Genes, Environmental Pollutants, and Endocrine System Disruption

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THESIS

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This thesis is dedicated to Sul-Ki Lee, without whom it would never have been accomplished.

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF TABLES (CONTINUED)

TABLE PAGE

LIST OF TABLES (CONTINUED)

LIST OF TABLES (CONTINUED)

LIST OF TABLES (CONTINUED)

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS

BKMR	Bayesian kernel machine regression
BMI	Body mass index
DDE	1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene
DDT	Dichloro-diphenyl-trichloroethane
GWAS	Genome-Wide Association Study
HCB	Hexachlorobenzene
HCHS/SOL	Hispanic Community Health Study / Study of Latinos
NHANES	National Health and Nutrition Examination Survey
NIDDM	Non-Insulin Dependent Diabetes Mellitus
LOD	Limit of Detection
PBB	Polybrominated Biphenyl
PBDE	Polybrominated Diphenyl Ethers
PC	Principal Component
PCA	Principal Component Analysis
PCB	Polychlorinated Biphenyl
PRS	Polygenic risk score
POP	Persistent Organic Pollutant
QC	Quality Control
QGCOMP	Quantile G-Computation
RAF	Risk Allele Frequency
SNP	Single Nucleotide Polymorphism
T2D	Type 2 Diabetes
T3	Triiodothyronine
T4	Thyroxine
TR	Thyroid Hormone Receptor
TSH	Thyroid Stimulating Hormone
WQS	Weighted Quantile Sum

SUMMARY

Endocrine-disrupting chemicals (EDCs) are exogenous agents, which, once absorbed, interfere with natural hormone functions such as hormone synthesis, secretion, binding, and elimination in hormonal pathways and organs. Evidence from previous studies suggest that the adverse impacts of EDCs on the endocrine system include diabetes, altered lipid metabolism, and changes in thyroid hormone levels and function. However, challenges remain in understanding these effects, including the interrelationships among chemical exposures and traditional risk factors including genetic predisposition; complex associations among lipid-soluble chemicals and lipid concentrations, and measuring collective effects of environmental factors as a mixture.

In this study, we aimed to explore the epidemiologic challenges identified above with the goal of expanding our understanding of endocrine disruption. First, we evaluated interactions between genetic polymorphisms and persistent organic pollutants (POPs) on hyperglycemic outcomes. We found associations of polygenic risk scores (PRS) for diabetes with incident overt diabetes and prediabetes, but not with POPs. However, we observed significant interactions between PRS and exposure to POPs, particularly among individuals with higher exposure to POPs. The adverse effects of polygenic risk of T2D on hyperglycemic outcomes were modified by greater exposure to POPs, which implied that managing modifiable risk factors, i.e. environmental exposures, can contribute to decrease risk of adverse health outcomes attributed to unmodifiable genetic risk.

Second, we assessed the associations between POPs and longitudinal changes in lipid profiles, to address potential reverse causality bias in cross-sectional study designs. We observed associations between POPs exposure and lower HDL cholesterol in longitudinal analyses, and the associations were attenuated after accounting for lipid-lowering medication use. Associations between POPs and total cholesterol, low-density lipoprotein cholesterol, and

triglycerides were observed in cross-sectional analyses. Our findings with longitudinal analyses deviated from cross-sectional analyses, which suggested that the results from cross-sectional analyses might be biased and emphasized the necessity of longitudinal studies in the area. It is also critical to considering the interrelationship among variables in the study design phase, especially considering the complex and multifactorial pathways underlying the associations between exposure and outcome.

Lastly, we investigated the association between exposure to multiple metals and thyroid hormones. We adopted quantile g-computation (QGCOMP), an advanced statistical method to assess the association of a mixture with outcomes, as well as linear regression models. We found monotonic and non-monotonic associations between individual metals and thyroid hormone profiles. Furthermore, we observed the associations of single metal with thyroid hormones may be modified after accounting for the concentrations of other metal exposures, which highlights the importance of extending traditional regressions with mixtures analysis in research of the health impacts of environmental pollutants.

We anticipate our findings will contribute to further understanding the associations of EDCs with health outcomes and current challenges in epidemiologic methods. From the standpoint of public health, our results emphasize the role of public health interventions to minimize exposure to environmental risk factors and guidance in public health.

