tr>
<td>Intake of NSAIDs (tablets/year), median (p25, p75)</td>
<td>1.3 (0.5, 2.0)</td>
<td>1.6 (1.0, 2.3)</td>
</tr>
<tr>
<td>Dietary factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kJ/day), mean ± SD</td>
<td>10515 ± 3368</td>
<td>8343 ± 1768</td>
</tr>
<tr>
<td>Protein (g/day), mean ± SD</td>
<td>90.6 ± 27.2</td>
<td>76.2 ± 14.5</td>
</tr>
<tr>
<td>Carbohydrates (g/day), mean ± SD</td>
<td>276.0 ± 94.1</td>
<td>221.5 ± 55.1</td>
</tr>
<tr>
<td>Fat (g/day), median (p25, p75)</td>
<td>99.7 (73.1; 113.5)</td>
<td>76.2 (65.5; 89.8)</td>
</tr>
<tr>
<td>Fruits (g/day), median (p25, p75)</td>
<td>149.3 (62.8; 253.0)</td>
<td>122.5 (84.7; 236.6)</td>
</tr>
<tr>
<td>Vegetables (g/day), median (p25, p75)</td>
<td>110.4 (87.0; 147.0)</td>
<td>118.6 (85.1; 157.6)</td>
</tr>
<tr>
<td>Fibre (g/day), median (p25, p75)</td>
<td>27.7 (22.1; 31.1)</td>
<td>21.7 (18.8; 26.9)</td>
</tr>
<tr>
<td>Meat (g/day), median (p25, p75)</td>
<td>129.4 (85.4; 154.0)</td>
<td>100.7 (60.7; 123.0)</td>
</tr>
<tr>
<td>Alcohol (glasses/week), median (p25, p75)</td>
<td>7.6 (3.0; 15.7)</td>
<td>3.7 (0.9; 14.2)</td>
</tr>
</tbody>
</table>

References:
1. Because of missing values, not all numbers sum up to 45 and 49, respectively.
2. All HNPCC family members were free of cancer themselves. Six (four male, two female) and three (one male, two female) of the HNPCC family members were known to carry a germline mutation in hMLH1 and hMSH2, respectively, whereas 24 (nine male, 15 female) were known to be no germline mutation carriers. One female subject classified as not having HNPCC had potentially late onset HNPCC, although her pedigree did not fulfill the Amsterdam criteria.
3. Of the non-HNPCC subjects, six (23.1%) of the females and three (13.0%) of the males had a positive first-degree family history of colorectal cancer. For one subject this was unknown.
4. Seven male and four female subjects had rectal adenomas.
5. In the year prior to recruitment.
GSTP1-1 [0.9%, 95% CI = (−18.4−24.7)] and GSH [−26.1%, 95% CI = (−97.1−1796.3)].

Consumption of brassica vegetables, which was associated with GST enzyme activity, was also associated with GSTM1-1 protein levels [difference between high and low consumption: 67.5%, 95% CI = (6.8–162.7)], but not with GST-α [−1.5%, 95% CI = (−24.4–28.4)].

GSTP1-1 [8.3%, 95% CI = (−17.3−41.8)] and GSH [177.7%, 95% CI = (−95.0–15405.8)]. Consumption of allium vegetables was not associated with GST enzyme activity, but was associated negatively with GSTP1-1 levels [difference between high and low consumption: −23.3%, 95% CI = (−35.5–8.6)]. Consumption of green leafy vegetables, which was also not associated with GST enzyme
activity, was positively associated with GSTM levels [133.3%, 95% CI = (11.7–387.4)], and negatively with GST-α levels [−26.5%, 95% CI = (−44.2; −3.3)]. These associations were not modified by GSTM1 and GSTT1 genotype, and remained after adding consumption of brassica vegetables to the model. Other associations of fruits and vegetables with GST isoenzyme and GSH levels were absent.

