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Research Letter

# High Levels of Vitamin C Intake Modify Effects of Phthalates on Metabolic Dysfunction-associated Steatotic Liver Disease: A Nationally Representative Study

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a growing cause of chronic liver diseases worldwide, with a global prevalence of 38%.[1] Patients with MASLD are at risk of developing liver-related complications, such as cirrhosis, hepatocellular carcinoma, and extra-hepatic adverse events.[1] In recent years, endocrine-disrupting chemicals (EDCs) have been linked to the development and progression of MASLD.[2] Given the role of oxidative stress and inflammation in EDC-induced effects on MASLD, antioxidant agents may have the potential to counteract the adverse impact of EDC exposure. In this study, we aimed to confirm the association between MASLD and exposure to phthalates, one of the most commonly used EDCs in industries and everyday household products, found in the air, solid materials, and especially in food.[3] We also investigated the potential of antioxidants to mitigate the risk of MASLD in a representative sample of the U.S. population.

Using seven cycles of the National Health and Nutrition Examination Surveys (NHANES) from 2005 to 2018, we included adult participants with available data on urinary phthalate metabolites, antioxidant intake, MASLD diagnosis, other clinically relevant covariates, and reliable energy intake records (600–5,000 kcal/day). MASLD was diagnosed based on hepatic steatosis (defined as a U.S. fatty liver index above 30) and the presence of at least one of the following cardiometabolic risk factors: (1) body mass index (BMI) ≥25 kg/m$^2$ or waist circumference over 94/80 cm (males/females); (2) fasting serum glucose ≥5.6 mmol/L (100 mg/dL), hemoglobin A1c ≥5.7% (39 mmol/L), type 2 diabetes, or current treatment for type 2 diabetes; (3) blood pressure ≥130/85 mmHg or use of antihypertensive medications; (4) plasma triglycerides ≥1.70 mmol/L (150 mg/dL) or use of lipid-lowering medications; (5) plasma high-density lipoprotein cholesterol ≤1.0 mmol/L (40 mg/dL, males) or ≤1.3 mmol/L (50 mg/dL, females) or use of lipid-lowering medications.[4] Participants with other causes of hepatic steatosis (e.g., alcohol use ≥140/210 g/week (females/males) or hepatitis B/C infection) were excluded. We analyzed 11 phthalate metabolites, including MEHP, MEHHP, MEOHP, MECPP, MiNP, MCOP, MCPP, MEP, MiBP, MnBP, and MBzP. Assessments of 15 dietary antioxidants were conducted by trained interviewers using a 24-h dietary recall. These antioxidants included vitamin A, thiamin (vitamin B1), riboflavin (vitamin B2), vitamin B6, vitamin C, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein + zeaxanthin, retinol, lycopene, food folate, selenium, iron, and zinc. To account for daily fluctuations, we used the mean values of two 24-h dietary recalls. Covariates included age (in quantiles: <34, 34–50, 50–64, >64), sex (male or female), race/ethnicity (Mexican/other Hispanic, Non-Hispanic White, Non-Hispanic Black, others), BMI categories (<25 kg/m$^2$, 25–30 kg/m$^2$, >30 kg/m$^2$), hypertension, diabetes, smoking status (never, former, and current smokers), drinking status (drinker, non-drinker), leisure-time physical activity (sufficient, insufficient), poverty-income ratio (<1.3, 1.3–3.5, >3.5, missing), education level (less than high school, high school graduate, college or higher), urinary creatinine, total energy intake (kcal/day), dietary supplement use (yes, no), healthy eating index-2020, and composite dietary antioxidant index (CDAI).