I. INTRODUCTION AND BROAD OVERVIEW OF THE RESEARCH

A. Background

Endocrine-disrupting chemicals (EDCs) are exogenous agents that interfere with natural hormone functions comprising hormone synthesis, secretion, transport, binding, or elimination [1]. Once absorbed into the body, EDCs mimic, block, or interrupt the normal pathways between hormones and cells to growth, reproduction, development, energy balance and metabolism [2]. Target hormonal pathways of EDCs include, but are not limited to, estrogen receptor pathways, antiandrogen activity, thyroid hormones, retinoid receptors, and progesterone [3, 4].

Recent findings have linked EDCs with type 2 diabetes (T2D). Of particular interest are the persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and other organochlorine (OC) pesticides [5-10]. It is known that POPs persist in the environment and accumulate in human and animal adipose tissue, predominantly through contaminated food [6, 11], and have long half-lives in humans. Though production and import of POPs are banned in most developed countries, humans are still exposed to POPs from various sources such as contaminated fish, dairy products, high-fat meats, and deteriorated building materials. After exposure, POPs can disrupt endocrine systems in the body by interfering with insulin secretion/sensitivity or mimicking natural steroid, thyroid and other hormonal pathways, and leading to metabolic disorders including dyslipidemia and glucose intolerance [8, 12]. Though the biological mechanism underlying the POPs – T2D association is plausible, results from previous studies are inconclusive [6, 8, 13]. The inconsistent results and non-linear relationships from previous epidemiologic studies suggest the importance of considering the interaction or mediation effects among traditional risk factors such as caloric intake and genetic predisposition [14, 15]. In addition, complex correlations among POP exposures also remains a challenge.

Despite increasing interest in the potential interaction between genetic polymorphisms and environmental chemicals, few studies have focused on the effects of gene-environmental pollutant interactions on health outcomes. In regard to diabetes, the vast majority of gene-environment interaction studies have focused on gene-lifestyle interactions [15-18]. Many of the gene variants related to diabetes risk are likely to be involved in lipid metabolism, insulin resistance, and obesity [19]. Since POPs have also been linked to disruption of glucose and lipid metabolism and obesity [6], investigating the interactive effects of diabetes risk loci and POPs may expand the understanding of the etiology of diabetes. While most of the previous research on gene-environment interactions with POPs has focused on cancer [20-22], a recent study found a synergetic interaction between a genetic polymorphism and beta-hexachlorocylcohexane (β-HCH) that increased the risk of T2D [23]. In this study, we propose to investigate the relationships between the single nucleotide polymorphisms (SNPs) previously reported to be associated with T2D and serum-levels of POP concentrations.

Altered lipid synthesis such as high total cholesterol and low-density lipoprotein (LDL) cholesterol, low high-density lipoprotein (HDL) cholesterol, and high triglycerides (TG) is a risk factor for cardiovascular disease [24-27]. It has been also thought that dyslipidemia is involved in the development of T2D through disruption of β-cell function and insulin secretion [28-30], although debates are arising from genetic studies using Mendelian randomization that reported no association of low HDL cholesterol and high TG with T2D [31, 32]. Previous studies suggested that exposure to POPs may be involved in the altered lipid composition in blood. In animal studies, POPs were associated with elevated levels of TG and cholesterol [33, 34]. Epidemiologic evidence from human studies also have shown associations between PCBs, OC pesticides and lipid concentrations [35-41]. Therefore, exposure to POPs may also be linked to cardiovascular disease and diabetes through altered lipid metabolism; however, there are remaining challenges to fully discover the associations between POPs and lipid profiles, including understanding non-linear relationships of POPs with lipid concentrations [42, 43].

Moreover, due to the lipid-solublity of POPs, the association between POPs and lipid profiles is challenging to evaluate in cross-sectional studies that incur potential reverse causality [6]. As a first step to understanding the complex associations among cardiometabolic disease, POPs and dyslipidemia, here we aimed to investigate the relationship between POP concentrations and dyslipidemia in a prospective study setting. Assessing longitudinal effects of POPs and lipid profiles will contribute to understanding of potential causal relationships between POPs and lipids.

