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Glyphosate Excretion is Associated With Steatohepatitis and Advanced Liver Fibrosis in Patients With Fatty Liver Disease

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease in developed countries [1] Patients with non-alcoholic steatohepatitis (NASH) are considered to be at a higher risk of fibrosis progression and development to cirrhosis and hepatocellular carcinoma.

Among potential environmental contributors to the pathophysiology of NAFLD are exposure to pesticides and herbicides [2]. Glyphosate, the primary weed-killing ingredient in Roundup®, is sprayed on genetically modified (GM) crops and on many non-GM grain crops and is found in these crops at harvest [3].

Rodents chronically fed with a low dosage of glyphosate exhibit signs of hepatotoxicity, liver congestion, necrosis, and DNA damage of the liver cells [456]. This study examined excretion levels of glyphosate and its primary metabolite aminomethylphosphonic acid (AMPA) in a well-characterized and prospectively recruited cohort of patients with biopsy-proven NAFLD.

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Author Contributions. Paul J. Mills: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis. Cyrielle Caussy: Study concept and design; acquisition of data; interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content. Rohit Loomba: Study concept and design; acquisition of data; interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

COIs. The authors report no conflicts of interest.

Methods

Participants were originally recruited as part of a larger study between September 2012 and March 2018 at the University of California at San Diego (UCSD) NAFLD Research Center. As previously described [7], patients with suspected NAFLD with a clinical indication for liver biopsy underwent a careful evaluation for other causes of hepatic steatosis and liver disease through a standardized research visit including detailed medical and alcohol use history as well as anthropometric and physical examination. Histologic scoring was done using the Nonalcoholic Steatohepatitis Clinical Research Network Histologic Scoring System. Prior to conducting statistical analyses, cases were grouped as definite NASH or NAFLD not NASH. This study was approved by the UCSD Institutional Review Board. Informed written consent was obtained from each study participant.

Each patient provided a fasting urine sample which was stored at −80° C. Urine samples were analyzed for glyphosate and AMPA using HPLC coupled with mass spectrometry. Using the formula [(glyphosate + 1.5) × AMPA] we calculated the glyphosate residue, which provides an estimate of dietary intake and exposure to residues.

ANOVA, ANCOVA, Chi-square, and multivariate general linear models covarying for age, gender and BMI were used (SPSS Version 24.0 software package (IBM, Armonk, NY)). Dependent variables were glyphosate, AMPA and glyphosate residue. Results were considered statistically significant at the $p \leq 0.05$ level. Prior to statistical analyses, data were tested for normality and homogeneity of variance.

Results

Patient characteristics are presented in Table 1. Neither age nor BMI were significantly related to glyphosate, AMPA or glyphosate residue. Similarly, neither diabetes status nor race / ethnicity were significantly related to glyphosate, AMPA or glyphosate residue. Glyphosate [women 0.373 μg/L (SD=0.41)] vs [men 0.215 μg/L (SD=0.17) (F=5.18; p=0.025)] and glyphosate residue [women 0.833 μg/L (SD=0.67) vs men 0.594 μg/L (SD=0.38) (F=4.09; p=0.046)] were elevated in women as compared to men.

In multivariate models adjusting for age, sex, and BMI, as compared to patients without NASH, AMPA (F=5.39; p=0.022) and glyphosate residue (F=7.43; p=0.008) were elevated in patients with definite NASH (Table 1). When compared to patients without advanced fibrosis (Stages 0 & 1), patients with advanced fibrosis (Stages 2, 3, & 4) had, respectively, elevated AMPA [0.196 μg/L (SD=0.20) vs 0.365 μg/L (SD=0.33) (F=9.44; p=0.003), glyphosate residue [0.525 μg/L (SD=0.38) vs 0.938 μg/L (SD=0.372) (F=11.9; p=0.001), and glyphosate [0.230 μg/L (SD=0.19) vs 0.351 μg/L (SD=0.45) (F=4.13; p=0.046).

Discussion

We report that glyphosate excretion is significantly higher in patients with NASH compared to patents without NASH. In addition, we also report a significant dose-dependent increase of glyphosate exposure with increase in fibrosis stages.

For individuals not working in the agricultural or horticultural industries, the primary route of glyphosate exposure is through ingestion of Roundup®-treated GM foods and/or non-GM crops such as wheat and oats [3]. Glyphosate excretion was elevated in women, which presumably reflected an increased exposure to glyphosate.

While there are strengths to this study, including the use of a well-characterized cohort using liver biopsy for the diagnosis of NASH and stage of liver fibrosis, we acknowledge limitations, including no information on dietary intake or occupation and no patients without NAFLD. We did not find an association between glyphosate excretion and BMI, suggesting that glyphosate intake was independent of total caloric intake.

As far as potential mechanisms of glyphosate on the liver, Mesnage et al. showed that rats fed glyphosate have disrupted liver mitochondrial oxidative phosphorylation leading to proteome disturbances reflecting peroxisomal proliferation, steatosis and necrosis, a profile consistent with NAFLD and its progression to NASH [4]. Other studies show that glyphosate inhibits fatty acid oxidation and increases fat and cholesteryl ester levels in mice livers, leading to increased lipid mass per gram of liver [8].

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Abbreviations.

