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Glutathione depletion and recovery after acute ethanol administration in the aging mouse

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ABSTRACT

Glutathione (GSH) plays an important role in the detoxification of ethanol (EtOH) and acute EtOH administration leads to GSH depletion in the liver and other tissues. Aging is also associated with a progressive decline in GSH levels and impairment in GSH biosynthesis in many tissues. Thus, the present study was designed to examine the effects of aging on EtOH-induced depletion and recovery of GSH in different tissues of the C57Bl/6NNIA mouse. EtOH (2–5 g/kg) or saline was administered i.p. to mice of ages 6 months (young), 12 months (mature), and 24 months (old); and GSH and cyst(e)ine concentrations were measured 0–24 h thereafter. EtOH administration (5 g/kg) depleted hepatic GSH levels >50% by 6 h in all animals. By 24 h, levels remained low in both young and old mice, but recovered to baseline levels in mature mice. At 6 h, the decrease in hepatic GSH was dose-dependent up to 3 g/kg EtOH, but not at higher doses. The extent of depletion at the 3 g/kg dose was dependent upon age, with old mice demonstrating significantly lower GSH levels than mature mice ($P < 0.001$). Altogether these results indicate that aging was associated with a greater degree of EtOH and fasting-induced GSH depletion and subsequent impaired recovery in liver. An impaired ability to recover was also observed in young animals. Further studies are required to determine if an inability to recover from GSH depletion by EtOH is associated with enhanced toxicity.

1. Introduction

Ethanol (EtOH) abuse in the elderly is an important public health concern [1–3]. Indeed, it has been estimated that as high as 45% of persons over age 60 have problems related to EtOH consumption [4]. The magnitude of this problem is compounded by the rapidly increasing proportion of the population represented by the elderly [5]. In addition, sensitivity of elderly subjects to the toxic effects of EtOH appears to be enhanced compared to younger persons [6–9]. Laboratory animal studies have also indicated that ethanol metabolism is decreased and toxicity increased with advancing age [10–13].

While little is known about the biochemical mechanisms responsible for the enhanced sensitivity to EtOH during aging, depletion of glutathione (GSH) may be involved based on its important role in detoxification of EtOH [14]. Hepatic GSH levels are depleted 6 h after acute EtOH administration in young animals [14–17]. Further, when GSH levels are depleted prior to ETOH administration, an increase in toxicity is observed [18]. On the other hand, when GSH levels are enhanced by administration of GSH or its precursors, the depletion of GSH levels by EtOH are prevented [19,20] and toxicity is diminished [21–26].

GSH depletion is also an important factor in the aging process. Previous results from this laboratory and others have

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demonstrated that a GSH deficiency is a common phenomenon of senescent organisms, including humans [27–31]. While the mechanisms responsible for this aging impairment are unknown, several studies indicate that a decrease in turnover of GSH and a loss of biosynthetic capacity may be involved [32–34]. Based on these results and on the numerous functions of GSH in cellular homeostasis and defense [35], a loss of GSH may represent a critical factor in the aging process [36].

The effect of aging on GSH status during EtOH intoxication has received little attention. In one study, old female Fischer 344 rats administered a dose of 4 g/kg EtOH exhibited a depletion of hepatic GSH comparable to that of mature and young rats [37]. However, in this study, only one dose of EtOH was examined and the confounding factor of fasting was not taken into account in the experimental design. Indeed, fasting is known to impact hepatic GSH levels and hepatotoxicity [38,39] and we have observed that fasting-induced GSH depletion was greater in old than in younger animals [34]. In another study, effects of age were examined in younger age groups, so that results reflected growth and maturational changes rather than the aging process per se [40].

The aim of the present study is to systematically examine the effects of aging on EtOH-induced GSH depletion and recovery in the mouse. To test this, GSH concentrations in various tissues were determined in young, mature and old mice throughout a 24 h time course after acute EtOH treatment, and after increasing doses of EtOH. Special attention was placed on comparison of EtOH-treated mice with time-matched controls, to account for the decreases in GSH resulting from food deprivation [34]. Also examined were the effects of EtOH on the tissue concentrations of GSH related metabolites, cyst(e)ine and glutathione disulfide (GSSG).

2. Materials and methods

2.1. Experimental animals

Male C57Bl/6NNIA mice of ages 6 months (young), 12 months (mature adults), and 24 months (old) were obtained from colonies administered by the National Institute on Aging, as described previously [34]. Prior to experimentation, which began at 8:00 a.m., mice had free access to food (NIH-07) and water. After dosing, food but not water was withheld until termination of the experiment. Mice from each of the age groups were utilized within an experimental period.