Statistical analyses accounted for NHANES's complex sampling design. Participant characteristics were presented as median (Q1, Q4) or as numbers and proportions. To explore the effects of phthalates on MASLD, weighted quantile sum (WQS) regression was used. We screened 15 dietary

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**Table 1. Associations between phthalate exposure and MASLD in the WQS model**

| Group | OR(95% CI) | *P*-value |
|---|---|---|
| Model 1 | 1.62(1.42,1.86) | <0.001 |
| Model 2 | 1.24(1.06,1.44) | 0.005 |
| Model 3 | 1.22(1.03,1.44) | 0.019 |

Statistical analysis was performed using the WQS model. Model 1 was adjusted for urinary creatinine, age, sex, and race. Model 2 was adjusted for urinary creatinine, age, sex, race, BMI, hypertension, diabetes, poverty-income ratio, and education level. Model 3 was adjusted for urinary creatinine, age, sex, race, BMI, hypertension, diabetes, poverty-income ratio, education level, smoking status, drinking status, leisure-time physical activity, and total energy intake. BMI, body mass index; CI, confidence interval; OR, odds ratio; MASLD, metabolic dysfunction-associated steatotic liver disease; WQS, weighted quantile sum.

antioxidants using multivariable logistic regressions across quartiles to assess their association with MASLD. Additionally, we evaluated the association between phthalate exposure and MASLD risk within antioxidant intake subgroups using WQS regression and restricted cubic spline models. Detailed methods are provided in the Supplementary File 1.

From NHANES 2005 to 2018, 3,961 participants were included, with 1,370 diagnosed with MASLD (weighted proportion: 32.6%). Compared to non-MASLD participants, those with MASLD were older and had higher BMIs. Significant differences were also observed in sex, race, smoking status, drinking status, and education level (Supplementary Table 1). The WQS model showed a significant association between phthalates and MASLD in both crude and adjusted models (OR = 1.22, 95% CI: 1.03–1.44, $P$ = 0.019 in the fully adjusted model) (Table 1). Among the entire population, MEHHP, MiBP, MnBP, and MECPP were the chemicals with significant weights in the mixture (Supplementary Fig. 1). Of the 15 commonly consumed antioxidants, vitamin C was the only one negatively associated with MASLD. Participants with the highest vitamin C intake had a lower MASLD risk compared to those with the lowest intake (Q4 vs Q1: OR = 0.67, 95% CI = 0.45–0.99, $P$ for trend = 0.011) (Supplementary Table 2). In WQS models, the association between MASLD and phthalate exposure was weakest in the highest vitamin C intake group when divided by tertiles (>95.6 mg/day: OR = 1.26, 95% CI = 0.97–1.64) (Table 2). Based on WQS regression results, we further analyzed the top-weighted phthalates using restricted cubic spline model, and observed a reduced increase in MASLD risk in the highest vitamin C intake group (>95.6 mg/day), particularly as MiBP concentrations increased (Fig. 1). To address potential recall bias, we conducted an additional analysis using serum vitamin C, which better reflects average exposure levels. Consistently, the weakened association was observed in participants with the highest serum vitamin C levels (Supplementary Fig. 2).

In this nationally representative study, we confirmed the adverse relationship between phthalate exposure and the risk of MASLD. Furthermore, we were the first to demonstrate a weakened association between phthalate exposure and MASLD risk in individuals with the highest vitamin C intake. This finding is consistent with a previous cross-sectional study, which reported a reduced MASLD risk in participants with higher serum vitamin C levels.[5] Our results are also supported by a randomized controlled trial showing that vitamin C intake (1 g/day), in combination with vitamin E and atorvastatin, alleviated hepatic steatosis.[6] However, two studies failed to establish a causal link between vitamin C levels and MASLD risk.[5,7] This discrepancy may be due to differences in the stage of MASLD or varying doses of vitamin C used in these studies. The mechanisms underlying the protective effect of high vitamin C intake on the phthalate-MASLD relationship are unclear but may be related to its antioxidant properties. Experimental studies have shown that phthalate exposure significantly increases reactive oxygen species levels, decreases superoxide dismutase activity, and leads to lipid accumulation and peroxidation in the liver.[8,9] Meanwhile, animal studies have demonstrated that high vitamin C intake alleviated oxidative stress in a mouse model of MASH.[7] Nevertheless, further evidence from both epidemiological studies and experiments is needed to clarify the counteracting effect of high vitamin C intake on phthalate-mediated MASLD risk.

