Total dioxin toxic equivalency is associated with altered serologic biomarkers of hepatic lipid metabolism, inflammation, fibrosis, and function in ACHS-II participants with suspected liver disease

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Introduction. Polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) may activate the aryl hydrocarbon receptor (AhR). These molecules are endocrine and metabolism disrupting chemicals (EDCs/MDCs) that may increase susceptibility to obesity-related metabolic diseases including nonalcoholic fatty liver disease (NAFLD) and diabetes (1). For example, PCB exposures have been associated with NAFLD in multiple epidemiologic studies (2-7).

Global NAFLD prevalence is 25%, and 8% of children are affected (8, 9). Dyslipidemia is the metabolic condition most commonly associated with NAFLD, and it is present in 69% of cases (8). NAFLD represents a pathologic spectrum of liver disease ranging from steatosis to steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (10). NAFLD may result in liver-related mortality or liver transplantation. Key NAFLD mechanisms include alterations in hepatic lipid metabolism promoting steatosis; increased Kupffer cell-derived pro-inflammatory cytokines such as tumor necrosis factors alpha (TNFα); and increased pro-fibrotic cytokines including transforming growth factor beta (TGF-β) (11). As liver disease progresses, it may be associated with a decline in hepatic synthetic function, and serum albumin may decrease.

At the 2016 Dioxin Meeting, we reported on the high prevalence of suspected fatty liver disease in Phase I participants in the Anniston Community Health Survey (ACHS). Liver disease was associated with exposures to multiple ortho-substituted PCB congeners. In the re-contact study, ACHS-II, new exposure biomarkers were measured including non-ortho PCBs, PCDDs, and PCDFs (12). The purpose of this study was to determine if a serologic biomarker of total exposure to dioxin-like chemicals was associated with mechanistic fatty liver disease biomarkers in ACHS-II participants with suspected liver disease.

Materials and Methods. The study design of ACHS-II was as previously reported (12). Informed consent was obtained from all participants. Analysis of de-identified archived serum samples and data was approved by the University of Louisville Institutional Review Board. Serologic exposure biomarkers were measured. The total dioxin toxic equivalency (TEQ, wet weight) was determined by summing the TEQs for the PCBs, PCDDs, and PCDFs. The TEQ for an individual congener was determined for each participant by multiplying that congener's wet weight concentration by its World Health Organization toxic equivalency factor (TEF) (13).

Disease biomarkers were measured by either ELISA or by multi-analyte chemiluminescent detection. Subjects were categorized as having suspected liver disease if serum cytokeratin 18 (CK18, a hepatocyte cell death biomarker) was

elevated beyond pre-defined cutoffs (CK18 M65 > 300 U/ML and/or CK18 M30 > 200 U/ML). CK18 M30 measures apoptosis and CK18 M65 measures total cell death. CK18 has been proposed to be a NAFLD biomarker because it correlates with histology (14). In subjects with suspected liver disease, adjusted beta coefficients were determined for associations between wet weight total dioxin TEQ and mechanistic liver disease biomarkers. Two statistical models were utilized. Model 1 adjusted for age, race, sex, BMI, smoking, and alcohol consumption. Model 2 adjusted for total lipids in addition to the aforementioned potential confounders. Statistical significance was set at $p<0.05$.

Results and Discussion. Demographic results are presented in Table 1. The high prevalence of suspected liver disease in ACHS Phase I was confirmed in ACHS-II. In ACHS-II, 62% of subjects had biomarker indicated liver disease. The mean BMI in the liver disease group was high (31.5±7.2 kg/m^2). Very few of these participants (9.8%) reported significant alcohol consumption. Certain demographic variables were significantly associated with the presence of suspected liver disease including sex and race/ethnicity. Males (p=0.02) and Non-Hispanic Whites (p=0.002) were more likely to have suspected liver disease. This was again consistent with data from ACHS Phase I. There was a trend towards increased diabetes in the suspected liver disease group (p=0.07). Thus, participants categorized in the suspected liver disease group were at risk for NAFLD based on these demographic findings.

Table 1. Demographic Information for ACHS-II Participants By Liver Disease Status

	Liver Disease Status					
Characteristic	No Liver Disease (n=131)		Liver Disease (n=214)		P-value	Total (n = 345)
	Mean	SD	Mean	SD		
Age (years)	63.8	12.7	62.3	13.3	0.30	62.9±13.1
BMI (kg/m^2)	32.0	9.5	31.5	7.2	0.54	31.7±8.1
Cytokeratin 18 M65 (U/dL)	231.8	46.0	485.9	238.5	**<0.001**	389.4±226.5
Cytokeratin 18 M30 (U/dL)	84.1	26.5	181.8	183.7	**<0.001**	144.7±153.1
Total lipids (mg/dL)	611.1	143.7	629.8	159.9	0.27	622.7±154.0
	N	%	N	%		
Gender					**0.02**	
Male	26	19.9	67	31.3		93 (27.0)
Female	105	80.2	147	68.7		252 (73.0)
Race/ethnicity					**0.002**	
Non-Hispanic White	50	38.2	118	55.1		168 (48.7)
African/American	81	61.8	96	44.9		177 (51.3)
Diabetes Status					0.07	
Ever Diabetic	44	33.6	93	43.5		137 (39.7)
Non-diabetic	87	66.4	121	56.5		208 (60.3)
Number of Alcoholic Drinks in Past 12 Months					0.54	
No drinks	56	42.8	82	38.3		138 (40.0)
Within defined limits[a]	66	50.4	111	51.9		177 (51.3)
More than limit	9	6.9	21	9.8		30 (8.7)
Current Smoker					0.61	

No	105	80.8	168	78.5	273 (79.4)
Yes	25	19.2	46	21.5	71 (20.6)
Missing	1				1

Data are n(%) or mean±SD. Not all percents add to 100% due to rounding. P-value is one-way ANOVA (means) or Pearson chi-square test, across liver disease categories.
[a] Limits are <= 1 drink/day for females and <=2 drinks/day for males.