2.2. Experimental protocols

For dose–response studies, mice were weighed and injected i.p. with 2, 3, 4, or 5 g/kg EtOH as a 20% (w/v) solution in 0.9% NaCl warmed to 37 °C. Control mice received i.p. injections of 20 ml/kg 0.9% NaCl, corresponding to the volume of the 4 g/kg EtOH dose. All dose–response data were obtained from mice sacrificed at 6 h after injection. Time course data were obtained from animals sacrificed at 0, 2, 6 and 24 h after administration of EtOH. Animals were anesthetized with diethyl ether and exsanguinated by cardiac puncture. Whole blood was collected into syringes containing 100 μl of 0.05 M disodium EDTA (Sigma, St. Louis, MO). Liver, kidneys and lung were excised quickly, trimmed, rinsed in ice-cold 0.9% (w/v) saline to remove blood, blotted dry and weighed.

2.3. Tissue processing

Whole blood was processed by the addition of four volumes of ice-cold 5% (w/v) metaphosphoric acid (MPA) (Mallinckrodt, Paris, KY). Liver, kidney and lung samples were homogenized (10%, w/v) in 5% MPA using an all-glass Ten-Broeck homogenizer, and centrifuged at 14,000 × *g* for 3 min. The protein-free supernatants of blood and tissues were stored at −70 °C until assayed for glutathione and cyst(e)ine. Additional tissue samples were homogenized in ice-cold 1.5% (w/v) KCl for immediate analysis of thiobarbituric acid-reactive substances (TBARS).

2.4. Analytical methods

Total glutathione (GSH and glutathione disulfide) was assayed enzymatically as previously described [41]. Analysis of selected samples by HPLC with electrochemical detection [42] demonstrated that total GSH was comprised of approximately 95% GSH and GSSG levels were low and unchanged as a result of EtOH administration. Cyst(e)ine (cysteine + cystine) was determined as previously described [43]. Thiobarbituric acid-reactive substances were determined as described previously and expressed as pmol/mg of protein [44].

2.5. Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). Differences between groups were considered significant at $P < 0.05$. Two-way analysis of variance (ANOVA) was employed to determine the main effects of age and time, or age and dose. Significant interactions of age by time or dose were followed up with one-way ANOVA and Scheffe's post hoc test to identify specific group effects. Analysis of covariance (ANCOVA) was also used to identify age effects that exist in time or dose trends. Linear and nonlinear regressions were utilized to model selected dose–response data. All statistical analyses were performed using SAS software.

3. Results

3.1. Time course of EtOH effects

The time course of EtOH-induced depletion and recovery of hepatic GSH in mice of different ages is shown in Table 1. By 6 h after administration of 5 g/kg EtOH, GSH levels were depleted 52, 57 and 63%, in mice of ages 6, 12 and 24 months, respectively ($P < 0.001$). While the extent of the drop was similar among all age groups, mice of different ages demonstrated divergent recovery patterns by 24 h. Only mature 12 months old mice displayed a significant increase in GSH from 6 h, reaching a level comparable to their initial GSH concentration by 24 h. GSH levels in young and old mice remained low, displaying no apparent recovery. Thus, at 24 h, hepatic GSH levels in mature ethanol-treated mice were significantly higher than those of the young or old mice ($P < 0.05$).

Table 1 – Effect of acute ethanol administration on hepatic glutathione levels

Time (h)	Glutathione (μmol/g tissue)					
	Age (6 months)		Age (12 months)		Age (24 months)	
	Control	Ethanol	Control	Ethanol	Control	Ethanol
0	8.84 ± 0.49		8.62 ± 0.24		8.54 ± 0.51	
2	8.80 ± 1.08	5.41 ± 0.71*	8.71 ± 0.21	6.46 ± 0.57*	8.46 ± 0.96	6.91 ± 0.65
6	8.08 ± 0.34	4.19 ± 0.50*	7.46 ± 0.41‡	4.22 ± 0.28*	6.01 ± 0.62‡	3.82 ± 0.36*
24	7.92 ± 0.33‡	5.55 ± 0.70*,†	5.62 ± 0.54‡	8.88 ± 0.36*	4.29 ± 0.51‡	5.53 ± 0.96†

Mice were administered 5 g/kg EtOH; values are mean ± S.E.M., $n = 4$.
* Significantly different from controls, $P < 0.05$.
† Significantly different from ethanol-treated 12 months group, $P < 0.05$.
‡ Significantly different from baseline (0 h) control, $P < 0.05$.

