

Glutathione S-Transferase Activity and Isoenzyme Concentrations in Obese A^{vy}/a and Lean a/a Mice (43696)

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Abstract. To assist in defining the mechanism(s) by which the activity of hepatic glutathione S-transferase (GST) is decreased in obese rodents, the cytosolic concentrations of individual GST isozymes were determined by high-performance liquid chromatography. For this purpose, liver cytosols from 8- and 16-week-old obese yellow A^{vy}/a and lean black a/a male and female mice of the inbred VY/WfC3H/Nctr- A^{vy} strain were assayed. Obese yellow males contained less hepatic GT-9.0 than lean black males; however, there were no differences between the obese and lean females. GT-9.0 concentration, which is induced by testosterone, was several-fold higher in males than in females, regardless of genotype or body weight. No differences in concentrations of other isozymes were detected. Hepatic GST activity towards 1-chloro-2,4-dinitrobenzene was significantly higher in lean males than in obese males; however, there were no differences between obese and lean females. Lean males had higher activity than lean females; but obese males and females had similar enzyme activities. These changes in enzyme activity can be accounted for by the changes in GT-9.0 concentration measured by HPLC. Lung and testes cytosols were also assayed for GST isozyme concentrations. No differences in any isozyme concentration were found between the sexes or the genotypes in the lung or between genotypes in the testes.

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Hepatic glutathione S-transferase (GST) activity towards the substrate 1-chloro-2,4-dinitrobenzene (CDNB) has been reported to be about 35%–50% less in obese mice of the *ob/ob* genotype (1) or of the $A^{vy}/-$ genotype (2, 3) than in the corresponding lean nonmutant siblings. Thus, obese mice, regardless of their genotype, are deficient in this important detoxicating enzyme activity. Could this relative deficiency be a factor in the increased likelihood that obese *ob/ob* and obese yellow $A^{vy}/-$ mice will develop more hepatocellular neoplasms than their lean *Ob/-* and non- A^{vy} siblings (reviewed in 4)?

In rats GST activity was also decreased by obesity or obesity-influenced factors. Hepatic GST activity towards four different substrates was assayed in obese Zucker rats and their lean littermates (5). The data indicated that transferases 3–3, 3–4, and 4–4, but not 5–5, were decreased in the obese rats.

No information has been available regarding the specific GST isozymes that are reduced in obese mice whether these changes are organ-specific. Therefore, as an initial step in the investigation of the mechanism underlying this apparent deficiency in enzyme, it was important to identify whether it arose from an inhibition of enzyme activity or from an absolute decrease in amount of enzyme. If the latter were the case, it is of interest to identify the specific isozyme(s) that are decreased. To this end, activities, as well as the concentrations, of individual isozymes were measured in liver and lung of both sexes of obese and lean mice, and in testes of obese and lean male mice. In CD-1 mice there are three major constitutive GST isozymes: GT-8.7, GT-9.0, and GT-10.6 (6) that correspond to the BALB/c mouse forms Yb1, Yf or Pi, and Ya3 of Hays *et al.* (7).

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Materials and Methods

Male and female obese yellow ($A^{vy/a}$) and lean black (a/a) mice of the inbred VY/WffC3Hf/Nctr- A^{vy} strain were aged to 8 or 16 weeks. At that time they were euthanized by inhalation of carbon dioxide. The livers were excised, labeled by cage/ear clip I.D., sex and genotype, immediately frozen in liquid nitrogen, and stored at -70°C . Cytosols were prepared as previously described (6). In the initial study with 8-week-old mice, the nine livers were pooled into three groups. The concentrations of the various GST isozymes were determined from the peak areas on HPLC chromatograms of GSH-Sepharose affinity purified (8) GST isozymes as previously described by Benson *et al.* (6). In our earlier work (6) retention times and peak areas of purified mouse GST isozymes were established. Linearity between amount of isozyme and HPLC peak area was observed for each isozyme. Enzymatic activity towards the substrate 1-chloro-2,4-dinitrobenzene (CDNB) was assayed by the method of Habig *et al.* (9). The data were evaluated by an analysis of variance (ANOVA) with two variables, sex and phenotype.

