Environmental Pollution 254 (2019) 112906

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring☆

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ARTICLE INFO

Article history:
Received 18 March 2019
Received in revised form
20 June 2019
Accepted 14 July 2019
Available online 20 July 2019

Keywords:
Roundup
Liver disease
Steatosis
Lipid metabolism
Hepatotoxicity

ABSTRACT

Glyphosate is the active ingredient in Roundup, one of the most popular herbicides in the world, and its toxicity has caused increasing concerns. The present study aims to investigate the toxic effects of prenatal exposure to pure glyphosate or Roundup on lipid metabolism in offspring. During gestational days (GDs), ICR mice (from Institute of Cancer Research) were given distilled water, 0.5% glyphosate solution (w/v, 0.5 g/100 ml) or 0.5%-glyphosate Roundup solution orally. The livers and serum samples of the offspring were collected on gestational day 19 (GD19), postnatal day 7 (PND7) and PND21. The results showed a significant decrease in the body weight and obvious hepatic steatosis with excessive lipid droplet formation in offspring. Moreover, the concentrations of lipids such as triglycerides (TGs), total cholesterol (T-CHO), and low-density lipoprotein cholesterols (LDL-C) increased to a significant extent in both the serum and livers. Furthermore, there were significant differences in the expression levels of the genes SREBP1C, SREBP2, Fasn, Hmgcr, Hmgcs and PPARα, which are related to lipid biosynthesis or catabolism in the liver. These results demonstrate that chronic prenatal exposure to glyphosate can result in lipid metabolism disruption in the offspring of mice, as glyphosate exerts a negative influence on the expression of lipogenesis genes.

1. Introduction

Glyphosate is the active ingredient in Roundup, which dominates the herbicide market. Since glyphosate is a highly effective weed killer, there is a growing demand for glyphosate-based herbicides, resulting in a sharp increase in the cultivation of glyphosate-tolerant products, also known as genetically modified crops. However, there is serious public concern about the safety of glyphosate, since glyphosate residue is often discovered in both animals and animal products, which are the main sources of human food. Additionally, the transgenerational inheritance capacity of glyphosate increases the risk of causing chronic toxicity. The transmission of glyphosate from females to their fetuses through pregnancy or lactation has been demonstrated in several studies. It has been discovered that glyphosate can pass through the placental barrier and be transferred through breast milk, according to its detection in the umbilical cord blood of pregnant females (Kongtip et al., 2017) and in the serum of maternally exposed offspring (Milesi et al., 2018). Maternally exposed offspring reveal many adverse effects, such as congenital defects (Antoniou et al., 2012; Krüger et al., 2014; Paganelli et al., 2010), neurotoxicity (Cattani et al., 2014; Gallegos et al., 2016; Roy et al., 2016) and endocrine disruption (de Souza et al., 2017). Therefore, the transgenerational feature of glyphosate is a major threat to the next generation.

In addition, dysbiosis has been observed in glyphosate-treated animals, appearing as decreased levels of *Firmicutes* (Yan et al., 2011), which includes probiotics that can alleviate liver disease (Velayudham et al., 2009) and hyperlipidemia (Li et al., 2014). Hepatotoxicity is a common symptom due to glyphosate exposure (Beuret et al., 2005), which suggests that glyphosate probably leads to hepatic pathological alteration through disruption of the balance of intestinal microbiota. It is believed that the biotoxicity of glyphosate is mainly due to its ability to inactivate Cytochromes P450 (CYPs) (Larsen et al., 2014) and inducing metal chelation (Krüger et al., 2013; McLaren et al., 2007). CYP enzymes inactivation can not only reduce the detoxification capacity of the liver and cause inflammation but also play an important role in controlling

☆ This paper has been recommended for acceptance by Dr. Haidong Kan.

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https://doi.org/10.1016/j.envpol.2019.07.074
0269-7491/

cholesterol metabolism (Pikuleva, 2006). Moreover, multiomics analysis has revealed that glyphosate could extremely likely cause non-alcoholic fatty liver disease (Mesnage et al., 2015; Mesnage et al., 2017).

Many studies of aquatic creatures have detected marked lipid vacuoles in the cytoplasm of hepatocytes (Albinati et al., 2007; Jiraungkoorskul et al., 2003; Langiano and Martinez, 2008; Szarek et al., 2000), which reaffirms glyphosate's threat to hepatic lipid metabolism.

There is a high possibility that mother-originated glyphosate in offspring functions in the same way as in their parents, causing dysbiosis and lipid metabolism disruption. However, research on these aspects of the progeny has received less attention, so we decided to explore the link between glyphosate exposure and hepatic lipid metabolism. Therefore, the following data were assessed: the serum biochemical indexes, histopathological observations, lipid concentrations, and mRNA gene expression levels, which are related to lipogenesis and lipid catabolism in the livers of prenatally exposed offspring.

2. Materials and methods

2.1. Animals

Ten-week-old female and male ICR mice were purchased from Nanjing Qinglongshan Experimental Animal Center (Nanjing, China). After one week of adaptation, one male and two female mice were housed in each cage from 5.00 p.m. to 8.00 a.m. daily to obtain pregnant mice. Pregnant mice were placed into separate cages once the pregnancy was confirmed by a vaginal smear the following morning. This day was defined as the first day of gestation. Animals were fed with water and feed *ad libitum*. The temperature and relative humidity in the animal house were controlled at 23 ± 2 °C and 50 ± 10%, respectively, and the animals were kept on a 12-h light/dark cycle. The animal experiments were approved by the Animal Welfare Committee of Nanjing Agricultural University (Nanjing, China) and implemented in accordance with the National Institutes of Health Guidelines for Animal Care and the Committee of Animal Research Institute.

