

SHORT COMMUNICATION

Age and gender dependent levels of glutathione and glutathione S-transferases in human lymphocytes

Esther M.M.van Lieshout and Wilbert H.M.Peters¹

Department of Gastroenterology, University Hospital St. Radboud, PO Box 9101, 6500 HB Nijmegen, The Netherlands

¹To whom correspondence should be addressed

Glutathione S-transferases (GSTs) are a family of enzymes involved in the detoxification of a wide range of chemicals including chemical carcinogens. Human cytosolic GSTs are divided into four major classes; α , μ , π and θ . This study was performed to evaluate the influence of age and gender on the GST isoenzyme expression and glutathione (GSH) content in lymphocytes. Blood was collected from 124 healthy controls, which were divided into age groups of 20–40 years (21 females, 20 males), 40–60 years (20 females, 21 males) and 60–80 years (20 females, 22 males). Lymphocytes were isolated by density centrifugation on Histopaque-1077. After homogenization, cytosolic fractions were isolated. Herein, GST isoenzyme levels were determined by densitometrical analysis of western blots after immunodetection with monoclonal antibodies. Total GSH content was determined by high performance liquid chromatography after conjugation with monobromobimane. Spearman rank correlation and Wilcoxon rank sum tests were used for statistical evaluation. Lymphocytic GST μ and π levels were not correlated with age or gender. GST α was not detectable in lymphocytes. GSH contents were not different in males and females, but decreased with age in both males and females. In age group 60–80, GSH content was significantly lower as compared with age groups 20–40 and 40–60 in both sexes. Since high GSH is an essential factor in the detoxification of many compounds, these data indicate that the detoxification potential of the GSH/GST system in lymphocytes may decrease with age in man.

Human cytosolic glutathione S-transferases (GSTs) are a family of dimeric enzymes, divided into the main classes α , μ , π and ϕ (1–3). GSTs catalyse the binding of a large variety of electrophiles to the sulphydryl group of glutathione (GSH), generally resulting in less harmful and more water soluble molecules (1). The GSH/GST system may be a critical factor in protecting cells and organs against toxicity and disease. GSH, an important factor in the normal functioning of the GSH/GST system, is involved in the detoxification of xenobiotics, carcinogens, free radicals and peroxides (1). Low GSH contents have been found in several pathological conditions, including alcoholic liver disease (4), acquired immunodeficiency syndrome (5), xenobiotic-induced oxidative stress and toxicity (6) and (pre)cancerous lesions (7). From these studies it may be concluded that the availability of GSH might be a key factor in the maintenance of health, and that GSH concentration may serve as a useful indicator of disease risk in humans.

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

Little is known about the differences in GST and GSH expression between men and women. Also data on GST and GSH levels with respect to aging in humans are scarce. Loguercio *et al.* (8) showed that GSH content in body and antrum of the stomach decreased with age. They did not find a sex dependency in GSH content. To obtain information of the lymphocytic GSH/GST system during aging, we investigated GST isoenzyme levels and GSH contents in human lymphocytes from 124 healthy subjects, aged 20–80 years.

Blood was collected by venapuncture into sterile siliconized EDTA K3 10 ml vacutainer tubes (Beckton Dickinson, San Jose, CA). Controls were divided into three age groups; 20–40 years (21 females, 20 males), 40–60 years (20 females, 21 males) and 60–80 years (20 females, 22 males). Lymphocytes were isolated by density centrifugation on Histopaque-1077, according to the manufacturers instructions (Sigma diagnostic, St Louis, MO). Lymphocytes were pelleted and stored at -20°C until use. For preparation of cytosolic fractions, lymphocytes were thawed slowly, homogenized in 100 μl of 20 mM Tris-HCl buffer pH 7.4, containing 1 mM dithiothreitol using a glass/glass potter. Homogenates were centrifuged at 12 000 g (4°C) for 20 min. Aliquots of the supernatant were stored at -20°C until use. The investigations were approved by the local ethical committee on human experimentation.

