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Blood BTEXS and Heavy Metal Levels Are Associated with Liver Injury and Systemic Inflammation in Gulf States Residents

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Abstract

Introduction—Exposures to volatile organic compounds and metals have previously been associated with liver diseases including steatohepatitis, although more data are needed. Benzene, toluene, ethylbenzene, xylenes, styrene (BTEXS) and metals were measured in blood samples collected between May 2012-July 2013 from volunteers participating in home visits for the Gulf Long-term Follow-up (GuLF) Study. This cross-sectional analysis evaluates associations of exposure biomarkers with serum liver injury and adipocytokine biomarkers in a sample of 214 men.

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Methods—Adult nonsmoking men without a history of liver disease or heavy alcohol consumption were included. The serologic disease biomarkers evaluated were the hepatocellular injury biomarker, cytokeratin 18 [whole (CK18 M65) and caspase-cleaved fragment (CK18 M30)]; and adipocytokines. Confounder-adjusted beta coefficients were determined using linear regression models for the overall sample (primary endpoints) and for obesity-classified sub-groups (secondary endpoints). A product interaction term between the exposure of interest and a dichotomized indicator of obesity was included to determine the disease modifying effects of obesity on the biomarker associations.

Results—The study sample was 57% white and 51% obese. In the overall sample, lead was positively associated with CK18 M30 (β=21.7±6.0 (SE), p=0.0004); IL-1β (β=32.8±5.2, p<.0001); IL-6 (β=72.8±18.3, p=0.0001); and IL-8 (β=140.8±42.2, p=0.001). Cadmium exposures were associated with increased IL-1β (β=77.8±26.3, p=0.003) and IL-8 (β=419.5±201.2, p=0.04). There were multiple significant interactions between obesity and exposure to lead, cadmium, benzene and toluene in relation to outcome biomarkers. Among obese participants (n=108), benzene, lead, and cadmium were each positively associated with CK18 M30, IL-1β, IL-6, and IL-8. In obese subjects, lead was also inversely associated with leptin, and toluene was positively associated with IL-1β.

Conclusion—For the overall sample, heavy metal exposures were associated with liver injury (lead only) and/or systemic inflammation (lead and cadmium). Obesity modified the associations between BTEXS and heavy metal exposures on several of the outcome variables. In the obesity subgroup, liver injury was positively associated with lead, cadmium and benzene exposures; systemic inflammation was increased with lead, cadmium, benzene, and toluene exposures; and leptin was inversely associated with lead exposures. The cross-sectional design of this study makes it difficult to determine causality, and all results should be interpreted cautiously. Nonetheless, the potential impact of exposures to lead, cadmium, benzene and toluene in steatohepatitis, an obesity-associated inflammatory liver disease, warrants further investigation.

Keywords

environmental liver disease; toxicant-associated steatohepatitis; nonalcoholic fatty liver disease; benzene; lead; cadmium; toluene

1. Introduction

Volatile organic compound (VOC) and metal exposures have previously been associated with liver disease and endocrine/metabolic disruption (Heindel et al., 2017). However, more data are required for a deeper understanding of these associations. The purpose of this cross-sectional study is to evaluate associations between blood biomarkers of exposure and serologic biomarkers of liver injury and adipocytokines in Gulf coast residents. Exposures to BTEXS (benzene, toluene, ethylbenzene, xylene, styrene) and metals (lead, cadmium and mercury) as well as exposure-obesity interactions are evaluated.

Liver diseases associated with occupational VOC exposures range from acute liver failure, to toxicant-associated fatty liver disease (TAFLD) with or without fibrosis, and liver cancer (Cave et al., 2011; EASL, 2019; Tolman and Dalpiaz, 2013; Wahlang et al., 2013; Wahlang

et al., 2019b). Despite significant knowledge gaps, clinical guidance in these areas has recently been published (EASL, 2019; Nerenz et al., 2015). VOC-related occupational hepatotoxicity and TAFLD have been of particular interest for the petrochemical industry (EASL, 2019). Abnormal liver enzymes and biopsy-proven nonalcoholic steatohepatitis (NASH) with fibrosis and cholestasis were reported in workers with BTXS and other VOC exposures at a petrochemical plant in Brazil (Barberino et al., 2005; Cotrim et al., 1999; Cotrim et al., 2005; Cotrim et al., 2004). The liver disease improved following removal from the workplace (Cotrim et al., 1999). BTXS-exposed petrol workers from Iran had higher mean alanine aminotransferase activity (ALT) than unexposed controls; and ALT correlated positively with workplace BTXS concentrations (Neghab et al., 2015; Salehpour et al., 2019). BTX-exposed petrochemical workers from Argentina had significantly increased odds for hypertransaminasemia compared to unexposed controls; and about half of affected workers had hepatic steatosis by ultrasound (Perez et al., 2006).

When compared to the occupational health literature, less is known about the liver health effects of environmental VOC exposures. Urinary VOC metabolites were not associated with ‘unexplained ALT elevation’, a surrogate composite NASH biomarker, in the adult National Health and Nutrition Examination Survey (NHANES) 2003–2004 (Cave et al., 2010a). However, positive associations were observed between specific liver biochemistries and BTEX/other VOC exposures measured by passive exposure monitors in NHANES 1999–2000 (Liu et al., 2009). Likewise in NHANES, metals (e.g., lead, cadmium, and mercury) were positively associated with ‘unexplained ALT elevation’, liver enzymes, hepatic steatosis and/or NASH (Cave et al., 2010a; Hyder et al., 2013). A urinary metabolite of vinyl chloride was associated with increased odds for nonalcoholic fatty liver disease (NAFLD) in children living near a petrochemical complex (Wang et al., 2019). Following a 2010 benzene release during a flaring incident at a Texas refinery, mean liver enzymes were increased in exposed community residents *vs.* unexposed controls (D’Andrea and Reddy, 2016a, b).

When compared to imaging or histologic biomarkers of TAFLD, routine liver biochemistries, such as ALT may be insensitive for the detection of this VOC-related liver disease (Anakwue et al., 2017; Cave et al., 2010b). Cytokeratin 18 (CK18) is a serologic biomarker of hepatocyte death. Both the whole protein (CK18 M65) and its caspase-cleaved fragment (CK18 M30) may be measured in serum by enzyme-linked immunosorbent assays (ELISA). CK18 M65 reflects total hepatocyte death, and CK18 M30 indicates hepatocyte apoptosis. CK18 correlated with the severity of both alcoholic and nonalcoholic steatohepatitis (Feldstein et al., 2009; Vatsalya et al., 2019). Likewise, this biomarker appeared more effective than ALT for VOC-induced liver toxicity and toxicant-associated steatohepatitis (TASH) (Cave et al., 2011; Cave et al., 2010b). More recently, serum CK18 and adipocytokines have been used to investigate environmental liver disease and endocrine/metabolic disruption related to polychlorinated biphenyl and perfluoroalkyl substance exposures (Bassler et al., 2019; Clair et al., 2018). In cohort studies, TASH has been associated with increased hepatocyte death, increased serum pro-inflammatory cytokines and abnormal adipokines (Cave et al., 2011; Cave et al., 2010b; Clair et al., 2018).