Results

Body Weights. Initial experiments were performed on 8-week-old animals. As indicated in Table I, the variability in body weight, particularly in the yellow obese phenotype, was large. Additional animals were aged to 16 weeks to allow full expression of the obese phenotype. At 16 weeks the yellow mice had gained an additional 12–14 g and were more uniform in weight, whereas the black mice had gained only 4 g (Table I).

Glutathione S-Transferase Activity. Hepatic glutathione S-transferase activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was significantly higher in the black males than in the yellow males at 8 and 16 weeks; however, there was no difference in activity between the black and yellow females in either age group (Table II). Black males had higher activity than

Table I. Age and Body Weights (g) of Strain VY Obese Yellow $A^{vy/a}$ and Lean Black a/a Male and Female Mice

Age	Males		Females	
	8 weeks	16 weeks	8 weeks	16 weeks
Yellow $A^{vy/a}$ obese				
mean	33.5	47.7	32.6	44.2
SE	3.0	0.7	3.0	1.2
<i>n</i>	9	10	9	10
Black a/a lean				
Mean	25.8	29.5	18.8	22.1
SE	1.7	0.3	1.4	0.4
<i>n</i>	9	10	9	10

Table II. Specific Activity ($\mu\text{moles CDNB}/\text{min}/\text{mg}$ protein) of Glutathione S-Transferase towards 1-Chloro-2,4-Dinitrobenzene (CDNB) in Liver of Yellow $A^{vy/a}$ and Black a/a Male Strain VY Mice as a Function of Age

Age	Males		Females	
	8 weeks	16 weeks	8 weeks	16 weeks
Yellow $A^{vy/a}$ obese				
Mean	3.29 ^a	1.31 ^b	1.77	0.92
SE	0.02	0.13	0.27	0.10
<i>n</i>	3	8	3	8
Black a/a lean				
Mean	5.72	2.81	1.88	1.05
SE	0.17	0.26	0.27	0.14
<i>n</i>	3	8	3	8

^a $P < 0.01$ Obese yellow males $<$ lean black males.

^b $P < 0.001$ Obese yellow males $<$ lean black males.

black females; such a difference was not observed between the yellow males and females (Table II).

In the 16-week-old mice, glutathione S-transferase activity towards CDNB in the lungs did not differ between yellow and black mice or between the sexes (data not shown). In the testes, there was no difference between the yellow and black males (data not shown).

Specific Isozyme Concentrations. Ninety-five percent of the CDNB enzyme activity applied to the GSH-Sepharose affinity column bound to the column, and essentially all the activity was recovered after washing the column with buffer and eluting with 5 mmolar GSH. Figure 1 shows the typical HPLC elution profile of affinity purified liver cytosol from (A) a 16-week-old obese yellow mouse and (B) a 16-week-old lean black littermate. Glutathione S-transferase isozymes GT-8.7 (Peak 1) and GT-10.6 (Peak 2) were very similar in amounts. Isozyme GT-9.0 (Peak 3) was several fold larger in the black mouse as compared with the yellow mouse. The GST isozymes eluted from the HPLC at the same retention times as those from the CD-1 mouse. Based on retention times of purified rat GST isozymes and amino acid sequence analysis of these mouse forms (data not shown), we have determined that mouse GT-8.7, GT-10.6, and GT-9.0 correspond to rat forms 3–3, 2–2, and 7–7.

Table III shows the concentrations of GT-9.0 in the liver as determined by HPLC for males and females of both genotypes and ages. There was no difference in GT-9.0 concentration between the yellow and black males at eight weeks of age; however, at 16 weeks of age this isozyme concentration was significantly lower in the obese yellow male than in the black male. In contrast, there was no difference in this isozyme between the black and yellow females at either age. GT-9.0 was significantly higher in the yellow and black males as compared with yellow and black females, respectively. The levels of GT-9.0 in lung and