Table II. Description of rectal GST enzyme activity according to GSTM1 and GSTT1 genotype, lifestyle and medical factors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>GST enzyme activity (nmol/min/mg protein)</th>
<th>Mean ± SD</th>
<th>Adjusted for HNPCC and current adenoma</th>
<th>Adjusted for HNPCC, current adenoma and current smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total group (n = 77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1- plus</td>
<td>38 (49.4%)</td>
<td>243 ± 73</td>
<td>9.8 (−28.6; 48.2)</td>
<td>21.3 (−16.9; 59.4)</td>
<td></td>
</tr>
<tr>
<td>GSTM1-null</td>
<td>39 (50.6%)</td>
<td>231 ± 84</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>GSTT1- plus</td>
<td>67 (87.0%)</td>
<td>242 ± 79</td>
<td>30.9 (−30.0; 91.9)</td>
<td>28.6 (−31.2; 88.4)</td>
<td></td>
</tr>
<tr>
<td>GSTT1-null</td>
<td>10 (13.0%)</td>
<td>202 ± 69</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>36 (46.8%)</td>
<td>239 ± 93</td>
<td>3.6 (−34.6; 41.8)</td>
<td>5.6 (−31.5; 42.6)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>41 (53.2%)</td>
<td>235 ± 63</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Age &lt; 50 years old</td>
<td>43 (55.8%)</td>
<td>238 ± 83</td>
<td>14.8 (−26.2; 55.7)</td>
<td>21.3 (−18.4; 61.1)</td>
<td></td>
</tr>
<tr>
<td>Age ≥ 50 years old</td>
<td>34 (44.2%)</td>
<td>235 ± 73</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Association between consumption of fruits, vegetables and rectal GST enzyme activity

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Consumption (g/day)</th>
<th>Difference in GST enzyme activity (nmol/min/mg protein) between individuals with a relatively high and those with a relatively low consumption</th>
<th>P-value of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits, vegetables, juices and apple sauce</td>
<td>347.8 (227.1; 516.9)</td>
<td>23.4 (−0.5; 47.3)</td>
<td>30.3 (−11.9; 72.5)</td>
</tr>
<tr>
<td>Fruits</td>
<td>122.9 (62.8; 244.3)</td>
<td>25.6 (5.0; 46.2)</td>
<td>27.3 (−7.5; 62.0)</td>
</tr>
<tr>
<td>Citrus fruits</td>
<td>30.0 (13.0; 76.0)</td>
<td>28.9 (9.3; 48.6)</td>
<td>24.3 (−2.6; 51.2)</td>
</tr>
<tr>
<td>Other fruits</td>
<td>92.9 (42.0; 175.9)</td>
<td>20.8 (−0.7; 42.2)</td>
<td>21.6 (−16.8; 59.9)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>105.3 (84.9; 149.0)</td>
<td>12.7 (13.8; 39.2)</td>
<td>36.5 (0.6; 72.4)</td>
</tr>
<tr>
<td>Brassica vegetables</td>
<td>19.2 (11.9; 31.2)</td>
<td>10.6 (−8.3; 29.4)</td>
<td>22.6 (0.2; 45.0)</td>
</tr>
<tr>
<td>Raw brassica vegetables</td>
<td>2.1 (0.6; 4.5)</td>
<td>7.4 (−4.2; 19.0)</td>
<td>18.7 (−0.2; 37.5)</td>
</tr>
<tr>
<td>Cooked brassica vegetables</td>
<td>16.2 (7.4; 28.3)</td>
<td>8.0 (−13.8; 29.8)</td>
<td>20.0 (−6.2; 46.2)</td>
</tr>
<tr>
<td>Allium vegetables</td>
<td>7.0 (3.5; 12.3)</td>
<td>3.9 (−13.9; 21.8)</td>
<td>3.9 (−22.3; 30.1)</td>
</tr>
<tr>
<td>Green leafy vegetables</td>
<td>13.2 (7.3; 25.5)</td>
<td>−10.4 (−36.2; 15.4)</td>
<td>−0.1 (−33.7; 33.4)</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>55.2 (44.0; 79.1)</td>
<td>8.6 (−11.9; 29.1)</td>
<td>24.3 (−6.4; 54.9)</td>
</tr>
<tr>
<td>Juices and apple sauce</td>
<td>61.5 (18.5; 146.6)</td>
<td>1.5 (−19.2; 22.2)</td>
<td>−3.2 (−30.0; 23.6)</td>
</tr>
<tr>
<td>Citrus fruit juices</td>
<td>28.8 (3.7; 69.2)</td>
<td>3.0 (−15.3; 21.2)</td>
<td>−2.8 (−24.9; 19.4)</td>
</tr>
<tr>
<td>Non-citrus fruit juices and apple sauce</td>
<td>30.8 (10.0; 69.8)</td>
<td>−2.4 (−23.9; 19.2)</td>
<td>−4.8 (−38.7; 29.0)</td>
</tr>
<tr>
<td>Vegetable juices</td>
<td>3.0 (0.6; 6.9)</td>
<td>2.4 (−6.8; 11.7)</td>
<td>1.3 (−12.4; 14.9)</td>
</tr>
</tbody>
</table>

*Adjusted for presence of adenomas at index endoscopy, HNPCC, total energy intake and current smoking.
Relatively high and relatively low are defined as the 75th and 25th percentile (p75, p25) of the consumption of each food group, respectively.
*One subject was excluded, because no information on smoking status was available.
*The consumption of fruits and vegetables did not differ between the subjects whose GST activity was assessed and all 94 subjects participating in the study.
Excluding potatoes and legumes.