One of the challenges in evaluating the associations between risk factors and health outcomes is that human beings are exposed to multiple risk factors simultaneously. It is known that metals influence the endocrine system and induce adverse health outcomes through various mechanisms of action [44, 45]. As metals may have synergetic or antagonistic effects with one another, it is important to assess the associations between metal mixtures on health outcomes rather than individual associations of single metals [46-48]. Evaluating the effects of simultaneous exposure to multiple metals is complicated by non-additive and non-linear relationships between mixtures and health outcomes and multicollinearity among metals [49]. To overcome these problems, various approaches have been proposed including weighted quantile sum regression (WQS) [50], principal component analysis (PCA), Bayesian kernel machine regression (BKMR) [51], and most recently, quantile g-computation (QGCOMP) [52]. As a supplement to traditional regression modeling, those approaches aim to evaluate the effects of metals as mixture by dimensional variable selection. In this study, we adopted QGCOMP as well as multivariable linear regression models to identify metals contributing to perturbation of thyroid hormones.

B. Specific Aims

The overarching aim of the study is to assess the associations between environmental pollutants and disruptions in the endocrine system such as incident diabetes and prediabetes,

changes in lipid profiles, and thyroid hormone disruption. Our focus is to explore epidemiologic challenges identified in other investigations with the goal of expanding our understanding of endocrine disruption. To investigate the impact of environmental pollutants along with other risk factors, we will evaluate the interaction between genetic polymorphisms and persistent organic pollutants on hyperglycemic outcomes. To overcome potential reverse causality bias in cross sectional study designs, we will model the impact of POPs exposure on longitudinal changes in lipid profiles. To understand the interrelationships among environmental pollutants on endocrine outcomes, we will explore the association between exposure to multiple metals and thyroid hormones. To achieve this goal, three aims were proposed as follows:

Specific Aim 1 is to investigate interactions of diabetes-related genetic polymorphisms and POPs exposures on the incidence of hyperglycemic outcomes, and HOMA measurements at V2 in the Hispanic Community Health Study / Study of Latinos (HCHS/SOL). The levels of 24 PCB congeners and 3 OC pesticides from the HCHS/SOL ancillary study (PI: Persky) were used. For genetic polymorphisms, 118 index single nucleotide polymorphisms (SNPs) evaluated previously in HCHS/SOL as well as previous genome-wide association studies (GWAS) were used to construct polygenic risk scores (PRS) for T2D and included in the analysis. We looked at the main effects of POPs congeners and PRS related to T2D traits as well as the interaction effects between POPs and PRS.

Specific Aim 2 is to investigate the associations between POP exposures and changes in serum lipids in HCHS/SOL participants. The levels of POPs were obtained from the same ancillary study in Aim 1. Serum lipid profiles were measured at baseline and approximately 6 years later, including total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (TG).

Specific Aim 3 is to investigate the associations of exposure to individual metals and metal mixtures with levels of thyroid hormones in adults from the NHANES 2007-2012 dataset. We assessed the associations between 12 types of metals and 5 thyroid hormones. Using

traditional linear regression, we modeled associations of individual and multiple with thyroid hormone profiles and evaluated the shapes of the dose response curves. We adopted Quantile G-Computation to assess the relative effect size of each metal and overall effect of the metal mixture on thyroid hormones.

With the results from the proposed research, we anticipate expanding understanding of the complex relationships among environmental pollutants, genetic polymorphisms, lipid profiles and endocrine system function.

II. LITERATURE REVIEW

A. Type 2 Diabetes

Increasing incidence and prevalence of diabetes is a global public health concern. The incidence of type 2 diabetes (T2D) in the United States has doubled between 1980 and 2012, and T2D currently affects over 29 million Americans [53]. The prevalence of diabetes is expected to increase with greater longevity [54, 55] as well as the global spread of obesity [54]. Globally, over 550 million people are projected to have diabetes by 2030 [56]. In the United States, the age-adjusted percentage of diagnosed diabetes among adults was 8.7% in 2015, and 12% of the Hispanic population had diagnosed diabetes, which was the second highest prevalence following the African-American population [57].