2.2. Chemicals and treatment

Pure glyphosate (N-(phosphonomethyl)glycine) and Roundup (as the isopropylamine salt) were provided by Shanghai Ryon Biological Technology Co., Ltd. (Shanghai, China) and Sinochem Crop Protection Products Co., Ltd. (Shanghai, China), respectively. Glyphosate and Roundup were diluted with distilled water to obtain 0.5% active ingredient solutions (w/v, 5 g glyphosate/1 L solution). Then, the subjects were administered the 0.5% glyphosate or Roundup solution through the drinking water (pH was controlled at 7.2 ± 0.2).

2.3. Animal treatment and sampling

A total of 30 pregnant mice were randomly divided into three groups: CON (control, n = 10), GLP (0.5% glyphosate treated, n = 10), and RU (0.5% Roundup treated, n = 10). Half of the pregnant mice (five from each group) were exposed throughout the first 19 days of pregnancy and were sacrificed on GD19.

The other half of the pregnant mice were exposed throughout the pregnancy period and given distilled water after giving birth. Weekly body weights of the offspring were recorded, and their anogenital distances were measured separately to identify their sexes. Seven and 21 days after birth, the prenatally exposed offspring were sacrificed (preferably two females and two males per mother) for blood and tissue analysis.

The water consumption of the pregnant mice was measured, and the real exposure dose of glyphosate in both GLP and RU groups was approximately 7 ml (Table 1). The serum was extracted through centrifugation (3500 rpm, 15 min, 4 °C) and was used to assay the biochemical indexes. Parts of the livers were stored at −80 °C for lipid concentration determination and reverse transcription-polymerase chain reaction (RT-PCR). Liver samples were either fixed in a 4% paraformaldehyde solution or embedded in optimal cutting temperature compound (O.C.T. compound) provided by Sakura Finetek Japan Co., Ltd. (Tokyo, Japan) prior to frozen sectioning for the histological observation of tissue sections.

2.4. Histological preparation

Some parts of the liver tissue were fixed in 4% paraformaldehyde solution for 24 h and then dehydrated, clarified, embedded with paraffin and sectioned. Tissue sections (5 μm) were used for hematoxylin–eosin (H&E) staining.

The remaining liver tissue was embedded with O.C.T. compound and sectioned using a microtome cryostat manufactured by Thermo Fisher Scientific Instrument Co., Ltd. (Shanghai, China) for Oil Red O staining.

2.5. Serum biochemical and liver lipid concentration assays

To preliminarily diagnose the liver injury and lipid content of the organisms, the following serum biochemical indexes were determined: aspartate transaminase (AST), alanine transaminase (ALT), triglyceride (TG), total cholesterol (T-CHO), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). Additionally, liver homogenate was centrifuged to obtain the supernatant (3500 rpm, 15 min, 4 °C) to measure the TG, T-CHO, LDL-C and HDL-C content.

Both the serum biochemical indexes and hepatic lipid content were assayed with commercial reagent kits purchased from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

2.6. Analysis of gene expression

Total RNA was extracted from liver tissue with the ISOGEN 2 reagent kit (from NIPPON GENE CO., LTD.) (Tokyo, Japan) according to the manufacturer's instructions. The concentration of the obtained RNA was determined by a spectrophotometer, and the purity was measured using a NanoDrop® 8000. Then, PrimeScriptTM RT Master Mix (from Takara Bio Inc.) was used to reverse transcribe RNA to cDNA, which acted as a template for the SYBR® Premix Ex Taq™ PCR kit (from Takara Bio Inc.) for real-time PCR. The expression levels of the genes SREBP1C (Sterol Regulatory Element Binding Protein 1C), SREBP2 (Sterol Regulatory Element Binding Protein 2), Fasn (Fatty acid synthase, which catalyzes fatty acid synthesis), Scd (Stearoyl-CoA Desaturase 1), Acc (Acetyl-CoA

Table 1
Effects of chronic glyphosate exposure on the performance of pregnant mice.

Items	CON	GLP	RU	*P*
Water consumption (ml)	9.69 ± 0.76[a]	7.88 ± 0.46[b]	7.45 ± 0.34[b]	0.023
Feed intake (g)	9.00 ± 0.16	8.32 ± 0.55	9.63 ± 0.72	0.261
Body weight gain (g)	32.60 ± 3.30	35.96 ± 2.92	29.84 ± 0.70	0.281
Number of fetuses (n)	10.80 ± 1.50	14.40 ± 1.57	12.60 ± 2.11	0.376
Average birth weight (g)	1.73 ± 0.13	1.65 ± 0.08	2.03 ± 0.72	0.220

Each value represents the mean ± SEM of the group (n = 5).
Different letters indicate statistically significant differences. a, b $p < 0.05$.

