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INTEGRATIVE APPROACH TO VARIOUS ENVIRONMENTAL TOXINS: EFFECTS AND INTERACTIONS

Higher urinary glyphosate exposure is associated with increased risk of liver dysfunction in adults: An analysis of NHANES, 2013–2016

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Abstract

Glyphosate (GLY) exposure, both exogenous and endogenous, is a global concern. Multiple studies of model systems in vitro and in vivo have demonstrated the potential toxic effects of GLY exposure on human organs, particularly the liver and renal system. However, there is currently limited epidemiological evidence establishing a link between GLY exposure and hepatorenal function in the general population. In this study, a multivariable linear regression model and forest plots were employed to evaluate the connection between urinary GLY and biomarkers of hepatorenal function in 2241 participants from the National Health and Nutrition Examination Survey 2013–2016. Additionally, subgroup analyses were conducted based on age, gender, race, BMI, and chronic kidney disease (CKD). Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT and fibrosis 4 score (FIB-4) all increased with elevated urinary GLY concentrations after adjusting for potential confounders, while albumin (ALB) exhibited the opposite trend, particularly among younger, female, non-Hispanic white, overweight, and CKD participants. Furthermore, individuals in the third tertile had a greater risk of liver dysfunction than those in the first tertile after categorizing urinary GLY concentrations. However, our study showed no proof that GLY exposure affects the ratio of urine albumin to creatinine (ACR) or serum creatinine levels. Overall, these results imply that GLY exposure may have adverse effects on human liver function.

Keywords Cross-sectional studies · Environmental poisons · Glyphosate · Hepatorenal function · National Health and Nutrition Examination Survey

Background

Glyphosate (GLY), an efficient herbicide recognized for its broad-spectrum activity, has been extensively used in agricultural production, urban greening, residential gardens, and even waterways to eliminate weeds and exotic species (Botero-Coy et al. 2013; Bento et al. 2016). Presently, there are hundreds of GBHs available on the market globally under a variety of labels (Williams et al. 2000). As a consequence, GLY is detectable in the air, water, food supply, and therefore in biological fluids, including urine, blood, and breast milk, due to its widespread usage (Gillezeau et al. 2019). Despite the assertion that GLY exclusively interferes with the shikimate pathway, which is present in plants, bacteria, fungi, and protozoa but absent in humans (Steinrücken and Amrhein 1980), accumulated studies have demonstrated adverse effects of GLY in aquatic species, vertebrates, and invertebrates (Mesnage et al. 2017; Milić

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et al. 2018; Le Du-Carrée et al. 2021; Mutwedu et al. 2021). Therefore, concerns have grown within the scientific community regarding the possible toxicity and consequences of GLY exposure to human health.

The toxicity of GLY in target organs has been extensively investigated in numerous recent studies. One study revealed that GLY exposure may induce liver damage due to the increased production of oxidative stress (Soudani et al. 2019). Additionally, a rat model study reported the nephrotoxicity of GLY that results in the apoptosis of tubular cells (Gao et al. 2019). Moreover, case−control studies also proved that GLY could pose an adverse effect on hepatic and renal function and work synergistically with other pollutants, in particular paraquat, to increase toxic effects, resulting in the epidemic of chronic kidney disease (CKD) of unknown etiology in some regions (Jayasumana et al. 2015; Zhang et al. 2017; Gunatilake et al. 2019). However, most of these studies were performed in workers or farmers with occupational exposure. There are few studies on the impacts of GLY exposure on hepatorenal function in the wider population.

The purpose of this study was to investigate the effects of varying levels of GLY exposure on hepatorenal function across a sizable population. To accomplish this objective, the current study examined the recently accessible data from the National Health and Nutrition Examination Survey (NHANES) (as of November 2022) to investigate potential associations between urinary GLY concentrations and hepatorenal function within a large population of the United States (US).

Methods

Study population

The NHANES is a population-based study that uses a complex, stepwise, and probabilistic sampling process to gather extensive data on nutrition and health in the general US population (Curtin et al. 2012). The 2013–2016 continuous cycle of the NHANES dataset was used for this study. The following patients were excluded from the 20146 eligible participants: 15408 with missing urine GLY data, 1237 under the age of 19, 101 with missing hepatorenal function or albumin to creatinine ratio (ACR) data, and 786 with hepatitis B or C (positive for HbsAg or HCV antibodies). In the end, 2241 individuals were involved in the study. Figure 1 depicts the process for sample selection.

Measurement of Urinary Glyphosate

After collecting the random urine samples, 2D-on-line ion chromatography, tandem mass spectrometry (IC-MS/MS), and isotope dilution quantification were used for analytical measurements, during which CLIA guidelines played a role in assuring accuracy and reliability (Schütze et al. 2021). The content of GLY in the urine had a 0.2 ng/mL lower limit of detection, and 1677 (74.83%) of the individuals were at or over the detection limit.