Type 2 diabetes is the most common form of diabetes in adults. Unlike type 1 diabetes, which is insulin dependent, the etiology of T2D is more complex [58]. Diagnosis of T2D depends on circulating glucose concentration, but the development of hyperglycemia and subsequent T2D involves the reciprocal activity of insulin sensitivity/resistance and disrupted insulin secretion, and β-cell function. It has been thought that development of T2D initiates with insulin resistance, which results in abnormal insulin hypersecretion, subsequent β-cell dysfunction due to increased demand on the β-cell from the insulin-mediated glucose disposal, β-cell failure, and eventually type 2 diabetes [59]. Fatty acids have also been implicated in β-cell failure [60, 61]. Recently, however, several human studies reported that hyperresponsiveness and impaired β-cell function is involved in development of glucose intolerance, followed by a subsequent massive decrease of β-cell mass during the later stage of T2D pathology [62, 63]. Although the plasma insulin level is associated more with hyperglycemia than with obesity, obesity has a strong relationship with insulin resistance [64]. In particular, with central obesity, overexpressed inflammatory cytokines can prompt insulin resistance in the pathway to T2D development [64, 65]. Thus, investigations on the progression to T2D have encompassed

dyslipidemia, insulin resistance, beta cell dysfunction and hyperglycemia, and efforts to identify risk factors for T2D have been largely focused on lifestyle factors related to these traits, such as high-fat dietary pattern, sedentary lifestyle, lack of physical activity, and obesity [58, 66].

B. Persistent Organic Pollutants and T2D

Persistent organic pollutants (POPs) are a group of halogenated toxic chemicals including aldrin, chlordane, 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), dichloro-diphenyl-trichloroethane (DDT), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), mirex, dioxins and furans. Due to their uses in pest control, crop production, and industry, POPs have been widely used in various fields before most of them were banned in the 1970s in the United States. However, some countries in Central or South America had longer duration of POPs use, and therefore, populations originated in those countries may have higher levels of POPs in the body, particularly OC pesticides [67, 68]. Though many developed countries have banned production and import of POPs, humans are still exposed to POPs since they persist for a long period of time in the environment and bioaccumulate up the food chain. POPs are lipid soluble, and, therefore, are transported within the body while bound to lipids and accumulate in adipose tissue. This results in some POPs having half-lives of 10 years or longer.

Persistent organic pollutants (POPs) may adversely influence human health through multiple mechanisms, including altering lipid metabolism, disrupting glucose transport and the insulin signaling pathway, and impacting synthesis, metabolism, transport and action of steroid and thyroid hormone activities. However, the toxicological mechanisms through which POPs impact these health outcomes vary by chemical structure and may differ for parent compounds and their metabolites. For example, PCBs are a group of chemicals consisting of 209 congeners with differing combinations of chlorine atoms on the biphenyl rings, and with diverse health effects and mechanisms of action [69, 70]. PCB congeners can be classified in several ways, based on the chlorine substitution degree [71], degree of enzyme induction [72]

(McFarland 1989), and estrogenic or anti-estrogenic properties [73]. Proposed PCB classification schemes related to toxicological mechanisms of action have been summarized by Warner et al. [73].

Early research on relationships between POPs and T2D were conducted in occupational or accidental exposure settings. One of the earliest studies with POPs and T2D examined US Air Force veterans exposed to Agent Orange, which was contaminated with dioxin [74], and found higher exposure to the herbicide was associated with early onset of T2D in later life. The positive association was consistent in other veteran groups who were exposed to Agent Orange in Vietnam [75, 76]. Several studies that investigated the effects of POPs in occupational settings did not demonstrate associations between POPs and T2D outcomes [77-80]. Even inverse associations between POPs and T2D were observed in some studies, such as a study with US workers exposed to TCDD [81], and another study conducted in Germany with workers exposed to trichlorophenol [82]. Therefore, although populations in occupational settings were exposed to POPs in higher doses compared to the general population, the impact on diabetes was inconclusive.