The present cross-sectional pilot study investigates associations between blood exposure (BTEXS and metals) biomarkers and serum disease (CK18/adipocytokine) biomarkers using

archived materials from a *DWH* oil spill response subcohort. Because steatohepatitis is an obesity-associated inflammatory liver disease, interactions between exposures and obesity impacting on the disease biomarkers are also investigated. The tested hypothesis is that the exposures are significantly associated with CK18 and adipocytokine levels and interact with obesity.

Subjects for this pilot study were from a subset of the Gulf Long-term Follow-up Study (GuLF Study) participants enrolled in a Chemical Biomonitoring Study (CBS) carried out between 2012 and 2013. Blood BTEXS and metal (lead, cadmium and mercury) levels were previously determined in CBS participants. While the parent GuLF Study was focused on health effects associated with oil spill response and clean-up exposures, because the blood specimens for the CBS participants were obtained several years after the spill and VOCs have short half-lives, the measured BTEXS levels are unlikely to be related to oil spill hydrocarbon exposures (Ashley and Prah, 1997; Chambers et al., 2018). Indeed, despite living in a region with prolific petrochemical and industrial operations, blood BTEX levels in this population were shown to be comparable to population levels measured in NHANES (Werder et al., 2018a). Thus, while these participants may have previously experienced short term high environmental and/or occupational BTEXS exposures related to the *DWH* oil spill and response, their exposures at the time of assessment were likely similar to the general U.S. residential population. Therefore, the data generated by this research is relevant to both occupational and environmental liver disease.

2. Materials and methods

Study Design and Participants

Data and archived serum samples were obtained from the GuLF Study, a prospective cohort of individuals (ages 21 and older) who participated in oil spill response activities and others who received safety training, but did not work on the spill, following the 2010 *Deepwater Horizon* disaster. Participants enrolled between 2011 and 2013. A detailed description of this study is available elsewhere (Kwok et al., 2017). Approximately 2–3 years after the disaster (May 2012-July 2013), a convenience sample of GuLF Study participants living in the Gulf region (N=1,055) were enrolled in the CBS (Werder et al., 2018a). CBS participants provided a blood sample used to measure levels of toxicants, including VOCs and metals, as part of a home visit for participants residing in this region. Participants provided written informed consent, and the Institutional Review Board of the National Institute of Environmental Health Sciences approved this study.

Eligible participants for this substudy were male, nonsmoking, reported no previous liver disease/hepatitis, typically consumed less than three alcoholic drinks per day, and those whose blood specimens were processed within three days of collection (n=401). We restricted to men to reduce heterogeneity in the sample. Inclusion was further restricted to participants with known measured values for blood levels of benzene, toluene, xylenes, and styrene (n=368). To facilitate internal comparisons, all participants from this sample who were also members of a distinct sub-cohort providing measured liver enzymes (n=86) were selected. For statistical efficiency, participants with the highest (n=66) and lowest (n=62) blood toluene levels were selected to maximize the exposure distribution. Toluene was

chosen because levels were universally detectable in this population. This ultimately resulted in a total pilot sample of 214 adult nonsmoking male participants with complete exposure, covariate, and outcome information (Figure 1).

BTEXS, Metals, and Cotinine Exposure Assessment

Blood collection tubes containing potassium oxalate and sodium fluoride anticoagulant were used to collect 10 mL of blood for measurement of benzene, toluene, ethylbenzene, ortho-xylene, meta-/para-xylene, styrene, cadmium, lead, and mercury. Tubes and stoppers were pre-treated by the Centers for Disease Control and Prevention (CDC) laboratory to remove VOC residues to minimize pre-collection contamination [27, 28]. Samples were stored in a 4°C refrigerator prior to being shipped overnight on cold packs in biweekly batches to the Division of Laboratory Sciences, National Center for Environmental Health, CDC in Atlanta, Georgia. VOCs were analyzed using equilibrium headspace solid-phase micro-extraction with benchtop gas chromatography/mass spectrometry following standard CDC procedures [29, 30]. Cotinine, a biomarker of exposure to tobacco smoke, was measured in serum that was stored in gas-phase nitrogen until analysis. Cotinine analysis was performed using liquid chromatography/mass spectrometry. The laboratories provided actual measured values below the limit of detection (LOD; benzene, 0.024 μg/L; toluene, 0.025 μg/L; ethylbenzene, 0.024 μg/L; ortho-xylene (o-xylene), 0.024 μg/L; meta-/para-xylene (m-/p-xylene), 0.034 μg/L; cotinine, 0.015 μg/L; cadmium, 0.16 μg/L; lead, 0.25 μg/dL; mercury, 0.16 μg/L). A portion of ethylbenzene measurements were excluded due to column interference, so analyses for this exposure are limited to 82 obese and 75 non-obese participants. Additionally, the total sample size for cadmium exposure analyses is 211 because blood cadmium levels were missing for three (non-obese) participants.

Serum Cytokeratin 18 and Adipocytokine Measurement

Serum CK18 M30 and M65 and adipocytokines were measured as previously published (Clair et al., 2018). Briefly CK18 was measured by ELISAs using separate kits (P10011 and P10020, Diapharma, West Chester, OH) according to the manufacturer's instructions. Adipocytokines were measured using multiplex bead arrays (Milliplex Human Adipokine Panels 1 and 2, EMD Millipore Corporation, Billerica, MA) run on a Luminex IS100 system. Interleukins 1β, 6, and 8 (IL-1β, IL-6, IL-8), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNFα), insulin, leptin, adiponectin, resistin, and total plasminogen activator inhibitor-1 (PAI-1) were measured. Analytes below the LOD (IL-1β < 1.3 pg/ml; IL-6 < 0.96 pg/ml, 4 records; insulin < 9.6 pg/ml, 4 records; IL8 < 0.64 pg/ml; MCP-1 < 1.3 pg/ml; TNFα < 0.64 pg/ml, 4 records) were set to half the LOD. Analytes greater than the upper limit of quantification were set to the upper standard (adiponectin >160 μg/ml, 3 records; MCP-1 > 2000pg/ml, 1 record). To address concerns about possible outliers and potentially implausible values, serum adipocytokines were measured a second time, in duplicate, for 24 participants. The average of the original and duplicated value from the second measurement was used in the statistical analyses of these adipocytokines.

Liver Enzyme Measurement

As part of a separate effort, routine serum liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin) were

previously measured by standard clinical chemistry on a Carolina Liquid Chemistry 720 analyzer (Winston-Salem, NC) for a sub-sample of participants (n=86). All reagents were purchased from Carolina Liquid Chemistry.

Statistical Methods

In primary analyses of all 214 participants, linear regression was used to estimate associations separately between each exposure of interest (BTEXS, cadmium, lead, and mercury) and the serologic disease endpoints. Associations were estimated for CK18 M65, CK18 M30, IL-1, IL-6, IL-8, TNFα, MCP-1, PAI-1, adiponectin, insulin, leptin, and resistin. Beta coefficients for the continuous difference in outcomes associated with a one-unit increase in each exposure, and their corresponding standard errors and p-values are reported. Exposures were additionally modeled as natural log-transformed values, but model fit was superior for raw concentrations, so only the results for non-transformed exposures are presented. Models were adjusted for age (<30, 30–45, >45 years), race (white, nonwhite), educational attainment (completed less than high school diploma or general equivalency degree (GED), completed high school diploma/GED, completed some college, college graduate), current daily alcohol consumption (0, 1, 2 drinks), continuous serum cotinine (ng/mL), body mass index (BMI) (<25, 25-<30, ≥30 kg/m^2), and diabetes (self-report of doctor diagnosis). Self-reported covariate information was obtained during the enrollment telephone interview, whereas body mass index and serum cotinine were measured at the time of the blood draw.

