Letter to the Editor

Effect of Maternal DEHP Exposure on Lipid Metabolism in Adult Male Rats and the Antagonistic Effect of Genistein*

ZHANG Yun Bo, LI Jiang Li, TIAN Jie, and NA Xiao Lin#

Lipid metabolism refers to the biochemical processes involved in synthesising, storing, utilising, and breaking down lipids in living organisms. Lipids are essential for various physiological functions, including energy storage, insulation, protection of organs, and the formation of cell membranes. Aberrations in lipid metabolism can lead to a number of health issues, such as atherosclerosis, obesity, and type 2 diabetes, etc.[1]. Environmental factors, genetics, and lifestyle factors are some of the factors that can contribute to the development of dyslipidemia. Currently, there is a growing academic interest in the impact of environmental factors.

Di (2-ethylhexyl) phthalate (DEHP), a common plasticiser with an annual global production of over 8 million tons, is used in a variety of consumer products, which consist of medical devices, food packaging, and children's toys. DEHP is recognised as an endocrine-disrupting chemical (EDC) that can mimic or interfere with the action of natural hormones in the body, resulting in harmful health effects, particularly developmental and reproductive toxicity in both genders. Both animal experiments and population surveys have revealed that exposure to DEHP may cause disruptions in lipid metabolism. Nevertheless, the literature on the effects of maternal exposure to DEHP on the lipid metabolism of offspring is still scarce. In a study conducted by Xu et al., it was observed that utero exposure to DEHP altered the lipid metabolome in the foetal brain with the dose of 1,500 mg/kg from gestational day (GD) 0 to GD 19[2]. In addition, a population survey showed that the increasing incidence of childhood obesity and diabetes in adolescents is linked to the influence of DEHP on lipid homeostasis in children[3]. However, the results were inconsistent.

Soybeans and soy-based foods contain genistein (GEN), a phytoestrogen, and isoflavone. Additionally, this compound has been found in a number of processed foods and other dietary sources. GEN's chemical structure allows it to interact with estrogen receptors, which has led to research analysing its effects on menopausal symptoms, osteoporosis, and cardiovascular disease. Because of its potential health benefits—such as its anti-inflammatory, anti-cancer, and antioxidant qualities—even in the maternal diet, it has garnered a lot of attention.

Therefore, the current study primarily aims to explore the potential effect of maternal DEHP exposure on lipid metabolism and the ameliorating effect of GEN in male offspring. Additionally, the study intends to elucidate the underlying mechanisms of these effects using transcriptomic analyses.

As shown in Figure 1, the overall process of the present study is as follows: female pregnant rats were randomly assigned to three groups: the CON group, the DEHP group (1,200 mg/kg·bw DEHP), and the DG group (1,200 mg/kg·bw DEHP + 900 mg/kg·diet GEN). The exposure dosage for this experiment was based on relevant literature and previous dosages employed by our research group[2,4]. In order to assess the toxic effects of maternal DEHP exposure on the offspring, lipid profiles, and oxidative stress levels in the serum were evaluated in the offspring upon reaching adulthood. The dietary consumption of male offspring was evaluated during their adult years and showed no statistical differences. Transcriptomic sequencing was utilised to delve into the underlying mechanisms. As illustrated in Figure 1, the findings revealed that maternal DEHP exposure in rats resulted in reduced HDL-C level in their male offspring at adult age, accompanied by decreased catalase (CAT) levels, while there were no significant changes in TC, TG, and LDL-C levels (data not shown). GEN, an endocrine disruptor modulator, did,

