Research Article
MASLD and Alcohol-Related Liver Diseases

JOURNAL OF HEPATOLOGY

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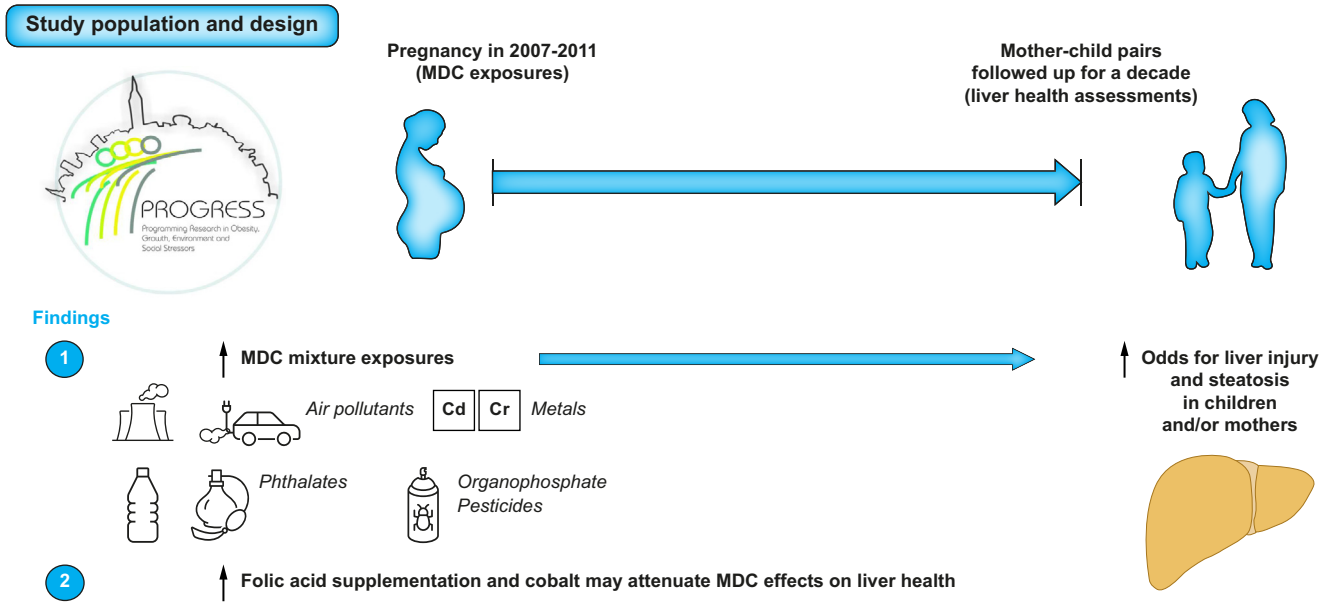
Graphical abstract



Highlights

- Higher prenatal MDC exposures increased the odds of liver injury in children.
- Weaker MDC-exposure associations with liver steatosis were observed in mothers.
- Maternal cobalt levels attenuated MDC associations with liver injury in children.
- Higher folic acid intakes attenuated the associations in both mothers and children.

Impact and implications

The effects of environmental chemical exposures on steatotic liver diseases are not well understood. In a parallel investigation of mothers and children, we found that pregnancy exposures to metabolism-disrupting chemicals may increase the risk of liver injury and steatosis, especially in the child, and that these associations could be attenuated by higher folic acid and/or cobalt levels. These findings can inform policies to decrease environmental chemical pollution and contribute to the design of clinical interventions addressing the metabolic dysfunction-associated steatotic liver disease epidemic.

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Impact of metabolism-disrupting chemicals and folic acid supplementation on liver injury and steatosis in mother-child pairs

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Background & Aims: Scarce knowledge about the impact of metabolism-disrupting chemicals (MDCs) on steatotic liver disease limits opportunities for intervention. We evaluated pregnancy MDC-mixture associations with liver outcomes, and effect modification by folic acid (FA) supplementation in mother-child pairs.

Methods: We studied ~200 mother-child pairs from the Mexican PROGRESS cohort, with 43 MDCs measured during pregnancy (estimated air pollutants, blood/urine metals or metalloids, urine high- and low-molecular-weight phthalate [HMWPs, LMWPs] and organophosphate-pesticide metabolites), and serum liver enzymes (ALT, AST) at ~9 years post-parturition. Outcomes included elevated liver enzymes in children and established clinical scores for steatosis and fibrosis in mothers (*i.e.*, AST:ALT, FLI, HSI, FIB-4). Bayesian-weighted quantile sum regression assessed MDC-mixture associations with liver outcomes. We further examined chemical-chemical interactions and effect modification by self-reported FA supplementation.

Results: In children, many MDC-mixtures were associated with liver injury. Per quartile HMWP-mixture increase, ALT increased by 10.1% (95% CI 1.67%, 19.4%) and AST by 5.27% (95% CI 0.80%, 10.1%). LMWP-mixtures and air pollutant-mixtures were associated with higher AST and ALT, respectively. Air pollutant and non-essential metal/element associations with liver enzymes were attenuated by maternal cobalt blood concentrations (*p*-interactions <0.05). In mothers, only the LMWP-mixture was associated with odds for steatosis (odds ratio = 1.53, 95% CI 1.01–2.28 for HSI >36, and odds ratio 1.62, 95% CI 1.05–2.49 for AST:ALT <1). In mothers and children, most associations were attenuated (null) at FA supplementation ≥600 μg/day (*p*-interactions <0.05).

Conclusions: Pregnancy MDC exposures may increase risk of liver injury and steatosis, particularly in children. Adequate FA supplementation and maternal cobalt levels may attenuate these associations.

Introduction

Steatotic liver diseases are on the rise worldwide. Over a third of the general adult population is affected by metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease.[1] MASLD is characterized by hepatic steatosis in conjunction with one cardiometabolic risk factor, and can progress to liver fibrosis and metabolic-dysfunction-associated steatohepatitis (MASH). Latin-Americans and Hispanics are disproportionally affected and are more likely to develop MASLD and advanced fibrosis compared to non-Hispanic Whites.[2,3] In Mexico, it is estimated that ~20% of young adults,[4] and up to 60% of children with obesity, may have MASLD.[5] Furthermore, the Mexican population has high exposure to heavy metals, particularly lead, due to common glazed ceramics use,[6] as well as to air pollutants in urban areas due to increased population and traffic-related pollution in metropolitan areas (particularly Mexico City).[7,8]

Beyond high-fructose diets and cardiometabolic disease, higher exposure to metabolism-disrupting chemicals (MDCs) (*e.g.*, phthalates, heavy metals, pesticides, and air pollutants) may also contribute to MASLD, as supported by recent epidemiological and toxicological studies.[9–11] MDC exposure in

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the sensitive pregnancy period is particularly concerning for both mothers and offspring, as it may alter endocrine and metabolic systems and fetal epigenetic programming leading to long-term cardiometabolic effects and MASLD in later life. However, knowledge about the impact of pregnancy MDC-mixture exposures on long-term liver health in mothers and children is limited. Furthermore, folate has been associated with lower MASLD risk,[12] and experimental[13] and epidemiological studies[14] suggest that folic acid (FA) supplementation may prevent or treat MASLD. FA attenuates the associations of MDCs with adverse birth and neurodevelopmental outcomes, but its potential modifying role in MDC associations with liver outcomes has not been studied.