While earlier studies were conducted in populations with high-dose exposures in relatively short-term exposure windows during war or occupational settings, later cross-sectional studies were performed among the general population with low to moderate dose and long-term exposure from predominantly dietary sources. In the National Health and Nutrition Examination Survey (NHANES) 1999-2002 survey cycles, a mixture of 6 POPs (PCBs, dioxin, oxychlordane, p,p′-DDT, and trans-nonachloride) was strongly associated with the prevalence of diabetes [5]. A study of Spanish adults also found that the sum of 4 PCB congeners (118, 138, 153, and 180) was positively associated with the prevalence of diabetes [83]. A recent study conducted in a South Asian population demonstrated 5-fold increased prevalence of T2D with participants who were exposed to higher levels of DDE, and more than 9-fold increased T2D prevalence among people with higher β-HCH concentrations [84].

Like other observational studies with cross-sectional designs, research focused on POPs and T2D are potentially subject to bias from reverse causality. Studies in cross-sectional settings assessed POPs as a risk factor for T2D, based on the hypothesis that elevated POPs concentration would disrupt the endocrine system and play a role in T2D development. However, if T2D disease progression altered the metabolism of POPs by slowing their excretion or increasing release of POPs from adipose tissue, prevalent T2D would appear to increase serum levels of POPs [85]. However, several lines of evidence reduce the likelihood that reverse causality is operative. These include prospective findings and an investigation that found the annual percent change of DDE and PCB congeners did not differ significantly between diabetic and non-diabetic participants [10]. Prospective studies have yielded somewhat inconsistent findings in terms of the types of POPs impacting diabetes incidence. In Turyk et al., associations between incident diabetes with POPs concentration among participants in The Great Lake Consortium were investigated. Higher DDE exposure was associated with increased risk of T2D development, whereas PCBs demonstrated null associations [10]. In the Nurses' Health Study, authors found up to a 3.1 fold increased risk of T2D among people with higher exposure to hexachlorobenzene, however, they did not observe statistically significant associations with PCBs, DDE, and DDT [86]. While those studies did not find any associations with PCBs and T2D, another study reported increased risk of T2D among people with higher concentrations of PCBs after 25 years of follow-up [87].

Some challenges in understanding the effects of POPs on T2D in epidemiologic research are related to the unique features of POPs. First, since POPs are lipophilic and accumulate in lipid-containing tissues, higher lipid concentrations in the body may play a role as a confounder, masking associations between POPs and health outcomes [6, 88]. On the other hand, since POPs are known to disrupt homeostatic mechanisms in metabolic and endocrine systems, elevated lipid levels might be a result in higher accumulation of POPs in the body [38] (see section E).

Second, POPs are always present as chemical mixtures. This is a particular issue among populations with low to modest exposure to POPs predominantly from dietary sources, compared to occupational or accidental exposures that may be dominated by a few POPs. Thus, investigation in isolation of a single POP does not reflect real life exposures, and is potentially misleading by ignoring synergistic or antagonistic interactions and confounding among different chemicals [6, 89]. This challenge is particularly critical considering that almost all human beings are exposed to at least minimal levels of POPs, therefore it is not possible to identify a control group without exposure to any POPs [6].

Third, non-linear and non-monotonic relationships between POPs and T2D also make it difficult to assess causal relationships between POPs exposure and T2D. In longitudinal studies conducted in the general population, non-linear relationships or inverted U-shape associations were found between POPs and T2D. A study investigated the relationships of PCBs, polybrominated biphenyls(PBBs), and OC pesticides with development of T2D in the Coronary Artery Risk Development in Young Adults (CARDIA) cohort, the highest risk was found in the second quartiles (OR=5.3) of 16 POPs, rather than the in highest quartile [7]. In another longitudinal study, the fourth quintile of 14 PCBs had a greater effect size (OR=8.8) compared to the fifth quintile (OR=7.7) [90].

C. Genes, Gene-Environmental Interactions and T2D

Genetic susceptibility has been widely explored for diabetes [19, 91]. It has been shown that heritability of T2D ranged from 20% to 80%, by family or twin studies [19]. Genes such as CAPN10 [92, 93] and TCF7L2 [94], which are involved in glucose metabolism, were identified through linkage studies. In candidate gene studies, genes already known to be involved in glucose metabolism, insulin secretion, insulin receptors, post-receptor signaling, and lipid metabolism were studied and additional genes associated with diabetes were identified, including PPARG, IRS1, IRS2, KCNJ11, WFS-1, and HNF families [95].