Study variables

The following factors were analyzed in the current study: age, gender, race, education level, body mass index (BMI), income-poverty ratio, lifetime smoking, alcohol drinking times in the last 12 months, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST/ALT ratio, fibrosis 4 score (FIB-4), serum creatinine, ACR, estimated glomerular filtration rate (eGFR, using the CKD Epidemiology Collaboration creatinine equation (Levey et al. 2009)), hypertension, and CKD. FIB-4 = (age (year) × AST (U/L)) / [PLT (10^9/L) × $ALT^{1/2}$(U/L)] (Hou et al. 2023). The younger group and elderly group were divided according to the 60-year-old cutoff and CKD was defined by either an eGFR < 60 ml/min/1.73 m^2 or a urine ACR ≥ 30 mg/g (Stevens and Levin 2013).

Statistical analysis

According to the recommendations of the Centers for Disease Control and Prevention (CDC), all statistical analyses were carried out using the appropriate NHANES sample weights, taking complicated multistage cluster surveys into account. Means with standard errors (SE) were used to summarize continuous variables, while proportions were used to display categorical characteristics. Either a weighted linear regression model or weighted chi-square test was employed for continuous/categorical variables, thus evaluating the variations between individuals as classified by GLY tertiles. Multivariate linear regression analysis was used to obtain β values and 95% confidence intervals (CIs). Three models were built as multivariate tests: Model 1: no variables adjusted; Model 2: age, gender, race, and education level adjusted; Model 3: age, gender, race, education level, income-poverty ratio, BMI, lifetime smoking, alcohol drinking times, hypertension, diabetes, and CKD.

Stratified analyses were conducted by age (< 60 or ≥ 60), gender (male or female), race (non-Hispanic white, non-Hispanic black, Hispanic or other groups), BMI (< 25 or ≥ 25 kg/m^2), and CKD (yes or no). To estimate the significance of interactions between urinary GLY concentrations and stratification variables, P values for each product term were recorded. R software (version 4.1.3) and EmpowerStats (version 2.0) were used during the statistical computing and graphics analysis process. When statistically significant, $P < 0.05$ was recorded.