Therefore, we applied a state-of-the-art data science framework to determine the mixture effects of 43 MDCs during pregnancy on liver injury and steatosis in mother-child pairs a decade later. We hypothesized that higher MDC exposures increase the risk of liver injury and steatosis, and that higher FA supplementation attenuates these associations.

Patients and methods

Design and population

We used data from the ongoing prospective, population-based, Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) cohort which enrolled 948 mother-newborn pairs from Mexico City (detailed in Supplementary Methods 1). MDC exposures were measured during pregnancy (2007-2011) and liver outcomes a decade later. This study included a representative subset of 234 mothers and 205 children (Table S1) who had measured serum liver enzymes. This study was approved by the Institutional Review Boards of Icahn School of Medicine at Mount Sinai (US) and National Institute of Public Health (Mexico).

Liver outcomes assessment

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) were measured in fasting blood collected from mothers and children ~9 years after childbirth (Supplemental Methods 2). In the mothers, we defined steatosis risk using established clinical non-invasive score cut-offs: AST:ALT ratio <1,[15,16] hepatic steatosis index (HSI) >36,[17] and fatty liver index (FLI) ≥60.[18] In children, we defined liver injury using the cohort's internal 90th-percentile for AST (≥32.5 U/L) and ALT (≥25.3 U/L), which was comparable to the 95% percentile of NHANES children,[19] and the clinical ALT cut-off of 25 U/L commonly used for North American children.[20] ALT and AST were also analyzed continuously to enhance power and allow for more direct results comparability between mothers and children. Fibrosis scores in mothers (*e.g.*, Fibrosis-4) and children (pediatric NAFLD fibrosis score) were considered as secondary outcomes.

MDC exposures assessment

MDC estimates (outdoor air pollutants) and biomarkers (metals/metalloids, pesticides, phthalates) in pregnant women were measured as previously detailed. Briefly, we estimated daily concentrations in the Mexico City Metropolitan Area at 1-km^2 grids for two air pollutants ($PM_{2.5}$, NO_2) based on household location using validated spatiotemporal models and satellite information,[21,22] and averaged concentrations of the three pregnancy trimesters.

MDC biomarker analysis is detailed in Supplementary Methods 3-5 and Table S2. We measured 15 metals and metalloids in mothers' urine (n = 234) and six metals or trace elements in mothers' blood (n = 232) collected during the 2nd and 3rd trimesters. Trace elements may also be protective, thus we evaluated essential metals/trace elements *vs*. non-essential metals/metalloids separately. A subset of mothers (n = 117) had seven organophosphate-pesticide (OP) metabolites measured in a single-spot urine sample collected during the 2nd pregnancy trimester. Last, 15 urine phthalate metabolites were measured during the 2nd and 3rd pregnancy trimesters. For metals and phthalates, we averaged the 2nd and 3rd trimester concentrations as a proxy of exposure throughout pregnancy. MDCs with concentrations above the detection limit (LOD) for at least 60% of samples were included in statistical analyses, after substituting values <LOD with LOD/$\sqrt{2}$. Phthalates, OPs, and metals/metalloids were corrected using specific gravity to account for urine dilution.[23]

The 43 MDCs under study included 2 air pollutants, 5 OP metabolites, 7 essential metals/trace elements,14 non-essential metals or elements, 5 low-molecular-weight phthalates (LMWPs) and 10 high-molecular-weight phthalates (HMWPs) (Table S3).

Additional variables

Sociodemographic and lifestyle variables were measured in PROGRESS as previously detailed.[24,25] Our analysis accounted for household socio-economic status (SES) during pregnancy, maternal age at partum, parity, self-reported maternal passive/active smoking status and alcohol intake during pregnancy, maternal pre-pregnancy BMI, self-reported FA intake during pregnancy (average of 2nd and 3rd trimesters), child's sex, daily sedentary time, sugar-sweetened beverages, and child's exact age and puberty status (Tanner stage) at the 9-year examination. Maternal fasting blood HbA1c, whole blood platelets, plasma triglycerides (Supplementary Methods 6) and anthropometry (waist circumference [WC] and BMI) measured at the 9-year examination were used to calculate maternal liver steatosis and fibrosis scores.

Statistical analyses

Few outliers (n <5) in liver enzyme levels were excluded using Rosner's test (Supplementary Methods 2). Logarithmic transformations normalized MDC exposure (log_2) for correlation analyses. We used Bayesian-weighted quantile sum (BWQS) regression models ("BWQS" R-package) to evaluate associations between MDC-mixtures and liver outcomes in mothers or children. BWQS does not assume *a priori* directionality of the exposure-outcome association.[26] The BWQS coefficients and their credible intervals (CIs) represent the mixture-outcome association and its precision, respectively. The BWQS estimated weights represent the relative contribution of the corresponding components (each chemical) to the mixture-outcome association. Weights closer to zero indicate lower contribution to the association. Continuous liver outcomes were ln-transformed to normalize distributions for association analyses. To facilitate interpretation, we transformed log (base=e) estimates to % change in the outcome per quartile

MDC-mixture increase. We conducted BWQS analyses separately by chemical class and for the overall MDC-mixture containing all chemicals.

We conducted a discovery-based analysis using an innovative machine-learning framework[27] that combines repeated hold-out signed iterative random forest (rh-SiRF) and regression approaches to identify potential chemical-chemical interactions within the overall MDC-mixture in association with liver outcomes (Supplementary Methods 7). To reduce false positive results, we conducted rh-SiRF analyses using primary liver outcomes, and in children only for whom we observed a compelling pattern of associations for multiple MDC-mixtures as well as the overall MDC-mixture. We then examined whether identified interactions in children were replicated in the mother sample.

We conducted exploratory stratified analyses by FA supplementation levels of 600 μg/day, which is equivalent to the recommended dietary folate equivalents during pregnancy, and the average of the recommended FA supplementation level for pregnant women according to clinical guidelines (400-800 μg/day).[28,29] Almost all study participants (~90%) reported FA supplementation above 400 μg/day. We tested effect modification by including a cross-product term between the dichotomized variable (FA ≥600 μg *vs*. FA <600 μg/day) and the BWQS weighted index mean in unstratified models. Stratified analyses in children were conducted using continuous liver outcomes only, to enhance power.

Covariates were selected *a priori*, based on clinical relevance, and/or statistical significance in our dataset. Maternal models were controlled for pre-pregnancy BMI, age at partum, SES, parity, passive or active smoking status and alcohol intake during pregnancy. Child models were adjusted for the above-mentioned covariates in addition to child's sex and age. Childhood obesity, puberty status, sugar-sweetened beverages, and sedentary time were evaluated in sensitivity analyses. Handling of missing values and secondary analyses are detailed in Supplementary Methods 8. All analyses were conducted in Rv4.3.0. The significance level was set at an alpha <0.05.

Results

Population description

Most mothers had low SES (53%) and overweight/obesity at pre-pregnancy (57%) and follow-up (82%) (Table 1). One-fifth of women reported FA ≥600 μg/day. Children had a mean (SD) age of 9.36 (0.86) years and most were at puberty (79%) (Table 1 and Table S4). Spearman correlations (p <0.05) between mothers and children were ρ = 0.18 for ALT and ρ = 0.23 for AST (Table S5). Only ~5% of mothers had ALT and AST levels above the upper limit of normal (ULN) (Table 1). Based on FLI and HSI scores and AST:ALT ratio, 43%, 54%, and 19% of women, respectively, were at risk of having steatosis. Based on FIB-4 (Fibrosis-4 index), APRI (AST-to-platelet ratio index) and NAFLD fibrosis scores, only five women were at risk of having moderate/advanced fibrosis. Stronger correlations were observed within MDC class (Fig. S1). MDC distributions are provided in Table S3.

