

SHORT COMMUNICATION

Effects of consumption of Brussels sprouts on plasma and urinary glutathione S-transferase class- α and - π in humansWim A.Nijhoff, Theo P.J.Mulder, Hans Verhagen¹, Geert van Poppel¹ and Wilbert H.M.Peters²Department of Gastroenterology, University Hospital St Radboud, Nijmegen and ¹TNO Nutrition and Food Research Institute, Zeist, The Netherlands²To whom correspondence should be addressed

The effects of consumption of glucosinolate-containing Brussels sprouts on plasma and urinary glutathione S-transferase (GST) class- α and - π were investigated. Five male and five female non-smoking volunteers were randomly assigned to two groups in a crossover design. Five persons started on a glucosinolate-free diet (control period), while the other five consumed 300 g of cooked Brussels sprouts per day, at the expense of 300 g of glucosinolate-free vegetables (sprouts period). Dietary regimes were reversed after 1 week. GST levels were measured by enzyme-linked immunoabsorbent assay. At the end of the sprouts period, a significant increase (1.5-fold) in plasma class- α GST levels was observed in males but not in females (control versus sprouts, paired *t*-test; *P*-values 0.031 and 0.317 respectively), while plasma GST class- π levels as well as secretion of urinary GST class- α and - π levels remained unchanged. We conclude that (i) increased plasma GST class- α levels in males originate probably solely from the liver and not from stomach, intestine or kidney; (ii) males are more susceptible for induction of hepatic GSTs than females; and (iii) urinary GST concentration seems less useful as a biomarker for hepatic GST induction.

Gastrointestinal tumours are among the most common malignancies in Western society (1). For this reason, research on prevention of gastrointestinal carcinogenesis is of the utmost importance (2). Epidemiological studies show the importance of dietary habits. Diets rich in cruciferous vegetables, such as Brussels sprouts (*Brassica oleraceae*), are associated with a lower risk (3). The anticarcinogenic properties of cruciferous vegetables have been mainly attributed to the degradation products of glucosinolates (4,5). A possible mechanism of the chemopreventive action of these constituents may be the induction of detoxification enzymes (6-9). Important drug-metabolizing or detoxification enzymes are glutathione S-transferases (GSTs*; EC 2.5.1.18), a family of enzymes with tissue-specific distribution. For instance, epithelial cells from the small intestine contain class- α and - π isozymes, while in liver only class- α is expressed (10,11). GSTs catalyse the reaction of a wide variety of electrophiles to glutathione. Since most of the reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, GSTs contribute considerably to carcinogen inactivation (12,13). Enhancement of such detoxifying enzyme systems could potentially increase the capacity to

*Abbreviations: GST, glutathione S-transferase; ELISA, enzyme-linked immunosorbent assay.

withstand the burden of toxicants and (pre)carcinogens we are exposed to daily (14). Recently Bogaards *et al.* (15) found elevated plasma GST class- α levels in males, as a result of consumption of Brussels sprouts, and stated that this may represent induced GST class- α levels in liver and other gastrointestinal organs, such as the intestine. Thus, changes in GST levels in plasma could be a useful biomarker for induction of GSTs in several organs including the intestine. The aims of the present study were (i) to elucidate the origin of elevated plasma GST class- α levels due to dietary intake of glucosinolate-containing Brussels sprouts; (ii) to reveal possible sex-specific induction of plasma GSTs; and (iii) to examine the effectiveness of urinary GST levels as a biomarker for GST induction by dietary components.

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. Ten healthy non-smoking volunteers (Table I) were randomly assigned to a crossover design. Five volunteers (three females, two males) started with a glucosinolate-free diet (control period), while the other five (two females, three males) consumed 300 g of cooked Brussels sprouts per day, at the expense of 300 g of glucosinolate-free vegetables (sprouts period). After 7 days the dietary regimen was changed for another week. All standardized dinners were prepared and eaten at the Academic Hospital Nijmegen. The Brussels sprouts used in this study were from the same batch as used previously by Bogaards *et al.* (15). Volunteers were asked to refrain from other glucosinolate-containing foods during the experiment. For their convenience a dietary exclusion list was provided. On day 7 of both periods (control and sprouts), blood was sampled by venepuncture into EDTA-containing tubes, and 24 h urine samples were collected. After centrifugation (2500 g, 10 min), plasma samples were stored at -20°C.