In addition to examining overall associations, secondary analyses assessing effect measure modification by obesity were performed. These models included a product interaction term between the exposure of interest and a dichotomized indicator of obesity (BMI ≥ 30.0 kg/m^2 *vs.* BMI < 30.0 kg/m^2), allowing estimation of stratum-specific exposure-outcome associations for obese (n=108) and non-obese (n=106) participants. The p-values for the exposure-obesity interaction terms are reported. We also conducted sensitivity analyses adjusting main effects in the overall sample for continuous BMI.

All statistical analyses were conducted using SAS 9.4 (Cary, NC, USA). All tests were two-sided with α=0.05.

3. Results

In this sample of adult nonsmoking men, approximately half (53%) of participants are age 45 or younger and half (50%) are obese (Table 1). The study sample is predominantly white (57%), while 37% are black and 6% are classified as other race. All participants reported that they were not current smokers and over half (54%) had non-detectable levels of serum cotinine. The detectable cotinine levels in the remaining sample suggest that participants may have had recent, appreciable exposure to environmental tobacco smoke. Most (90%) of participants participated in *DWH* oil spill response and/or cleanup. Consistent with previous investigations of the source population (Werder et al., 2018a; Werder et al., 2018b), no differences in exposure biomarker levels are observed between *DWH* cleanup workers and nonworkers (data not shown). Mean exposure biomarker levels BTEXS ranged from 0.07 μg/L (ethylbenzene and o-xylene) to 0.39 μg/L (toluene). Metals ranged from 0.40 μg/L

(cadmium) to 1.82 μg/dL (lead). Levels of both BTEXS and metals in blood were similar to general population levels. While mercury varied independently of all other exposures, there were strong correlations among BTEXS, and modest correlations between cadmium, lead, and BTEXS (Supplemental Table 1).

Associations between lead exposure with liver injury and inflammation biomarkers were observed, as well as evidence that obesity modified these relationships. Increasing blood lead was positively associated CK18 M30 in the overall sample (β=21.7±6.0, p=0.0004) and among obese participants (β=57.7±8.4, p<.0001), with a statistically significant interaction between lead level and obesity (p-interaction<.0001) (Table 2). Similar patterns were observed for lead and IL-6 (overall, β=72.8±18.3, p=0.0001; obese, β=169.6±25.4, p<.0001; p-interaction<.0001), IL-8 (overall, β=140.8±42.2, p=0.001; obese, β=360.9±58.5, p<.0001; p-interaction<.0001), and IL-1β (overall, β=32.8±5.2, p<.0001; obese, β=76.3±6.5, <=.0001; p-interaction<.0001). An inverse association was observed between lead and leptin among obese participants (β=−851.1±386.1, p=0.03; p-interaction=0.2). There were no significant associations between lead and any endpoint among non-obese participants. Indeed, the only statistically significant association observed among non-obese participants was a positive association between mercury and MCP-1 (β=36.6±14.8, p=0.01), though the interaction was not significant (p-interaction=0.1), and this association was not observed with mercury in the overall sample. Blood mercury was not associated with any other disease biomarkers. Unadjusted correlations between BTEXS and metals and liver endpoint biomarkers were generally low, with few statistically significant correlations (Supplemental Table 2).

Blood cadmium was positively associated with IL-8 in the overall sample population (β=419.5±201.2, p=0.04) and among obese participants (β=822.8±285.9, p=0.004; p-interaction=0.1). Similarly, cadmium was significantly associated with IL-1β in the overall sample (β=77.8±26.3, p=0.003) and among obese participants (β=150.6±37.2, p<.0001; p-interaction=0.01). The positive associations between cadmium and CK18 M30 (β=100.8±41.8, p=0.02; p-interaction=0.1) and IL-6 (β=307.4±126.3, p=0.02; p-interaction=0.03) were observed among obese participants only.

Among analyses of BTEXS exposures, benzene was the only chemical for which consistent associations were observed. The same disease biomarkers that emerged as relevant to blood metal exposures (CK18 M30, IL-6, IL-8 and IL-1β) were also associated with blood benzene levels, though all associations with benzene were limited to obese participants only (Table 3). Among obese participants, benzene was associated with CK18 M30 (β=253.6±112.8, p=0.03; p-interaction=0.04), IL-6 (β=685.3±338.7, p=0.04; p-interaction=0.04), IL-8 (β=1713.0±769.8, p=0.03; p-interaction=0.04), and IL-1β (β=336.1±100.5, p=0.001; p-interaction=0.004).

Although associations were not significant for toluene or ethylbenzene and CK18 M30, IL6, or IL8, directional heterogeneity between obese and non-obese participants was observed. Similarly, directional heterogeneity by obesity was observed between toluene and ethylbenzene and IL-1β, although the association was only significant for toluene (obese, β=59.0±27.7, p=0.03; p-interaction=0.03) (Table 3).

In sensitivity analyses modeling log-transformed BTEXS and metal exposures, results and interpretations were similar to primary analyses (data not shown). When adjusting for continuous BMI, results in the overall population were similar to estimates obtained in primary analyses adjusted for categorical BMI (Supplemental Table 3 and 4). In post hoc analyses of CK18 M30 and metals (cadmium, lead), we mutually adjusted for all other liver endpoints which were significantly associated with these exposures (IL-6, IL-8, and IL-1β). In these mutually adjusted models, the association between cadmium and CK18 M30 among obese participants was no longer statistically significant, but the associations with lead among obese participants remained unchanged (data not shown). These results are consistent with the expectation that lever cell death does not occur independently of cytokine levels.

Pairwise Spearman correlations were examined among the liver injury biomarkers (CK18 M30 and CK18 M65) and liver biochemistries (ALT, AST, ALP, and bilirubin) in the sub-sample of 86 participants for whom these levels were available (data not shown). The highest correlations were between CK18 M30 and CK18 M65 ($r=0.56$, $p<0.0001$) and ALT and AST ($r=0.55$, $p<0.0001$). Additionally, CK18 M65 and AST were significantly, albeit modestly, correlated ($r=0.25$, $p=0.02$). No other correlation coefficients exceeded 0.2 or had statistically significant p-values.