Protein was assayed in triplicate by the method of Lowry *et al.* (9), using bovine serum albumin as a standard. Specific GST isoenzyme levels were determined as described previously (10). In short, cytosolic fractions were subjected to SDS-PAGE [11% acrylamide (w/v)], and subsequently to western blotting, using a semi-dry blotting system (Novablot II, Pharmacia, Uppsala, Sweden). Western blots were incubated with monoclonal antibodies against human GST classes α , μ and π . Class α antibodies react with human GST A1-1, GST A1-2 and GST A2-2 (10). Class μ antibodies recognize human GST M1a-1a, GST M1a-1b and GST M1b-1b (11,12). Class π antibodies react with human GST P1-1 (13). The specific binding of the monoclonal antibodies to the isoenzymes was demonstrated by incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dakopatts, Glostrup, Denmark) and subsequent staining with 4-chloro-1-naphthol and hydrogen peroxide. Staining intensity on the immunoblots was quantified using a laser densitometer (Ultrosan XL, LKB, Bromma, Sweden). Known amounts of purified GSTs were run in parallel with the experimental samples and served as standards for the calculation of the isoenzyme levels in the cytosolic fractions. The detection limit of this assay is ~ 50 ng/mg protein. Total GSH was quantified by high performance liquid chromatography after reaction with monobromobimane, as described previously (14). In this assay, oxidized GSH present is reduced by adding sodium borohydride to the reaction mixture. A Spearman rank correlation test was used to correlate lymphocytic GSH content and GST isoenzyme expression with age and gender. A Wilcoxon rank sum test was used to assess statistical significance of differences between age

Table I. GSH content and GST α , μ and π isoenzyme levels in human lymphocytes

Age group		GSH (nmol/mg protein)	GST α (ng/mg protein)	GST μ (ng/mg protein)	GST π (ng/mg protein)
20–40	Total ($n = 41$)	21.5 \pm 4.1 (4.7–43.7)**	ND	368 \pm 76 (0–1766)	883 \pm 97 (0–2743)
	Males ($n = 20$)	19.4 \pm 1.8 (4.7–43.7)***	ND	278 \pm 99 (0–1199)	892 \pm 199 (0–2743)
	Females ($n = 21$)	23.8 \pm 3.2 (5.1–25.4)**	ND	454 \pm 97 (0–1766)	874 \pm 95 (0–2377)
40–60	Total ($n = 41$)	17.9 \pm 1.1 (5.5–41.7)**	ND	383 \pm 90 (0–2864)	871 \pm 102 (0–3025)
	Males ($n = 21$)	16.8 \pm 1.2 (5.5–26.7)*	ND	376 \pm 146 (0–2864)	969 \pm 182 (0–3025)
	Females ($n = 20$)	19.0 \pm 1.8 (8.9–41.7)**	ND	389 \pm 96 (0–1230)	769 \pm 97 (0–1701)
60–80	Total ($n = 42$)	12.3 \pm 0.6 (2.4–26.3)	ND	363 \pm 55 (0–917)	791 \pm 57 (207–1816)
	Males ($n = 22$)	13.3 \pm 0.9 (8.2–26.3)	ND	357 \pm 70 (0–872)	879 \pm 70 (409–1618)
	Females ($n = 20$)	11.2 \pm 0.9 (2.4–18.7)	ND	370 \pm 88 (0–917)	695 \pm 90 (217–1816)

GSH content and GST isoenzyme levels in lymphocytes were determined as described in the text.

Values are given as mean \pm SEM. Ranges are indicated in parentheses. ND, not detectable.

Wilcoxon rank sum test was used for statistical evaluation: * $P < 0.02$, ** $P < 0.005$ and *** $P < 0.002$ as compared with age group 60–80 years.

groups. Table I shows the GSH contents and GST isoenzyme levels of all age groups studied.