Carboxylase), Hmgcr (3-hydroxy-3-methyl-glutaryl-CoA reductase), Hmgcs1 (3-hydroxy-3-methylglutaryl-CoA synthase 1), Hmgcs2 (3-hydroxy-3-methylglutaryl-CoA synthase 2) and PPARα (Peroxisome proliferator-activated receptor alpha) were determined. The relative expression levels of the above genes were normalized to β-actin expression. All primers were designed and supplied by GenScript Bio-Tech Co., Ltd. (Nanjing, China).

2.7. Data analysis

The software packages SPSS Statistics 20.0 and GraphPad Prism (GraphPad Software, San Diego, CA, USA) were utilized to analyze the data. One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed. Values are expressed as the mean ± standard error of the mean (SEM), and statistical significance was set as $p < 0.05$.

3. Results

3.1. Physical and organ development

From GD19 to PND21, the body weight in both GLP and RU groups decreased and finally saw a statistically significant reduction on PND21 ($p < 0.05$) (Fig. 1). When separated according to sex, offspring showed no significant differences in either body weight or weight gain among the three groups (Table 2).

3.2. Liver histological observation

In both GLP and RU groups, relatively elevated numbers of vacuoles exhibiting hepatic lipid droplets were observed within the hepatocytes of both female and male offspring (Fig. 2B, C, E, H, I, K, L), when compared with the CON group. Additionally, the red areas observed in the Oil Red O stained sections represent lipid substances (Fig. 3B, C, E, H, K, L). In females, there tended to be more lipid droplets in the GLP group than in the other two groups. In contrast, in males, both the GLP and RU groups showed excessive lipid deposits.

In addition, there were several clusters of monocytes in both the GLP and RU groups of PND7 females. It appears that glyphosate could cause inflammation in early-aged female mice.

3.3. Serum biochemical index

Compared with the CON group, TG levels showed a significant increase in the GLP group in both GD19 fetuses ($p < 0.01$) (Table 3) and PND21 female mice ($p < 0.05$) (Table 4). With respect to T-CHO levels, GLP mice showed a remarkable increase in both PND7 males ($p < 0.01$) and PND21 females ($p < 0.05$) compared with CON mice. LDL-C levels also increased in PND7 mice in both the GLP and RU groups ($p < 0.05$) (Table 4). The increased lipid content reflects the adverse effects of glyphosate on lipid metabolism, although this disturbed effect was not detected in every individual.

Furthermore, significantly elevated AST levels in PND7 females in the RU group ($p < 0.01$) are theoretically considered to be a result of an injured liver.

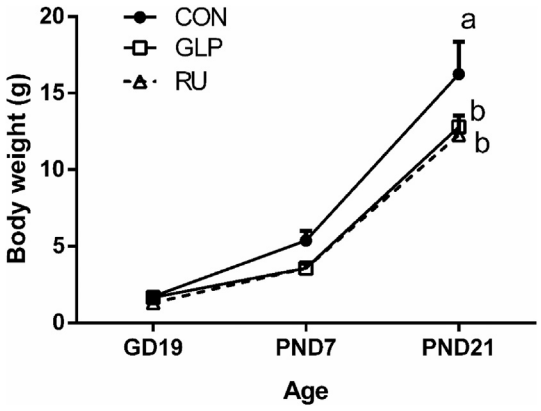

Fig. 1. Effects of chronic glyphosate exposure to pregnant mice on the body weights of offspring at the ages of GD19, PND7 and PND21 (mean ± SEM). Different letters indicate statistically significant differences, $p < 0.05$.

3.4. Lipid concentration in the liver

Compared with that in CON mice, TG levels in the RU group significantly increased in GD19 fetuses and PND7 female offspring ($p < 0.05$) (Fig. 4A and B). Moreover, T-CHO levels of both PND7 and PND21 offspring increased in the GLP or RU groups ($p < 0.05$) (Fig. 4B–E). Elevated TG and T-CHO levels in the liver can probably cause lipid deposits.

The LDL-C levels of PND21 males showed a noticeable increase in both the GLP and RU groups ($p < 0.05$) (Fig. 4E), and the HDL-C levels in PND7 males were elevated in the RU group ($p < 0.05$) (Fig. 4C). Both low-density and high-density lipoproteins can transport cholesterol in the extracellular environment. The elevated level of these proteins in serum is considered to be the result of increased cholesterol levels.

3.5. Expression levels of genes related to lipid metabolism in the liver

The relative expression levels of the genes SREBP1C, SREBP2, Fasn, Acc, Scd, Hmgcr, Hmgcs1 and Hmgcs2 in the GLP and RU groups showed a significant increase in GD19 fetuses and PND7 and PND21 offspring ($p < 0.05$) (Fig. 5). These genes are closely related to hepatic lipid production, so their elevation contributes to increased fat storage. However, this kind of increase does not match well to the trend in serum lipid content alteration. The levels of PPARα in PND7 males and PND21 females increased remarkably in both the GLP and RU groups, which is likely due to the growing demand for lipid catabolism caused by the increased lipid content.

4. Discussion

Previous studies have found that glyphosate could cause fatty liver disease at the level of transcriptome and proteome (Mesnage et al., 2015; Mesnage et al., 2017). The transgenerational potential of glyphosate between dams and their litter successfully raises concerns about the hepatotoxicity of glyphosate in the progeny. The present study was designed to study the toxic effects of chronic prenatal glyphosate exposure on lipid metabolism in the livers of offspring. The results suggested that chronic maternal exposure to glyphosate can lead to the disruption of lipid metabolism in the next generation.