4. Discussion

In this sample of 214 adult males living in the Gulf coast region, we examined cross-sectional associations between blood BTEXS and metals and serum liver injury biomarkers and adipocytokines. In the overall sample, positive associations were observed between lead exposure with hepatocyte apoptosis (CK18 M30) and pro-inflammatory cytokines (IL-1β, IL-6 and IL-8); and for cadmium exposures with IL-1β and IL-8. Thus, heavy metal exposures were associated with liver injury (lead only) and/or systemic inflammation (lead and cadmium) in the overall sample. Significant obesity x pollutant interactions were observed for: (*i*) lead or benzene exposures with CK18 M30 and the pro-inflammatory cytokines, IL-1β, IL-6 and IL-8; (*ii*) cadmium or toluene exposures with IL-1β; and (*iii*) cadmium exposures with IL-6. Thus, obesity status modified the associations between lead, cadmium, benzene or toluene exposures with pro-inflammatory cytokines; and between benzene or lead exposures with liver injury. In the obesity subgroup, positive associations were noted between lead, cadmium or benzene exposures with CK18 M30 and the pro-inflammatory cytokines, IL-1β, IL-6 and IL-8. In this subgroup, lead was inversely associated with leptin levels and toluene was positively associated with IL-1β. Thus, in the obesity subgroup: (*i*) lead exposures were associated with liver injury, systemic inflammation, and endocrine disruption; (*ii*) cadmium or benzene exposures were associated with liver injury and systemic inflammation; and (*iii*) toluene exposures were associated with systemic inflammation. Mercury was positively associated with MCP-1 in the non-obese subgroup. Otherwise no significant associations between mercury, ethylbenzene, xylene, and styrene exposure and the outcomes were observed. Neither the CK18 M65, TNFα, PAI-1, insulin, adiponectin nor resistin outcome biomarkers were associated with any of the measured exposure biomarkers. The mechanism of the hepatocyte cell death associated with lead, cadmium, and benzene exposures was apoptosis rather than necrosis.

A major limitation of cross-sectional studies is causality determination. However, if the observed significant relationships for the obesity subgroup are causal, then they would be consistent with a worsening of diet-induced NAFLD by benzene, toluene, lead, or cadmium exposures, *via* exposure-induced liver cell death, hepatic inflammation and endocrine disruption.

In this study, most of the significant associations noted were with the assessed heavy metals. Lead, cadmium and mercury are endocrine and metabolism disrupting chemicals which have previously been associated with abnormal liver enzymes in cohort studies and with fatty liver disease in animal models [reviewed in (Heindel et al., 2017; Wahlang et al., 2013)]. In these GuLF Study participants, lead was positively associated with liver injury (CK18 M30) and systemic inflammation (IL-1β, IL-6 and IL-8) in both the overall sample and the obesity subgroup, and also with endocrine disruption (leptin) in the obesity subgroup. Cadmium was associated with systemic inflammation in both the overall sample (IL-1β and IL-8) and obesity subgroup (IL-1β, IL-6 and IL-8), and also with liver injury (CK18 M30) in the obesity subgroup. Mercury was positively associated with systemic inflammation (MCP-1) only in the non-obese subgroup.

Given the extensive evaluations on the human health effects of lead, surprisingly little is known about the impact of lead exposures on liver disease. Nonetheless, lead has been associated with hepatic hyperplasia and NAFLD (Cave et al., 2010a; Lin et al., 2017; Mudipalli, 2007; Zhai et al., 2017). While Lin et al. reported that heavy metal exposure correlated with fatty liver disease, especially in lean male subjects (Lin et al., 2017), a significant interaction between lead and obesity was observed in the current study. Lead was positively associated with CK18 M30 in the overall sample and the obese subgroup, but not in the non-obese subgroup. Multiple studies have investigated the relationship of early life lead exposures with BMI and obesity with inconclusive results. However, few studies have reported on effects of adult lead exposure on obesity, especially in the context of liver disease. However, a recent study demonstrated that lead increased hepatic lipid content, insulin resistance and glucose intolerance in rats fed a high fat diet (Sun et al., 2017). While insulin was not associated with lead in the present study, leptin (inverse relationship) and several interleukins (positive relationship) were. The leptin and IL-8 results are consistent with those from a pediatric cohort study (Yang et al., 2014).

Cadmium is a well-known carcinogenic and metabolism disrupting chemical. Cadmium has a whole-body half-life of between 15 and 30 years and accumulates in the kidney and the liver (Chen et al., 2018). It has been suggested that cadmium is a risk factor for obesity-associated diseases because of its ability to alter systemic metabolism (Tinkov et al., 2018). While a recent animal study has shown that low-dose cadmium exposure in combination with a high fat diet affected essential metal homeostasis leading to increased cadmium accumulation (Young et al., 2019); in human epidemiological studies, associations between cadmium, obesity and disease have not been demonstrated (Borne et al., 2014; Kuo et al., 2013; Moon, 2013). Urinary cadmium levels positively correlated with NAFLD and NASH in humans (Hyder et al., 2013). Moreover, an integrated ‘omics analysis in mice identified critical pathways associated with cadmium-induced metabolic disruption and NAFLD, such as oxidative phosphorylation, apoptosis and pro-inflammatory pathways (Go et al., 2015).

Likewise in mice, cadmium caused: (*i*) hepatic steatosis; (*ii*) activated the NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome and caspase-1; (*iii*) increased serum IL-1β, IL-6 and TNFα as well as histologic liver inflammation; and (*iv*) increased liver oxidative stress and cell death (Cao et al., 2017). The increased liver apoptosis and pro-inflammatory cytokines observed in the present cross-sectional study are consistent with the data from these animal models, except that TNFα was not associated with cadmium exposures here.

BTEXS are well-known for bone marrow toxicity, neurotoxicity, carcinogenesis and, more recently, cardiovascular effects (Abplanalp et al., 2017). While it is known that BTEXS are also hepatotoxic, their role in liver disease is not well-appreciated. Multiple published occupational health studies have demonstrated associations between occupational BTEXS exposures with liver enzymes and NAFLD (Barberino et al., 2005; Cotrim et al., 1999; Cotrim et al., 2005; Cotrim et al., 2004; Neghab et al., 2015; Perez et al., 2006; Salehpour et al., 2019). While VOC hepatotoxicity, in general, is believed to be due to reactive intermediates, the modes of action for BTEXS in liver disease are unknown. There is an increased understanding of the importance of ambient and transient BTEXS exposures (Bolden and Kwiatkowski, 2016; Werder et al., 2019). However, data on environmental BTEXS exposures and liver injury are currently limited, with the strongest data being for benzene (Cave et al., 2010a; D'Andrea and Reddy, 2016a, b; Liu et al., 2009). In the present study, the associations between BTEXS and the outcome biomarkers were modified by obesity. Significant associations for the BTEXS only occurred in the obesity subgroup. With obesity, benzene was positively associated with hepatocyte apoptosis and systemic inflammation (i.e., CK18 M30, IL-1β, IL-6 and IL-8); while toluene was positively associated with systemic inflammation (IL-1β).

The BTEXS results point to a possible role of benzene and toluene exposures in obesity-associated NAFLD and systemic inflammation. Potential modes of action include hepatocyte apoptosis and pro-inflammatory cytokine activation. Data in these areas are limited, and in some cases conflicting with the observed results. Pathway analysis of transcriptomics data from peripheral blood mononuclear cells from benzene-exposed *vs.* unexposed human subjects demonstrated differences in genes involved in apoptosis and lipid metabolism (Zhang et al., 2010). A population-based study demonstrated that increased caspase-dependent apoptosis in benzene-induced hematotoxicity, and that this toxicity was mediated by the benzene metabolite, 1,4-benzoquinone (Chen et al., 2016). Likewise, benzene metabolites increased IL-8 production from endothelial cells *in vitro* (Bironaite et al., 2004). However, a benzene metabolite inhibited conversion of pre-IL-1β to active IL-1β in bone marrow stromal macrophages *in vitro* (Kalf et al., 1996).