Main associations between pregnancy MDC-mixtures and liver outcomes

In children, higher exposure to mixtures of LMWPs, HMWPs, air pollutants, and the overall MDC-mixture, were associated with increases in ALT and/or AST levels (Fig S2). Per 1-quartile increase in gestational HMWP biomarker-mixtures, we observed increases of 10.1% in ALT (95% CI 1.67%–19.4%) (Fig. 1A) and 5.27% in AST (95% CI 0.80%–10.1%) (Fig. 1E). Similarly, per quartile increase in the HMWP-mixture, children had a 94% greater likelihood of having elevated ALT (OR_{HMWP} = 1.94; 95% CI 1.11-3.56) (Fig. 1C). Per 1-quartile increase in gestational LMWP biomarker-mixtures, AST levels increased on average by 4.98% (95% CI 0.73%–9.75%) (Fig. 1E). A marginally significant association in the same direction was observed between LMWPs and ALT (6.45%, 95% CI -1.67% to 15.5%) (Fig. 1A). Top chemical contributors to these phthalate-mixture group associations were MECPP (10.9%) and MCOP (11.4%) for ALT (Fig. 1B,D; Table S6), and MiBP (23.1%) and MCOP (11.1%) for AST (Fig. 1F; Table S7). In addition, per 1-quartile increase in gestational exposure to the air pollutant-mixture, ALT levels increased by 9.66% (95% CI 1.05%–19.6%) (Fig. 1A). Associations for non-essential metals/trace element and OP pesticide biomarker-mixtures with liver outcomes tended to be positive, although not statistically significant. The overall MDC exposure-mixture was also positively associated with higher ALT (β = 14.6%, 95% CI 0.94%–31.3%) and AST levels (β = 7.54%, 95% CI 0.52%–15.4%) (Fig. S2A); top chemicals contributing to these associations were $PM_{2.5}$ (2.6%-2.9%), Cr (2.7%-2.8%), TCP (2.5%-2.7%), and MiBP (2.5%-2.6%) (Fig. S2B,C; Table S8).

In mothers, we found positive associations only for the LMWP-mixture (Fig. 2). One-quartile increase in the LMWP-mixture was associated with greater likelihood of having steatosis (OR_{LMWP} = 1.62; 95% CI 1.05, 2.49 for an AST:ALT ratio below 1, and OR_{LMWP} = 1.53; 95% CI 1.01, 2.28 for an HSI >36) (Fig. 2C). We did not find an association between MDC-mixtures and FLI, FIB-4, or other continuous liver outcomes (Fig. S3). Furthermore, no association was observed between the overall MDC-mixture and liver outcomes (Fig. S4).

Discovery analysis of potential chemical-chemical interactions associated with liver enzymes

We performed chemical-chemical interaction analyses in children for ALT and AST (Fig. 3), which were associated with the overall MDC-mixture (Fig. S2A). Using the BWQS rh-SiRF algorithm, we identified three top two-way interactions (Supplementary Methods 5) between Co and three other chemicals (NO_2, Tl, MECPTP) in association with higher ALT levels in children (p-interaction <0.05) (Fig. 3A). Chemical combination thresholds for two-way interactions were: lower concentrations of Co (≤40th-percentile, equal to ≤0.22 μg/L) combined with either higher levels of NO_2 (≥35th-percentile, ≥29.7 μg/m^3), or higher concentrations of Tl (≥40th-percentile, ≥0.34 μg/L), or lower/medium concentrations of MECPTP (≤80th-percentile, ≤7.1 ng/ml). We also observed two top two-way interactions between Co (≤80th and ≤65th-percentiles, respectively) and either $PM_{2.5}$ (≥70th-percentile) or MEP (≤50th-percentile) in association with higher AST levels (Fig. 3B), and a two-way interaction between Cs and Sr in association with AST. A three-way interaction was also found between Co (≤80th-percentile), MEP (≤75th-percentile), and $PM_{2.5}$ (≥70th-percentile) with AST levels. We defined 'low-low', 'low-high', 'high-low' and 'high-high' exposure groups based on identified two-way chemical combinations using rh-SiRF cut-off

Table 1. Characteristics of PROGRESS mothers and children.

Variable	Mean (SD) [Range: min, max] or n (%)
Mothers	**n = 234**[a]
Age at partum (years)	28.1 (5.39) [19.0, 44.0]
SES index	
Low	125 (53.4%)
Medium	86 (36.8%)
High	23 (9.8%)
Smoking exposure (active/passive) during pregnancy	
No	160 (68.4%)
Yes	74 (31.6%)
Parity at baseline (including index pregnancy)	
1 pregnancy	94 (40.2%)
2 pregnancies	80 (34.2%)
3+ pregnancies	60 (25.6%)
Pre-pregnancy BMI (kg/m^2)	26.5 (4.27) [18.6, 40.5]
Pre-pregnancy overweight (BMI ≥25 kg/m^2)	133 (56.8%)
Alcohol during pregnancy[b]	
No	193 (82.5%)
Yes	41 (17.5%)
Pregnancy FA intake (μg/day)[c]	525 (246) [7.50, 2,600]
Pregnancy FA intake ≥400 μg/day[c]	207 (88.5%)
Pregnancy FA intake ≥600 μg/day[c]	47 (20.1%)
Variables 9 years after parturition:	
Age[d]	37.5 (5.44) [28.0, 53.0]
BMI (kg/m^2)[e]	29.3 (5.45) [16.1, 53.7]
Overweight (BMI ≥25)[e]	184 (82.1%)
Triglycerides (mg/dl)[f]	131 (75.3) [30.8, 725]
Waist circumference (cm)[g]	97.5 (13.4) [62.9, 168]
Platelet count (10^3/μl)[h]	289 (66.6) [133, 538]
Diabetes (HbA1c % ≥6.5)[i]	5 (2.3%)
ALT (U/L)[j]	15.4 (10.2) [3.20, 87.3]
ALT elevation (≥ULN = 35 U/L)[j]	11 (4.8%)
AST (U/L)[j]	18.7 (7.84) [4.80, 68.7]
AST elevation (≥ULN = 31 U/L)[j]	11 (4.8%)
AST:ALT ratio <1[j]	44 (19.4%)
GGT (U/L)[k]	24.7 (18.5) [3.50, 157]
FLI[l]	53.1 (28.9) [1.32, 100]
FLI ≥60[l]	91 (43.1%)
HSI[m]	37.6 (6.66) [20.2, 62.6]
HSI >36[m]	117 (54.4%)
FIB-4[n]	0.70 (0.28) [0.20, 1.59]
FIB-4 ≥1.30 and ≤2.67[n]	5 (2.5%)
FIB-4 ≥2.67[n]	0 (0.0%)
APRI ≥0.84[o]	1 (0.5%)
NFS ≥0.676[p]	1 (0.5%)
Children	**n = 205**[a]
Age (years)	9.36 (0.86) [8.08, 12.1]
Sex	
Female	99 (48.3%)
Male	106 (51.7%)
Puberty[q]	
Pre-puberty (Tanner stage = 1)	43 (21.0%)
Puberty (Tanner stages = 2-5)	162 (79.0%)
zBMI[e]	0.91 (1.27) [-2.39, 3.54]
Overweight[r]	92 (45.1%)
ALT (U/L)[s]	14.0 (10.7) [3.00, 79.8]
AST (U/L)[t]	25.0 (8.44) [9.20, 74.1]
GGT (U/L)[u]	13.2 (5.30) [4.10, 40.0]
ALT elevation (≥25.3 U/L)[s,v]	20 (9.9%)
ALT elevation (≥25 U/L)[s]	22 (10.7%)
AST elevation (≥32.5 U/L)[t,v]	21 (10.8%)
PNFS[w]	2.57 (1.32) [0.77, 10.5]
PNFS (≥8)[w,x]	1 (0.5%)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FA, folic acid; FLI, fatty liver index; GGT, gamma-glutamyltransferase; HSI, hepatic steatosis index; PNFS, Pediatric NAFLD Fibrosis Score; PROGRESS, Programming Research in Obesity, Growth, Environment and Social Stressors; ULN, upper limit of normal.