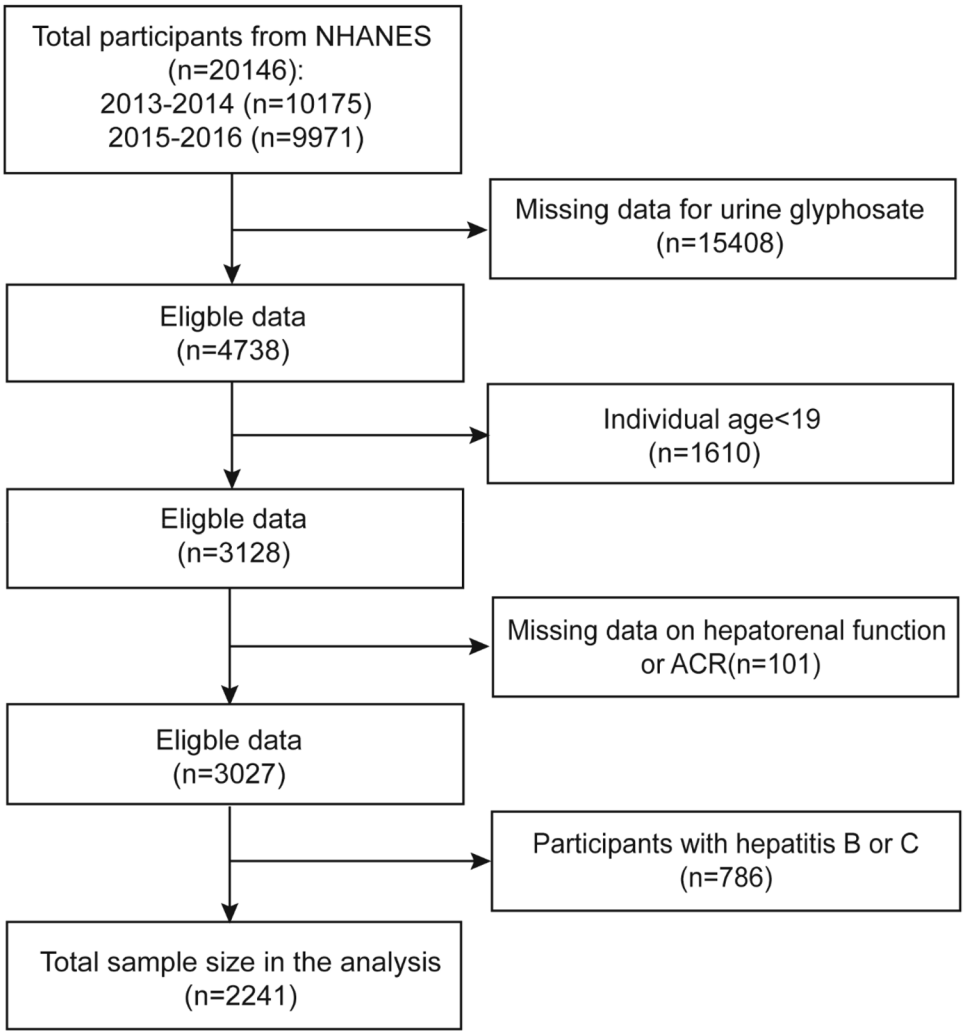

Fig. 1 Flow chart of participant selection

Results

Demographic and clinical characteristics of participants

Based on the above criteria, a total of 2241 adults were included in this study, with an average age of 50.09 ± 16.75 years. Among these participants, 49.15% were men, and 50.85% were women; 66.60% were non-Hispanic white, 10.42% were non-Hispanic black, 6.38% were Hispanic, and 16.61% were from other races; 14.77% were less than high school, 22.04% were high school, and 63.19% were more than high school. Based on the levels of urinary GLY concentration, the individuals' weighted features were split into three tertiles (Tertile 1: ≤ 0.262 ng/ml; Tertile 2: 0.263 ~ 0.495 ng/ml; Tertile 3: ≥ 0.496 ng/ml), as shown in Table 1. Of all the participants, the data revealed significant differences ($p < 0.05$) between individuals among the three tertiles in the distribution of age, gender, alcohol drinking times, hypertension, diabetes, income-poverty ratio, BMI, ALB, ALP, ALT, AST, FIB-4, serum creatinine, and eGFR.

Higher urinary glyphosate exposure is associated with a higher likelihood of liver dysfunction

Regarding the relationship between several biomarkers of hepatorenal function and GLY, the findings of the multivariate regression analyses are shown in Table 2. In the unadjusted model, albumin had a negative relationship with GLY (β = -0.424, 95% CI [-0.644, -0.204], $P < 0.001$). This strong correlation persisted even after covariate adjustment in Model 2 (β = -0.349, 95% CI [-0.555, -0.144], $P < 0.001$) and Model 3 (β = -0.560, 95% CI [-0.813, -0.307], $P < 0.001$). Similarly, although not statistically significant, inverse relationships were identified between levels of serum creatinine, ACR, and urinary GLY levels after adjusting for all covariates (β = -0.02, 95% CI [-0.04, 0.00], $P = 0.052$; β = -27.70, 95% CI [-59.45, 4.04], $P = 0.087$). In contrast, ALP was positively linked with GLY in the unadjusted model (β = 2.99,

Table 1 Baseline characteristics of the study population from NHANES (2013–2016)

Variables	Total ($n=2241$)	Tertile 1[a] ($n=746$)	Tertile 2[a] ($n=748$)	Tertile 3[a] ($n=747$)	*P* value
Age(years)[b]	50.09 ± 16.75	48.69 ± 16.36	49.20 ± 16.41	52.54 ± 17.25	< 0.001
Gender(%)[c]					< 0.001
Men	49.15	44.31	49.29	54.33	
Women	50.85	55.69	50.71	45.67	
Race (%)[c]					0.178
Non-Hispanic White	66.60	67.23	65.21	67.33	
Non-Hispanic Black	10.42	8.15	11.91	11.39	
Hispanic	6.38	6.98	5.84	6.26	
Other groups	16.61	17.64	17.05	15.03	
Education level (%)[c]					0.238
< High school	14.77	12.67	16.85	14.96	
High school	22.04	22.89	20.96	22.22	
> High school	63.19	64.44	62.19	62.82	
Lifetime smoking (%)[c]					0.983
< 100 cigarettes	45.15	44.94	45.41	45.1	
≥ 100 cigarettes	54.85	55.06	54.59	54.9	
Alcohol drinking times (%)[c]					0.004
0	17.96	13.54	20.63	20.26	
1–3	52.34	53.66	52.39	50.81	
≥ 4	29.70	32.81	26.98	28.93	
Hypertension (%)[c]					0.002
Yes	35.99	32.08	35.38	40.91	
No	64.01	67.92	64.62	59.09	
Diabetes (%)[c]					< 0.001
Yes	10.90	7.29	11.25	14.53	
No	89.10	92.71	88.75	85.47	
CKD(%)[c]					0.054
Yes	12.21	10.65	11.56	14.58	
No	87.79	89.35	88.44	85.42	
Income-poverty ratio[b]	3.06 ± 1.65	3.21 ± 1.63	2.96 ± 1.67	3.00 ± 1.65	0.009
BMI(kg/m^2)[b]	29.61 ± 7.02	28.52 ± 6.92	29.96 ± 6.90	30.46 ± 7.08	< 0.001
Laboratory features					
ALB(g/L)[b]	43.02 ± 3.35	43.50 ± 3.26	42.79 ± 3.40	42.73 ± 3.33	< 0.001
ALP(IU/L)[b]	66.57 ± 21.27	64.69 ± 19.52	66.99 ± 20.54	68.22 ± 23.57	0.005
ALT(IU/L)[b]	25.68 ± 18.15	24.88 ± 14.73	25.12 ± 17.07	27.13 ± 22.13	0.033
AST(IU/L)[b]	25.72 ± 15.22	25.23 ± 9.90	24.68 ± 9.65	27.33 ± 22.72	0.002
AST/ALT[b]	1.11 ± 0.35	1.13 ± 0.34	1.10 ± 0.33	1.11 ± 0.37	0.274
FIB-4[b]	1.22 ± 0.82	1.16 ± 0.70	1.16 ± 0.70	1.35 ± 1.01	< 0.001
Serum creatinine (mg/dL)[b]	0.89 ± 0.30	0.85 ± 0.24	0.91 ± 0.37	0.90 ± 0.26	< 0.001
ACR(mg/g)[b]	46.14 ± 373.85	57.78 ± 520.48	49.06 ± 332.39	30.34 ± 157.44	0.351
eGFR(mL/min/1.73 m$^{2)}$ [b]	110.40 ± 23.86	113.28 ± 25.27	109.81 ± 22.52	107.82 ± 23.25	< 0.001