Table 2 – Effect of acute ethanol administration on renal glutathione levels

Time (h)	Glutathione (μmol/g tissue)					
	Age (6 months)		Age (12 months)		Age (24 months)	
	Control	Ethanol	Control	Ethanol	Control	Ethanol
0	3.49 ± 0.19		3.14 ± 0.24		2.44 ± 0.21‡	
2	3.66 ± 0.22	2.90 ± 0.25	3.29 ± 0.19	2.60 ± 0.25	2.38 ± 0.20	2.47 ± 0.15
6	3.27 ± 0.18	2.51 ± 0.17†	3.10 ± 0.16	2.35 ± 0.16†	2.57 ± 0.19	1.98 ± 0.14†
24	3.65 ± 0.20	3.31 ± 0.27	2.88 ± 0.20	4.18 ± 0.22*	2.35 ± 0.23	2.75 ± 0.22

Mice were administered 5 g/kg EtOH; values are mean ± S.E.M., $n = 4$.
* Significantly different from controls, $P < 0.05$.
† Significantly different from baseline (0 h) controls, $P < 0.05$.
‡ Significantly different from 6 months control, $P < 0.05$.

In these experiments, absolute GSH values represent the effects of both EtOH and food deprivation, as food was withheld throughout the 24 h to prevent confounding effects of differential food consumption. As we observed previously, changes in GSH levels due to fasting alone were observed for all age groups (Table 1). By 24 h in control mice, GSH levels decreased 10, 35 and 50% in 6, 12 and 24 months old mice, respectively ($P < 0.05$). Decreases were observed after only 6 h in both mature and old age groups. Therefore, in order to identify effects due specifically to EtOH, GSH concentrations in EtOH-treated mice were examined relative to saline-injected, age-matched controls at each time point (Fig. 1). Mice of all ages exhibited a similar decrease in GSH due to EtOH exposure with a maximum decrease of 62, 52 and 70% at 6 h in young, mature and old mice, respectively. This decrease in GSH was maintained though 24 h in young mice where levels were 30% lower than fasted controls ($P < 0.05$). However, in mature mice GSH levels rebounded to 158% of fasted controls by 24 h ($P < 0.05$), while in old mice levels were not significantly different from those in fasted controls after 24 h.

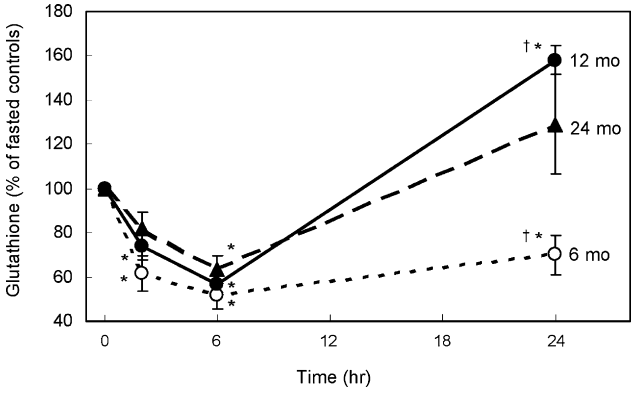

Fig. 1 – Time course of hepatic glutathione depletion and recovery after ethanol administration. Mice were sacrificed 2, 6 and 24 h after i.p. administration of 5 g/kg EtOH. Total glutathione (GSH + GSSG) was expressed as μeq. GSH/g tissue. Results are shown as mean ± S.E. of 4–10 mice per age group. *Significantly different from 0 time point, $P < 0.05$; †significantly different from 6 h time point, $P < 0.05$; ‡significantly different from 12 months age group, $P < 0.05$.

The time course of EtOH-induced GSH depletion and recovery in the kidney is presented in Table 2. By 6 h after administration of 5 g/kg EtOH, GSH levels were depleted by 28, 25 and 19% in mice of ages 6, 12 and 24 months, respectively ($P < 0.05$). A complete recovery of GSH levels occurred in all mice by 24 h with the greatest increase being observed in mature animals. Thus, in a manner similar to that of liver, renal GSH levels were significantly greater in mature mice than in either young or old animals 24 h after EtOH administration ($P < 0.05$).