Human subjects with high-level occupational VOC exposures (e.g., vinyl chloride or acrylonitrile, butadiene, and styrene) or environmental PCB exposures had liver disease characterized by hepatocyte necrosis, rather than apoptosis, which was associated with pro-inflammatory cytokine elevation; while environmental perfluoroalkyl substance exposures were positively associated with hepatocyte apoptosis and inversely associated pro-inflammatory cytokines (Bassler et al., 2019; Cave et al., 2011; Cave et al., 2010b; Clair et al., 2018). The observed differences in the associations between the exposure and outcome

variables between the present study and these could be related to the specific chemical exposures, doses, and mixtures, or demographic differences (e.g., obesity rates). Indeed, while high-level occupational vinyl chloride exposures in non-obese chemical plant workers were associated with liver necrosis, vinyl chloride metabolite levels in mice with diet-induced obesity were associated with liver apoptosis (Anders et al., 2016; Cave et al., 2010b). However, benzene exposures resulted in elevated transaminase levels even in lean mice (Park et al., 2008). No differences were observed with respect to NAFLD histology or presentation in chemical workers exposed to benzene, xylene and vinyl chloride, whether or not they were obese (Cotrim et al., 2004). While previous studies have suggested that higher body fat mass may increase levels of BTEX compounds in the blood via increased retention after exposure due to their lipophilic properties (Lin et al., 2008), there are no other studies to date examining BTEXS and obesity in liver injury. Therefore, interactions between VOCs and underlying liver diseases such as obesity-related NAFLD could be a new paradigm for risk, although more data are required (Lang and Beier, 2018).

Given BTEXS's short half-life *in vivo*, the timing of the exposure assessment, which was performed 2–3 years following the 2010 *DWH* oil spill, precludes this study's ability to determine transient CK18 and adipocytokine abnormalities associated with potentially heightened BTEXS exposures related to the oil spill and cleanup activities. Indeed, blood levels of BTEX in this population were similar to those in NHANES (Werder et al., 2018a). Furthermore, work on oil spill response and cleanup-related activities was not associated with differences in blood metals or VOCs in this sample or previous Gulf Study investigations (Werder et al., 2018a; Werder et al., 2018b). A general limitation in the study of BTEXS is that the components are also precursors to other pollutants (Bolden and Kwiatkowski, 2016; Fu et al., 2010) such as tropospheric ozone, polycyclic aromatic hydrocarbons, particulate matter, and ultrafine particles, which have been connected to myriad health effects, many of which may contribute to the observed injury.

This study has several other potential limitations. First, the cross-sectional design makes determination of causality difficult. Moreover, only men were examined, and sex-differences may exist. For example, male mice were more susceptible to vinyl chloride-related hepatotoxicity and steatohepatitis than female mice (Wahlang et al., 2019a). Because this was an exploratory study, correction for multiple comparison testing was not performed. This may increase the possibility for false positive results, though the consistency in results for cadmium and lead with other studies is reassuring. Confirmatory studies with a larger sample size, prospectively collected data, and correction for multiple comparisons are required. Liver enzymes were available for only a subset of the examined sample. As expected, CK18 M30 correlated reasonably well with CK18 M65, and to a lesser degree with AST and ALT. Because CK18 is a biomarker of hepatocyte death, it did not correlate with the biomarkers of cholestatic liver injury, alkaline phosphatase and bilirubin. Prior studies demonstrated that the NAFLD associated with BTEXS exposures may have cholestatic features (Cotrim et al., 1999; Cotrim et al., 2004). Because a future manuscript will report on the associations between exposures and routine liver biochemistries, including the cholestatic biomarkers, in the largest possible sample of CBS participants, these preliminary associations were not reported here. Finally, because neither liver histology nor

liver imaging are available in the GuLF Study, it is impossible to conclude with absolute certainty that the observed positive associations with CK18 M30 were due to NAFLD.

In conclusion, for this sample of GuLF Study participants with exposure levels similar to those reported in NHANES and corresponding with the general population, heavy metal exposures were associated with liver injury (lead only) and/or systemic inflammation (lead and cadmium). Obesity modified the associations between BTEXS and heavy metal exposures on several of the outcomes. In the obesity subgroup, liver injury was positively associated with lead, cadmium and benzene exposures; systemic inflammation was increased with lead, cadmium, benzene, and toluene exposures; and leptin was inversely associated with lead exposures. The potential impact of exposures to lead, cadmium, benzene and toluene in steatohepatitis, an obesity-associated inflammatory liver disease, warrants further investigation. Given the correlated exposures and possible shared exposure sources, future analyses should employ mixtures approaches where possible. If the observed associations are causal, then they could be consistent with a worsening of diet-induced NAFLD *via* exposure-induced liver cell death, hepatic inflammation and endocrine disruption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate transaminase
BMI	body mass index
BTEXS	benzene, toluene, ethylbenzene, xylene, styrene
CBS	Chemical Biomonitoring Study
CDC	Centers for Disease Control and Prevention
CK18 M30	caspase-cleaved cytokeratin 18 fragment
CK18 M65	cytokeratin 18 whole protein
DWH	Deepwater Horizon
ELISA	enzyme-linked immunosorbent assay

GED	general equivalency degree
GuLF Study	Gulf Long-term Follow-up Study
IL-1β	interleukin 1β
IL-6	interleukin 6
IL-8	interleukin 8
LOD	limit of detection
MCP-1	monocyte chemotactic protein-1
NAFLD	non-alcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NHANES	National Health and Nutrition Examination Survey
NLRP3	NOD-like receptor pyrin domain containing 3
PAI-1	total plasminogen activator inhibitor-1
TAFLD	toxicant-associated fatty liver disease
TASH	toxicant-associated steatohepatitis
TNFα	tumor necrosis factor α
VOC	volatile organic compound

