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

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Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease in developed countries.[1] Patients with nonalcoholic 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 (Monsanto, St Louis, MO), is sprayed on genetically modified crops and on many non–genetically modified 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.[4–6] 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.

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 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. Before conducting statistical analyses, cases were grouped as definite nonalcoholic steatohepatitis (NASH) or NAFLD not NASH. This study was approved by the University of California at San Diego Institutional Review Board. Informed written consent was obtained from each study participant.

Each patient provided a fasting urine sample that was stored at −80°C. Urine samples were analyzed for glyphosate and AMPA by using high-performance liquid chromatography 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.

Analysis of variance, analysis of covariance, χ^2, and multivariate general linear models covarying for age, gender, and body mass index 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 $P \leq .05$ level. Before statistical analyses, data were tested for normality and homogeneity of variance.

Results

Patient characteristics are presented in Table 1. Neither age nor body mass index was significantly related to glyphosate, AMPA, or glyphosate residue. Similarly, neither diabetes status nor race/ethnicity was significantly related to glyphosate, AMPA, or glyphosate residue. Glyphosate (women, 0.373 μg/L; standard deviation [SD], 0.41 vs men, 0.215 μg/L; SD, 0.17) ($F = 5.18$; $P = .025$) and glyphosate residue (women, 0.833 μg/L; SD, 0.67 vs men, 0.594 μg/L; SD, 0.38) ($F = 4.09$; $P = .046$) were elevated in women as compared with men.

In multivariate models adjusting for age, sex, and body mass index, as compared with patients without NASH, AMPA ($F = 5.39$; $P = .022$) and glyphosate residue ($F = 7.43$; $P = .008$) were elevated in patients with definite NASH (Table 1). When compared with patients without advanced fibrosis (stages 0 and 1), patients with advanced fibrosis (stages 2, 3, and 4) had elevated AMPA

Abbreviations used in this paper: AMPA, aminomethylphosphonic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SD, standard deviation.

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Table 1. Patient Characteristics (Mean ± Standard Deviation)

	All (n = 97)	Not NASH (n = 34)	Definite NASH (n = 63)	*P* value[a]
Demographics				
Age (*y*)	50.5 (13.2) (range, 19–74)	47.3 (12.5) (range, 21–72)	51.9 (13.8) (range, 19–74)	.249
Male (%)	41.2	53.3	36.5	.124
White (%)	42.2	39.6	47.1	.27
Hispanic or Latino (%)	35.0	35.2	34.9	.81
Body mass index (kg/m^2)	31.8 (7.0)	29.2 (3.5)	33.0 (7.8)	**.014**
Clinical				
Type 2 diabetes (%)	38.10	17.6	44.4	**.008**
Biological data				
AST (*U/L*)	47.0 (31.7)	35.4 (13.2)	52.5 (36.2)	**.014**
ALT (*U/L*)	65.1 (43.2)	56.3 (24.8)	69.3 (48.3)	.179
Hemoglobin A1c (%)	6.19 (1.2)	5.71 (0.9)	6.45 (1.2)	**.002**
Triglycerides (*mg/dL*)	148.4 (66.3)	168.0 (78.5)	143.3 (58.8)	.813
Total cholesterol (*mg/dL*)	185.0 (46.8)	195.3 (31.8)	180.2 (51.9)	.153
HDL cholesterol (*mg/dL*)	44.8 (12.9)	44.2 (10.4)	45.1 (13.9)	.753
LDL cholesterol (*mg/dL*)	106.7 (29.7)	117.7 (25.0)	101.8 (30.4)	**.020**
Platelet count ($10^3/\mu L$)	238,880 (72,375)	238,566 (83,977)	239,032 (66,794)	.977
Histology				
Fibrosis (%)	(n = 74)	(n = 27)	(n = 47)	**<.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)	**.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)	**.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)	**<.001**
Stage 0	38.7	84.7	13.3	
Stage 1	46.3	15.3	63.4	
Stage 2	15.0	0	23.3	
Glyphosate excretion				
Glyphosate (*μg/L*)	0.308 (0.34)	0.241 (0.18)	0.344 (0.40)	.164
AMPA (*μg/L*)	0.284 (0.28)	0.197 (0.18)	0.331 (0.31)	**.022**
Glyphosate residue (*μg/L*)	0.735 (0.58)	0.538 (0.35)	0.841 (0.65)	**.008**

NOTE. Data are provided as mean values ± standard deviation or %. Not NASH group defined as patients with NAFL (n = 24) and borderline NASH (n = 10) as opposed to patients with definite NASH.

ALT, alanine aminotransferase; AMPA, aminomethylphosphonic acid; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a]*P* values determined by comparing characteristics of definite NASH as compared with not NASH, using analysis of variance and analysis of covariance or χ^2 test when appropriate to compare categorical variables.

(0.196 μg/L; SD, 0.20 vs 0.365 μg/L; SD, 0.33) (F = 9.44; *P* = .003), glyphosate residue (0.525 μg/L; SD, 0.38 vs 0.938 μg/L; SD, 0.372) (F = 11.9; *P* = .001), and glyphosate (0.230 μg/L; SD, 0.19 vs 0.351 μg/L; SD, 0.45) (F = 4.13; *P* = .046), respectively.

Discussion

We report that glyphosate excretion is significantly higher in patients with NASH compared with 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 genetically modified foods and/or non–genetically modified crops such as wheat and oats.[3] Glyphosate excretion was elevated in women, which presumably reflected an increased exposure to glyphosate.

Although 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 body mass index, suggesting that glyphosate intake was independent of total caloric intake.

As far as potential mechanisms of glyphosate on the liver, Mesnage et al[4] 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. 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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Conflicts of interest
The authors disclose no conflicts.

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