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Department of Environmental Hygiene, Public Health College, Harbin Medical University, Harbin 150081, Heilongjiang, China

however, partially mitigate the damage caused by DEHP. HDL-C is a type of cholesterol that is carried in the blood by high-density lipoprotein particles. HDL-C helps remove excess cholesterol from arteries, which in turn prevents atherosclerosis and reduces cardiovascular risk. Low levels of HDL-C are associated with an increased risk of atherosclerosis, heart attacks, as well as strokes. The results of a previous study showed a strong negative correlation between the levels of DEHP in Wistar rats given DEHP at 0, 5, 50, and 500 mg/kg per day for eight weeks and the plasma HDL-C level[5]. A cross-sectional study demonstrated that DEHP metabolites showed independent negative correlations with HDL-C in the National Health and Nutrition Examination Surveys (NHANES) database[6]. CAT plays a crucial role in protecting cells from damage caused by reactive oxygen species (ROS)[7]. A drop in CAT levels can disrupt the delicate balance between the production and elimination of ROS, which can result in cellular damage and an increased risk of various health problems, including those related to lipid metabolism. According to the current study's findings, maternal exposure to DEHP may trigger disruptions in lipid metabolism through inducing oxidative stress by destroying the integrity of cell membranes and causing lipid peroxidation within cells. It was unexpected to learn that GEN might somewhat counteract the lowering effect of DEHP exposure on HDL-C. These findings suggested that GEN may hold promise as a potential therapeutic agent for lipid metabolism-related metabolic disorders.

Transcriptomic sequencing results showed 162 differentially expressed genes (DEGs) between the DEHP and CON groups, 32 genes between the DEHP

