**Effect of BPA Exposure on Cardiometabolic Risk: Longitudinal Studies in Three Biracial Cohorts**

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Obesity, diabetes and cardiovascular disease (CVD) are the leading cause of morbidity and mortality among all ethnic groups. Although genetic predisposition, overeating and sedentary lifestyle are undoubtedly key causal factors, these commonly held perceptions do not fully explain the alarming rise in obesity and diabetes. A growing body of evidence suggests that endocrine-disrupting chemicals (EDCs) such as bisphenol A (BPA) that is ubiquitous in our environment may contribute to the development of cardiometabolic disorders by interfering with adipocyte formation, glucose/lipid homeostasis and energy balance. Recent studies also indicate that environmental endocrine disruptors may induce aberrant epigenetic changes, resulting in altered gene expression thus contributing to disease susceptibility. However, existing studies are primarily experimental, and data from human epidemiologic studies are scarce and inconsistent. This uncertainty greatly hampers our ability to implement early intervention, prevention and treatment for these debilitating disorders.

Our *long-term goal* is to understand the role of toxic chemical exposures in the development of obesity, diabetes and related cardiometabolic risk factors and to identify biological pathways through which toxic chemical exposures increase risk for these metabolic disorders. *The overall objective* here is to elucidate the role of BPA exposure in disrupting metabolic homeostasis, thereby increasing risk of obesity, diabetes and subclinical atherosclerosis. *Our central hypothesis* is that exposure to BPA is associated with an increased cardiometabolic risk, and that epigenetic factors play a critical role in mediating this association. The *rationale* for the proposed research is that, once we know the chemical exposures that are involved in obesity,diabetes, and coronary atherosclerosis, these toxic chemicals could be removed from our diet or other sources and biomarkers of exposure could be developed for risk stratification. Moreover, identification of epigenetic pathways through which environmental chemicals contribute to cardiometabolic risk will potentially lead to novel therapeutic targets for prevention, intervention and treatment of these chronic disorders that are tailored to individual patients. Our ultimate goal is to mitigate the current rising tides of obesity, diabetes and their associated disorders. By leveraging the unique resoures of three well-characterized prospective cohorts and the repeatedly measured DNA methylation data on the same Illumina 450K Methylation Arrays, we plan to test our central hypothesis and, thereby, attain the objective of this application by pursuing the following three *specific aims*:

**Aim 1. To investigate the association of changes in urinary BPA concentrations with changes in cardiometabolic risk measures in three prospective biracial cohorts.**

**Discovery cohort:** We will repeatedly measure urinary BPA concentrations in 1,400 participants (900 white and 500 blacks, aged 34-58 years) who attended baseline clinical exam (2006-2008) and followed through 2016 in the Bogalusa Heart Study (BHS). BPA concentrations in spot urine collected at two time points (8-10 years apart) will be quantified by HPLC at the Center for Disease Control and Prevention (CDC). DNA methylation assayed on Illumina 450K methylation arrays at both baseline and follow-up in all BHS participants have been available for the current project. Statistical analyses will be conducted to examine: (1) prospective association between changes in BPA concentration and changes in cardiometabolic risk measures, e.g., BMI, glucose homeostasis, insulin resistance, dyslipidemia, inflammatory biomarkers, blood pressure, and subclinical atherosclerosis (e.g., arterial stiffness, cIMT), and (2) prognostic value of baseline BPA concentration in predicting future risk for cardiometabolic phenotypes, including weight gain, progression in diabetes and subclinical atherosclerosis. Sex- and race-specific effects of BPA exposure on cardiometabolic risk will also be evaluated.

**Replications in CARDIA & HeartBeat!:** Findings from the BHS will be replicated in two independent biracial prospective cohorts: the CARDIA participants and the HeartBeat!. BPA concentrations in urine samples collected at two time points ( ~5 year apart) in both cohorts will be repeatedly measured using the same lab protocols (i.e., HPLC) by CDC. Longitudinal DNA methylation data measured by Illumina 450K methylation in genomic DNA collected at same time points in both CARDIA participants (n=678, 508 whites and 170 blacks) and HeatBeat! (n=678, 508 whites and 170 blacks) will have been available by the time of this grant is funded. Statistical analysis will be conducted within each cohort first, and then meta-analysis will be performed to combine results across all three cohorts. The DNA methylation data in CARDIA and HeartBeat were recently funded by the AHA Center Grant. Dr. Lifang Hou is the PI of the methylation project and she will serve as the Multi-PD/PI on this ancillary study proposal.

**2. To determine the extent to which DNA methylation variation mediates the association between BPA exposure and cardiometabolic risk.**

Exposure to BPA may induce epigenetic changes, which in turn affect gene expression and subsequent disease risk. By leveraging the longitudinal DNA methylation data in three cohorts (BHS, CARDIA, and HeartBeat!), we will conduct statistical analyses to identify genes/genomic regions that are sensitive to BPA exposure, to investigate the association of these BPA-induced epigenetic alterations with cardiometabolic risk, and to determine the extent to which this association is mediated by DNA methylation. Sex-and race-specific mediation effect of DNA methylation on the relationship between BPA exposure and cardiometabolic risk will also be assessed.

**3. To identify genotype-specific effects of BPA exposure on DNA methylation and cardiometabolic risk.**

DNA methylation variation, and perhaps interindividual response to BPA exposure as well, may be under genetic control. By taking the advantage of available GWAS data in both three biracial cohorts (BHS, CARDIA & HeartBeat), this aim is to perform integrated genetic and epigenetic analyses to identify genetic variants influencing the interindividual variation of urinary BPA concentrations, and to investigate genotype/haplotype-specific effects of BPA exposure on DNA methylation and cardiometabolic risk.

The work proposed is anticipated to identify BPA-responsive methylome and novel biological pathways linking BPA exposure to cardiometabolic risk. Such results are expected to have an important positive impact, because these findings will provide valuable information on toxic chemical exposures that could be removed from our diet or other sources, and are highly likely to provide targets for prevention and therapeutic intervention in addition to fundamentally advancing the fields of BPA methylome with cardiometabolic disorders.

**Data request from BHS**

We plan to submit the proposed research as an R01 application to NIEHS in the coming deadlines. To strengthen this application, we request the following data for statistical analyses. Results will be included as preliminary data for the grant proposal, and if possible, will be published in peer-reviewed journals as well:

a) Cardiometabolic phenotypes collected at baseline (2006-2008) and follow-up, e.g., adiposity, glucose homeostasis, lipids, blood pressure, subclinical atherosclerosis (e.g, arterial stiffness, cIMT), and inflammatory biomarkers.

b) Covariates: Demographics, lifestyle, and laboratory parameters including creatinine.

c) DNA methylation data at baseline and follow-up

**Sample request from BHS**

For the proposed BPA analysis, we will request spot urine sample collected at both baseline and follow-up (1ml at each time point) for all BHS participants. To examine the potential role of metabolism in mediating the effect of BPA on cardiometabolic risk, urine or blood (serum or plasma) sample (~0.5ml per subject at each time point) may also be requested for metabolomics analysis.

Of note, this is a collaborative study between BHS and CARDIA&HeartBeat! The final investigative team of the grant proposal needs to be discussed with CARDIA and HeatBeat investigators.