In the present study, 19 or 21 gestational days was typically chosen as the exposure period, and offspring were collected on gestational day 19 and after birth on PND7 and PND21. The average water consumption in the GLP and RU groups was approximately 7 ml, which means that the pregnant mice took an average of 35 mg glyphosate per day. Thus, the real glyphosate exposure dose in fetuses was probably less than 35 mg per day. To our knowledge, the median lethal dose (LD_{50}) of glyphosate in mice is 5000 mg/kg body weight (bw), and the nonobserved adverse effect level (NOAEL) is 500 mg/kg bw. Although the true administered dose of glyphosate that the offspring received in the present study is far lower than the

Table 2
Effects of chronic glyphosate exposure to pregnant mice on the physical development of the offspring (g).

Items	Female				Male			
	CON	GLP	RU	*P*	CON	GLP	RU	*P*
PND7								
Body weight	5.72 ± 0.61	4.40 ± 0.28	4.29 ± 0.59	0.429	5.24 ± 1.00	4.80 ± 0.30	4.89 ± 0.88	0.918
Body weight gain	3.19 ± 0.80	2.74 ± 0.25	3.08 ± 0.50	0.222	3.51 ± 0.88	3.15 ± 0.29	2.87 ± 0.45	0.115
PND21								
Body weight	14.69 ± 2.44	13.63 ± 0.78	13.26 ± 1.06	0.786	15.79 ± 2.21	15.10 ± 0.85	13.86 ± 1.82	0.732
Body weight gain	12.04 ± 2.01	11.98 ± 0.76	11.24 ± 0.66	0.704	14.06 ± 2.09	13.45 ± 0.82	11.84 ± 1.33	0.243

Each value represents the mean ± SEM of the group (n = 7–10).

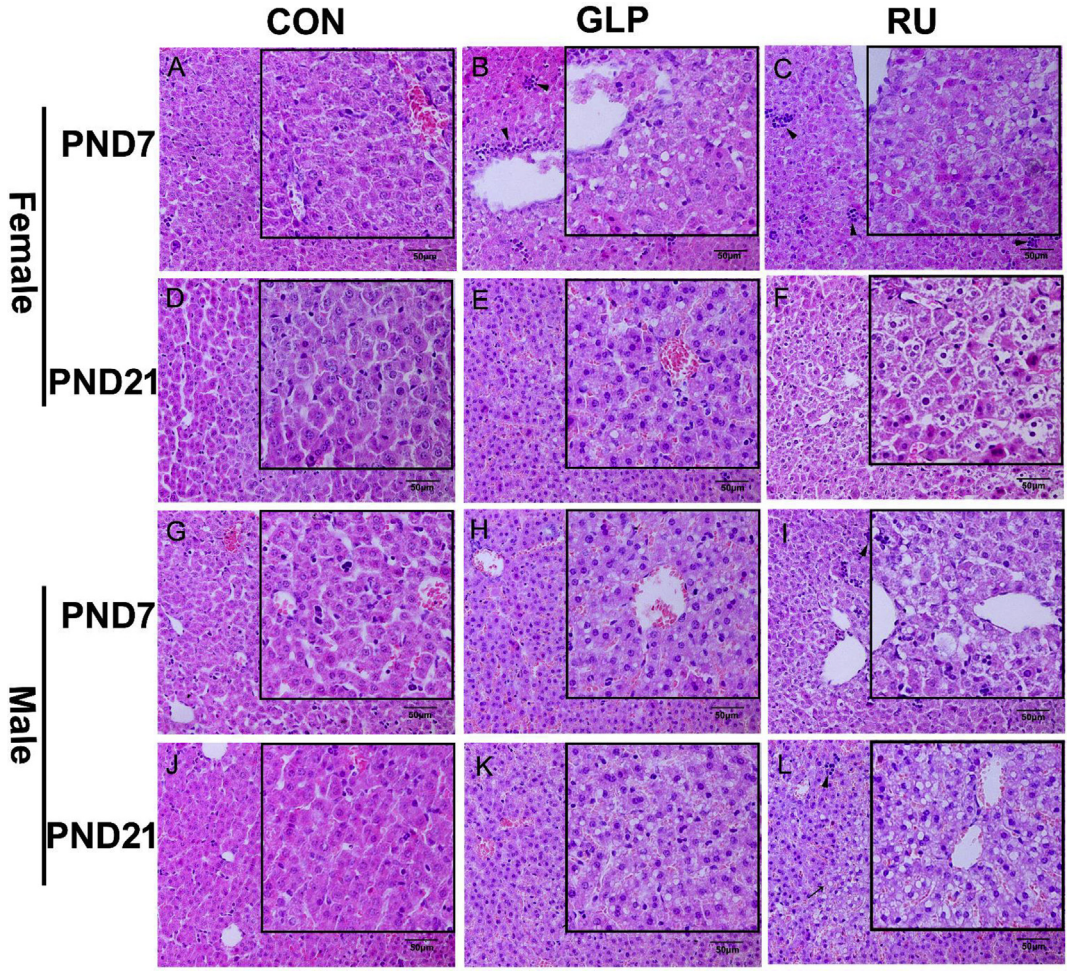

Fig. 2. Photomicrographs of H&E-stained liver sections of chronic prenatal glyphosate-treated offspring. Enlarged portions show the lipid vacuoles within the cells where the lipids have been cleared by the stain. (B), (C), (D), (H), (I) and (J) show a slightly increased number of lipid vacuoles compared with (A), (D) and (G). (K) and (L) show an obvious increase in the number of vacuoles when compared with (J). The arrowheads in (B), (C), (I) and (L) show clusters of monocytes representing inflammatory infiltration. (H&E × 400).