[a] Tertile 1: ≤ 0.262 ng/ml; Tertile2: 0.263 ~ 0.495 ng/ml; Tertile3: ≥ 0.496 ng/ml

[b] Mean ± SD for continuous variables: P value was calculated by the weighted linear regression model

[c] % for categorical variables: P value was calculated by weighted chi-square test

Abbreviations: NHANES, National Health and Nutrition Examination Survey; CKD, chronic kidney disease; BMI, body mass index; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; FIB-4, fibrosis 4 score; ACR, albumin to creatinine ratio; eGFR, estimated glomerular filtration rate (mL/min/1.73 m^2)

Table 2 Relationship between biomarkers of hepatorenal function and GLY

	Model 1[a] β (95% CI) *P* value	Model 2[a] β (95% CI) *P* value	Model 3[a] β (95% CI) *P* value
ALB	-0.42 (-0.64, -0.20)	-0.35 (-0.56, -0.14)	-0.56 (-0.81, -0.31)
	< 0.001	< 0.001	< 0.001
ALP	2.99 (1.59, 4.39)	2.82 (1.44, 4.20)	3.27 (1.55, 4.98)
	< 0.001	< 0.001	< 0.001
ALT	2.12 (0.93, 3.32)	2.42 (1.26, 3.58)	2.25 (0.85, 3.65)
	< 0.001	< 0.001	0.002
AST	4.44 (3.46, 5.43)	4.52 (3.53, 5.51)	6.80 (5.50, 8.11)
	< 0.001	< 0.001	< 0.001
AST/ALT	0.02 (-0.00, 0.04)	0.01 (-0.01, 0.03)	0.05 (0.03, 0.08)
	0.120	0.278	< 0.001
FIB-4	0.23 (0.18, 0.28)	0.14 (0.10, 0.19)	0.23 (0.17, 0.28)
	< 0.001	< 0.001	< 0.001
Serum creatinine	0.01 (-0.01, 0.03)	-0.00 (-0.02, 0.01)	-0.02 (-0.04, 0.00)
	0.160	0.757	0.052
ACR	-11.98(-36.60, 12.64)	-13.42(-38.16, 11.32)	-27.70 (-59.45, 4.04)
	0.340	0.288	0.087

[a] Model 1: no covariates were adjusted; Model 2: age, gender, race, and education level adjusted; Model 3: age, gender, race, education level, income-poverty ratio, BMI, lifetime smoking, alcohol drinking times, hypertension, diabetes, and CKD were adjusted

95% CI [1.59, 4.39], $P < 0.001$), model 2 (β = 2.82, 95% CI [1.44, 4.20], $P < 0.001$) and model 3 (β = 3.27, 95% CI [1.55, 4.98], $P < 0.001$). Similarly, ALT, AST, AST/ALT and FIB-4 showed significantly positive links with GLY in these three models, and the figures in model 3 are listed (β = 2.25, 95% CI [0.85, 3.65], P = 0.002; β = 6.80, 95% CI [5.50, 8.11], $P < 0.001$; β = 0.05, 95% CI [0.03, 0.08], $P < 0.001$; β = 0.23, 95% CI [0.17, 0.28], $P < 0.001$).