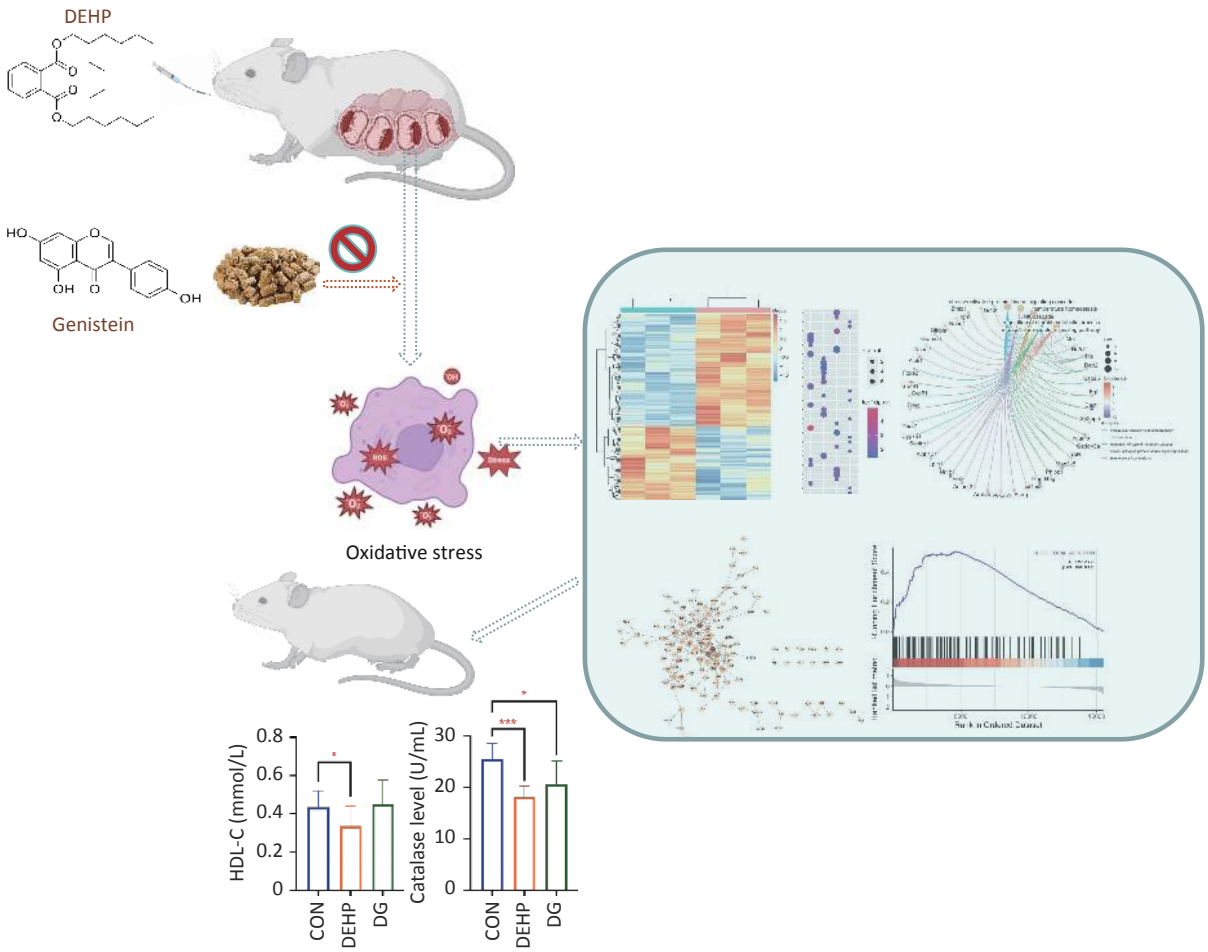

Figure 1. Experimental design for maternal DEHP exposure on adult male offspring. CON: Control group; DEHP: 1,200 mg/kg·bw DEHP; DG: 1,200 mg/kg·bw DEHP + 900 mg/kg·diet GEN. *** $P < 0.001$ and * $P < 0.05$ compared with CON group. GEN, genistein; DEHP, Di (2-ethylhexyl) phthalate; HDL-C: High-density lipoprotein cholesterol; CAT: Catalase.

and DG groups, and 42 genes between the DG and CON groups. Among the DEGs, 13 genes were found in both DEHP *vs.* CON and DG *vs.* DEHP, 4 genes were found in both DG *vs.* DEHP and DG *vs.* CON, while 6 genes were found in both DEHP *vs.* CON and DG *vs.* CON. In addition, RNA-seq and Real-time PCR demonstrated a largely consistent direction and magnitude of gene alterations by detecting 6 genes. For the DEHP *vs.* CON, GO enrichment analysis revealed several pathways, including response to stress, phosphorus metabolic process, positive regulation of the apoptotic process, lipid biosynthetic process and cellular response to external stimulus (Figure 2A). All the pathways make great contributions to the toxicity of maternal DEHP exposure. Furthermore, the five most prominent pathways were individually enriched to display the genes associated with each pathway (Figure 2B).

These pathways shed light on the important role of oxidative stress induced by DEHP exposure in lipid metabolism, which is in line with the changes observed in blood indicators. Phosphorus is an essential element for maintaining normal cellular function and is involved in various metabolic processes[8], which involve ATP formation, intracellular signalling pathway regulation, cell membrane formation, bone mineralisation, and DNA/RNA synthesis. The phosphorus metabolic pathway is involved in ATP-dependent energy transactions, phosphorylation events in signal transduction, and the formation of phospholipids necessary for membrane structure and function. These processes all contribute to the synthesis, breakdown, and regulation of lipids in lipid metabolism. The present study found that maternal DEHP exposure could disturb the phosphorus metabolic process, eventually leading to dysregulation of lipid metabolism. Meanwhile, GEN could alleviate the toxicity through glycerophospholipid metabolism as well as