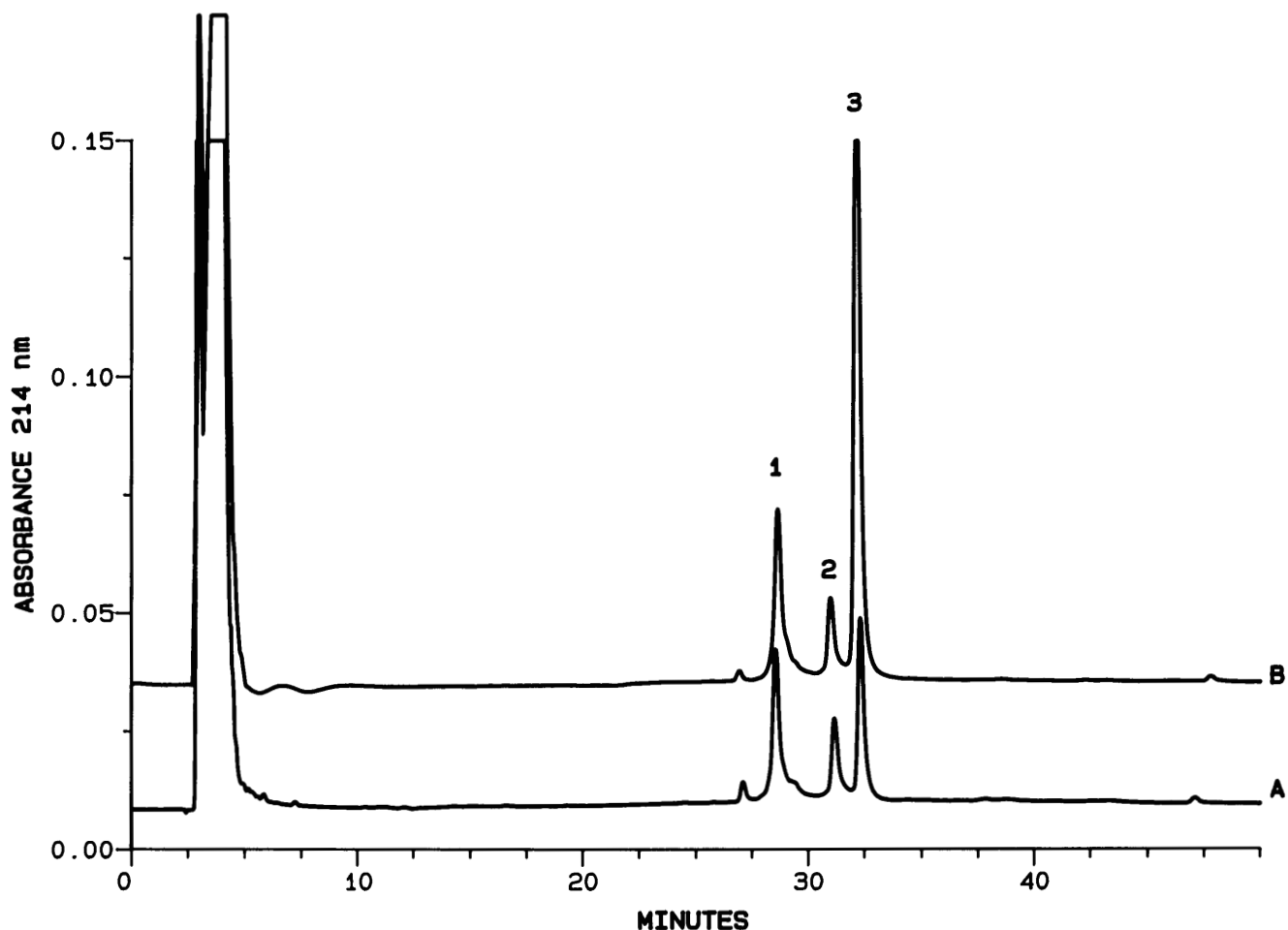


Figure 1. HPLC profile of affinity purified glutathione transferase isozymes from (A) obese yellow $A^{vy/a}$ mouse liver and (B) lean black a/a mouse liver. Liver GSTs were affinity purified on GSH-Sepharose. In both chromatograms an amount of affinity purified protein equivalent to 0.56 mg of cytosolic protein was injected and separated on a Vydak C4 column using a gradient of 50%–100% buffer B over 50 min at a flow rate of 1 ml/min. Buffer B contained 60% acetonitrile in 0.07% aqueous trifluoroacetic acid (TFA). Buffer A was 0.07% aqueous TFA. Peaks 1, 2, and 3 represent glutathione S-transferase isozymes GT-8.7, GT-10.6 and GT-9.0, respectively, as determined by cochromatography with purified isozymes.

testes were low (0.3–0.8 $\mu\text{g}/\text{mg}$ protein) and showed no significant differences between sexes or phenotypes.