NOAEL, several phenomena still revealed excessive lipid production. Additionally, according to the classification criteria of the EU and the OECD Globally Harmonized System, glyphosate is not listed as an acute oral toxin based on 145 studies. In chronic exposure research, the "overall NOAEL" was assessed to be 100 mg/kg bw per day based on human beings, which is also higher than the present study's dose. Taken together, a dose lower than overall NOAEL can still cause hepatic lipid metabolism disruption, although the consistency is not perfect among the tested parameters and groups in this study.

Physical development is used as an important indicator of the health state of organisms. The body weights of the prenatal glyphosate-treated offspring showed a reduction compared to that of the CON group, which is in accordance with the previous results (Milesi et al., 2018). The adverse effects on the physical development of offspring could be explained by the decreased body weight of the pregnant mice (Ren et al., 2018), while the feed intake of the pregnant mice showed no statistically significant changes. Therefore, the reduced body weight might be the result of energy consumption for the purpose of detoxification instead of for physical development. (Peixoto, 2005).

The liver is the most important detoxification organ that works to metabolize xenobiotics and can reflect the risk of the xenobiotics to the body system to some extent. To observe the liver state of the

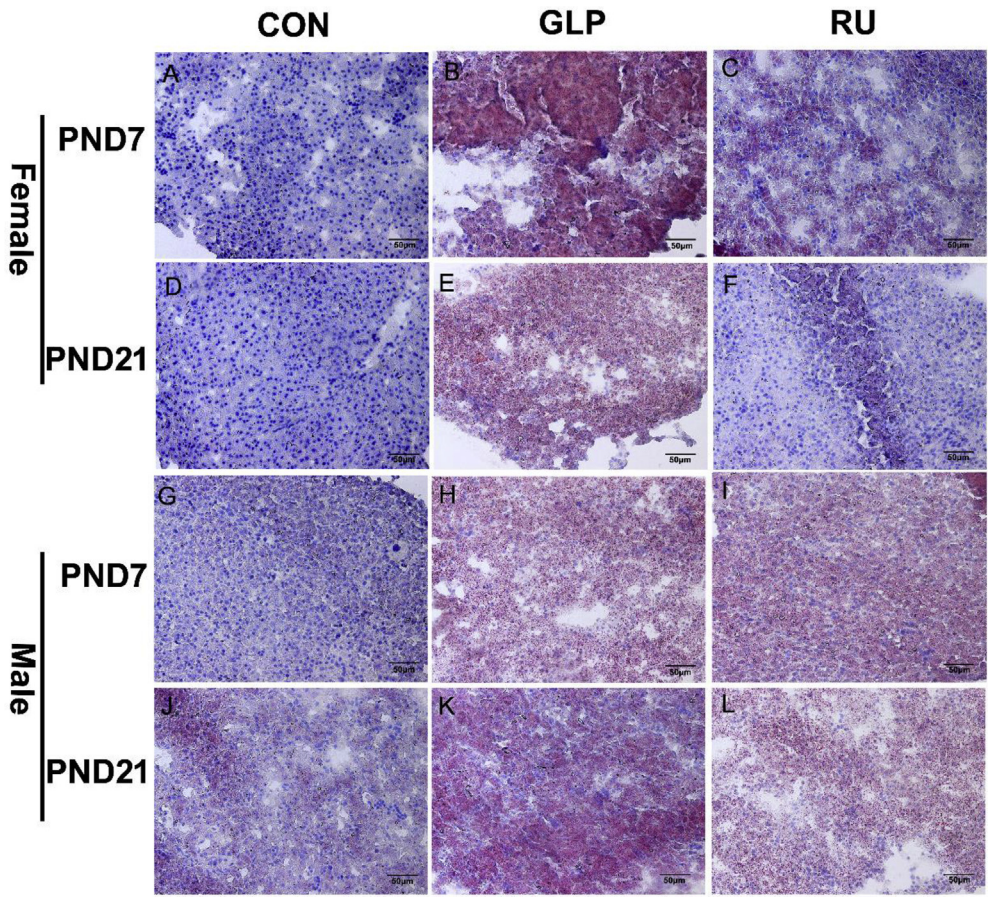

Fig. 3. Photomicrographs of liver sections stained with Oil Red O of chronic prenatal glyphosate-treated offspring. The red areas stained by Oil Red O represent the lipid deposits. (A) and (D) show the normal liver tissue of the female offspring. (B) and (E) show substantial lipid deposits from the GLP group, while there are slight lipid deposits in (C) and (F) from the RU group. (G) and (J) show the normal liver tissue with few lipid substances in the CON group. (H), (I) and (K) show more lipid deposits than do (G) and (J). (Oil-red O × 400). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Effects of chronic glyphosate exposure to pregnant mice on the blood biochemical indexes in fetuses.