[a]Distributions of liver outcomes in original scale (not ln-transformed).

[b]Heavy alcohol drinkers were excluded from enrollment. n=23 imputed missing values.

thresholds for downstream regression analysis (Fig. 4). These results further supported that maternal blood Co concentrations may attenuate air pollutant (NO_2 and $PM_{2.5}$) and Tl associations with liver injury outcomes, and that Cs and Sr could have synergistic effects on AST levels in children. None of the chemical-chemical interactions observed in children were evident in mothers (Fig. S5).

Effect modification by FA supplementation in the MDC-liver outcome associations

We observed a consistent pattern of positive associations between MDC-mixtures and serum liver enzymes only in the subgroup of children whose mothers reported daily FA supplementation below 600 μg, but not in children whose mothers had higher FA supplement intakes (Table 2). Specifically, air pollutant- and OP-mixture associations with ALT and AST were positive in children at lower FA supplement intake but tended to be negative in children with FA supplementation at or above 600 μg/day (p-interactions <0.05). In mothers, a similar pattern of effect modification by FA supplementation (p-interactions <0.05) was observed for air pollutants and the HSI, AST:ALT ratio (Table 3), and continuous ALT levels (Table S9). Furthermore, higher FA supplementation attenuated the LMWP- and overall MDC-mixture associations with an AST:ALT ratio below 1 in mothers (Table 3).

Secondary analyses

Sex or puberty status did not modify associations in children (Tables S10,11). Similarly, we found no consistent evidence for effect modification by overweight/obesity status in children (Table S12) or mothers (Table S13). Analyses in children using an ALT cut-off of ≥25 yielded consistent results compared to primary analyses using the 90th-percentile cut-off (Table S14). Analyses restricted to participants with available pesticide data yielded comparable results to the imputed pesticide analyses

[c]n = 20 imputed missing values.

[d]n = 13 missing values.

[e]n=10 missing values.

[f]n = 13 missing values.

[g]n = 19 missing values.

[h]n = 25 missing values.

[i]n = 12 missing values.

[j]n = 4 outliers excluded and n = 7 missing values when ALT and AST data is combined to construct AST:ALT ratio.

[k]n = 1 outlier excluded.

[l]n = 23 missing values. Index calculated as: $(e^{0.953*\ln(TGs)+0.139*BMI+0.718*\ln(GGT)+0.053*WC-15.745})/(1+e^{0.953*\ln(TGs)+0.139*BMI+0.718*\ln(GGT)+0.053*WC-15.745})*100$.

[m]n = 19 missing values. Index calculated as: 8×(ALT/AST ratio)+BMI(+2, if female; +2, if diabetes mellitus defined as an HbA1c % ≥6.5).

[n]n = 33 missing values. Index calculated as: age(during the 9-year follow-up visit)*AST/(platelet count*√ALT).

[o]n = 28 missing values. Index calculated as: [(AST/ULN)/platelet count]×100.

[p]n = 36 missing values. Index calculated as: −1.675+0.037*age(years)+0.094*BMI+1.13*(impaired fasting glycemia or diabetes mellitus defined as an HbA1c % ≥6.5 [yes=1, no=0])+0.99*(AST/ALT)−0.013*platelet count−0.66*albumin.

[q]n = 9 imputed missing values.

[r]n = 1 missing value. BMI sex-and-age-specific z-scores calculated using the WHO Growth Reference. Overweight defined as a BMI z-score >1SD.

[s]n = 2 outliers excluded.

[t]n = 1 outlier excluded.

[u]n = 3 outliers excluded.

[v]Above 90th-percentile of liver enzymes levels in PROGRESS children.

[w]n = 16 missing values. Index calculated as: z = 1.1+0.34*√ALT+0.002*(Alkaline phosphatase) -1.1*log(platelets)-0.02*GGT. PFNS probability = $[e^z/(1+e^z)]*100$.

[x]At risk of fibrosis.