In contrast to liver, no consistent changes in renal glutathione levels were observed due to fasting alone (Table 2). Thus, when the effects of EtOH on renal GSH were also analyzed relative to fasted controls, similar results were observed (Fig. 2). At 6 h, GSH levels were significantly lower in

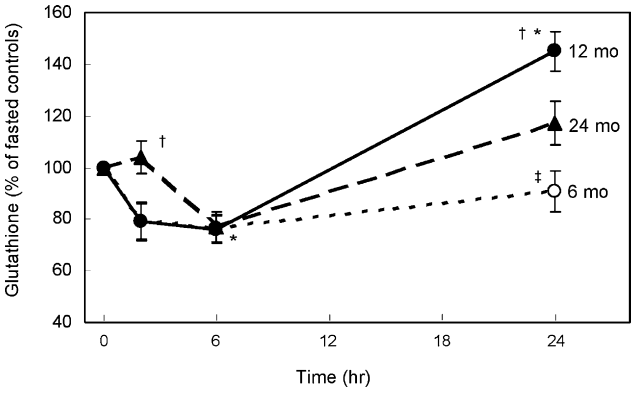

Fig. 2 – Time course of renal glutathione depletion and recovery after ethanol administration. Mice were sacrificed 2, 6 and 24 h after i.p. administration of 5 g/kg EtOH. Total glutathione (GSH + GSSG) was expressed as μeq. GSH/g tissue. Results are shown as mean ± S.E. of 4–10 mice per age group. *Significantly different from 0 time point, $P < 0.05$; †significantly different from 6 h time point, $P < 0.05$; ‡significantly different from 12 months age group, $P < 0.05$.

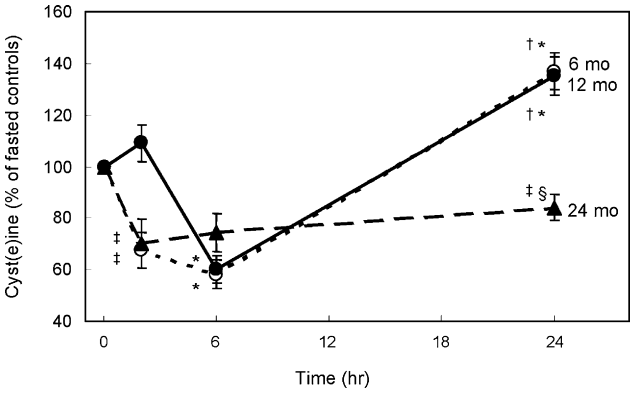

Fig. 3 – Time course of renal cyst(e)ine depletion and recovery after ethanol administration. Mice were sacrificed 2, 6 and 24 h after i.p. administration of 5 g/kg EtOH. Cyst(e)ine (cysteine + cystine) was expressed as μeq. Cys/g tissue. Results are shown as mean ± S.E. of 4–10 mice per age group. *Significantly different from 0 time point, $P < 0.05$; †significantly different from 6 h time point, $P < 0.05$; ‡significantly different from 12 months age group, $P < 0.05$; §significantly different from 6 months age group, $P < 0.05$.

EtOH-treated mice than fasted controls in all age groups ($P < 0.05$). Similar to liver, an overshoot of GSH was observed in mature animals at 24 h compared to fasted mice ($P < 0.05$), whereas no differences were observed in either young or mature animals due to EtOH.

Hepatic cyst(e)ine levels did not differ according to time or age in control or EtOH-treated mice (data not shown). However, aging, fasting and EtOH specific changes in renal cyst(e)ine concentrations were observed (Table 3). Initial Cys levels in mature and old mice were 22 and 21% lower, respectively, than in young mice ($P < 0.05$). Mice of all ages displayed different renal Cys profiles in response to fasting. In young mice, Cys levels were unchanged through 6 h and subsequently decreased to 64% of initial levels after 24 h ($P < 0.05$). In mature mice, Cys levels increased 17% after 6 h ($P < 0.05$) and returned to baseline values by 24 h. Old mice displayed declining Cys concentration throughout the 24 h time period, ending with a level 70% of that of initial levels ($P < 0.05$).

A depletion in renal Cys levels after EtOH administration was observed in only young animals after 2 and 6 h ($P < 0.05$) (Table 3). By 24 h, increased Cys levels were evident in both young and mature animals compared to 6 h values ($P < 0.05$). In old mice, renal cyst(e)ine levels decreased throughout the entire time course to levels 42% lower than initial values and lower than that of the other two age groups ($P < 0.05$). When renal cyst(e)ine levels were examined relative to fasted, saline-injected controls (Fig. 3), similar patterns were observed. In the young and mature mice, cyst(e)ine levels were 40% lower than controls at 6 h and 40% greater than control at 24 h ($P < 0.05$). In old mice, renal cyst(e)ine levels were 20–30% lower than fasted controls at all time points ($P < 0.05$).