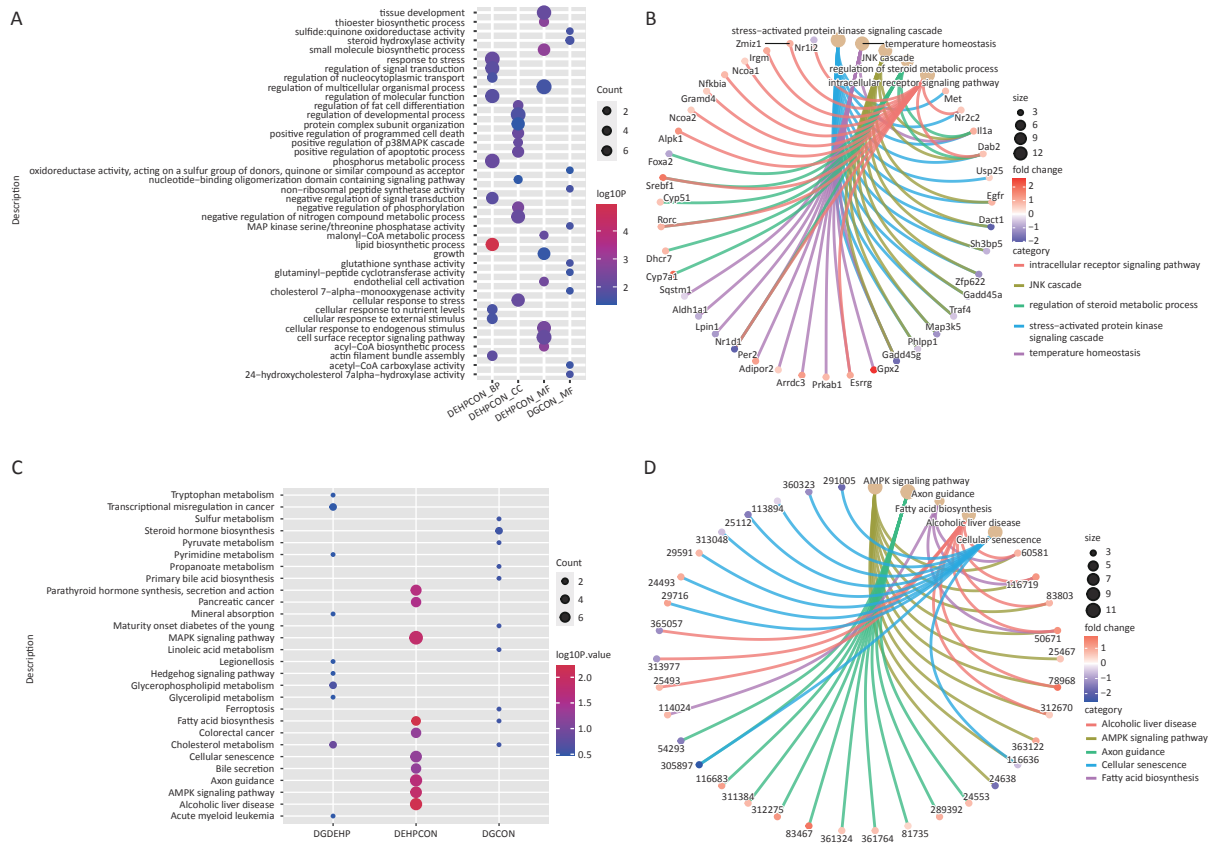

Figure 2. GO and KEGG enrichment analysis with differential expressed genes. (A) GO enrichment analysis in DEHP *vs.* CON group; (B) top five GO enrichment pathways displayed the involved genes. (C) KEGG enrichment analysis in DEHP *vs.* CON group; (D) Top five KEGG enrichment pathways displayed the involved genes. BP: Biological processes; MF: Molecular function; CC: Cellular component; CON: Control group; DEHP: 1,200 mg/kg·bw DEHP; DG: 1,200 mg/kg·bw DEHP + 900 mg/kg·diet GEN.

cholesterol metabolism pathways. KEGG enrichment analysis pointed out that parathyroid hormone synthesis, secretion and action, MAPK signalling pathway, AMPK signalling pathway, and fatty acid biosynthesis pathways were involved in the toxic effects of DEHP exposure (Figure 2C). Moreover, the five most prominent pathways were individually enriched to display the genes involved within each pathway (Figure 2D). Meanwhile, glycerophospholipid metabolism and cholesterol metabolism pathways were enriched in the group of DG *vs.* DEHP.