NAFLD	nonalcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis

References

1. Loomba R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol. 2013;10(11): 686–90. [PubMed: 24042449]
2. Yang JS, Park Y. Insecticide Exposure and Development of Nonalcoholic Fatty Liver Disease. J Agric Food Chem. 2018;66(39):10132–8. Epub 2018/09/08. [PubMed: 30193066]
3. Myers JP, Antoniou MN, Blumberg B, et al. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ Health. 2016;15:19. Epub 2016/02/18. [PubMed: 26883814]
4. Mesnage R, Renney G, Seralini GE, et al. Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. Sci Rep. 2017;7:39328. Epub 2017/01/10. [PubMed: 28067231]
5. Mesnage R, Arno M, Costanzo M, et al. Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. Environ Health. 2015;14:70. [PubMed: 26302742]
6. Milic M, Zunec S, Micek V, et al. Oxidative stress, cholinesterase activity, and DNA damage in the liver, whole blood, and plasma of Wistar rats following a 28-day exposure to glyphosate. Arh Hig Rada Toksikol. 2018;69(2):154–68. Epub 2018/07/11. [PubMed: 29990293]
7. Caussy C, Hsu C, Lo MT, et al. Link between gut-microbiome derived metabolite and shared gene-effects with hepatic steatosis and fibrosis in NAFLD. Hepatology. 2018. Epub 2018/03/25.

8. Bonvallot N, Canlet C, Blas YEF, et al. Metabolome disruption of pregnant rats and their offspring resulting from repeated exposure to a pesticide mixture representative of environmental contamination in Brittany. PLoS One. 2018;13(6):e0198448. Epub 2018/06/21. [PubMed: 29924815]

Table 1.

Patient Characteristics (Mean ± SD)

	All (n=97)	Not NASH (n=34)	Definite NASH (n=63)	p-value*
Demographics				
Age (years)	50.5 (13.2) (range 19 – 74)	47.3 (12.5) (range 21 – 72)	51.9 (13.8) (range 19 – 74)	0.249
Male (%)	41.2	53.3	36.5	0.124
White (%)	42.2	39.6	47.1	0.27
Hispanic or Latino (%)	35.0	35.2	34.9	0.81
BMI (kg/m^2)	31.8 (7.0)	29.2 (3.5)	33.0 (7.8)	**0.014**
Clinical				
Type 2 Diabetes (%)	38.10	17.6	44.4	**0.008**
Biological data				
AST (U/L)	47.0 (31.7)	35.4 (13.2)	52.5 (36.2)	**0.014**
ALT (U/L)	65.1 (43.2)	56.3 (24.8)	69.3 (48.3)	0.179
Hemoglobin A1c (%)	6.19 (1.2)	5.71 (0.9)	6.45 (1.2)	**0.002**
Triglycerides (mg/dL)	148.4 (66.3)	168.0 (78.5)	143.3 (58.8)	0.813
Total cholesterol (mg/dL)	185.0 (46.8)	195.3 (31.8)	180.2 (51.9)	0.153
HDL-cholesterol (mg/dL)	44.8 (12.9)	44.2 (10.4)	45.1 913.9)	0.753
LDL-cholesterol (mg/dL)	106.7 (29.7)	117.7 (25.0)	101.8 (30.4)	**0.020**
Platelet count ($10^3\mu L$)	238880 (72375)	238566 (83977)	239032 (66794)	0.977
Histology				
Fibrosis (%)	(n=74)	(n=27)	(n=47)	**<0.001**
Stage 0	39.0	88.9	10.6	
Stage 1	5.40	3.70	6.4	
Stage 2	27.1	3.70	40.4	
Stage 3	23.1	3.70	34.1	
Stage 4	5.40	0	8.5	
Steatosis (%)	(n=97)	(n=34)	(n=63)	**0.017**
Stage 0	0.1	0	1.5	
Stage 1	37.7	60.1	26.6	
Stage 2	39.3	23.3	47.4	
Stage 3	22.9	16.6	24.5	
Lobular inflammation (%)	(n=90)	(n=32)	(n=58)	**0.003**
Stage 0	4.5	13.3	0	
Stage 1	47.7	60.0	41.4	
Stage 2	45.5	23.4	56.9	
Stage 3	2.3	3.3	1.7	
Ballooning (%)	(n=97)	(n=34)	(n=63)	**<0.001**
Stage 0	38.7	84.7	13.3	

	All (n=97)	Not NASH (n=34)	Definite NASH (n=63)	p-value*
Stage 1	46.3	15.3	63.4	
Stage 2	15.0	0	23.3	
Glyphosate Excretion				
Glyphosate (μ/L)	0.308 (0.34)	0.241 (0.18)	0.344 (0.40)	0.164
AMPA (μ/L)	0.284 (0.28)	0.197 (0.18)	0.331 (0.31)	**0.022**
Glyphosate Residue (μ/L)	0.735 (0.58)	0.538 (0.35)	0.841 (0.65)	**0.008**

Data are provided as mean values ± standard deviation or %. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein. Not NASH group defined as patients with NAFL (n=24) and borderline NASH (n= 10) as opposed to patients with definite NASH.

* P-values determined by comparing characteristics of definite NASH as compared to not NASH, using ANOVA and ANCOVA or Chi-square test when appropriate to compare categorical variables.