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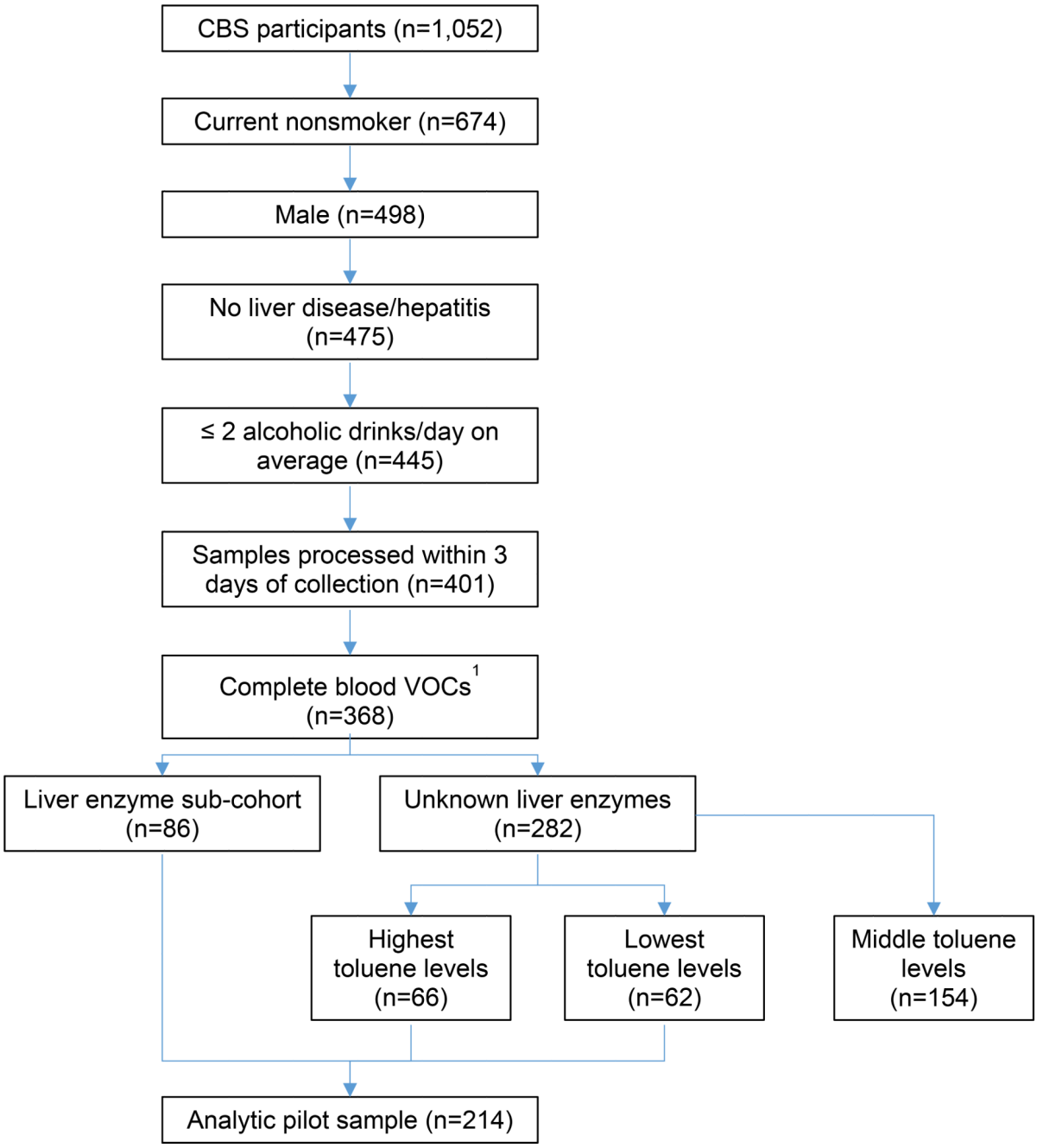

Figure 1.

Flow chart of inclusion criteria between Chemical Biomonitoring Study (CBS) and analytic pilot sample (n=214).

Table 1.

Characteristics and exposures in study sample population (N=214).

Characteristics			**N (%)**
Age		<30	37 (17)
		30–45	76 (36)
		>45	101 (47)
Race		White	121 (57)
		Black	80 (37)
		Other	13 (6)
BMI (kg/m^2)		<25	43 (20)
		25–<30	63 (29)
		≥30	108 (50)
Diabetes diagnosis from physician (self-reported)			27 (13)
Education		< HS/GED	47 (22)
		HS/GED	69 (32)
		Some college	71 (33)
		≥ College	27 (13)
Number of daily alcoholic drinks typically consumed		0	153 (72)
		1	45 (21)
		2	16 (7)
Serum cotinine (μg/L)		non-detect	115 (54)
		≤10	24 (11)
		>10	75 (35)
Exposures		% < LOD	Mean (SD)
Blood metals (μg/L)	Cadmium	17	0.40 (0.41)
	Lead (μg/dL)	0	1.82 (1.76)
	Mercury	3	2.17 (3.01)
Blood VOCs (μg/L)	Benzene	59	0.09 (0.18)
	Toluene	1	0.39 (1.68)
	Ethylbenzene[1]	50	0.07 (0.14)
	o-Xylene	53	0.07 (0.24)
	m-/p-Xylene	27	0.19 (0.55)
	Styrene	44	0.33 (1.16)

BMI, body mass index; HS, high school; GED, general equivalency degree; VOCs, volatile organic compounds

[1]A portion of ethylbenzene measurements were excluded due to column interference, so sample size for this exposure is limited to 82 obese and 75 non-obese participants.

Table 2.

Associations between continuous blood metals and continuous liver endpoints (n=214).

Disease Biomarker		Overall (N=214)[1]		Obese (n=108)[2]		Not obese (n=106)[2]		Interaction p-value[3]
	Exposure	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value	
CK18 M65	Cadmium	36.5 (34.5)	0.3	18.3 (50.3)	0.7	46.2 (43.4)	0.3	0.7
	Lead	2.4 (7.7)	0.8	14.9 (11.4)	0.2	−2.9 (9.9)	0.8	0.2
	Mercury	−2.1 (4.5)	0.6	−1.8 (7.3)	0.8	−0.5 (5.6)	0.9	0.9
CK18 M30	Cadmium	51.8 (28.8)	0.07	100.8 (41.8)	**0.02**	15.7 (36.1)	0.7	0.1
	Lead	21.7 (6.0)	**0.0004**	57.7 (8.4)	**<.0001**	−3.2 (7.3)	0.7	**<.0001**
	Mercury	−3.9 (3.7)	0.3	−2.6 (5.9)	0.7	−2.1 (4.5)	0.6	0.9
IL-6	Cadmium	117.3 (88.9)	0.2	307.4 (126.3)	**0.02**	−36.4 (109.0)	0.7	**0.03**
	Lead	72.8 (18.3)	**0.0001**	169.6 (25.4)	**<.0001**	−2.6 (21.9)	0.9	**<.0001**
	Mercury	4.0 (11.2)	0.7	−9.3 (17.7)	0.6	14.2 (13.5)	0.3	0.3
IL-8	Cadmium	419.5 (201.2)	**0.04**	822.8 (285.9)	**0.004**	140.3 (246.7)	0.6	0.1
	Lead	140.8 (42.2)	**0.001**	360.9 (58.5)	**<.0001**	−18.4 (50.5)	0.7	**<.0001**
	Mercury	21.7 (25.5)	0.4	−6.7 (40.2)	0.9	43.4 (30.6)	0.2	0.3
IL-1β	Cadmium	77.8 (26.3)	**0.003**	150.6 (37.2)	**<.0001**	21.4 (32.1)	0.5	**0.01**
	Lead	32.8 (5.2)	**<.0001**	76.3 (6.5)	**<.0001**	−0.6 (5.6)	0.9	**<.0001**
	Mercury	−0.6 (3.4)	0.9	−1.2 (5.3)	0.8	−0.1 (4.1)	1.0	0.9
TNFα	Cadmium	−3.3 (4.1)	0.4	−1.5 (6.0)	0.8	−5.7 (5.2)	0.3	0.6
	Lead	0.8 (0.9)	0.4	1.1 (1.3)	0.4	0.3 (1.1)	0.8	0.6
	Mercury	0.2 (0.5)	0.7	−0.4 (0.8)	0.6	0.6 (0.6)	0.3	0.3
MCP-1	Cadmium	3.1 (97.9)	1.0	58.0 (142.0)	0.7	10.1 (122.6)	0.9	0.8
	Lead	−8.3 (20.9)	0.7	3.6 (31.0)	0.9	−16.0 (26.8)	0.6	0.6
	Mercury	17.6 (12.3)	0.2	−3.2 (19.4)	0.9	36.6 (14.8)	**0.01**	0.1
PAI-1	Cadmium	9.0 (8.7)	0.3	−2.8 (12.5)	0.8	16.3 (10.8)	0.1	0.2
	Lead	−0.5 (1.9)	0.8	−1.4 (2.7)	0.6	0.2 (2.4)	0.9	0.7
	Mercury	−0.8 (1.1)	0.4	−0.05 (1.7)	1.0	−1.1 (1.3)	0.4	0.6