Our study has limitations. First, although quality control measures, such as excluding participants with unreliable energy intake and averaging data from two questionnaires, improve reliability, using a 24-h recall to assess antioxidant intake may still compromise accuracy due to recall bias. More precise methods, such as measuring circulating concentrations of vitamin C, should be considered. Second, our findings could not establish causality between high vitamin C intake and reduced MASLD risk due to the cross-sectional design of the NHANES dataset, which needs to be confirmed through prospective cohort studies or randomized controlled trials. Additionally, although we adjusted for healthy eating and antioxidant exposure, the interactions between vitamin C and other antioxidants or dietary patterns were not accounted for, which may oversimplify the effect of vitamin C. Finally, our analysis was limited to NHANES data from the United States, so the findings require further investigation in other nations/regions or among different races and ethnicities.

In conclusion, this study revealed an association between phthalate exposure and increased MASLD risk, which can be attenuated by high vitamin C intake. Our findings have potential implications given the rising prevalence of MASLD and the increasing use of endocrine-disrupting chemicals in daily life. However, further research is needed to confirm the beneficial effect of high-dose vitamin C in mitigating the MASLD risk associated with endocrine-disrupting chemical exposure.

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## Conflict of interest

YS has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2022. The other authors have no conflict of interests related to this publication.

## Author contributions

Conceptualization (DX), investigation, formal analysis, writing - original draft (RZ), conceptualization, writing - review & editing (YW), conceptualization, writing - review & editing, supervision (YS), formal analysis, writing - review & editing (YW), methodology (XL), and methodology (XY). All authors have approved the final version and publication of the manuscript.