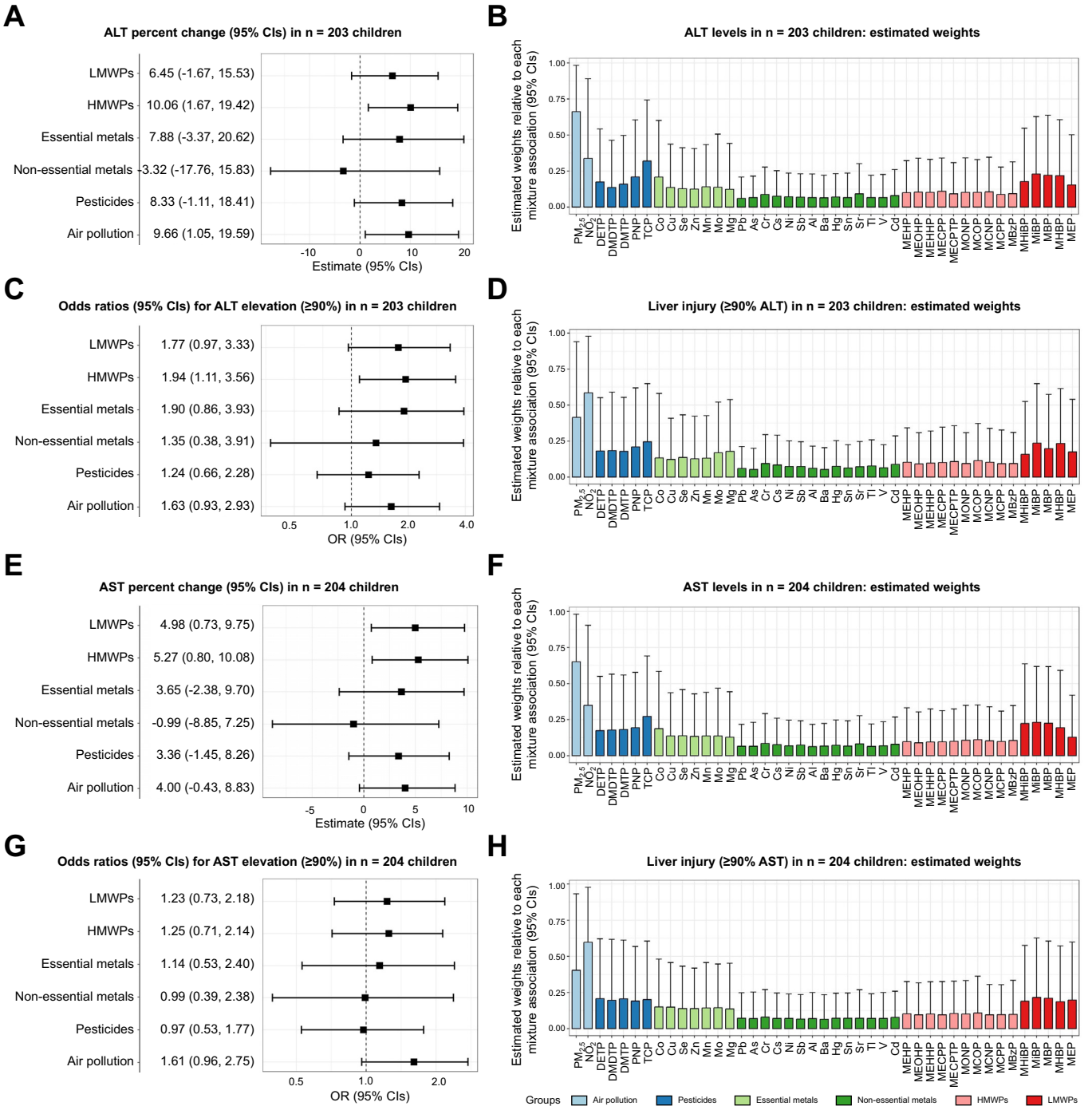

Fig. 1. Associations between pregnancy MDC-mixtures and liver outcomes in PROGRESS children. On the left-hand side, forest plots (A, C, E, G) represent effect estimates from covariate-adjusted BWQS regression (% change in continuous outcome or OR per MDC-mixture quartile increase). On the right-hand side, graphs (B, D, F, H) represent the estimated weight (contribution) of each chemical to the MDC-mixture association. The significance level was set at an alpha <0.05, which is denoted by credible intervals (95% CI) not crossing the vertical dotted line. See full abbreviation list at end of manuscript.

(Table S15). Lastly, adjusting for sugar-sweetened beverages and sedentary time did not meaningfully change associations in children (Table S16).

Discussion

This is the first study to comprehensively examine the associations between MDC-mixture exposures and liver injury and steatosis in mother-child pairs, and to consider potential effect modification by FA supplementation during pregnancy. Our findings showed that pregnancy exposures to air pollutants, phthalates, and/or OPs may increase the odds of liver injury and steatosis, particularly for the offspring. FA supplementation above 600 μg/day attenuated MDC associations with liver outcomes in both mothers and children. Furthermore, higher maternal Co concentrations during pregnancy attenuated

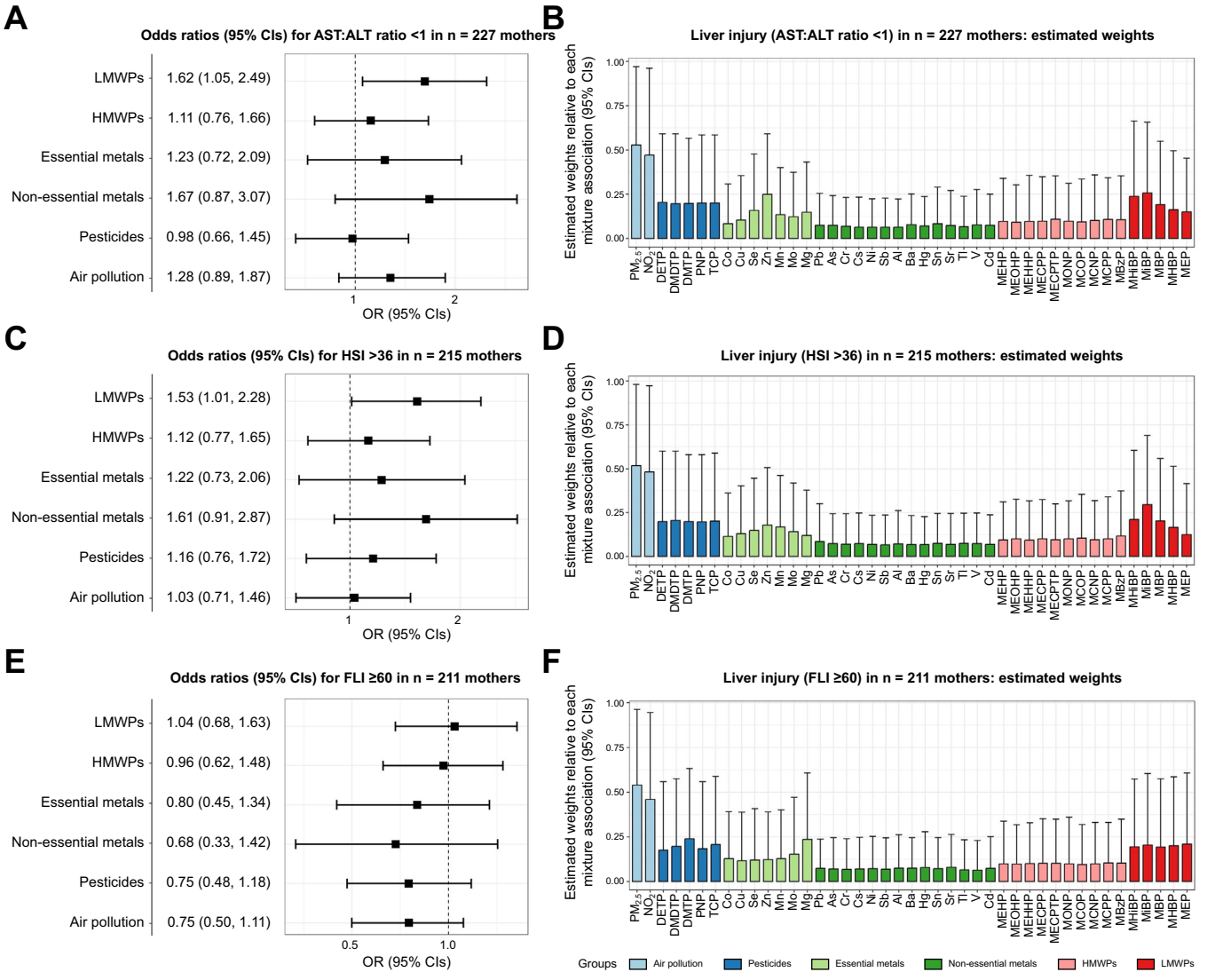

Fig. 2. Associations between pregnancy MDC-mixtures and liver outcomes in PROGRESS mothers. On the left-hand side, forest plots (A, C, E) represent effect estimates from covariate-adjusted BWQS regression (OR per MDC-mixture quartile increase). On the right-hand side, graphs (B, D, F) represent the estimated weight (contribution) of each chemical to the MDC-mixture association. The significance level was set at an alpha <0.05, which is denoted by credible intervals (95% CI) not crossing the vertical dotted line. See full abbreviation list at end of manuscript.

associations of air pollutants, and non-essential metal Tl, with liver injury in children. These findings suggest potential benefits of nutritional interventions promoting FA and cobalamin (vitamin B_{12}) intake during pregnancy (or treating folate and Co deficiencies when present) in mitigating detrimental effects of MDCs on liver health, particularly for children.