Disease Biomarker		Overall (N=214)[1]		Obese (n=108)[2]		Not obese (n=106)[2]		Interaction p-value[3]
	Exposure	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value	
Insulin	Cadmium	−344.4 (284.1)	0.2	−599.7 (405.4)	0.1	−113.4 (349.9)	0.7	0.3
	Lead	−44.6 (60.7)	0.5	−66.8 (88.9)	0.5	−30.2 (76.8)	0.7	0.8
	Mercury	−6.0 (35.8)	0.9	−68.0 (56.2)	0.2	27.9 (42.9)	0.5	0.2
Leptin	Cadmium	321.9 (1200.3)	0.8	−942.9 (1745.3)	0.6	882.9 (1506.2)	0.6	0.4
	Lead	−426.4 (260.0)	0.1	−851.1 (386.1)	**0.03**	−182.1 (333.4)	0.6	0.2
	Mercury	2.3 (154.3)	1.0	−37.2 (247.8)	0.9	1.8 (188.9)	1.0	0.9
Adiponectin	Cadmium	−2.0 (6.3)	0.8	−8.6 (9.0)	0.3	2.7 (7.8)	0.7	0.3
	Lead	1.1 (1.4)	0.4	1.7 (2.0)	0.4	0.5 (1.7)	0.8	0.7
	Mercury	−0.8 (0.8)	0.3	−0.2 (1.2)	0.8	−1.1 (1.0)	0.2	0.6
Resistin	Cadmium	3.1 (6.6)	0.6	−7.6 (9.6)	0.4	11.2 (8.3)	0.2	0.1
	Lead	−1.3 (1.4)	0.3	−0.5 (2.1)	0.8	−2.0 (1.8)	0.3	0.6
	Mercury	0.8 (0.8)	0.4	0.6 (1.3)	0.7	0.8 (1.0)	0.4	0.9

Cadmium and mercury (μg/L); lead (μg/dL)

[1] Adjusted for age (<30, 30–45, >45), race (white, nonwhite), typical alcohol consumption (0, 1, 2 drinks/day), serum cotinine (continuous), BMI (<25, 25–<30, ≥30 kg/m^2), diabetes diagnosis, education (<high school diploma, high school diploma/equivalent, some college, college degree); sample size is 211 for all associations with cadmium due to missing exposure data for three participants

[2] Adjusted as above, except BMI is dichotomized at the threshold for obesity (<30 vs ≥30 kg/m^2) and an interaction term is added between the exposure of interest and the dichotomous obesity term; sample size is 103 for all associations with cadmium among non-obese sample due to missing exposure data for three participants

[3] P-value associated with interaction term (exposure × obesity)

Table 3.

Associations between continuous blood VOCs and continuous liver endpoints (n=214).

Disease Biomarker		Overall (N=214)[1]		Obese (n=108)[2]		Not obese (n=106)[2]		Interaction p-value[3]
	Exposure	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value	
CK18 M65	Benzene	−10.4 (79.2)	0.9	83.3 (140.7)	0.6	−37.6 (91.7)	0.7	0.5
	Toluene	−10.2 (8.0)	0.2	−30.8 (38.1)	0.4	−8.1 (8.0)	0.3	0.6
	Ethylbenzene	−185.4 (126.8)	0.1	−106.8 (340.4)	0.8	−169.8 (132.8)	0.2	0.9
	o-Xylene	−84.8 (56.5)	0.1	−79.5 (89.1)	0.4	−77.6 (70.9)	0.3	1.0
	m-/p-Xylene	−35.8 (24.5)	0.1	−30.6 (33.8)	0.4	−35.6 (33.9)	0.3	0.9
	Styrene	−8.9 (11.4)	0.4	−4.6 (11.9)	0.7	−14.1 (34.2)	0.7	0.8
CK18 M30	Benzene	59.3 (63.7)	0.4	**253.6 (112.8)**	**0.03**	−19.5 (73.5)	0.8	**0.04**
	Toluene	−1.7 (6.4)	0.8	41.8 (30.8)	0.2	−3.2 (6.5)	0.6	0.2
	Ethylbenzene	−13.5 (109.1)	0.9	373.8 (290.9)	0.2	−70.1 (113.5)	0.5	0.2
	o-Xylene	−23.7 (45.7)	0.6	−10.6 (72.6)	0.9	−27.6 (57.7)	0.6	0.9
	m-/p-Xylene	−8.3 (19.8)	0.7	−1.5 (27.5)	1	−12.5 (27.6)	0.6	0.8
	Styrene	−2.7 (9.2)	0.8	−1.3 (9.7)	0.9	−3.1 (27.8)	0.9	0.9
IL6	Benzene	131.6 (194.9)	0.5	685.3 (338.7)	**0.04**	−123.9 (220.8)	0.6	**0.04**
	Toluene	−3.9 (19.7)	0.8	112.3 (92.4)	0.2	−10.5 (19.5)	0.6	0.2
	Ethylbenzene	−58.1 (311.0)	0.9	385.4 (822.0)	0.6	−168.2 (320.6)	0.6	0.5
	o-Xylene	−73.4 (139.8)	0.6	−42.9 (218.0)	0.8	−117.2 (173.1)	0.5	0.8
	m-/p-Xylene	−33.5 (60.5)	0.6	−14.6 (82.5)	0.9	−66.1 (82.8)	0.4	0.7
	Styrene	−6.0 (28.0)	0.8	−1.4 (29.0)	1.0	−16.2 (83.3)	0.8	0.9
IL8	Benzene	431.6 (443.9)	0.3	1713.0 (769.8)	**0.03**	−125.5 (501.9)	0.8	**0.04**
	Toluene	−9.6 (44.9)	0.8	265.4 (210.4)	0.2	−24.3 (44.5)	0.6	0.2
	Ethylbenzene	−54.3 (731.5)	0.9	1152.6 (1929.0)	0.6	−286.9 (752.4)	0.7	0.5
	o-Xylene	−215.8 (318.6)	0.5	−129.2 (496.0)	0.8	−297.4 (393.8)	0.5	0.8
	m-/p-Xylene	−91.1 (137.9)	0.5	−42.8 (187.7)	0.8	−149.9 (188.4)	0.4	0.7
	Styrene	34.7 (63.9)	0.6	6.5 (65.6)	0.9	292.9 (188.5)	0.1	0.2
IL1β	Benzene	98.6 (58.2)	0.1	336.1 (100.5)	**0.001**	−4.9 (65.5)	0.9	**0.004**