Items	CON	GLP	RU	*P*
TG (mmol/L)	0.15 ± 0.10[b]	0.79 ± 0.21[a]	0.30 ± 0.10[b]	0.002
T-CHO(mmol/L)	2.15 ± 0.49	2.56 ± 0.52	1.22 ± 0.06	0.165
LDL-C (mmol/L)	1.03 ± 0.09	1.08 ± 0.04	0.91 ± 0.05	0.185
HDL-C (mmol/L)	0.14 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.433
AST (IU/L)	67.88 ± 14.17	103.20 ± 10.30	76.21 ± 10.55	0.134
ALT (IU/L)	20.71 ± 1.02	47.54 ± 10.20	32.09 ± 2.35	0.091

Each value represents the mean ± SEM of the group (n = 20).
Different letters indicate statistically significant differences. a, b $p < 0.05$.

prenatally exposed offspring at the level of histology, H&E and Oil Red O staining were implemented in the present study. A relative increase in the number of fat vacuoles can be seen in several photomicrographs in the GLP or RU groups compared with the CON group, which is probably due to the elevated TG and T-CHO levels determined in both the serum and liver. Lipid production and storage in organisms are mainly controlled by liver lipogenesis and catabolism. We learned from the related gene expression level data that the genes related to these two biological processes showed increased expression levels in the GLP and RU groups compared with the CON group. SREBPs are essential activators for the synthesis of fatty acids and cholesterol (Horton et al., 2002) once combined with a lipid synthesis promoter. SREBP1c, one of the isoforms of SREBP1, regulates the process of lipogenesis that increases from the early age of GD19, while SREBP2 is responsible for cholesterol biosynthesis. Furthermore, the increasing expression levels of downstream genes, such as Fasn, Scd, Acc, Hmgcr and Hmgcrs, contribute to higher fatty acid and cholesterol biosynthesis, which could result in inevitable hepatic fat storage. For lipid catabolism, PPARα plays a crucial role in mitochondrial β-oxidation and peroxisomal fatty acid oxidation, both of which act as important biological reactions in the degradation of liver lipids (Kersten, 2014). Increased PPARα levels in the prenatally exposed offspring might be due to the growing demand for lipid catabolism caused by the rising TG or T-CHO levels. However, the alterations in gene expression are not completely consistent with TG or T-CHO synthesis proteins level or fat storage in the liver sections. This finding might be due to the limited number of subjects, and we will try to explore more detailed reasons and explain these reasons in our future studies. Taken together, we believe that glyphosate could affect lipid production by disturbing lipid metabolism-related gene expression.

Previous research had detected a time-dependent enhancement in triglyceride and cholesterol levels as glyphosate-treated subjects aged (El-Shenawy, 2009; Mesnage et al., 2017), whereas there were contradictory results shown in bullfrog tadpoles (Dornelles and Oliveira, 2016). To explore more mechanistic insights, a lipidomic profiling experiment was conducted, and 62 distinct lipid species were identified as altered, including triglycerides and cholesteryl esters (Ford et al., 2017). Additionally, glyphosate is found to be

Table 4
Effects of chronic glyphosate exposure to pregnant mice on the blood biochemical indexes of PND7 and PND21 offspring.

Items	Female				Male			
	CON	GLP	RU	*P*	CON	GLP	RU	*P*
PND7								
TG (mmol/L)	1.46 ± 0.07	1.79 ± 0.26	1.60 ± 0.08	0.324	1.11 ± 0.14	1.45 ± 0.14	1.45 ± 0.20	0.240
T-CHO (mmol/L)	1.46 ± 0.07	1.60 ± 0.27	1.42 ± 0.19	0.792	2.01 ± 0.23^{b}	2.92 ± 0.19^{a}	1.97 ± 0.11^{b}	0.007
LDL-C (mmol/L)	1.30 ± 0.18^{b}	1.73 ± 0.07^{a}	1.42 ± 0.07^{ab}	0.047	0.58 ± 0.07^{c}	0.99 ± 0.12^{b}	1.75 ± 0.11^{a}	0.000
HDL-C (mmol/L)	1.12 ± 0.11	1.18 ± 0.09	0.86 ± 0.04	0.074	0.86 ± 0.12	0.88 ± 0.04	1.13 ± 0.11	0.189
AST (IU/L)	28.03 ± 6.14^{b}	29.09 ± 6.22^{b}	63.84 ± 8.03^{a}	0.008	65.17 ± 7.65	72.54 ± 10.41	81.71 ± 10.52	0.503
ALT (IU/L)	30.93 ± 3.77	40.51 ± 4.86	39.89 ± 4.08	0.245	29.22 ± 2.39	32.01 ± 2.89	39.27 ± 2.29	0.058
PND21								
TG (mmol/L)	1.30 ± 0.18^{b}	2.41 ± 0.37^{a}	1.36 ± 0.15^{ab}	0.022	1.01 ± 0.11	1.10 ± 0.37	1.19 ± 0.38	0.741
T-CHO (mmol/L)	1.30 ± 0.18^{b}	2.06 ± 0.16^{a}	1.37 ± 0.15^{b}	0.028	0.92 ± 0.07	1.01 ± 0.06	1.05 ± 0.21	0.778
LDL-C (mmol/L)	2.26 ± 0.26	2.23 ± 0.12	2.65 ± 0.18	0.293	1.24 ± 0.19	1.08 ± 0.27	0.64 ± 0.13	0.115
HDL-C (mmol/L)	1.41 ± 0.28	1.65 ± 0.31	1.29 ± 0.10	0.570	1.56 ± 0.31	1.49 ± 0.17	1.22 ± 0.29	0.713
AST (IU/L)	41.14 ± 12.38	70.64 ± 9.87	50.04 ± 12.01	0.286	39.37 ± 9.28	54.06 ± 8.10	22.56 ± 8.90	0.091
ALT (IU/L)	19.02 ± 0.43	22.50 ± 4.61	19.48 ± 3.20	0.722	14.27 ± 4.06	16.01 ± 2.34	16.34 ± 3.04	0.890

Each value represents the mean ± SEM of the group (n = 7–10).
Different letters indicate statistically significant differences. a, b $p < 0.05$.