We found associations primarily in children compared to mothers, suggesting that the developing fetus is particularly sensitive to the hepatotoxic effects of MDCs. Few recent studies also indicated that exposure to MDCs, particularly during gestation, may increase risk of liver injury in children and adolescents.[10,30–32] These studies also identified associations between phthalates and air pollutants with liver injury in children. We did not find an association for the metal/metalloid mixture, contrary to other previous studies in children,[10,33] but the non-essential metals Cr and Sr were among the top contributors in our overall MDC-mixture associations. One previous study in a European pediatric population (HELIX)[10] found an association between gestational exposure to phthalate mixtures and lower odds of liver injury, contrary to the positive association observed in the present study, despite comparable phthalate distributions. Differences in sociodemographic factors and potential effect modifiers (*i.e.* FA) could explain this discrepancy. Nevertheless, the European study of predominantly White participants also showed positive associations for certain metals and OPs with pediatric liver injury, consistent with our findings in Mexican children.

Associations in mothers were weaker compared to children, supporting enhanced susceptibility to adverse liver effects of *in utero* MDC exposures. PROGRESS mothers were overall young with no evident liver fibrosis based on non-invasive screening tests. However, based on clinical scores, up to 54% of women were classified as possibly having steatosis, a higher prevalence than previously reported in pregnant

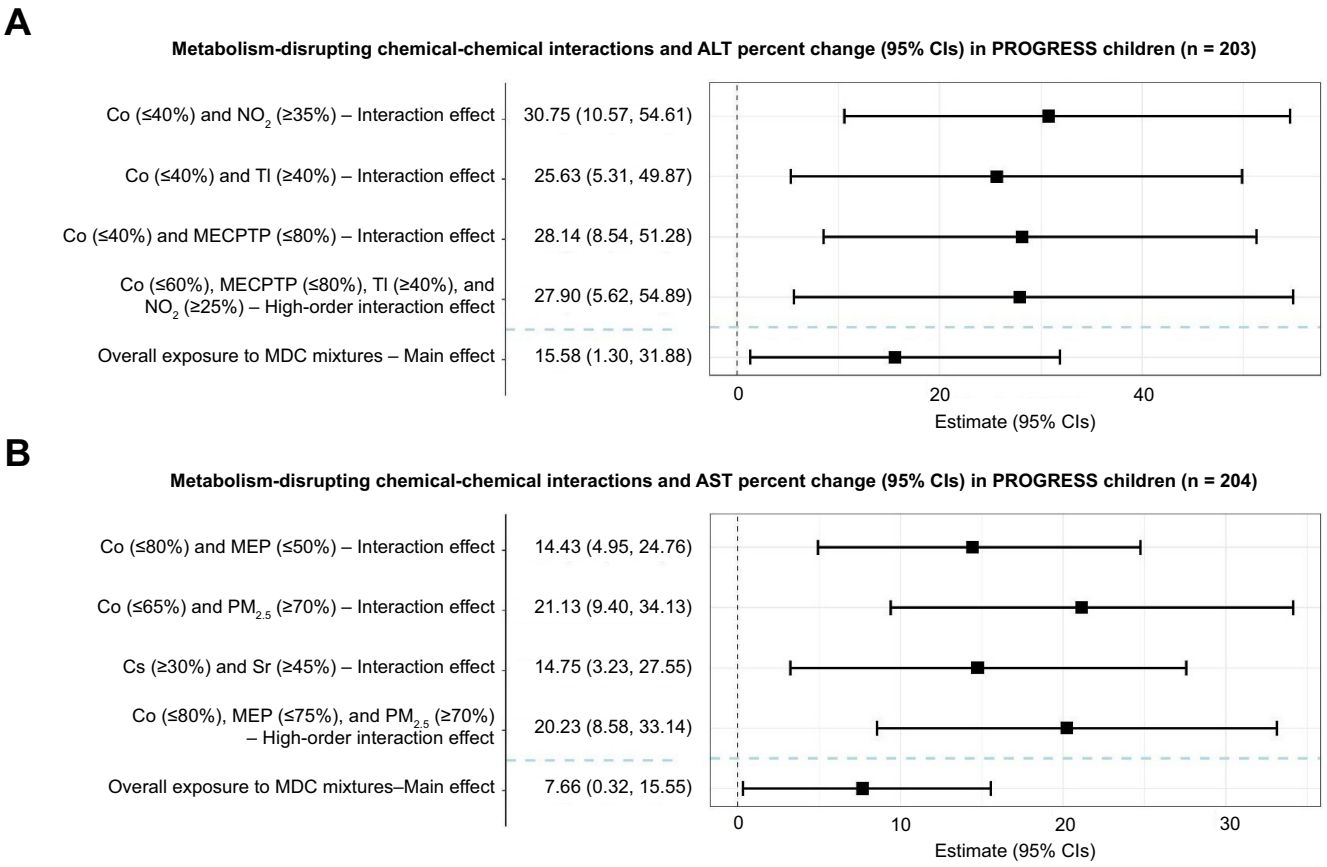

Fig. 3. Chemical-chemical interactions in relation to serum liver enzymes in PROGRESS children identified using rh-SiRF. Discovered interaction indicators in association with continuous ALT and AST outcomes are shown in forest plots A and B, respectively. Effect estimates from covariate-adjusted linear regression are expressed as % change in the outcome in the group of children within the defined clique group vs. the reference group (*i.e.*, all other threshold combinations between the same two chemicals). The significance level was set at an alpha <0.05, which is denoted by confidence intervals (95% CI) not crossing the vertical dotted line. See full abbreviation list at end of manuscript.

Mexican women.[34] Only few studies have evaluated MDC-mixtures and MASLD in adults.[35,36] A US NHANES study showed a link between heavy metals, phthalates, or PFAS and MASLD, while an Asian population study showed elevated HSI and FLI associated with a combined mixture of PFAS, phthalates, phenols, parabens, and pesticides. These findings are in partial agreement with the observed association between LMWPs and elevated HSI in PROGRESS mothers.

Toxicological studies indicate both direct effects in the liver as well as indirect effects through alterations in other metabolic tissues (*i.e.* adipose, pancreas) from MDC exposures. HMWPs promote mitochondrial dysfunction, glucose metabolism disorders,[37] and lipid peroxidation in rat liver.[38] Furthermore, experimental studies have shown HMWP-induced lipid accumulation in the liver via oxidative stress, peroxisome proliferator activated receptor disruption, and apoptosis.[39,40] In our study, HMWP were associated with liver outcomes in children whereas only LMWP associated with steatosis in the mothers, indicating potentially different implicated mechanisms. Although evidence is limited, our results suggest that HMWP in particular could lead to long-term liver effects via alterations in fetal metabolic programming. This hypothesis is supported by research showing that gestational HMWP exposure can promote liver histological damage in rat offspring,[41,42] and epidemiological studies in children linking *in utero* HMWP with alterations in DNA methylation in offspring.[43,44] In addition, metals can alter steroid receptors, increase oxidative stress, and induce enzymatic liver activity imbalance.[45–48] OPs may induce toxicity through depletion of anti-oxidant systems.[49] Air pollution also increases reactive oxygen species and inflammation in tandem with increased liver enzymes and steatosis in *in vitro* liver cells.[50] Potential mechanisms may involve upregulation of tumor-necrosis-factor-alpha exacerbating dyslipidemia and leading to hepatic-function loss. Our findings on air pollution and liver injury are also consistent with previous epidemiological studies.[30,51]

The novel findings about interactions by FA and Co in the MDC associations with liver injury and steatosis could have important clinical implications in mitigating MDC effects. Co is a trace essential element found in diet (*e.g.*, green leafy vegetables, fish, meat, nuts) and an essential ring component of cobalamin (vitamin B_{12}). FA (the synthetic form of vitamin B_9/folate) is a common supplement for pregnant women that prevents neural tube defects and poor birth outcomes. Folate