Disease Biomarker		Overall (N=214)[1]		Obese (n=108)[2]		Not obese (n=106)[2]		Interaction p-value[3]
	Exposure	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value	
	Toluene	1.2 (5.9)	0.8	59.0 (27.7)	**0.03**	−1.9 (5.8)	0.7	**0.03**
	Ethylbenzene	27.8 (101.3)	0.8	316.0 (267.7)	0.2	−32.9 (104.4)	0.8	0.2
	o-Xylene	−11.9 (42.0)	0.8	−5.7 (65.7)	0.9	−23.1 (52.2)	0.7	0.8
	m-/p-Xylene	−4.3 (18.2)	0.8	−0.3 (24.9)	1.0	−12.1 (25.0)	0.6	0.7
	Styrene	0.5 (8.4)	0.9	2.5 (8.7)	0.8	3.1 (25.1)	0.9	1.0
TNFα	Benzene	−10.2 (9.0)	0.3	−9.5 (15.9)	0.5	−13.0 (10.4)	0.2	0.8
	Toluene	−0.6 (0.9)	0.5	−3.1 (4.3)	0.5	−0.6 (0.9)	0.5	0.6
	Ethylbenzene	−8.6 (12.8)	0.5	−19.4 (34.0)	0.6	−7.7 (13.3)	0.6	0.7
	o-Xylene	−3.5 (6.5)	0.6	−2.9 (10.2)	0.8	−5.6 (8.1)	0.5	0.8
	m-/p-Xylene	−1.8 (2.8)	0.5	−1.7 (3.8)	0.7	−2.8 (3.9)	0.5	0.8
	Styrene	−0.4 (1.3)	0.8	−0.6 (1.4)	0.7	1.4 (3.9)	0.7	0.6
MCP1	Benzene	211.0 (214.2)	0.3	49.5 (379.0)	0.9	252.7 (247.1)	0.3	0.6
	Toluene	24.7 (21.6)	0.3	26.8 (102.8)	0.8	18.9 (21.7)	0.4	0.9
	Ethylbenzene	230.8 (273.3)	0.4	−117.8 (719.4)	0.9	307.5 (280.6)	0.3	0.6
	o-Xylene	165.2 (153.5)	0.3	100.8 (241.9)	0.7	131.3 (192.0)	0.5	0.9
	m-/p-Xylene	65.9 (66.5)	0.3	33.8 (91.6)	0.7	60.8 (91.9)	0.5	0.8
	Styrene	−11.5 (30.9)	0.7	−22.5 (32.1)	0.5	−11.4 (92.4)	0.9	0.9
PAI1	Benzene	−4.4 (19.0)	0.8	−23.0 (33.6)	0.5	1.9 (21.9)	0.9	0.5
	Toluene	−1.8 (1.9)	0.3	−5.7 (9.1)	0.5	−2.1 (1.9)	0.3	0.7
	Ethylbenzene	−14.9 (24.1)	0.5	22.4 (64.0)	0.7	−26.9 (24.9)	0.3	0.5
	o-Xylene	−17.9 (13.6)	0.2	−20.0 (21.4)	0.4	−21.7 (16.9)	0.2	1.0
	m-/p-Xylene	−7.3 (5.9)	0.2	−7.3 (8.1)	0.4	−10.1 (8.1)	0.2	0.8
	Styrene	−1.3 (2.7)	0.6	−0.1 (2.8)	1.0	−6.0 (8.2)	0.5	0.5
Insulin	Benzene	−123.8 (624.1)	0.8	193.2 (1089.3)	0.9	−193.4 (710.1)	0.8	0.8
	Toluene	−27.9 (62.9)	0.7	1.8 (295.3)	1.0	−26.6 (62.4)	0.7	0.9
	Ethylbenzene	−551.4 (1000.0)	0.6	−1257.9 (2620.0)	0.6	−297.9 (1021.9)	0.8	0.7
	o-Xylene	−338.4 (446.8)	0.4	−464.1 (693.5)	0.5	−219.4 (550.6)	0.7	0.8
	m-/p-Xylene	−156.1 (193.3)	0.4	−186.8 (262.5)	0.5	−100.8 (263.3)	0.7	0.8

Disease Biomarker		Overall (N=214)[1]		Obese (n=108)[2]		Not obese (n=106)[2]		Interaction p-value[3]
	Exposure	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value	
	Styrene	6.6 (89.7)	0.9	43.2 (91.8)	0.6	−328.8 (264.0)	0.2	0.2
Leptin	Benzene	−2379.5 (2683.5)	0.4	−6891.1 (4756.2)	0.1	−648.9 (3100.8)	0.8	0.3
	Toluene	−113.9 (271.1)	0.7	−1857.1 (1289.7)	0.2	−68.0 (272.5)	0.8	0.2
	Ethylbenzene	482.5 (3742.8)	0.9	6269.1 (9844.5)	0.5	−768.0 (3839.6)	0.8	0.5
	o-Xylene	−1599.0 (1924.0)	0.4	−3372.9 (3073.6)	0.3	−748.0 (2411.6)	0.8	0.5
	m-/p-Xylene	−840.5 (831.9)	0.3	−1326.7 (1149.2)	0.2	−469.0 (1153.1)	0.7	0.6
	Styrene	−2.6 (386.4)	1.0	−3.7 (404.3)	1.0	−631.9 (1162.5)	0.6	0.6
Adiponectin	Benzene	−9.0 (13.9)	0.5	−17.2 (24.1)	0.5	−6.3 (15.7)	0.7	0.7
	Toluene	−0.6 (1.4)	0.7	−7.2 (6.5)	0.3	−0.4 (1.4)	0.8	0.3
	Ethylbenzene	−21.3 (19.6)	0.3	−29.8 (51.0)	0.6	−19.3 (19.9)	0.3	0.8
	o-Xylene	−6.4 (9.9)	0.5	−8.8 (15.4)	0.6	−5.1 (12.2)	0.7	0.8
	m-/p-Xylene	−4.1 (4.3)	0.3	−4.0 (5.8)	0.5	−4.0 (5.8)	0.5	1.0
	Styrene	−1.7 (2.0)	0.4	−0.6 (2.0)	0.8	−9.1 (5.8)	0.1	0.2
Resistin	Benzene	5.2 (14.4)	0.7	−25.0 (25.6)	0.3	17.4 (16.7)	0.3	0.2
	Toluene	0.1 (1.5)	0.9	−3.0 (7.0)	0.7	−0.1 (1.5)	0.9	0.7
	Ethylbenzene	−8.9 (20.7)	0.7	−58.5 (54.7)	0.3	−10.7 (21.3)	0.6	0.4
	o-Xylene	1.5 (10.3)	0.9	3.0 (16.4)	0.9	−5.2 (13.0)	0.7	0.7
	m-/p-Xylene	0.4 (4.5)	0.9	0.3 (6.2)	1.0	−2.9 (6.2)	0.6	0.7
	Styrene	−1.8 (2.1)	0.4	−0.8 (2.2)	0.7	−5.3 (6.3)	0.4	0.5

BTEXS (μg/L)

[1]Adjusted for age (<30, 30–45, >45), race (white, nonwhite), typical alcohol consumption (0, 1, 2 drinks/day), serum cotinine (continuous), BMI (<25, 25–<30, ≥30 kg/m^2), diabetes diagnosis, education (<high school diploma, high school diploma/equivalent, some college, college degree); sample size is 157 for all associations with ethylbenzene due to missing exposure data

[2]Adjusted as above, except BMI is dichotomized at the threshold for obesity (<30 vs ≥30 kg/m^2) and an interaction term is added between the exposure of interest and the dichotomous obesity term; sample size for all associations with ethylbenzene is 82 obese and 75 non-obese participants due to missing exposure data

[3]P-value associated with interaction term (exposure × obesity)