Fasting and EtOH administration had no effect on whole blood GSH levels (data not shown). While an over all significant effect of time was observed by ANOVA ($P < 0.03$), no differences between specific time points could be discerned by post hoc analyses.

Table 3 – Effect of acute ethanol administration on renal cyst(e)ine levels

Time (h)	Cyst(e)ine (μeq. Cys/g tissue)					
	Age (6 months)		Age (12 months)		Age (24 months)	
	Control	Ethanol	Control	Ethanol	Control	Ethanol
0	1.23 ± 0.059		0.964 ± 0.077†		0.845 ± 0.071†	
2	1.18 ± 0.075	0.796 ± 0.080*	0.840 ± 0.096	0.916 ± 0.062	0.890 ± 0.090†	0.621 ± 0.084*,§
6	1.32 ± 0.093	0.768 ± 0.073*	1.13 ± 0.087*	0.679 ± 0.059	0.831 ± 0.095†	0.619 ± 0.062*
24	0.783 ± 0.047*	1.07 ± 0.058‡	0.835 ± 0.093	1.13 ± 0.062‡	0.623 ± 0.104*,†	0.523 ± 0.032*,†,§

Mice were administered 5 g/kg EtOH; values are mean ± S.E.M., $n = 4$.
* Significantly different from baseline (0 h) control, $P < 0.05$.
† Significantly different from 6 months., $P < 0.05$.
‡ Significantly different from 6 h, $P < 0.05$.
§ Significantly different from 12 months., $P < 0.05$.

Table 4 – Time course of liver TBARS after ethanol administration

Time (h)	TBARS (pmol/mg protein)		
	Age (6 months)	Age (12 months)	Age (24 months)
0	421 ± 36.1[**]	604 ± 71.1[§]	763 ± 74.1[§,**]
2	440 ± 40.8	589 ± 64.2	819 ± 64.0
6	462 ± 39.4	671 ± 60.2	903 ± 86.6[*]
24	651 ± 74.0[*,†,‡]	784 ± 50.6[*,†,‡]	965 ± 90.9[*,†]

Mice were administered 5 g/kg EtOH; values are mean ± S.E.M., $n = 4–10$.
[*] Significantly different from baseline (0 h) control, $P < 0.05$.
[**] Significantly different from 12 months, $P < 0.05$.
[†] Significantly different from 2 h, $P < 0.05$.
[‡] Significantly different from 6 h, $P < 0.05$.
[§] Significantly different from 6 months, $P < 0.05$.

Effects of EtOH administration on liver levels of TBARS are presented in Table 4. In control rats, levels of TBARS were increased with advancing age across all age groups ($P < 0.05$). For both 6 and 12 months old rats, an increase in TBARS was observed 24 h after EtOH administration ($P < 0.05$). In old rats, an increase in TBARS was observed 6 and 24 h after EtOH administration ($P < 0.05$).

3.2. *Dose–response of GSH to EtOH*

Liver and kidney GSH concentrations 6 h after administration of increasing doses of EtOH are presented in Fig. 4. The 6 h time point was selected as the time of maximal depletion of GSH after EtOH administration. In the liver, EtOH administration resulted in an overall depletion of GSH in all three age groups. For the young and mature mice, significant decreases were observed for EtOH doses of 2, 3, 4, and 5 g/kg, while in old mice, significant decreases occurred only at the 3 and 5 g/kg doses ($P < 0.05$). GSH levels in most groups tended to decrease with increasing dose of EtOH through 3 g/kg with the lowest GSH level observed for the old 24 months mice administered 3 g/kg EtOH. At doses above 3 g/kg, GSH concentrations did not further diminish, and mice of all three ages displayed comparable levels of GSH at 4 and 5 g/kg EtOH. While age was a significant covariate in the depletion of GSH by EtOH ($P < 0.02$), specific differences between age groups were only observed at the 3 g/kg EtOH dose where lower levels in both young and old mice were observed compared to mature mice ($P < 0.05$).