GST class- α and - π levels were determined in duplicate using an enzyme-linked immunoabsorbent assay (ELISA). All samples were determined in the same run. Polystyrene 96 well plates (Greiner BV, Alphen a/d Rijn, The Netherlands) were incubated with 100 μ l of PBS containing mouse anti-human

Table I. General data pertaining to the volunteers

Volunteer	Sex	Age (years)	Quetelet index (kg/m ²)
1	female	27	20.4
2	male	22	25.2
3	female	23	21.6
4	male	24	24.4
5	female	23	19.3
6	male	26	25.8
7	female	22	22.1
8	male	26	21.5
9	female	29	19.4
10	male	21	19.9

GST- α (16) or - π (17) monoclonal antibody (10 μ g/ml) at 4 °C overnight. After removal of the free anti-GST- α or - π antibody the plates were incubated with PBS containing 1% BSA for 2 h at room temperature in order to block non-specific binding. The plates were washed five times with PBS containing 0.05% Tween 20 (buffer A) and then incubated overnight at room temperature with various amounts of purified GST- α and - π (0.2–100 ng/ml in buffer B; 20 mM EDTA and 10% heated normal human plasma in buffer A) or test plasma (diluted with buffer B). After washing five times, the plates were incubated for 3 h with rabbit anti-human GST- α or - π antibody, both prepared at our laboratory by routine immunization protocol, in PBS containing 1% BSA (diluted 4000-fold). The plates were washed again, and incubated for 3 h with swine anti-rabbit IgG antibody conjugated with peroxidase (Dakopatts, Glostrup, Denmark), diluted 2000-fold with PBS containing

1% BSA. After washing, the peroxidase substrate *ortho*-phenylenediamine (2.2 mM) in phosphate citrate buffer (51 mM Na₂HPO₄, 24 mM citric acid, pH 5.0) containing 0.01% H₂O₂ was added and the plates were incubated at room temperature for 15 min. Colour reaction was stopped by adding 100 μ l 4 N H₂SO₄ to each well. The colour intensity of each well was read at 492 nm with a background subtraction at 620 nm. Concentrations were calculated using a non-linear four-parameter logistic model. The detection limits were 0.05 and 0.2 ng/ml for GST class- α and - π respectively. Paired *t*-test or independent *t*-test were used as appropriate to assess statistical significance of differences; $P < 0.050$ is considered significant.

Male and female plasma GST class- α and class- π levels (means \pm SEM) as measured at the end of both dietary periods are given in Figure 1. GST class- π levels were ~15 times higher as compared to GST class- α levels for both males and females ($P = 0.0025$ and $P = 0.0005$, respectively). Plasma