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Dietary fibre, vitamin A, β-carotene and folate did not affect the parameter estimates, whereas the effect of vitamin C could not be disentangled from the contribution of citrus fruits due to their high correlation ($r = 0.76$).

**Discussion**

We observed that consumption of fruits, in particular citrus fruits, is positively associated with higher rectal GST enzyme activity. Consumption of vegetables, in particular brassica vegetables, was positively associated with GST enzyme activity, although this was limited to GSTM1-plus genotype carriers. Consumption of brassica vegetables was also positively associated with GSTM1-1 protein levels. No indication was found of an association between consumption of allium vegetables and GST enzyme activity, but allium vegetables were negatively associated with GSTP1-1 protein levels.

We requested subjects to report consumption of fruits and vegetables according to the year preceding endoscopy by filling out a food-frequency questionnaire. While the validity of the assessment was moderate, the reproducibility of the assessment was high. Although the assessment, like all assessments, is not perfect and may be subject to bias, this method allows assessing habitual consumption with minimal intrusion upon the participants. However, it is hard to determine whether we have assessed consumption in the time window relevant to GST induction. In rat hepatoma cells, GST induction was first observed 8 h after induction, with highest values after 24–48 h (30). In humans, rectal GST induction is likely to be a delayed process, as bioactive compounds have to reach the intestinal tissue, either via blood or the gastrointestinal tract. Thereupon, the higher activity might persist for some time for biological reasons or prolonged exposure. Indeed, Szarka and co-workers reported that human rectal GST enzyme activity was stable over a 2–4 week interval in another cross-sectional study (31).

We included subjects undergoing endoscopy for medical reasons instead of healthy subjects. This is unlikely to cause problems as subjects with colorectal cancer and inflammatory bowel diseases were excluded since these conditions may influence metabolism and gene expression; meanwhile controlling for self-reported bowel complaints did not importantly alter the estimates. Nevertheless, given subject selection on HNPPC or adenoma status, we adjusted all parameter estimates for these conditions, although this did not influence them. Likewise, these selection criteria modify none of the examined associations.

Our study design allowed us to evaluate the association between GST enzyme activity and adenoma presence, sex, age, HNPPC and genetic polymorphisms in GSTs. In line with the postulated role of GST induction in cancer prevention and previously published results (31), the presence of adenomas at index endoscopy, was associated with a lower GST enzyme activity in normal rectal tissue compared with rectal tissue of patients without adenomas. Cancer-free members of HNPPC families had lower rectal GST-α and GSH protein levels than those who did not belong to a HNPPC family. These differences, which could not be attributed to differences in GSTM1 and GSTT1 genotype, were unexpected and have not been reported before; therefore, these findings should be treated with caution. Interestingly, smoking was associated with a higher GST enzyme activity (Table II) and higher GST-α and GSTP1-1 protein levels. Indeed, rodent studies showed that several polycyclic aromatic hydrocarbons present in cigarette smoke induce GST enzyme activity as part of an adaptive response mechanism to chemical stress (3).

The main hypothesis concerned the association between GST, vegetables and GST enzyme activity, isoenzyme levels and GSH. First, fruits, in particular citrus fruits, were associated positively with GST enzyme activity. This might be attributed to the coumarin auraptene and to limonoids (highly oxidized triterpenoids), which are present in high concentrations in citrus fruit tissues (17,32). Indeed, auraptene increased GST enzyme activity in rat colon tissue (33) and limonoids induced GST enzyme activity in small intestinal mucosa and liver of mice (34). However, auraptene and limonoids are also present in citrus fruit juices (17), which were not associated with GST enzyme activity in this study. As processed juices comprise the larger part of the intake of juices, compounds that are lost during the processing of citrus fruits to juices may be responsible for these findings. Additionally, bioavailability of phytochemicals might differ between whole fruits and fruit juices. Other compounds present in citrus fruits may also account for the higher GST enzyme activity, such as citrus flavonoids and d-limonene (35). The latter enhanced colonic GST enzyme activity in rats (36), albeit at doses that far exceed human exposure.