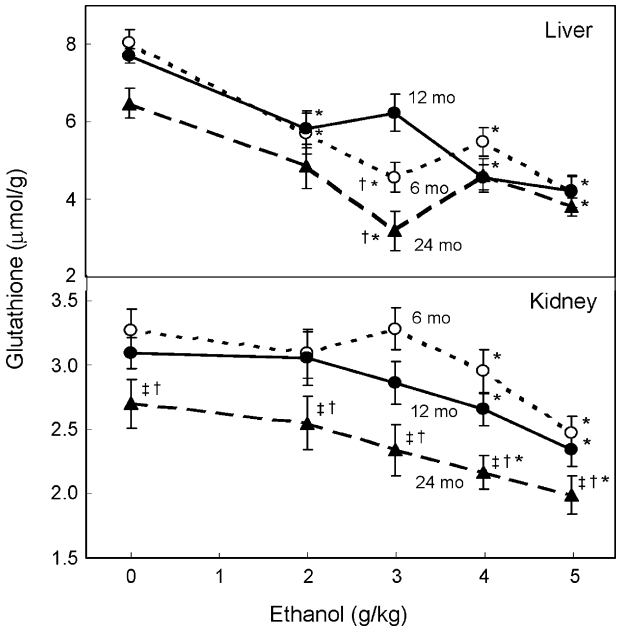

Fig. 4 – Response of hepatic and renal glutathione to increasing doses of ethanol. Mice were sacrificed 6 h after administration of ethanol i.p. on a body weight basis. Total GSH (GSH + GSSG) was expressed as μmol GSH/g liver or kidney. Results are shown as mean ± S.E.M. of 4–6 mice per age group. [*]Significantly different from saline-injected controls, $P < 0.05$; [†]significantly different from 12 months age group, $P < 0.05$; [‡]significantly different from 6 months age group, $P < 0.05$.

Table 5 – Response of cyst(e)ine concentrations in liver and kidney to increasing doses of ethanol

Dose (g/kg)	Cyst(e)ine (μeq. Cys/g tissue)		
	Age (6 months)	Age (12 months)	Age (24 months)
Liver			
0	0.25 ± 0.04	0.38 ± 0.04	0.29 ± 0.04
2	0.13 ± 0.05[†]	0.16 ± 0.05[†]	0.17 ± 0.05[*]
3	0.17 ± 0.04	0.22 ± 0.05	0.24 ± 0.05
4	0.16 ± 0.05	0.23 ± 0.03	0.23 ± 0.04
5	0.18 ± 0.04	0.24 ± 0.04	0.23 ± 0.04
Kidney			
0	1.32 ± 0.08	1.13 ± 0.07	0.84 ± 0.07[*]
2	1.47 ± 0.10	0.86 ± 0.10	0.53 ± 0.10
3	1.15 ± 0.08	1.07 ± 0.08	0.79 ± 11
4	0.93 ± 0.09	0.70 ± 0.07[†]	0.63 ± 0.07
5	0.77 ± 0.07[†]	0.68 ± 0.06[†]	0.62 ± 0.07

Mice were administered EtOH at the indicated doses and sacrificed after 6 h; values are mean ± S.E.M., $n = 4–10$.
[*] Significantly different from young mice, $P < 0.05$.
[†] Significantly different from saline-injected control, $P < 0.05$.

Table 6 – Response of glutathione concentrations in lung and blood to increasing doses of ethanol

Dose (g/kg)	GSH (μmol/g tissue)		
	Age (6 months)	Age (12 months)	Age (24 months)
Lung			
0	2.34 ± 0.16	2.44 ± 0.09	1.79 ± 0.16*
3	2.43 ± 0.07	1.91 ± 0.05	1.65 ± 0.17
4	2.59 ± 0.11	1.94 ± 0.10	1.89 ± 0.07
5	2.43 ± 0.11	1.89 ± 0.09	1.69 ± 0.06
Whole blood			
0	1.29 ± 0.09	1.11 ± 0.07	0.79 ± 0.09†
3	1.27 ± 0.10	1.18 ± 0.10	1.01 ± 0.16†
4	1.24 ± 0.09	1.20 ± 0.07	0.84 ± 0.08†
5	1.18 ± 0.07	1.08 ± 0.08	0.89 ± 0.09†

Mice were administered 5 g/kg EtOH; values are mean ± S.E.M., $n = 4–10$.
* Significantly different from mature mice, $P < 0.05$.
† Significantly different from 6 months age group, $P < 0.05$.

In the kidney, both age and EtOH dose-dependent decreases were observed for GSH levels 6 h after administration of EtOH. Levels of GSH tended to decrease with increasing dose of EtOH ($P < 0.0001$), particularly in the higher dose groups of 4 and 5 g/kg where mean values were significantly lower than saline-injected controls ($P < 0.05$). The kidney was less sensitive to GSH depletion by EtOH than the liver, as GSH values were lowered to only 75% of control values at the highest dose of EtOH administered. Age was also in important factor with old mice having significantly lower levels than either of the two younger age groups at each dose of EtOH ($P < 0.05$). Age was also a highly significant covariate in determining renal GSH levels by ANCOVA ($P < 0.0001$).