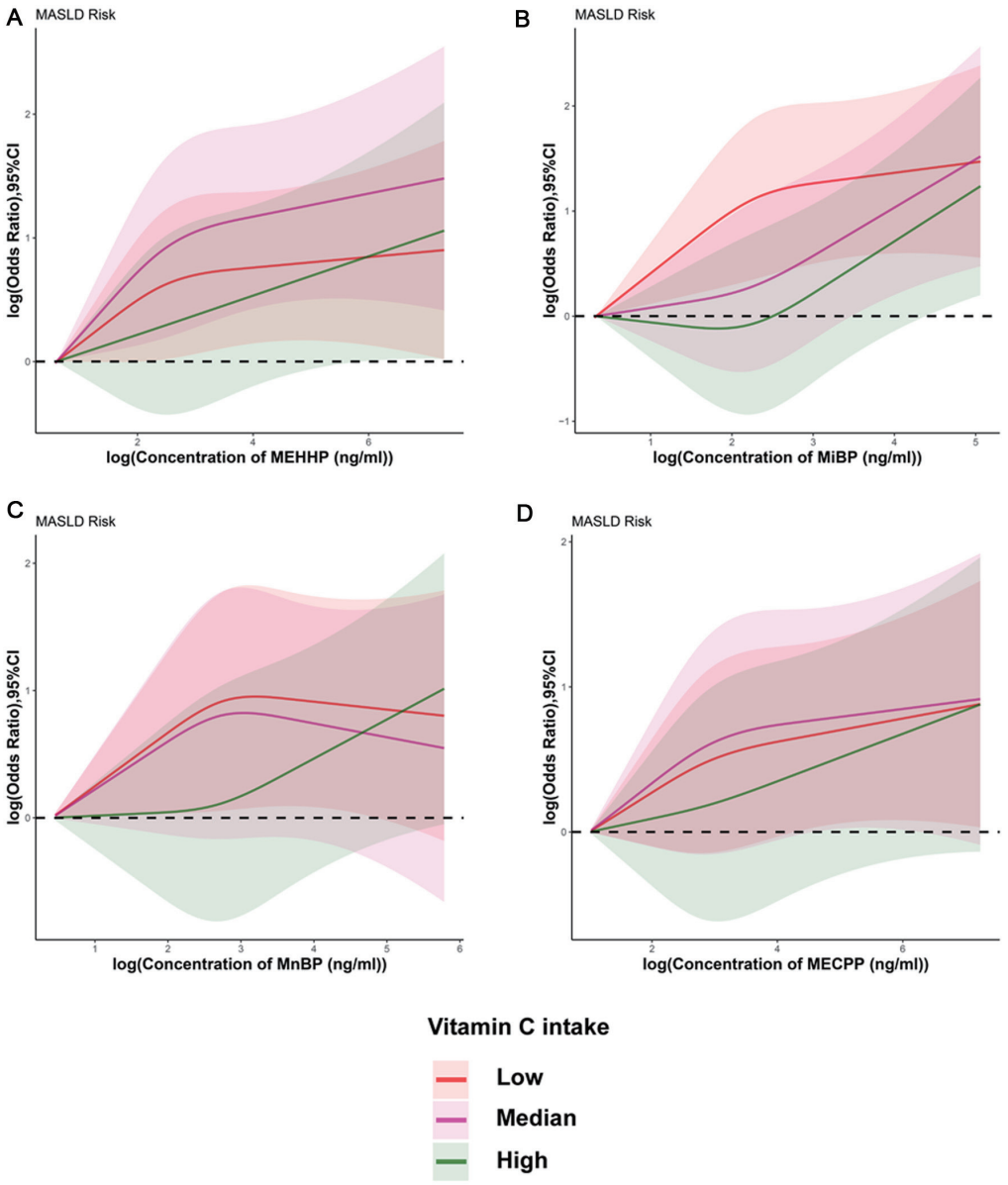


**Fig. 1. Association between phthalates and MASLD at different vitamin C intake levels.** Restricted cubic splines of different vitamin C intake levels: MEHHP (A), MiBP (B), MnBP (C), MECPP (D). The restricted cubic spline model was adjusted for urinary creatinine, age, sex, race, BMI, hypertension, diabetes, poverty-income ratio, education level, smoking status, drinking status, leisure-time physical activity, total energy intake, dietary supplement usage, HEI-2020, and CDAI. The shaded area represents the 95% confidence interval. BMI, body mass index; CDAI, composite dietary antioxidant index; CI, confidence interval; HEI-2020, healthy eating index-2020; OR, odds ratio; MASLD, metabolic dysfunction-associated steatotic liver disease; WQS, weighted quantile sum.

**Table 2. Associations between phthalates and MASLD at different vitamin C intake levels in WQS model**

| Group | Model 1 (OR,95% CI) | Model 2 (OR,95% CI) | Model 3 (OR,95% CI) |
|---|---|---|---|
| Vitamin C intake (by tertiles) | | | |
| Low (<42.6mg/day) | 1.69(1.34,2.16) | 1.32(0.97,1.80) | 1.29(0.95,1.76) |
| Median (42.6–95.6mg/day) | 1.56(1.25,1.97) | 1.36(1.01,1.85) | 1.34(0.97,1.85) |
| High (>95.6mg/day) | 1.54(1.23,1.93) | 1.26(0.98,1.62) | 1.26(0.97,1.64) |

Statistical analysis was performed using the WQS model in vitamin C intake subgroups. Model 1 was adjusted for creatinine, age, sex, and race. Model 2 was adjusted for creatinine, age, sex, race, BMI, diabetes, hypertension, poverty-income ratio, and education level. Model 3 was adjusted for creatinine, age, sex, race, BMI, diabetes, hypertension, poverty-income ratio, education level, smoking status, drinking status, leisure-time physical activity, energy intake, supplement usage, HEI-2020, and CDAI. BMI, body mass index; CDAI, composite dietary antioxidant index; CI, confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; HEI-2020, healthy eating index-2020; OR, odds ratio; WQS, weighted quantile sum.

## Ethical statement

This study was conducted following NHANES protocols. Participants were de-identified, and the data came from a public database. All NHANES participants provided informed consent.

## Data sharing statement

Data will be available upon request and approval from the corresponding author.

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