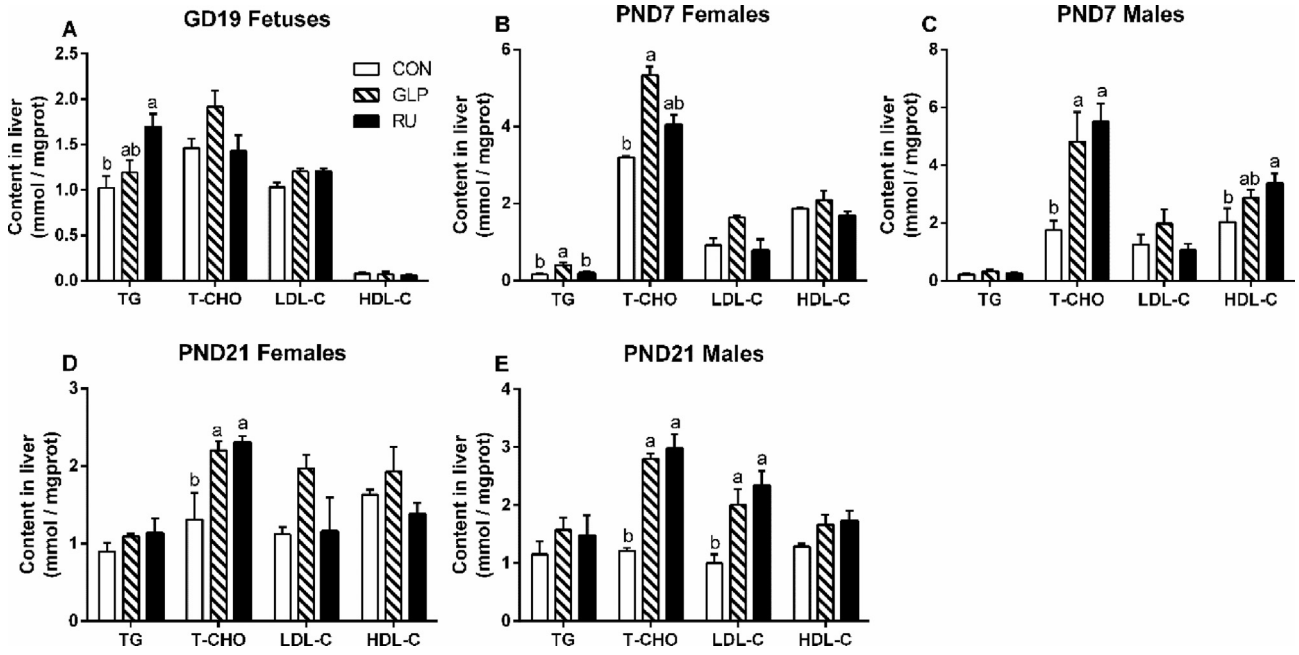

Fig. 4. Effects of chronic prenatal glyphosate exposure on the lipid content in the livers of the offspring (mean ± SEM). (A) shows the TG, T-CHO, LDL-C and HDL-C content in livers of GD19 fetuses, and (B), (C), (D) and (E) show these parameters in PND7 and PND21 females and males, respectively. Different letters indicate statistically significant differences. a, b $p < 0.05$.

metabolized into glyoxylate (Ford et al., 2017), which inhibits fatty acid oxidation enzymes, all of which are considered to be tightly associated with hepatic lipid dysregulation. Together with the reported impairment of mitochondrial oxidative phosphorylation and enzymatic activity (Larsen et al., 2014; Peixoto, 2005) after glyphosate treatment, the efficiency of the respiratory rate and tricarboxylic acid cycle will inevitably decline, which could further induce the diversion of redundant fatty acids into other lipid metabolism pathways (Klipsic et al., 2015; Lee et al., 2016).

There have been many studies on glyphosate-induced chromosome and DNA aberrations spanning more than 20 years (Lioi et al., 1998; Manas et al., 2009; Monroy et al., 2005). A great variety of species as well as different doses of glyphosate were used in these studies (Cavalcante et al., 2008; Kaya et al., 2000; Poletta et al., 2008). The reactive oxygen species and oxidative injury caused by glyphosate or its metabolites are commonly thought to be the reason for the genotoxicity (Cadet et al., 2003; Cavalcante et al., 2008; Cavas and Konen, 2007). In the present study, glyphosate's genotoxicity was highly likely reflected in the disruption of lipogenesis.