Secondly, consumption of vegetables was positively associated with GST enzyme activity among those with the GSTM1-plus genotype, but not those with the GSTM1-null genotype. This association was most evident for brassica vegetables, which are rich in glucosinolates. Isothiocyanates may be primarily responsible for this GST inducing capacity (37), although glucosinolate content may depend on species, maturity and processing of the plants (38).

So far, only one study examined consumption of brassica vegetables in relation to rectal GST enzyme activity. In this study, 10 human volunteers consumed 300 g/day of Brussels sprouts for 7 days. No effect upon rectal GST enzyme activity and GSH levels was found, while GSTA and GSTP1-1 levels increased by 30 and 15%, respectively (11). In our study, consumption of brassica vegetables was not associated with GST-α and GSTP1-1 levels. However, it was associated with GSTM1 level, which is in line with the observed association between brassica vegetables and GST enzyme activity among GSTM1-plus genotype carriers.

Furthermore, the synthetic dithiolethione oltipraz—dithiolethiones are a class of chemical compounds, which also occur naturally in brassica vegetables—increased colonic mucosal GST enzyme activity at single oral doses of 125 and 250, but not at 500 or 1000 mg/m$^2$, in 24 subjects having a family history of colorectal cancer or a personal history of colorectal polyps or carcinomas (39). However, this could not be confirmed in two other trials (40,41). Other studies have evaluated the effect of brassica vegetables upon GST enzyme activity in other tissues (11–15), but because of organ-specific patterns of expression (7) and differences in bioavailability of bioactive compounds able to induce GST, it is hard to compare their results to those of the present study.

Although compounds of allium vegetables did positively affect the GSH/GST detoxification system in many *in vitro* or *in vivo* studies (3), as well as in a human study on lymphocytes (12), we observed a negative association between allium vegetables and GSTP1-1 levels. However, a previous study in which six healthy volunteers were given 250 g/day of mixed vegetables (among which allium vegetables) for a period of 2140
3 weeks reported decreased GSTP1 protein levels in five of the six subjects, which is in line with our observation (42).

Assessing overall GST enzyme activity, no correlation between GST isoenzyme levels and GST enzyme activity appeared. This could be partially attributed to our substrate CDNB, which reacts with moderate activity to GSTA1, GSTA2 and GSTP1, and with high activity to GSTM1 and GSTM2 (3,43). The GST-μ and GST-π classes belong to the most important GST isoenzymes in the rectum (11). While this makes CDNB a good general substrate, it is not highly specific to a particular isoenzyme, which might be related to the lack of correlation between GST isoenzymes and GST enzyme activity. Besides, GSTM2 protein levels were not assessed, while GSTM2 contributes to GST enzyme activity. As GSTM2 is expressed in the human colon and is inducible (8), induction of GSTM2 might further explain the lack of correlation. Alternatively, the assessment of GST enzyme activity might be slightly biased, as GSTs also serve as binding and transport proteins, resulting in a temporary loss of enzyme activity (3,43).

Associations with fruits and vegetables appeared to be different for GST isoenzymes and GST enzyme activity. For citrus fruits, we found an association with GST enzyme activity, but this could not be attributed to any of the assessed isoenzymes. Theoretically, we cannot exclude the possibility that citrus fruits induce GSTM2 exclusively. However, it seems more likely that effects on specific isoenzymes are too small to be detected, whereas effects on GST enzyme activity—which reflects isoenzyme levels of GSTA, GSTM and GSTP1-1 in combination with their specific activity—can be detected. This might also explain why consumption of green leafy vegetables was associated with GSTM1-1 and GST-α levels, while no association with GST enzyme activity was found. As these associations have not been examined before, the actual nature of the relationships remains to be established.

The underlying idea of this study is that high GST enzyme activity is believed to be beneficial to cancer prevention. GST enzyme activity in normal mucosa along the gastrointestinal tract was inversely correlated with tumour incidence (6); inhibition of rat bowel carcinogenesis was shown to correlate with induction of GST (33); and compounds that induce GST generally counteract cytogenetic damage (44). However, it should be noted that certain food components [e.g. sulforaphane and indole 3-carbinol present in broccoli (45,46)] may affect phase I as well as phase II enzymes; the balance between induction and inhibition of different detoxification systems is of utmost relevance (47).

In conclusion, our study shows positive associations of habitual levels of consumption of fruits and vegetables with rectal GST enzyme activity. This is especially true for citrus fruits and brassica vegetables, but positive associations with the latter only existed for GSTM1-plus individuals.

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References

validity and reproducibility for food groups. Int. J. Epidemiol., 26 (suppl 1), S37-S48.


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