Protein-Protein Interaction (PPI) was utilised for investigating the network interaction relationships between DEHP exposure and CON groups. As illustrated in Figure 3, there exist close network interaction relationships among numerous genes, with the genes Fatty Acid Synthase (Fasn), Srebf1 and Egfr having the highest number of interaction connections, with degrees of 23, 21, and 20, respectively. Fasn was characterised as a target gene in the toxicity of DEHP exposure. An essential component of the synthesis of long-chain fatty acids is the enzyme fasn. It catalyses a series of reactions that form saturated fatty acids, which are essential components of various lipid molecules, including triglycerides and phospholipids[9]. Furthermore, Fasn can protect cells from ROS-induced damage by affecting the lipid biosynthetic process[10]. In the current investigation, we discovered that the fasn is the major gene regulating low HDL-C levels in adult male offspring following maternal exposure to DEHP. Fasn was also not included in the DEGs of DEHP and DG groups. As a result, the Fasn gene may be a significant specific target for the toxic effects of DEHP exposure.

The relationships between alterations in gene expression under various circumstances and biological pathways, functions, and physiological processes were found using GSEA enrichment analysis. As shown in Supplementary Figure S1, available in www.besjournal.com, exposure to DEHP might have the potential to influence Focal adhesion, Axon guidance, Gamma R mediated phagocytosis, Toll-like receptor signalling, Phosphatidylinositol signalling pathways, et al. which indicated that exposure to DEHP may potentially