Following the transformation of urine GLY concentrations from a continuous to a categorical variable (tertiles) for sensitivity analysis, the findings are shown in Table 3. Compared with the lowest GLY tertile (Tertile 1), participants in the top GLY tertile had 0.52 g/L lower ALB, 3.09 IU/L higher ALP, 2.33 IU/L higher AST and 0.12 higher FIB-4 than those in the bottom GLY tertile, and the P for trends were 0.012, 0.015, 0.005 and < 0.001, respectively. However, the association between ALT and GLY tertiles did not meet statistical significance (β = 1.36, 95% CI [-0.59, 3.31], P = 0.170). No significant difference between AST/ALT and urinary GLY concentrations was observed (β = 0.02, 95% CI [-0.02, 0.05], $P = 0.396$).

Subgroup analysis

The robustness of the relationship between several biomarkers of liver function and GLY was assessed by using subgroup analysis stratified by age, gender, race, BMI, and CKD (Fig. 2). In the younger subgroup stratified by age, only ALB exhibited a negative correlation with urinary GLY concentrations (β = -0.69, 95% CI [-1.01, -0.36], $P < 0.001$), while ALP, ALT, AST, AST/ALT and FIB-4 showed positive associations (all $P < 0.05$, Supplementary table). In addition, there was a positive association between FIB-4 and urinary GLY concentrations in the elderly group (β = 0.23, 95% CI [0.08, 0.38], $P = 0.025$).

In terms of subgroup analyses stratified by female and race, the negative correlation of ALB with urinary GLY concentrations was maintained in females (β = -0.78, 95% CI [-1.14, -0.42], $P < 0.001$) but not in males (β = -0.23, 95% CI [-0.58, 0.11], $P = 0.186$), as well as in non-Hispanic white individuals (β = -0.69, 95% CI [-1.03, -0.34], $P < 0.001$), but not in other races. However, our results showed that there was a positive association between urinary GLY and ALP, ALT, AST, AST/ALT and FIB-4 (all $P < 0.05$, Supplementary table).

With regard to subgroup analyses stratified by BMI, ALB was negatively linked with urinary GLY concentrations in both the low BMI group (β = -0.69, 95% CI [-1.16, -0.22], $P = 0.004$) and the high BMI group (β = -0.52, 95% CI [-0.82, -0.22], $P < 0.001$). Positive associations of ALP, ALT, AST, AST/ALT, FIB-4 and urinary GLY concentrations were observed in the high BMI group (β = 4.93, 95% CI [2.85, 7.01], $P < 0.001$; β = 3.13, 95% CI [1.25, 5.00], $P < 0.001$; β = 10.00, 95% CI [8.30, 11.69], $P < 0.001$; β = 0.07, 95% CI [0.04, 0.10], $P < 0.001$; β = 0.35, 95% CI [0.28, 0.42], $P < 0.001$).

With reference to CKD-stratified subgroup analyses, there was a negative correlation between ALB and urinary

Table 3 Relationship between biomarkers of liver function and GLY

	Tertile 1[a]	Tertile 2[a]	Tertile 3[a]	P for trend
ALB				
Model 1[b]	Ref	-0.70 (-1.01, -0.39)	-0.78 (-1.11, -0.44)	< 0.001
		< 0.001	< 0.001	
Model 2[b]	Ref	-0.54 (-0.89, -0.18)	-0.72 (-1.04, -0.41)	< 0.001
		0.003	< 0.001	
Model 3[b]	Ref	-0.54 (-0.89, -0.18)	-0.52 (-0.87, -0.17)	0.012
		0.003	0.004	
ALP				
Model 1[b]	Ref	2.30 (0.16, 4.43)	3.53 (1.38, 5.67)	0.002
		0.035	0.001	
Model 2[b]	Ref	2.23 (0.13, 4.34)	3.27 (1.14, 5.40)	0.005
		0.037	0.003	
Model 3[b]	Ref	1.74 (-0.65, 4.14)	3.09 (0.70, 5.47)	0.015
		0.154	0.011	
ALT				
Model 1[b]	Ref	0.24 (-1.58, 2.07)	2.25 (0.42, 4.08)	0.011
		0.794	0.016	
Model 2[b]	Ref	0.22 (-1.55, 1.99)	2.23 (0.44, 4.02)	0.010
		0.806	0.015	
Model 3[b]	Ref	-0.54 (-2.49, 1.42)	1.36 (-0.59, 3.31)	0.109
		0.592	0.170	
AST				
Model 1[b]	Ref	-0.55 (-2.08, 0.98)	2.11 (0.57, 3.64)	0.002
		0.480	0.007	
Model 2[b]	Ref	-0.55 (-2.08, 0.97)	2.03 (0.49, 3.58)	0.003
		0.478	0.010	
Model 3[b]	Ref	-0.61 (-2.47, 1.26)	2.33 (0.47, 4.19)	0.005
		0.525	0.014	
AST/ALT				
Model 1[b]	Ref	-0.03 (-0.06, 0.01)	-0.02 (-0.05, 0.02)	0.496
		0.110	0.355	
Model 2[b]	Ref	-0.03 (-0.06, 0.01)	-0.01 (-0.05, 0.02)	0.527
		0.115	0.380	
Model 3[b]	Ref	-0.00 (-0.04, 0.03)	0.02 (-0.02, 0.05)	0.346
		0.923	0.396	
FIB-4				
Model 1[b]	Ref	0.00 (-0.08, 0.09)	0.19 (0.11, 0.28)	< 0.001
			0.914	< 0.001
Model 2[b]	Ref	0.02 (-0.08, 0.05)	0.07 (0.00, 0.13)	0.020
			0.617	0.040
Model 3[b]	Ref	-0.01 (-0.08, 0.07)	0.12 (0.04, 0.20)	< 0.001
			0.893	0.002