GST α was not detectable in lymphocytes. In 54 (44%) of the samples, no GST μ protein was found, in 51% (60% males, 43% females) of the age group 20–40 years, in 34% (38% males, 30% females) of the age group 40–60 years and in 45% (41% males, 50% females) of age group 60–80 years. GST μ null phenotype was not related to gender or age. GST π expression was equally distributed among the different age groups, as well as in males and females. GSH content in lymphocytes was similar in male and female controls, but expression declined with age: females $R_s = -0.36$ (95% CI -0.56 – 0.12 ; $P = 0.004$); males $R_s = -0.42$ (95% CI -0.61 – 0.20 ; $P = 0.0005$). This significant decline in GSH content with age persisted when males and females were combined: $R_s = -0.39$ (95% CI -0.53 – 0.23 ; $P < 0.0001$). GSH content in age group 60–80 years was significantly reduced as compared with the age groups 20–40 and 40–60 years in both sexes, whereas no difference was observed when comparing GSH contents in age groups 20–40 and 40–60 years.

GSTs are involved in the protection against potentially harmful chemical compounds (1). Little data are available concerning age-related changes in the levels of GSH and GST isoenzymes in humans. This may be relevant with respect to the possible role of the GSH/GST system in the enhanced cancer rates at increased age. We now showed that the expression of the GST α , μ and π isoenzymes in human lymphocytes did not change with age, although in females there was a tendency for lower levels of GST π at older age ($P = 0.06$; 20–40 versus 60–80 year age group). No gender-related effects were found. Unfortunately, due to lack of sufficient material we were unable to measure GST enzyme activity and levels of the most recently discovered GST ϕ forms. Cebalos-Picot *et al.* (15) demonstrated a negative correlation between age and GST activity ($R = 0.58$, $P < 0.001$) in human erythrocytes (120 females, 65 males), but they found no gender-related differences.

Several studies have suggested that GSH might be a critical factor in protecting cells and organs against toxicity and disease, since GSH as an important factor in the GST/GSH peroxidase systems is involved in the detoxification of xenobiotics, carcinogens, free radicals and peroxides (1). However, no information is available on the variation of GSH in lymphocytes in relation to gender and age in healthy subjects. We noticed no differences in GSH levels of human lymphocytes between males and females. Similar results were found previously in human blood by Richie *et al.* (16) (484

males, 231 females) and Michelet *et al.* (17) (107 males, 94 females), in plasma by Yang *et al.* (18) (125 males, 157 females), and in body and antrum of the stomach by Loguercio *et al.* (8) (12 males, 10 females). We showed that GSH levels in human lymphocytes decreased with age in both males and females. Similar results were found by Lang *et al.* (19), who found a significant increase ($P < 0.001$) in the proportion of elderly individuals with low blood GSH values compared with younger adults. Also plasma GSH levels in Chinese male and female volunteers were found to decrease with increasing age (18). Loguercio *et al.* (8) showed that GSH content in body and antrum of the stomach decreased with age.

In summary, we demonstrated that GST expression in human lymphocytes was not related to sex or age. GSH content was similar in males and females and decreased with age in both sexes. Since GSH is correlated with protection against cellular or cytogenetic damage, reduction of GSH content during aging may be a factor of relevance for the increased risk of developing diseases such as cancer at an older age.

Acknowledgement

This work was supported by grant 94-715 (EMMvL) from the Dutch Cancer Society.