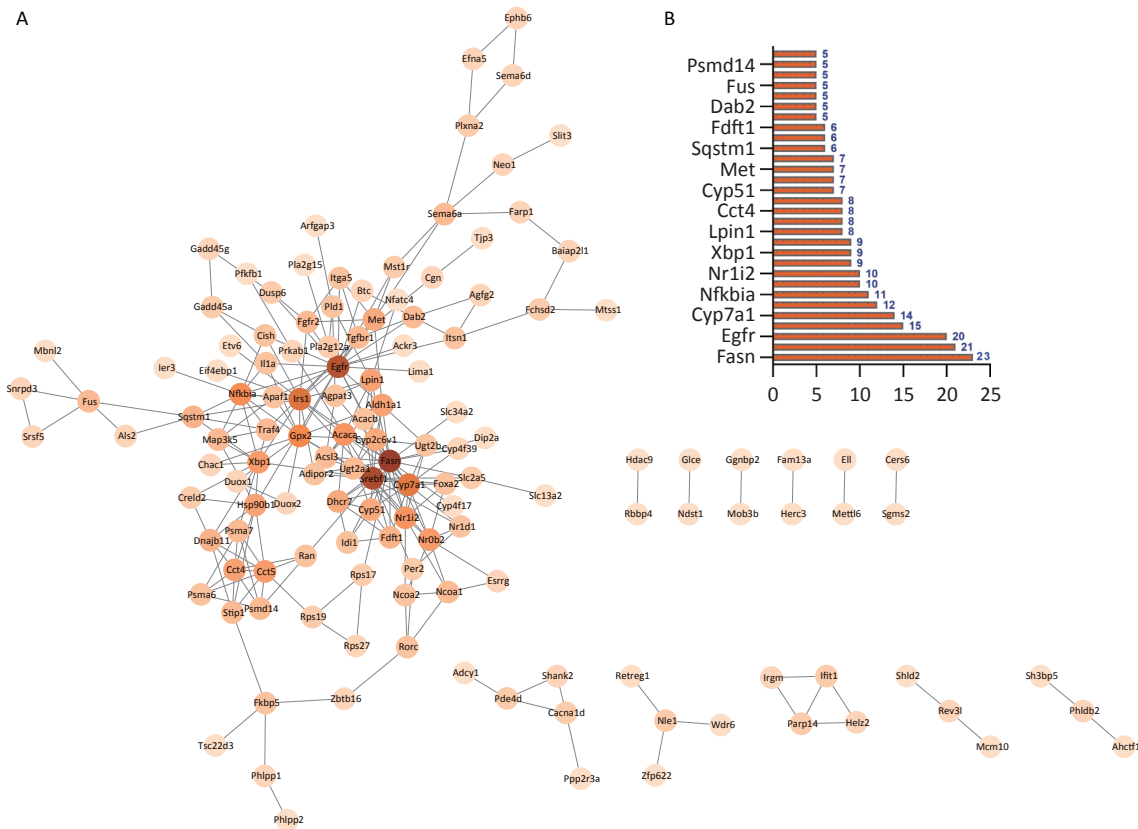

Figure 3. PPI network analysis with differential expressed genes in DEHP *vs.* CON group. A: each color in node represents the degree of its connections, with darker colors indicating higher degrees; B: the details degree enriched in the PPI analysis. PPI, Protein-Protein Interaction.

affect cellular signal transduction, immune response, cell adhesion, and neural system development. However, more thorough experimental research is required to delve deeper into the specific implications. In contrast, GEN exhibited a significant down-regulating of these effects.

In conclusion, the findings of the present study demonstrated that maternal DEHP exposure could lead to dysregulation in lipid metabolism and trigger oxidative stress in adult male offspring. Nevertheless, GEN was able to partially alleviate the damage caused by DEHP exposure, indicating that GEN may have a protective impact against the adverse effects induced by maternal DEHP exposure. Further research is required to confirm these findings.

Conflicts of Interest No potential conflicts of interest were disclosed.

[#]Correspondence should be addressed to NA Xiao Lin, PhD, Tel: 86-451-8750-2839; E-mail: naxiaolin1495@sohu.com

Biographical note of the first author: ZHANG Yun Bo, female, born in 1982, PhD, majoring in environmental health.

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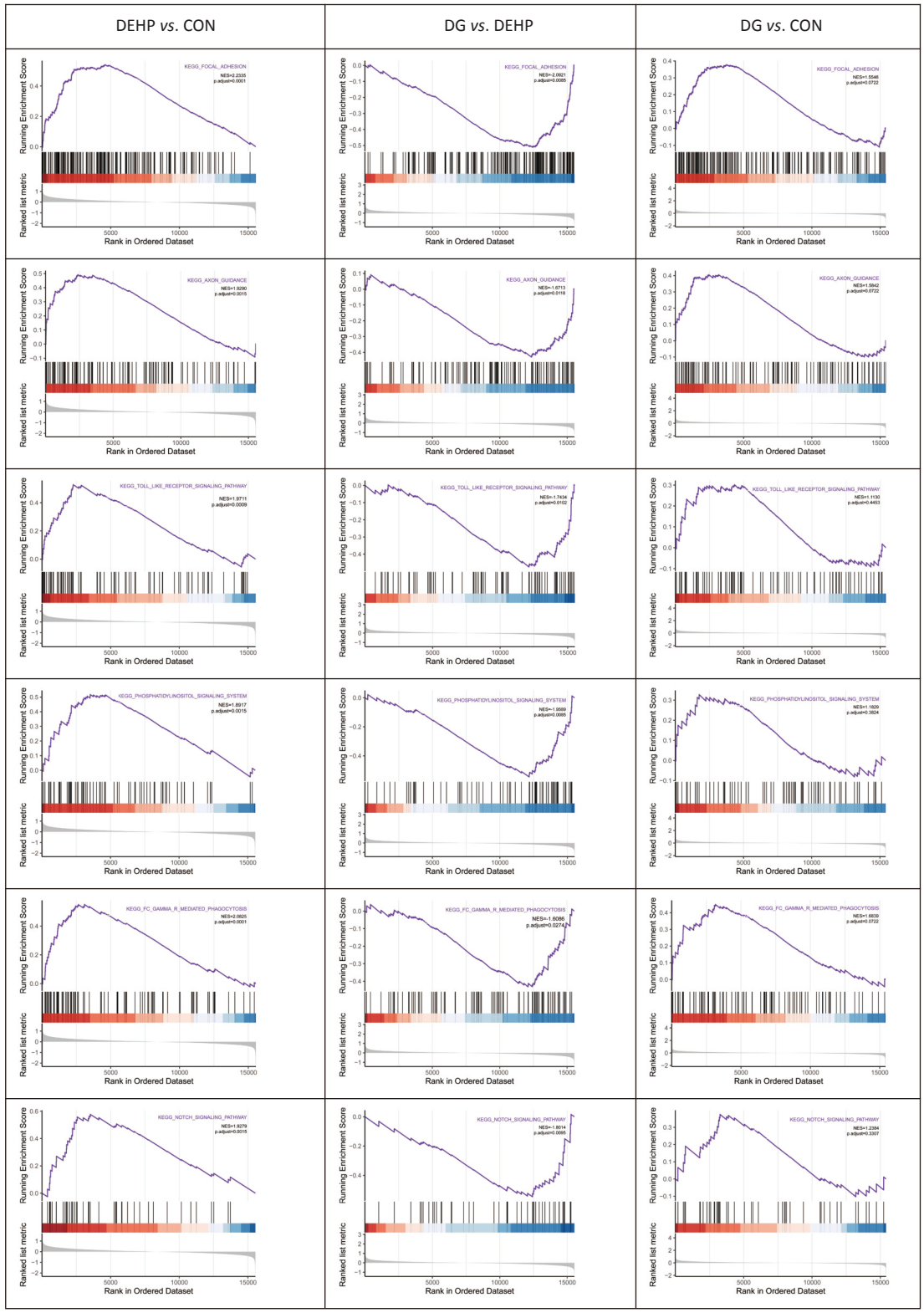
REFERENCES