Next, the subgroup with suspected liver disease (n=214) was examined further. Associations were determined between total dioxin TEQ and serologic biomarkers for NAFLD mechanisms. These included biomarkers of lipid metabolism, inflammation, fibrosis, and hepatic synthetic function. The results of these analyses are presented in Table 2.

Table 2. Associations between toxic equivalency (wet weight) and serologic biomarkers of hepatic lipid metabolism, inflammation, fibrosis, and synthetic function in ACHS-II subjects with liver disease (n=214)

Category	**Biomarker**	**Model 1[1]**		**Model 2[2]**	
		Beta±SD	P-value	Beta±SD	P-value
Lipid metabolism	LDL	0.08±0.04	**0.03**	-0.04±0.03	0.20
	HDL	0.01±0.03	0.74	0.01±0.03	0.80
	VLDL	0.21±0.05	**<0.001**	0.03±0.04	0.43
Inflammation	TNFα	0.16±0.06	**0.005**	0.19±0.06	**0.002**
Fibrosis	TGFβ	0.19±0.04	**<0.001**	0.16±0.05	**<0.001**
Synthetic function	Albumin	-0.01±0.01	0.43	-0.02±0.01	**0.02**

[1] Adjusted for age, race, sex, BMI, smoking, and alcohol drinking.
[2] Adjusted for age, race, sex, BMI, smoking, alcohol consumption, and total lipids.

Total dioxin TEQ was associated with significantly increased LDL and VLDL as well as biomarkers of hepatic inflammation (TNFα) and fibrosis (TGFβ). The associations between total dioxin TEQ and LDL/VLDL cholesterol were significant only in Model 1 which did not adjust for total serum lipids. No associations between exposure and HDL cholesterol were seen in either model. Total dioxin TEQ was associated with decreased hepatic synthetic function (albumin) only in Model 2.

These results confirm the high prevalence of suspected liver disease previously reported in ACHS Phase I. More importantly, the results suggest that AhR activation by environmental chemicals could potentially impact disease progression in individuals with liver disease. All the major mechanisms of NAFLD pathogenesis (abnormal lipid metabolism, inflammation, and fibrosis) were associated with exposure. Because ACHS-II is an epidemiological study, liver biopsies were not available. This is an important limitation of this study. The impact of dioxin exposures on liver pathology in NAFLD requires future investigation. Likewise, the potential role of sex and race/ethnicity on toxicant associated fatty liver diseases warrants future investigation.

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References.

1. Heindel JJ, Blumberg B, Cave M, Machtinger R, Mantovani A, Mendez MA, Nadal A, et al. Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol 2016.
2. Cave M, Appana S, Patel M, Falkner KC, McClain CJ, Brock G. Polychlorinated biphenyls, lead, and mercury are associated with liver disease in American adults: NHANES 2003-2004. Environ Health Perspect 2010;118:1735-1742.
3. Serdar B, LeBlanc WG, Norris JM, Dickinson LM. Potential effects of polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs) on immune cells and blood biochemistry measures: a cross-sectional assessment of the NHANES 2003-2004 data. Environ Health 2014;13:114.
4. Kumar J, Lind L, Salihovic S, van Bavel B, Ingelsson E, Lind PM. Persistent organic pollutants and liver dysfunction biomarkers in a population-based human sample of men and women. Environ Res 2014;134:251-256.
5. Yorita Christensen KL, Carrico CK, Sanyal AJ, Gennings C. Multiple classes of environmental chemicals are associated with liver disease: NHANES 2003-2004. Int J Hyg Environ Health 2013.
6. Kim MJ, Marchand P, Henegar C, Antignac JP, Alili R, Poitou C, Bouillot JL, et al. Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss. Environ Health Perspect 2011;119:377-383.
7. Rantakokko P, Mannisto V, Airaksinen R, Koponen J, Viluksela M, Kiviranta H, Pihlajamaki J. Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort study. Environ Health 2015;14:79.
8. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global Epidemiology of Non-Alcoholic Fatty Liver Disease-Meta-Analytic Assessment of Prevalence, Incidence and Outcomes. Hepatology 2015.
9. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. PLoS One 2015;10:e0140908.
10. Wahlang B, Beier JI, Clair HB, Bellis-Jones HJ, Falkner KC, McClain CJ, Cave MC. Toxicant-associated steatohepatitis. Toxicol Pathol 2013;41:343-360.
11. Joshi-Barve S, Kirpich I, Cave MC, Marsano L, McClain CJ. Alcoholic, Non-alcoholic and Toxicant-Associated Steatohepatitis: Mechanistic Similarities and Differences. *Cellular and Molecular Gastroenterology and Hepatology* 2015;1:356-367.
12. Birnbaum LS, Dutton ND, Cusack C, Mennemeyer ST, Pavuk M. Anniston community health survey: Follow-up and dioxin analyses (ACHS-II)-methods. Environ Sci Pollut Res Int 2015.
13. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, et al. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 2006;93:223-241.
14. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology 2009;50:1072-1078.