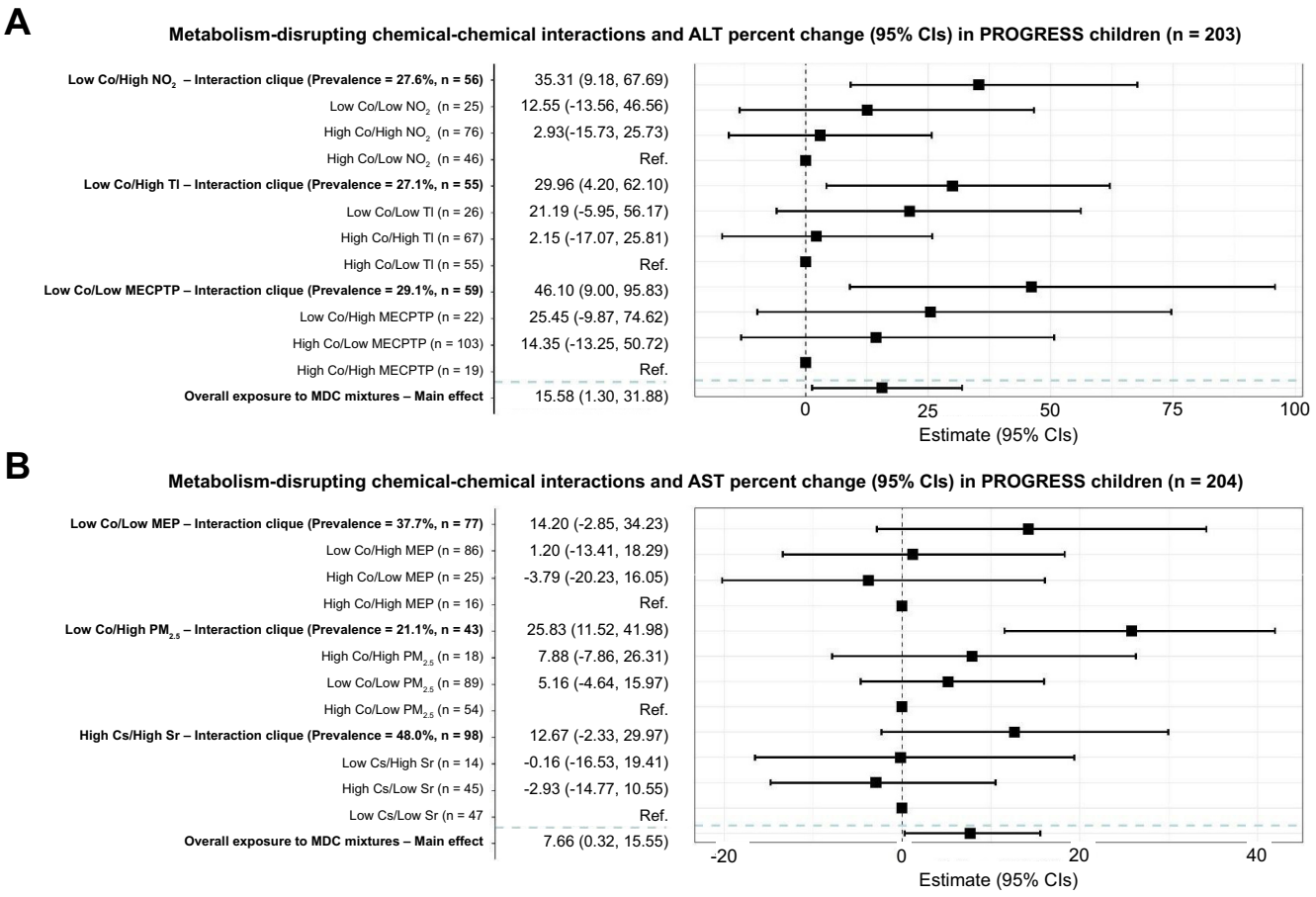

Fig. 4. Estimates for the associations between two chemical combination subgroups identified using rh-SiRF and serum liver enzymes in PROGRESS children. We used chemical thresholds identified by rh-SiRF (Fig. 3) to define 'low-low', 'low-high', 'high-low' and 'high-high' exposure groups based on identified two-way chemical-chemical interactions. Effect estimates from covariate-adjusted linear regression are expressed as % change in outcome in one exposure group compared to the reference group. The significance level was set at an alpha<0.05, which is denoted by confidence intervals (95% CI) not crossing the vertical dotted line. See full abbreviation list at end of manuscript.

and cobalamin, as methyl-nutrients, are implicated in epigenetic programming and regulation and have been suggested as therapeutic agents against MASH through decreasing inflammation and fibrosis in the liver of mice.[52] Folate deficiency may further promote liver disease through its effects on methionine metabolism, DNA synthesis and stability.[53] Similarly, Co-vitamin B_{12} deficiency increased lipid peroxidation and accumulation, branched-chain fatty acids, and histopathologic lesions, and decreased alpha-tocopherol concentrations in the liver of sheep.[54–56] In line with toxicological data, patients with steatotic liver disease have significantly decreased serum levels of folate and vitamin B_{12}.[57,58] B vitamin supplementation has been shown to attenuate the association between ambient fine particles and epigenetic effects in epidemiological research.[59] Our findings, together with prior evidence, point to gestational nutritional interventions promoting FA and B_{12} vitamin supplementation as a potential way to prevent long-term effects of MDCs on liver health.

We relied on non-invasive liver function tests that have limited diagnostic accuracy. Future studies with liver imaging and/or gold-standard biopsy methods can address this limitation. Self-reported FA data is another limitation. We did not account for the PNPLA3 variant, a genetic risk factor for MASLD in Mexicans, however, we expect that >75% of our cohort has this polymorphism.[60] Furthermore, the sample size may have reduced our ability to detect associations, especially for OPs. Lastly, results from the discovery-based chemical-chemical interaction analysis need to be corroborated in other populations. Study strengths included the prospective cohort design that minimized reverse causation bias, the numerous MDCs assessed, the state-of-the-art data science framework used for chemical mixture and interaction analyses, and our focus on a Mexican population disproportionally affected by MASLD. Additional strengths include the parallel investigation of associations in mother-child pairs that allowed us to further corroborate certain findings, and the novelty of considering FA supplementation as a modifier in MDC-liver associations.

Our findings support the premise of pregnancy as a sensitive window for long-term liver health in children, but also open an

Table 2. Effect modification by FA supplementation during pregnancy in the association between MDC-mixtures and liver outcomes in PROGRESS children.