plasma

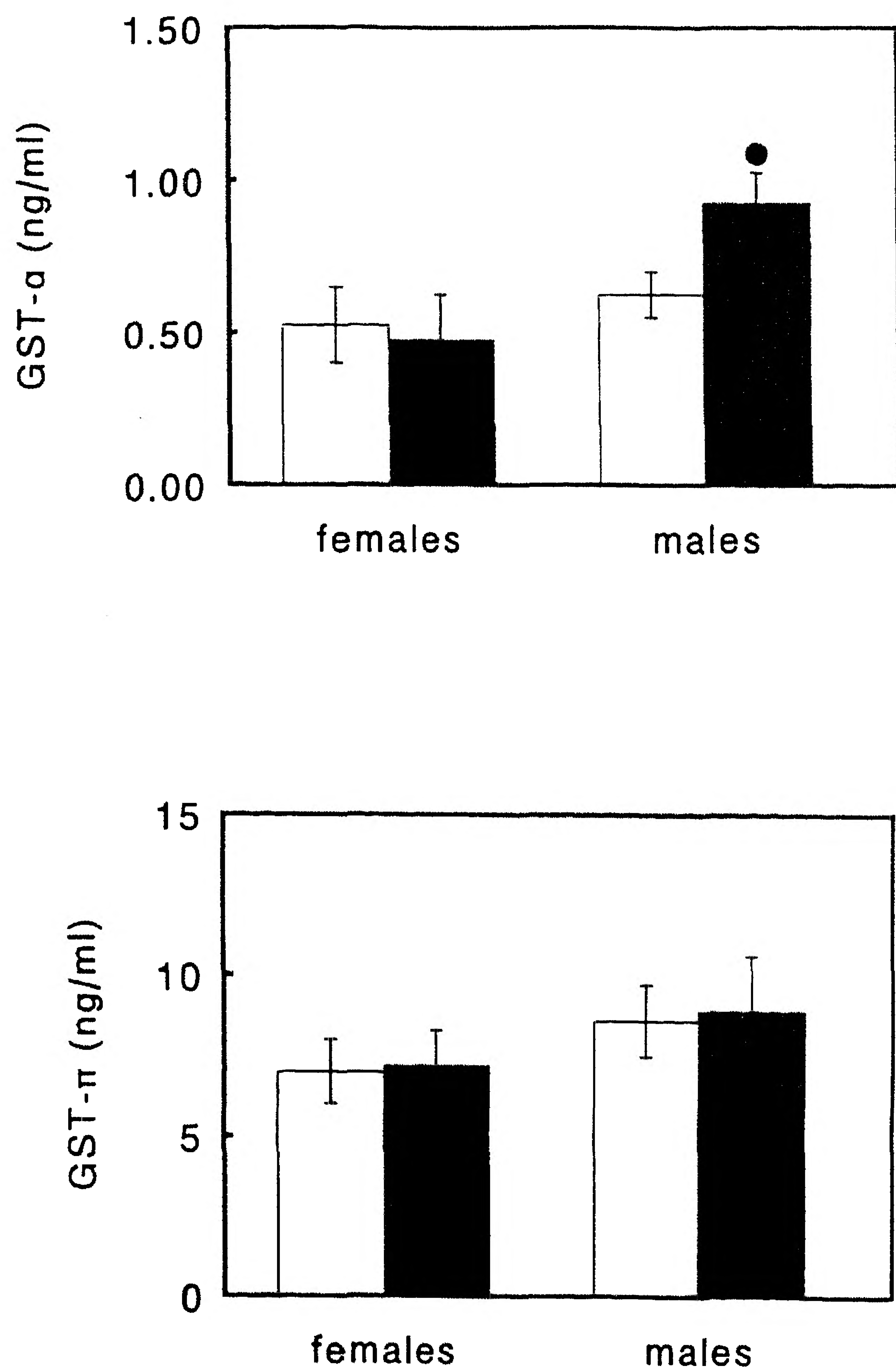


Fig. 1. Effects of consumption of glucosinolate-containing Brussels sprouts on plasma GST class- α and - π levels in females and males. Bars represent plasma GST levels (ng/ml, mean \pm SEM) for both sexes in control period (open bar) and sprouts period (closed bar) as measured by ELISA. During the control period, volunteers consumed a glucosinolate-free diet, while during the sprouts period they consumed 300 g of cooked Brussels sprouts per day, at the expense of glucosinolate-free vegetables. At the end of the sprouts period compared with control period, a significant induction (1.5-fold) in plasma GST class- α levels was observed in males but not in females. Paired *t*-test; P values 0.031 (filled circle) and 0.317 respectively. Plasma GST class- π levels remained unchanged for males and females.