In the 16-week-old mice, there were no statistically significant differences in the amounts of the other two major constitutive GST isozymes, GT-8.7 and GT-10.6, in testes or lung as a function of sex or genotype (data not shown).

Discussion

In the present study, both yellow male and female mice were not uniformly obese at eight weeks of age. Hepatic GST activity with the substrate CDNB was significantly decreased in the male yellow mouse as compared with the lean black male; however, identification, by HPLC analysis, of a decrease in a specific GST isozyme which would account for the decrease in enzyme activity was not possible with the available data (Table II). Based on these suggestive data another group of mice were aged for 16 weeks to allow full expression of the obese phenotype.

At 16 weeks of age, the yellow mice were distinctly obese compared with the black mice (Table I). Both the enzymatic activity and the GT-9.0 isozyme concentrations were decreased in the yellow males when compared with the lean black males as graphically indicated in Figure 1. Since the other GST isozymes were not significantly different between the black and yellow mice, the decreased GST CDNB activity in the male mice can be accounted for by the specific decrease in the GT-9.0 isozyme. This decreased GT-9.0 concentration may reflect an inhibitory effect of obesity or obesity-influenced factors on induction of GT-9.0 by testosterone (10) since this form decreases in the male liver after castration (10).

In the previous study (2), yellow obese $A^{vy/a}$ ($Y \times VY$) F_1 hybrid females exhibited significantly lower hepatic glutathione S-transferase (GST) activity than either their lean pseudoagouti $A^{vy/a}$ or black a/a littermates at 13 and 18 months of age. Since the obese yellow and lean pseudoagouti $A^{vy/a}$ mice were genet-

Table III. Concentrations ($\mu\text{g}/\text{mg}$ cytosol protein) of Glutathione S-Transferase Isozyme GT-9.0 in Livers of Obese Yellow A^{vy}/a and Lean Black a/a Male and Female Strain VY Mice as a Function of Age as Determined by HPLC of Affinity Purified Cytosols

Age	Males		Females	
	8 weeks	16 weeks	8 weeks	16 weeks
Yellow A^{vy}/a obese				
Mean	29.04 ^a	19.94 ^{a,b}	3.78	5.88
SE	1.47	2.28	0.14	0.60
<i>n</i>	3	7	3	8
Black a/a lean				
Mean	40.96 ^a	52.69 ^a	5.37	8.26
SE	4.62	5.30	0.84	0.71
<i>n</i>	3	8	3	7

^a $P < 0.001$ Black males $>$ black females; yellow males $>$ yellow females.

^b $P < 0.001$ Black males $>$ yellow males.

ically identical, even though phenotypically very different (11), the decreased hepatic GST activity was interpreted as due to obesity or obesity-influenced factors, *e.g.*, altered hormonal balance, rather than as a direct effect of the A^{vy} gene.

Since obesity had no effect on GST activity or GST isozyme levels in the 8- and 16-week-old yellow female mice (Tables II and III), the decreased GST activity previously reported in the 13- and 18-month-old yellow female mice (2) may require an age-related change in physiologic or metabolic parameter(s) other than obesity. The available data are insufficient to suggest the identity of this additional factor(s).

Prohaska (1) used only 12-week-old male obese C57BL/6J-*ob/ob* mice and their lean *Ob/-* littermates to determine hepatic enzyme activities. No published data on GST activity in female *ob/ob* mice appear to be available. It should be noted that the time of onset of obesity and related characteristics of *ob/ob* mice are quite different from those of the yellow mice (4). Thus, the present data suggest that in *ob/ob* mice as well, obesity-influenced factors of as yet unknown identity are involved in the decreased hepatic GST activity towards CDNB.