[a] Tertile 1: ≤ 0.262 ng/ml; Tertile2: 0.263 ~ 0.495 ng/ml; Tertile3: ≥ 0.496 ng/ml

[b] Model 1: no covariates were adjusted; Model 2: age, gender, race, and education level adjusted; Model 3: age, gender, race, education level, income-poverty ratio, BMI, lifetime smoking, alcohol drinking times, hypertension, diabetes, and CKD were adjusted

GLY concentrations in both the CKD and non-CKD groups (β = -0.32, 95% CI [-0.60, -0.04], P = 0.025; β = -1.46, 95% CI [-2.03, -0.89], $P < 0.001$), while FIB-4 showed a positive correlation with urinary GLY (β = 0.08, 95% CI [0.03, 0.14], $P = 0.004$; β = 0.68, 95% CI [0.53, 0.84], $P < 0.001$). For ALP, ALT, AST, and AST/ALT, this statistical significance was observed only in the CKD group (β = 4.93, 95% CI [2.85, 7.01], $P < 0.001$; β = 3.13, 95% CI [1.25, 5.00], $P < 0.001$; β = 10.00, 95% CI [8.30, 11.69], $P < 0.001$; β = 0.07, 95% CI [0.04, 0.10], $P < 0.001$).

In conclusion, the substantial connection with the p for interaction suggested that this association between biomarkers of liver function and GLY was certainly dependent on age, gender, race, BMI, and CKD (p for interaction < 0.05, Supplementary table).

Discussion

In our nationally representative sample of US adults, urinary GLY concentrations were positively connected with liver function but showed no significant association with renal function. In particular, our findings revealed that elevated GLY concentrations in urine were strongly linked to an increased risk of liver injury in specific subgroups, including younger participants, women, non-Hispanic whites, overweight individuals, and CKD patients.

The liver is the major organ affected by xenobiotic exposure. ALT, AST, and ALP activities serve as hepatotoxicity markers (McGill 2016). Previous studies illustrated that GLY could induce hepatic oxidative stress with altered AST, ALT, and ALP, evidenced by the upregulation of malondialdehyde, hydrogen peroxide and the downregulation of superoxide dismutase, glutathione, and vitamin C (Soudani et al. 2019; Abdelmagid et al. 2022). Transcriptome profile analysis also proved that the alterations in gene expression