urine

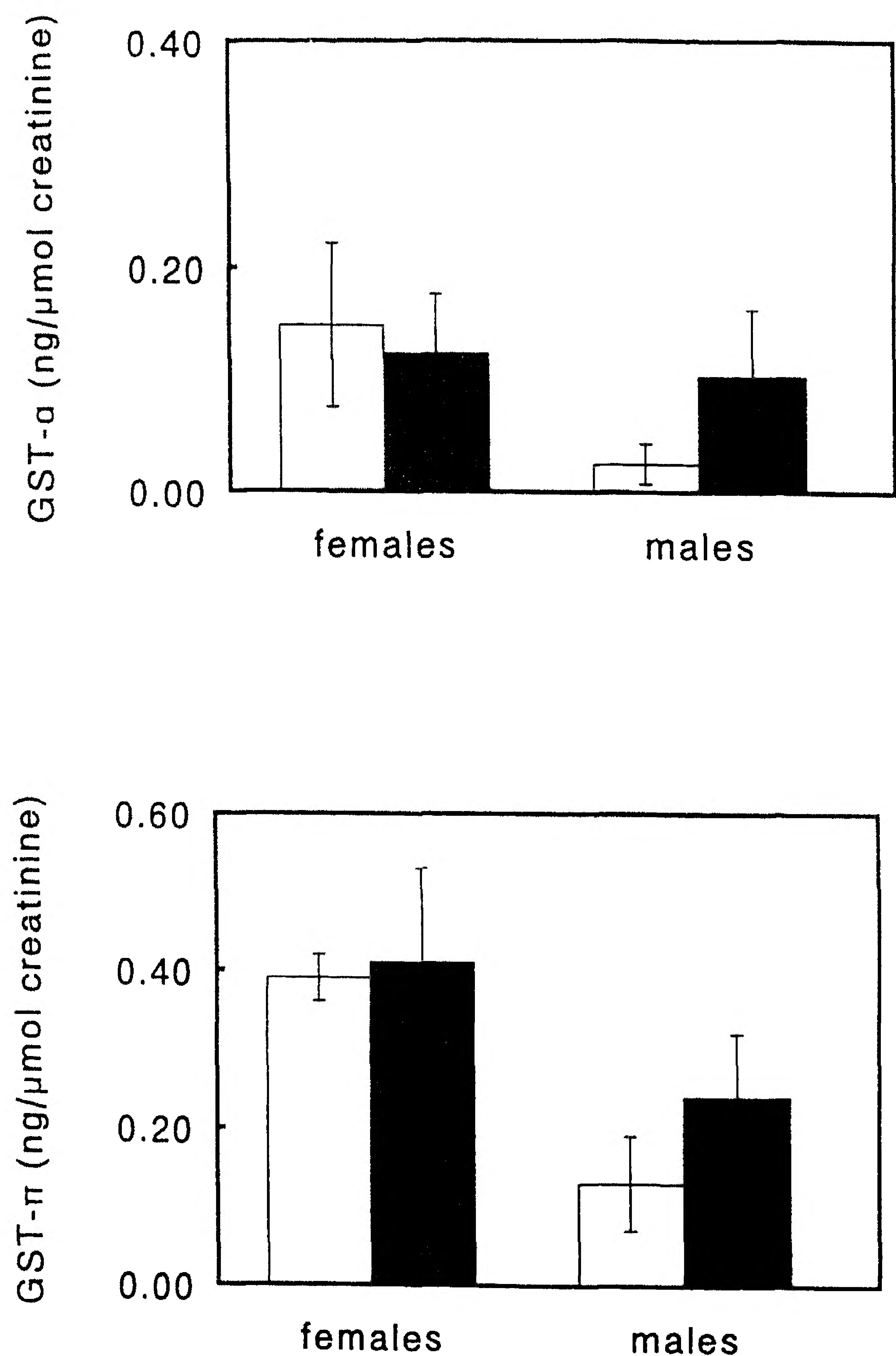


Fig. 2. Effects of consumption of glucosinolate-containing Brussels sprouts on urinary GST class- α and - π levels in females and males. Bars represent urinary GST values (ng/μmol creatinine, mean \pm SEM) for both sexes in control period (open bar) and sprouts period (closed bar) as measured by ELISA. During the control period volunteers consumed a glucosinolate-free diet, while during the sprouts period they consumed 300 g of cooked Brussels sprouts per day, at the expense of glucosinolate-free vegetables. No statistically significant difference was observed between control and sprouts period for either GST class- α or - π level in both sexes.

GST class- α levels at the end of the sprouts period were induced 1.5-fold in males but not in females (P values 0.031 and 0.317 respectively). Male and female urinary class- α or - π levels between the two periods were uninfluenced (Figure 2); although GST class- α and class- π levels in males appeared to be increased the change did not acquire statistical significance. Interestingly, in urine no differences in basal levels between GST class- α and - π were observed. Urinary GST class- π levels in females are three times higher as compared to those found in males ($P = 0.006$). This difference could reflect sex-preferable expression in addition to the already known tissue-specific distribution of GSTs.

In accordance with the results obtained in males after a 2 week Brussels sprouts dietary regimen by Bogaards *et al.* (15), we also found increased plasma GST class- α levels (1.5-fold) in males after consumption of Brussels sprouts for just 1 week. This suggests that dietary intervention periods, in order to study GST induction, need not be as long as those generally used in human or animal studies. Furthermore, consumption of glucosinolate-containing Brussels sprouts did not alter GST class- α levels in females. So there seems to be a sex-specific induction of plasma GST class- α levels in humans after consumption of glucosinolate-containing Brussels sprouts. In rodents, sex-specific or sex-preferable induction of GSTs by dietary components has already been reported (18–20). In humans class- α GSTs are most abundant in stomach, liver, small intestine and kidney, whereas class- π is present in considerable amounts in stomach, small and large intestine, kidney and blood cells (10,11). To elucidate the origin of the elevated plasma GST class- α levels in males, we studied plasma GST class- π levels and urinary GST class- α and class- π excretion. No concomitant change in plasma GST class- π levels was observed in males or females. Urinary GST levels seemed not to respond to the dietary regimen. Therefore, we conclude that (i) urinary GST levels seem less useful as a biomarker for gastrointestinal GST induction; and (ii) increased plasma GST class- α levels most probably originate from liver and not from stomach, intestine or kidney. In addition, no change in liver function parameters (alanine aminotransferase, aspartate aminotransferase and γ -glutamyltransferase) were observed between the two periods (data not shown). This strongly suggests that the increased plasma GST class- α levels are the result of normal hepatic cell turnover, with higher GST levels in the liver cell at the end of the sprouts period. We hypothesize that this increased hepatic detoxification capacity may reflect a lower susceptibility towards toxicants for males, and not for females, after consumption of glucosinolate-containing Brussels sprouts.

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