Glyphosate also caused obvious liver damage that appeared in the hematological parameters and histopathological alterations. The increased serum ALT and AST levels in prenatally exposed offspring (Abarikwu et al., 2015; Cavusoglu et al., 2011; Jasper et al., 2012) demonstrated severe liver damage, similar to a reported study (El-Shenawy, 2009). The leakage of liver enzymes is a remarkable indicator of hepatic injury owing to xenobiotics (El-Demerdash et al., 2001; El-Sakka et al., 2002). Additionally, the discovery of clusters of monocytes suggested the presence of inflammatory infiltration and immune responses (Caglar and Kolankaya, 2008), but it was not widely observed in all treatment groups. Apart from the liver lesions presented in our study, leukocyte infiltration, necrosis, blood congestion, hydropic degeneration and sinusoid dilation, which could enhance the risk of

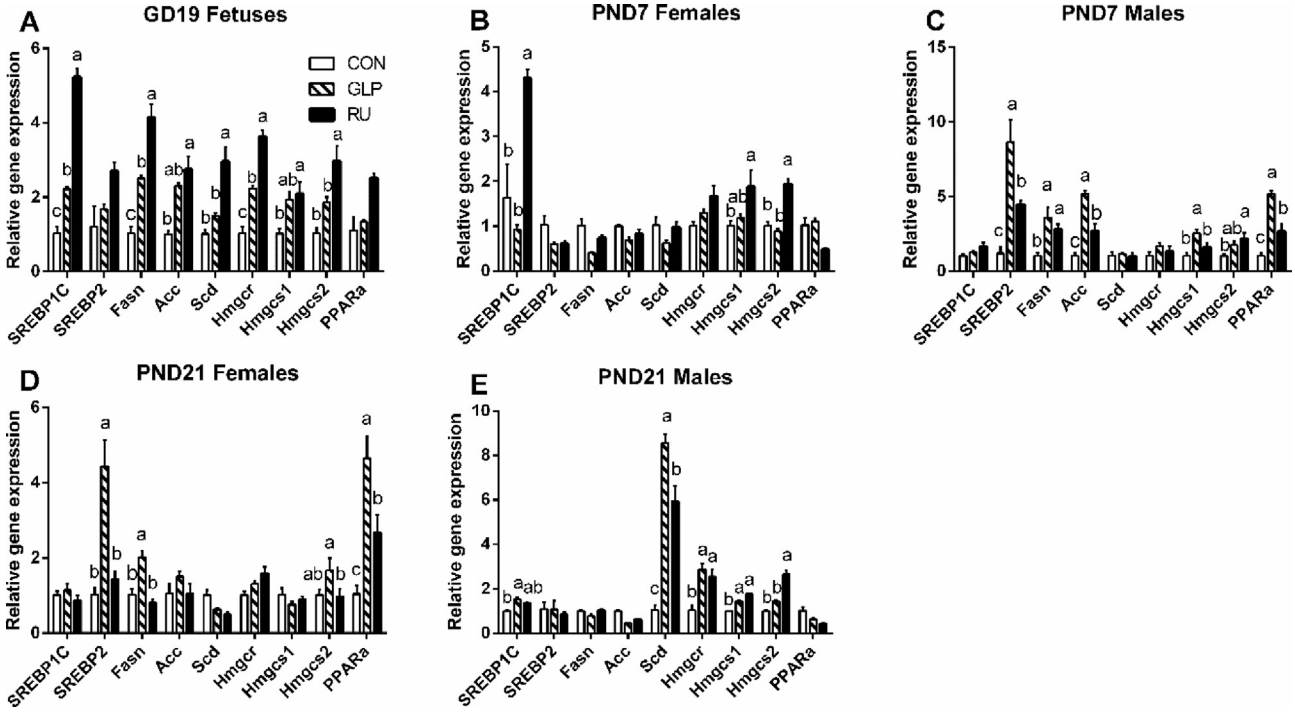

Fig. 5. Effects of chronic prenatal glyphosate exposure on relative mRNA expression levels in the livers of the offspring (mean ± SEM). The relative expression levels of the genes SREBP1C, SREBP2, Fasn, Acc, Scd, Hmgcr, Hmgcs1, Hmgcs2 and PPARα genes in the livers of (A) fetuses, (B) PND7 female offspring, (C) PND7 male offspring, (D) PND21 female offspring, and (E) PND21 male offspring are shown. Different letters indicate statistically significant differences. a, b $p < 0.05$.

glyphosate-induced steatosis progression into fatty liver disease, has been observed in hepatocytes in other experiments (Hued et al., 2012; Meshkini et al., 2018). Therefore, it is probable that glyphosate-induced lipid metabolism disruption could progress into steatosis if no recovery or interference therapy was performed.

Although significant differences were observed between some groups, the changes in the histopathological alterations, blood biochemical indexes and expression levels of lipid metabolism-related genes among the groups showed imperfect consistency. This result is highly likely caused by the limited quantity and individual variation of the subjects. Additionally, there are other factors that could have affected the results, such as sex, age and glyphosate source. Considering that the main aim of the present study was to explore the hepatotoxicity of glyphosate exposure, especially during pregnancy, on the lipid metabolism of offspring, these other factors will be future projects and will be studied in future research.

5. Conclusion

Chronic prenatal glyphosate exposure can probably cause lipid metabolism disruption in offspring, accompanied by an elevated lipid content in both serum and liver tissue. These alterations in hepatic lipid metabolism might result from rising lipogenesis in hepatocytes through increasing related gene expression.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 31772648).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.07.074.

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