	FA supplementation group[a]		
	Percent change (95% CIs)	Percent change (95% CIs)	
MDC-mixture	**FA <600 μg**	**FA ≥600 μg**	***p* for interaction[b]**
Liver outcome – ALT (n = 203)	**n = 165**	**n = 38**	
LMWP	9.13 (-0.16, 19.9)	2.18 (-18.7, 29.8)	0.754
HMWP	9.83 (0.72, 19.9)*	11.5 (-11.1, 41.5)	0.689
Non-essential metals	5.64 (-6.52, 19.6)	10.6 (-18.4, 48.7)	0.406
Essential metals	-2.20 (-16.2, 16.0)	-20.2 (-45.3, 17.9)	0.875
Pesticides	16.6 (5.75, 28.9)*	-17.1 (-34.3, 4.41)	0.003*
Non-imputed pesticides[c]	10.5 (-4.88, 28.2)	-34.5 (-56.4, 1.87)	0.003*
Air pollution	13.0 (3.06, 23.2)*	-4.84 (-22.8, 17.0)	0.043*
Overall MDC-mixtures	17.6 (1.57, 36.5)*	3.05 (-26.5, 42.4)	0.690
Liver outcome – AST (n = 204)	**n = 166**	**n = 38**	
LMWP	4.78 (-0.04, 9.77)	7.66 (-7.66, 26.1)	0.363
HMWP	5.68 (0.84, 10.6)*	2.72 (-10.6, 18.4)	0.864
Non-essential metals	3.67 (-2.49, 10.2)	4.99 (-11.8, 24.8)	0.640
Essential metals	0.01 (-7.43, 7.71)	-15.9 (-34.4, 9.81)	0.464
Pesticides	6.12 (0.81, 11.4)*	-4.72 (-18.2, 11.7)	0.019*
Non-imputed pesticides[c]	3.65 (-3.63, 11.3)	-4.22 (-28.0, 27.5)	0.376
Air pollution	7.39 (2.65, 12.2)*	-4.33 (-16.1, 9.43)	0.005*
Overall MDC-mixtures	9.07 (1.02, 17.7)*	2.71 (-16.4, 26.5)	0.613

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FA, folic acid; HMWP, high-molecular-weight phthalates; HSI, hepatic steatosis index; LOD, limit of detection; LMWP, low-molecular-weight phthalates; MDC, metabolism-disrupting chemical; PROGRESS, Programming Research in Obesity, Growth, Environment and Social Stressors; SES, socio-economic status.

*p value <0.05.

[a]Percent change (95% credible intervals, CIs) in ALT or AST per quartile MDC-mixture increase, stratified by FA supplementation, and adjusted for child age, sex, maternal pre-pregnancy BMI, SES, parity, smoking exposure and alcohol intake during pregnancy, and age at parturition.

[b]p value from linear regression models for the cross-product term between the dichotomized FA and the continuous MDC-mixtures variables.

[c]Sensitivity analysis without imputing organophosphate pesticides metabolite data (n = 104).

Table 3. Effect modification by FA supplementation during pregnancy in the association between MDC-mixtures with liver outcomes in PROGRESS mothers.

	FA supplementation group[a]		
	OR (95% CIs)	OR (95% CIs)	
MDC-mixture	**FA <600 μg**	**FA ≥600 μg**	***p* for interaction[b]**
Liver outcome - AST:ALT <1 (n = 227)	**n = 181**	**n = 46**	
LMWP	2.10 (1.30, 3.53)*	0.82 (0.22, 2.97)	0.024*
HMWP	1.41 (0.89, 2.28)	0.48 (0.13, 1.50)	0.051
Non-essential metals	1.61 (0.91, 2.99)	0.42 (0.09, 1.76)	0.116
Essential metals	1.84 (0.84, 3.64)	0.92 (0.11, 6.06)	0.346
Pesticides	1.11 (0.67, 1.78)	0.48 (0.11, 1.93)	0.338
Air pollution	1.63 (1.06, 2.66)*	0.53 (0.19, 1.38)	0.032*
Overall MDC-mixtures	2.19 (1.10, 4.48)*	0.32 (0.05, 1.97)	0.035*
Liver outcome - HSI >36 (n = 215)	n = 171	n = 44	
LMWP	1.47 (0.93, 2.33)	1.72 (0.46, 6.72)	0.850
HMWP	1.13 (0.74, 1.78)	2.37 (0.67, 9.71)	0.744
Non-essential metals	1.23 (0.67, 2.40)	3.01 (0.57, 19.2)	0.751
Essential metals	1.46 (0.70, 2.97)	3.28 (0.60, 23.5)	0.370
Pesticides	1.15 (0.72, 1.91)	1.28 (0.38, 4.83)	0.767
Air pollution	1.45 (0.94, 2.34)	0.40 (0.12, 1.07)	0.004*
Overall MDC-mixtures	1.49 (0.75, 3.09)	4.83 (0.68, 64.8)	0.811

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FA, folic acid; HMWP, high-molecular-weight phthalates; HSI, hepatic steatosis index; LOD, limit of detection; LMWP, low-molecular-weight phthalates; MDC, metabolism-disrupting chemical; PROGRESS, Programming Research in Obesity, Growth, Environment and Social Stressors; SES, socio-economic status.

*p value <0.05.

[a]Odds ratio (95% credible intervals, CIs) per quartile MDC-mixture increase, stratified by FA supplementation, and adjusted for maternal pre-pregnancy BMI, SES, parity, smoking exposure and alcohol intake during pregnancy, and age at parturition.

[b]p value from linear regression models for the cross-product term between the dichotomized FA and the continuous MDC-mixtures variables.

avenue of research examining pregnancy as a sensitive window for maternal liver health. Importantly, our findings suggest that nutritional interventions addressing folate and cobalt deficiencies in pregnant women and promoting adequate vitamin supplementation during pregnancy could help prevent the harmful effects of MDCs in the liver. Further research is needed to fully characterize the hepatotoxic effects of MDC exposures in vulnerable populations and inform intervention strategies to address the MASLD epidemic, starting early in life, as pediatric liver injury can persist towards adulthood.

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Abbreviations

Al, aluminum; ALT, alanine aminotransferase; As, arsenic; AST, aspartate aminotransferase; Ba, barium; BWQS, Bayesian-weighted quantile regression; Cd, cadmium; Co, cobalt; Cr, chromium; Cs, cesium; Cu, copper; DEDTP, diethyldithiophosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMTP, dimethylthiophosphate; FA, folic acid; FLI, fatty liver index; GGT, gamma-glutamyltransferase; Hg, mercury; HMWP, high-molecular-weight phthalates; HSI, hepatic steatosis index; LOD, limit of detection; LMWP, low-molecular-weight phthalates; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MBP, mono-n-butyl phthalate; MBzP, monobenzyl phthalate; MCNP, mono-carboxyisononyl phthalate; MCOP, mono(carboxy-isooctyl) phthalate; MCPP, mono-3-carboxypropyl phthalate; MDA, malathion dicarboxylic acid; MDCs, metabolism-disrupting chemicals; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MECPTP, mono-2-ethyl-5-carboxypentyl terephthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; MEP, monoethyl phthalate; Mg, magnesium; MHBP, mono-hydroxybutyl phthalate; MHiBP, mono-hydroxyisobutyl phthalate; MONP, monooxononyl phthalate; MiBP, mono-isobutyl phthalate; Mn, manganese; Mo, molybdenum; Ni, nickel; NO_2, nitrogen dioxide; OP, organophosphate; OR, odds ratio; Pb, lead; $PM_{2.5}$, particulate matter 2.5; PNP, nitrophenol; PROGRESS, Programming Research in Obesity, Growth, Environment and Social Stressors; Sb, antimony; Se, selenium; SES, socio-economic status; Sn, tin; Sr, strontium; rh-SiRF, repeated hold-out signed iterative random forest; TCP, 3,5,6-trichloro-2-pyridinol; Tl, thallium ULN, the upper limit of normal; V, vanadium; WC, waist circumference; Zn, zinc.

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Conflict of interest

The authors of this study declare that they do not have any conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

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Data availability statement

Data available only upon request.

Other disclosures

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute of Health and the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

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Supplementary data

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