A recent study (12) of hepatic drug metabolizing enzymes in obese A^{vy}/A (C3H \times VY)F1 hybrid male mice indicated differences between heavier (56.8 ± 0.8 g) and lighter (43.0 ± 1.3 g) yellow mice in the inducibility of glutathione S-transferase activity by sodium phenobarbital. Enzyme activity towards both 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene was induced by phenobarbital; however, the degree of induction was significantly higher in the heavier mice than in the lighter mice. Constituent activities towards both substrates were similar in the lighter and heavier mice.

Previous workers (1, 2, 10, 12) have utilized enzyme activity with different substrates as a measure of isoenzyme composition; however, since there is no one "specific" substrate for each isozyme, one can only draw general conclusions in most cases. A direct measure of each isozyme amount as reported in this study is more accurate in identifying which isozyme may be changing in response to an experimental stimulus.

The accumulated data suggest that unidentified factors, influenced by obesity, sex, and age, modulate the synthesis, activity, and inducibility of hepatic glutathione S-transferase activity. Future studies should be directed towards identification of these factors and their specific effects.

1. Prohaska JR, Wittmers LE Jr., Haller EW. Influence of genetic obesity, food intake, and adrenalectomy in mice on selected trace element-dependent protective enzymes. *J Nutr* **118**:739-746, 1988.
2. Wolff GL, Suber RL. Hepatic glutathione S-transferase activity in mice: Effects of A^{vy} -genotype, obesity, lindane treatment, and sex. *Proc Soc Exptl Biol Med* **181**:535-541, 1986.
3. Wolff GL, Gaylor DW, Frith CH, Suber RL. Controlled genetic variation in a subchronic toxicity assay: Susceptibility to induction of bladder hyperplasia in mice by 2-acetylaminofluorene. *J Toxicol Environ Health* **12**:255-265, 1983.
4. Wolff GL. Body weight and cancer. *Am J Clin Nutr* **45**:168-180, 1987.
5. Murray M, Zaluzny L. Comparative effects of genetic obesity and streptozotocin-diabetes on rat liver cytosolic glutathione S-transferase activities. *Nutr Res* **9**:1151-1160, 1989.
6. Benson AM, Hunkeler MJ, York JL. Mouse hepatic glutathione transferase isoenzymes and their differential induction by anticarcinogens. Specificities of butylated hydroxyanisole and bisethylxanthogen as inducers of glutathione transferases in male and female CD-1 mice. *Biochem J* **261**:1023-1029, 1989.
7. Hayes JD, Kerr LA, Peacock SD, Cronshaw AD, and McLellan LI. Hepatic glutathione S-transferases in mice fed on a diet containing the anticarcinogenic antioxidant butylated hydroxyanisole. *Biochem J* **277**:501-512, 1991.
8. Meyer DJ, Lalor E, Coles B, Kispert A, Alin P, Mannervik B, Ketterer B. Single-step purification and h.p.l.c. analysis of glutathione transferase 8-8 in rat tissues. *Biochem J* **260**:785-788, 1989.
9. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. First enzymatic step in mercapturic acid formation. *J Biol Chem* **249**:7130-7139, 1974.
10. Hatayama I, Satch K, Sato K. Developmental and hormonal regulation of the major form of hepatic glutathione S-transferase in male mice. *Biochem Biophys Res Comm* **140**:581-588, 1986.
11. Wolff GL. Obesity, cancer, and the viable yellow (A^{vy}/a) mouse: Discrimination among pathophysiologic effects of the expression of the A^{vy}/a genotype in obese and lean mice. In: Oomura Y et al., Eds. *Progress in Obesity Research 1990*. London: John Libbey, pp445-448, 1991.
12. Wolff GL, Leakey JEA, Bazare J, Harmon JR, Webb PJ, Law MG. Susceptibility to phenobarbital promotion of hepatocarcinogenesis: Correlation with differential expression and induction of hepatic drug metabolizing enzymes in heavy and light male (C3H \times VY) F1 hybrid mice. *Carcinogenesis* **12**:911-915, 1991.