1. Petrenko V, Sinturel F, Riezman H, et al. Lipid metabolism around the body clocks. Prog Lipid Res, 2023; 91, 101235.
2. Xu Y, Agrawal S, Cook TJ, et al. Di-(2-ethylhexyl)-phthalate affects lipid profiling in fetal rat brain upon maternal exposure. Arch Toxicol, 2007; 81, 57−62.
3. Zeng F, Zhang LD, Deng F, et al. Early-life exposure to di (2-ethyl-hexyl) phthalate: Role in children with endocrine disorders. Front Cell Dev Biol, 2023; 11, 1115229.
4. Dong XW, Dong J, Zhao Y, et al. Effects of long-term in vivo exposure to di-2-ethylhexylphthalate on thyroid hormones and the TSH/TSHR signaling pathways in Wistar Rats. Int J Environ Res Public Health, 2017; 14, 44.
5. Ding S, Qi W, Xu Q, et al. Relationships between di-(2-ethylhexyl) phthalate exposure and lipid metabolism in adolescents: Human data and experimental rat model analyses. Environ Pollut, 2021; 286, 117570.
6. Zhu X, Cheang I, Chen ZQ, et al. Associations of urinary di(2-ethylhexyl) phthalate metabolites with lipid profiles among US general adult population. Heliyon, 2023; 9, e20343.
7. Baker A, Lin CC, Lett C, et al. Catalase: A critical node in the regulation of cell fate. Free Radic Biol Med, 2023; 199, 56−66.
8. Wong SK. A review of current evidence on the relationship between phosphate metabolism and metabolic syndrome. Nutrients, 2022; 14, 4525.
9. Singh S, Karthikeyan C, Moorthy NSHN. Fatty Acid Synthase (FASN): a patent review since 2016-present. Recent Pat Anticancer Drug Discov, 2024; 19, 37−56.
10. Li Y, Yang WJ, Zheng YY, et al. Targeting fatty acid synthase modulates sensitivity of hepatocellular carcinoma to sorafenib via ferroptosis. J Exp Clin Cancer Res, 2023; 42, 6.



Supplementary Figure S1. GSEA analysis in DEHP *vs*. CON, DG *vs*. DEHP and DG *vs*. CON groups. CON: Control group; DEHP: 1,200 mg/kg·bw DEHP; DG: 1,200 mg/kg·bw DEHP + 900 mg/kg·diet GEN.