References

- Hayes, J.D. and Pulford, D.J. (1995) The glutathione S-transferase supergene family: regulation of GST and contribution of the isoenzymes to cancer chemoprevention and drug resistance. *Crit. Rev. Biochem. Mol. Biol.*, **31**, 445–600.
- Pemble, S., Schroeder, K.R., Spencer, S.R., Meyer, D.J., Hallier, E., Bolt, H.M., Ketterer, B. and Taylor, J.B. (1994) Human glutathione S-transferase theta (GSTT1) cDNA cloning and the characterization of a genetic polymorphism. *Biochem. J.*, **300**, 271–276.
- Tan, K.L., Webb, G.C., Baker, G.C. and Board, P.G. (1995) Molecular cloning of a cDNA and chromosomal localization of a human theta class glutathione S-transferase gene (GSTT2) to chromosome 22. *Genomics*, **25**, 381–387.
- MacDonald, C.M., Dow, J. and Moore, M.R. (1977) A possible protective role for sulphhydryl compounds in acute alcoholic liver injury. *Biochem. Pharmacol.*, **26**, 1529–1531.
- Staal, F.J., Ela, S.W., Roederer, M., Anderson, M.T. and Herzenberg, L.A. (1992) Glutathione deficiency and human immunodeficiency virus infection. *Lancet*, **339**, 909–912.
- DiSimplicio, P., Dolara, P. and Lodovici, M. (1984) Blood glutathione as a measure of exposure to toxic compounds. *J. Appl. Toxicol.*, **4**, 227–229.
- Peters, W.H.M., Roelofs, H.M.J., Hectors, M.P.C., Nagengast, F.M. and Jansen, J.B.M.J. (1993) Glutathione and glutathione S-transferases in Barrett's epithelium. *Br. J. Cancer*, **67**, 1413–1417.
- Loguercio, C., Taranto, D., Beneduce, F., Vitale, L.M. and Delle-Cava, M. (1996) Age affects glutathione content and glutathione transferase activity in human gastric mucosa. *Ital. J. Gastroenterol.*, **28**, 477–481.

9. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randal, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
10. Peters, W.H.M., Boon, C.E.W., Roelofs, H.M.J., Wobbes, Th., Nagengast, F.M. and Kremers, P.G. (1992) Expression of drug metabolizing enzymes and P-170 glycoprotein in colorectal carcinoma and normal mucosa. *Gastroenterology*, **103**, 448–455.
11. Peters, W.H.M., Kock, L., Nagengast, F.M. and Roelofs, H.M.J. (1990) Immunodetection with a monoclonal antibody of glutathione S-transferase mu in patients with and without carcinomas. *Biochem. Pharmacol.*, **39**, 591–597.
12. Van Ommen, B., Bogaards, J.J.P., Peters, W.H.M., Blaauwboer, B. and Van Bladeren, P.J. (1990) Quantification of human hepatic glutathione S-transferases. *Biochem. J.*, **269**, 609–613.
13. Peters, W.H.M., Nagengast, F.M. and Wobbes, Th. (1989) Glutathione S-transferases in normal and cancerous human colon tissue. *Carcinogenesis*, **10**, 2371–2374.
14. Nijhoff, W.A., Groen, G.M. and Peters, W.H.M. (1993) Induction of rat hepatic and intestinal glutathione S-transferases and glutathione by dietary naturally occurring anticarcinogens. *Int. J. Oncol.*, **3**, 1131–1139.
15. Ceballos-Picot, J., Trivier, J.M., Nicole, A., Sinet, P.M. and Thevenin, M. (1992) Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. *Clin. Chem.*, **38**, 66–70.
16. Richie, J.P., Skowronski, L., Abraham, P. and Leutzinger, Y. (1996) Blood glutathione concentrations in a large-scale human study. *Clin. Chem.*, **42**, 64–70.
17. Michelet, F., Gueguen, R., Leroy, P., Wellman, M., Nicolas, A. and Siest, G. (1995) Blood and plasma glutathione measured in healthy subjects by HPLC: relation to sex, aging, biological variables, and life habits. *Clin. Chem.*, **41**, 1509–1517.
18. Yang, C.S., Chou, S.T., Liu, L., Tsai, P.J. and Kuo, J.S. (1995) Effect of ageing on human plasma glutathione concentrations as determined by high-performance liquid chromatography with fluorimetric detection. *J. Chromatogr. B. Biomed. Appl.*, **674**, 23–30.
19. Lang, C.A., Naryshkin, S., Schneider, D.L., Mills, B.J. and Lindeman, R.D. (1992) Low blood glutathione levels in healthy aging adults. *J. Lab. Clin. Med.*, **120**, 720–725.

Received on April 29, 1998; revised on June 5, 1998; accepted on June 5, 1998