The response of cyst(e)ine levels in liver to increasing doses of EtOH is presented in Table 5. Significant decreases in hepatic cyst(e)ine levels were observed in all mice 6 h after administration of 2 g/kg EtOH ($P < 0.05$). While mean levels were 18–42% lower at higher doses, differences did not reach the level of significance. An overall effect of age was also apparent with lower levels being observed in younger mice ($P < 0.02$).

Renal cyst(e)ine concentrations were reduced by increasing doses of EtOH in young and mature mice (Table 5) ($P < 0.0001$). Significant decreases from controls were observed at 5 g/kg in young mice, and at both 4 and 5 g/kg in the mature animals ($P < 0.05$). No significant differences were observed in old mice at any dose of EtOH. However, initial cyst(e)ine levels in old mice were significantly lower than in young or mature animals ($P < 0.05$).

GSH levels in blood and lung tissue in animals of different ages 6 h after EtOH administration are reported in Table 6. In lung, aging resulted in a significantly lower baseline concentration of GSH ($P < 0.03$). There were no significant dose–response effects attributable to EtOH. In blood, GSH concentrations were dependent upon age with each age group differing significantly from each other ($P < 0.05$). Again, there were no significant changes in GSH concentration due to EtOH.

4. Discussion

This is the first systematic examination of the effects of EtOH on GSH status in mice throughout their life span, with special emphasis on the aging period. The approach included time course studies in order to examine both EtOH-induced GSH depletion and recovery phases. Depletion likely results from the intracellular utilization of GSH as a result of EtOH exposure, as well as, in the liver, efflux of GSH from hepatocytes into the blood. The recovery period likely results from enhanced hepatic GSH biosynthesis, but is also affected by continued efflux from the liver. An important design characteristic of our studies was the withholding of food throughout the 24 h experimental period. This constraint was applied in order to avoid differential amounts of food consumed by animals of different ages in different states of intoxication, a possibility not commonly accounted for in previous studies. Hepatic GSH levels are greatly affected by diet and fasting alone severely depletes hepatic GSH stores in as little as 6 h [34]. Fasting-induced GSH depletion in the liver is thought to result from a reduction in its de novo biosynthesis due to decreased levels of circulating Cys, its rate-limiting precursor. Consequently, changes in GSH levels after administration of intoxicating doses of EtOH will likely represent the combined effects of both EtOH and food deprivation.

Administration of 5 g/kg EtOH resulted in a significant 37–48% depletion of hepatic GSH in mice of all ages after 6 h, consistent with previous studies in young animals [14]. Further, a significantly lower absolute concentration of GSH was obtained in old mice after administration of 3 g/kg EtOH compared to younger animals. In animals of all ages, the depletion was dose-dependent only up to 3 g/kg, with no further decline observed at higher doses perhaps as a result of hepatotoxicity since glutathione S-transferases (GSTs) are impaired at these higher doses [45]. Previous reports concerning age effects on the extent of EtOH-induced GSH depletion have been conflicting. The degree of depletion of hepatic GSH by p.o. administration of 5 g/kg EtOH was lower in mature compared to young Wistar rats [46], however, this difference could have resulted from an overnight fasting. Rikans and Snowden [37] reported equivalent depletion of hepatic GSH after 4 g/kg EtOH (i.p.) in young, mature and old female F344 rats. The present results are consistent with these reports as mature animals were depleted less extensively than young mice at selected doses (3 g/kg), while at higher doses (5 g/kg) the degree of depletion was similar across age groups.

Chronic EtOH administration also depleted hepatic GSH levels to a greater extent in old (18 months) rats compared to young (3 months) animals [47].

The mechanism of EtOH-induced depletion of GSH may involve the direct conjugation of GSH with acetaldehyde (AcH), the reactive intermediate of EtOH oxidation [48,49]. While GSH–AcH conjugates have not always been observed in vitro [50,51], high concentrations of GSH in hepatocytes makes this reaction a likely candidate to explain GSH protection against EtOH toxicity. Adduct formation of AcH with Cys or cysteinylglycine rather than GSH has also been suggested as a mechanism of EtOH-induced GSH depletion [50], however, the steady state levels of these thiols are much lower that for GSH in the liver. However, in the kidney where steady state levels of Cys are significantly higher, Cys conjugation may play a more important role.

Other mechanisms for EtOH-induced hepatic GSH depletion may involve increased efflux of GSH from the liver as observed previously [52,53] or enhanced utilization of GSH for the detoxification of free radicals and oxidants produced as a result of EtOH exposure [17,54–56]. The role of GSH in protection against EtOH-induced oxidative stress is well-established [56–58] and human studies have suggested that alterations in GSH metabolism in erythrocytes of alcoholics are principally due to this protective function [59]. However, while the present findings suggest that lipid peroxidation was enhanced after EtOH administration, as assessed by liver TBARS, this increase was not apparent until after GSH levels had been depleted.