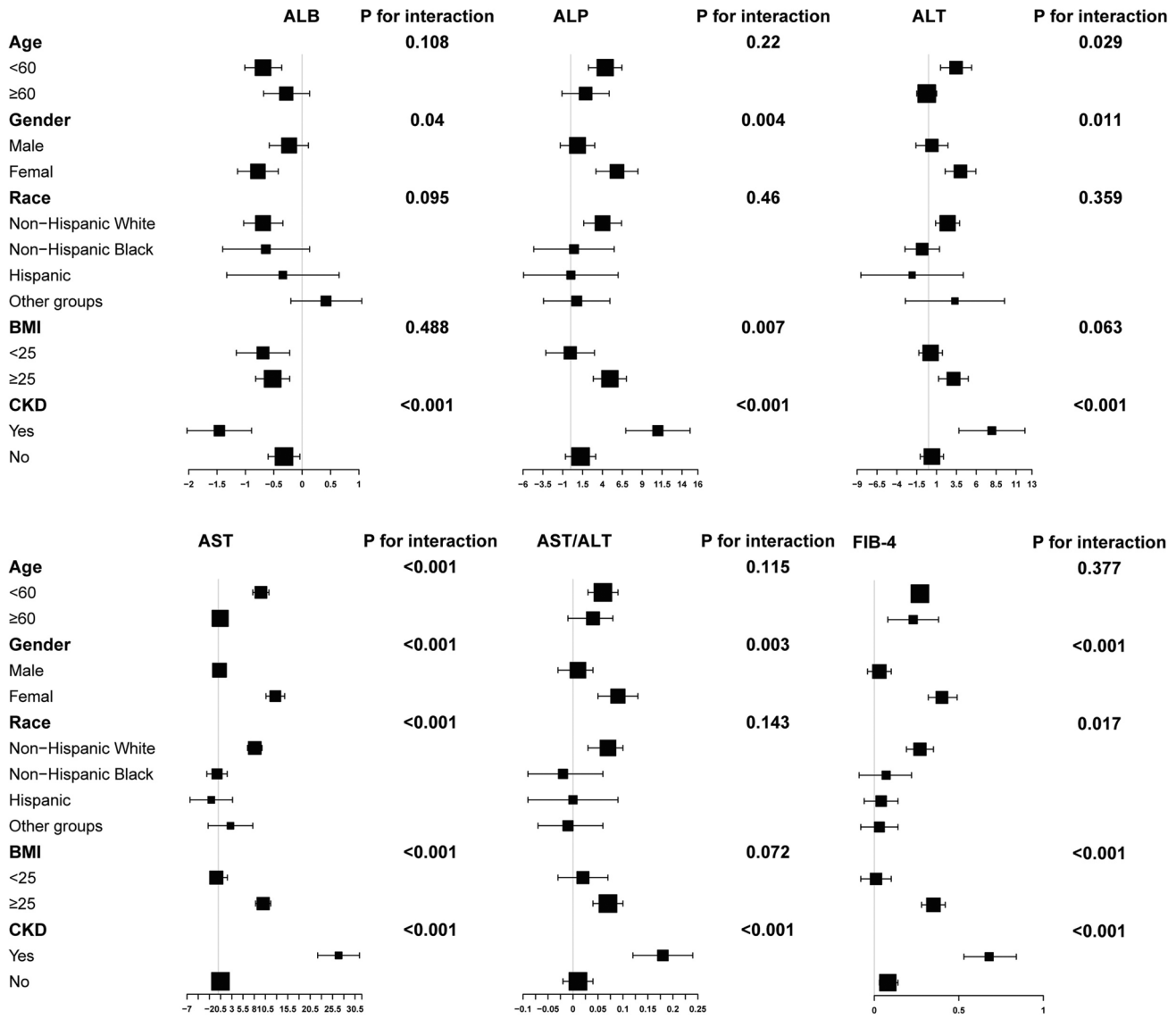

Fig. 2 Subgroup analyses of the association between GLY and ALB, ALP, ALT, AST, AST/ALT, FIB-4. Each subgroup analysis adjusted for all the factors (age, gender, race, education level, income-poverty ratio, BMI, lifetime smoking, alcohol drinking times, hypertension, diabetes, and CKD) except the stratification factor itself

of the liver were consistent with fibrosis, necrosis, phospholipidosis, and mitochondrial membrane dysfunction following chronic low-dose GLY exposure (Mesnage et al. 2015). These studies may serve as useful information for explaining the results of the present study. The AST/ALT ratio was helpful in the detection of liver fibrosis or damage (Mansoor et al. 2015), and a high AST/ALT ratio may indicate hepatocyte necrosis (Fu et al. 2019). We found that GLY was significantly correlated with the AST/ALT ratio, which indicated a positive association between prolonged exposure to GLY and hepatocyte necrosis. A previous study showed that liver pathology confirmed the presence of inflammatory reaction with pathology involving hepatocyte necrosis in rats (Hamdaoui et al. 2019). FIB-4 is another diagnostic tool for predicting the stage of liver fibrosis (Itakura et al. 2021; Xu et al. 2022). As previously described, the notable dose-dependent rise in glyphosate exposure was along with an increase in fibrosis phases (Mills et al. 2020), that consistent with the positive relationship between urinary GLY concentrations and FIB-4 in our study. In addition, ALB is a marker of hepatic synthesis function and is involved in physiological processes, such as endothelium stabilization, molecular transportation, antioxidant, anti-inflammatory, and modulating capillary permeability (Sun et al. 2019). Our findings supported previous research that showed a negative relationship between serum albumin concentrations and GLY (Abdelmagid et al. 2022). In addition, a higher concentration of GLY altered the structure of albumin while

significantly decreasing its antioxidant capability (Movaghati et al. 2023). More investigations are required to comprehend the mechanisms underlying the link between GLY exposure and albumin.