The most striking aging differences were observed in the recovery phase in the liver as both young and old mice failed to replenish GSH levels after EtOH treatment combined with food deprivation. These finding suggest that the biosynthetic capacity for replenishing GSH stores may be altered during aging, affecting the recovery of GSH after depletion by EtOH. A reduction in GSH biosynthetic capacity during aging has been implicated in previous studies [33]. The decreased levels of Cys observed in the kidney after 24 h in old mice may be indicative of a reduction in hepatic GSH biosynthesis and efflux in these animals. Renal Cys levels have been utilized as an indirect indicator of efflux since the majority of GSH transported from the sinusoidal membrane of the liver into the plasma ends up being degraded in the kidney by the enzyme γ-glutamyl transpeptidase resulting in the release of Cys [60]. Administration of radiolabelled GSH monoethylester resulted in quantitative increases in renal Cys concentration and in the labelling of the renal Cys pool to a similar specific activity as that of administered GSH [61]. Additionally, others have reported significant increases in renal Cys after i.p. or i.v. administration of GSH [62,63]. The rebound of renal Cys levels in young and mature mice after 24 h may indicate that hepatic efflux has returned to baseline levels, while the reduction of Cys in old mice may reflect a lower rate of GSH synthesis and turnover, possibly as a result of fasting as previously observed in old animals [64].

Our observation that the recovery of hepatic GSH levels after EtOH administration in young mice is less than complete is consistent with a pervious finding in mice [22] but contrasts with other studies in young rats, in which hepatic GSH levels were fully recovered by 18 h [14]. While this discrepancy may represent differential species-specific responses to EtOH, it could also be the result of differences in food consumption or availability between studies as hepatic GSH levels are dependent upon precursor amino acids for GSH synthesis. Additionally, an overnight fasting period prior to ethanol administration was included in many of these studies, which likely altered GSH homeostasis [65,66]. Since, in the present study, the loss of GSH due to starvation was minimal in young mice, the inability to recover from EtOH-induced GSH depletion was due to specific long-lasting effects of EtOH. One possible explanation for this age-related difference may be that young mice have an increased requirement for Cys, the rate-limiting precursor of GSH. Protein synthesis is known to compete with GSH synthesis for limited concentrations of Cys [67]. Metallothionein, a protein in which Cys comprises 30% of the amino acid residues, is induced 10-fold at 24 h after acute EtOH administration in young rats and mice [68–70]. Additionally, ethanol induces the rapid synthesis of stress proteins in young animals [71,72] while production of stress proteins is diminished in aging animals [73–75]. Thus, it is possible that in young animals, but less so in mature and old, an increase in protein synthesis occurs after exposure to high doses of EtOH, at the expense of GSH synthesis. This is consistent with other reports in which incorporation of radiolabelled amino acids into GSH was diminished after EtOH administration in young animals [76,77].

Alterations in GSH content and efflux as a result of EtOH exposure may, in part, explain the increased susceptibility of old organisms to EtOH. The lower absolute levels of GSH observed after EtOH administration in old mice compared to younger animals may result in a greater extent of EtOH-induced liver damage, as reactive oxygen species or intermediates are detoxified to a lesser extent by GSH. During GSH depletion and recovery periods, vulnerability to the adverse effects of other compounds is increased, as observed with acetaminophen, aflatoxin B1, and cocaine after EtOH exposure [78–80]. Additionally, the decreased rate of GSH efflux from the liver suggested in this study may enhance EtOH-mediated damage to other organ systems. Aging-related decreases in brain GSH content, as have been documented earlier [78], may contribute to the aging-related enhancement of neurological and psychomotor sensitivity to ethanol [7,8,81]. Indeed, GSH and GSSG have been found to interact with the *N*-methyl-D-aspartate (NMDA) receptor and antagonize EtOH-induced hypnosis [82,83]. Thus, GSH depletion could be an important factor in the neurological toxicity of EtOH displayed by the elderly.

In summary, animal age was found to significantly impact upon GSH depletion and recovery at selected times after EtOH administration. These age effects were most evident in the recovery of GSH by 24 h after initiation of EtOH treatment and food deprivation. Overall, a more severe depletion and impaired recovery of hepatic GSH may represent a possible biological basis for greater susceptibility of old organisms to the adverse effects of EtOH.

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