In the subgroup, we further investigated the association between GLY exposure and liver dysfunction. Age constitutes a pivotal risk factor contributing to an elevated prevalence of liver injury in the elderly population (Maeso-Díaz et al. 2018); however, our findings demonstrated that GLY exposure conferred a greater susceptibility to liver dysfunction in younger participants compared to elders, potentially attributable to heightened GLY exposure resulting from disparities in lifestyle habits and metabolic processes (Grau et al. 2022). The evidence from our study also strongly pointed to a greater risk for females to develop liver dysfunction than males, and the disparity was also discovered in other comparable studies (Colantoni et al. 2000; Wahlang et al. 2023). Recent epidemiological studies and studies performed in animals suggested that GLY could have endocrine-disrupting potentiality targeting sex hormones, which may be a significant contributing reason to women's increased susceptibility to liver dysfunction following GLY exposure (Lesseur et al. 2021; Maddalon et al. 2021; Geier and Geier 2023). Similar associations were also observed between GLY and biomarkers of liver function in non-Hispanic white individuals and the risks correlated with GLY exposure varied significantly by racial difference. It is anticipated that the observed substantial correlations might be impacted by genetic and/or biochemical variances (Strnad et al. 2010; Cirulli et al. 2019). However, no previous studies have demonstrated that liver dysfunction with regard to levels of GLY is attributed to racial differences and future studies are needed to focus on this issue. In addition, this study also revealed that the risk of liver dysfunction from GLY exposure was greater for overweight individuals. Overweight, especially progressing to obesity, is involved in the development of diseases linked to metabolic syndrome, which includes nonalcoholic fatty liver disease with an accumulation of fat in the liver. Hepatic steatosis subsequently causes inflammation and hepatocyte injury and even progresses to fibrosis (Weiß et al. 2014). Therefore, overweight individuals with exposure to GLY were more susceptible to liver injury and could even develop more severe fibrosis (Mills et al. 2020).

Although previous studies in humans and animals have reported that chronic exposure to GLY is nephrotoxic (Jayasumana et al. 2015; Zhang et al. 2017; Gunatilake et al. 2019), serum creatinine and ACR were not found to have significant associations with GLY exposure in this study. As a possible explanation, participants were those from general populations, not as observation subjects suffering from long-term uninterrupted GLY exposure in previous studies; thus, the average concentration of GLY in the recruited participants may not be high enough to cause impaired renal function (Gillezeau et al. 2019). However, we found that participants with CKD had a higher risk of liver dysfunction than non-CKD participants. This phenomenon could be explained by the fact that the liver is one of the main organs where ingested GLY accumulates (Williams et al. 2000), and its clearance capacity decreases in the presence of renal impairment (Tokunaga et al. 2020). Therefore, more attention should be given to the risk of GLY for individuals with CKD.

The current study has several limitations that need to be acknowledged. First, as this analysis was cross-sectional, we cannot establish causality or determine the temporal relationship between variables. To address this limitation, future research should consider longitudinal designs. Second, we were unable to completely control for all potential confounding variables, such as dietary habits and living and working environments. Therefore, caution should be exercised when interpreting our results. Future studies should aim to include a more comprehensive assessment of confounding factors. Third, the measurement of GLY exposure was based on the concentration of GLY in random urine samples. This method may introduce variability in the test outcomes, and first-morning void or 24-h urine samples might yield more precise statistical data. Future studies should consider using these alternative sampling methods to improve the accuracy of GLY exposure measurements. In addition, the equation for estimation of eGFR only incorporates creatinine while omitting cystatin C in the current study. However, the eGFR equation with the combination of creatinine and cystatin C provides a more accurate assessment than the equation with creatinine alone. Furthermore, cystatin C levels exhibit an earlier increase than creatinine levels, making it a more sensitive marker for diagnosing kidney repairment in the early stages. Whereas, there are no assay values for cystatin C in the 2013–2016 NHANES datasets. Last, glyphosate-based herbicide (GBH) formulations encompass additional chemicals in addition to glyphosate. The precise attribution of adverse effects to either glyphosate or the combined components remains uncertain. Consequently, NHANES should furnish comprehensive information concerning GBH formulations to facilitate further investigation into their potential impact on human health. In spite of these constraints, our research presented several benefits. First, we utilized a nationally representative sample, generalizability of our findings to the diverse adult population in the US, encompassing various ethnicities and genders. Additionally, the inclusion of a significant number of participants in our study enabled us to conduct subgroup analysis, providing valuable insights into specific subpopulations.

Conclusions

This study revealed significant associations between GLY exposure and biomarkers of liver dysfunction among US adults, suggesting that avoiding exposure can assist in

lessening the harmful effects of GLY on health. Considering the limited availability of data in the literature and the constraints of our study, additional research is required to validate our findings concerning the effects of environmental GLY exposure on liver injury.

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Data availability Both the data and analysis materials are freely downloaded from National Health and Nutritional Examination Survey. (https://www.cdc.gov/nchs/nhanes).

Declarations

Ethics approval and consent to participate The CDC/NCHS Ethics Review Board approved the NHANES study and all participants provided written informed consent.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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