In large-scale genome-wide association studies (GWAS), more than 60 susceptibility loci have been identified for diabetes [96-101]. The mechanisms of the gene activity and etiology of diabetes have not been fully discovered, though many of the identified genes are related to β-cell dysfunction or insulin secretion. Interestingly, genes identified in linkage analyses or candidate gene studies that were involved in the insulin signaling pathway were rarely replicated in GWAS [94]. In addition, the weak effect size has remained an issue in establishing the effect of genetic components on diabetes risk. The odds ratios per allele of the identified variants is prone to be smaller than 1.3 [91, 94]. Furthermore, all the risk alleles across the susceptibility loci that have been identified to date only explain about 10% of the heritability of diabetes [94]. Under the circumstances, gene and environment interaction (GxE) studies have been performed in order to expand understanding the etiology of diabetes by evaluating the interdependent effects of genetic polymorphisms and other risk factors, such as lifestyle factors, and to a lesser extent environmental chemical exposures.

To date, the vast majority of research in GxE study with T2D-related genes or gene have focused on lifestyle factors [15-18]. A study explored the interactions between genes and multiple lifestyle factors, and demonstrated notable interaction effects between T2D genes and BMI and migration, as well as marginal effects with alcohol consumption and smoking [18]. Several studies have been conducted in light of gene-diet interactions. In NHANES, it appeared that dietary carbohydrates modified the associations between T2D-related SNPs (rs471253, rs8050136, rs1092398, and rs7961581) and T2D [15]. In the Nurses' Health Study, strong dose-response relationships between number of risk alleles in TCF7L2 gene and risk of T2D were observed in participants who had highest glycemic load and glycemic index. Among the people with highest glycemic load, having one more risk allele of TCF7L2 was associated with 1.62 times of OR for risk of T2D (95% CI=1.32-2.00). In the group of people with the highest glycemic index, having one more risk allele of TCF7L2 increased the risk of T2D by 1.5 times (95% CI=1.24-1.92) [16].

Physical activity has also been investigated in GxE research with T2D. In a large-scale meta-analysis with 218,666 adults and 19,268 children, physical activity attenuated the influence of the variants of FTO gene, a well-known gene associated with obesity and diabetes. The association of the FTO risk allele with the odds of obesity was attenuated by 27% in adults who performed active exercise [17]. In a large-scale cohort study in Sweden, physical activity modified the associations of polymorphisms on CDKN2A/B (rs10811661), HNF1B (rs4430796), and PPARG (rs1801282) with impaired glucose regulation [102]. In the study, rs4430896 on HNF1B also showed a significant interaction effect with physical activity in association with T2D. Another study demonstrated synergistic interaction effects between a physical activity intervention and protective genotype of CDKN2A on improved β-cell function [103].

In spite of emerging interests in the interactive effects between genetic polymorphisms and environmental chemicals, relatively few studies have focused on the effects of gene-environmental pollutant interactions on T2D. Some studies have been conducted to assess GxE effects with POPs, however outcomes of the studies were health conditions other than diabetes, such as bladder cancer, preterm birth, and endometriosis [20-22]. Though GxE with POPs and T2D has not been widely investigated, interactive effects with POPs on T2D is biologically plausible as the majority of the identified T2D variants are likely to be involved in lipid metabolism, insulin resistance, and obesity [19], and many endocrine-disrupting chemicals have also been linked to these pathways. A recent study found interactions between SNPs on ADIPOQ gene, which encodes adiponectin, a protein hormone which is secreted from adipose tissue, and β-HCH in association with increased the risk of T2D [23]. In the study, the number of risk alleles in three SNPs on ADIPOQ gene (rs182052, rs266729, and rs6810075) showed synergetic interactive effects with the level of β-HCB (interaction OR range=1.07-2.13). Data from a Swiss cohort study demonstrated that additive genetic scores constructed with risk alleles for 63 T2D-related SNPs modified the associations between the exposure to particulate matter (PM_{10}) and T2D risk. To date, the number of studies with environmental pollutants and

gene interaction are sparse, therefore more research is needed for deeper understanding in this field.

D. Polygenic Risk Scores

It has been pointed out that the effect of a single genetic variant is typically small for most chronic diseases, and therefore not sufficient to explain the effect of the genetic component on health outcomes [104]. Evidence from previous genome-wide association studies (GWAS) implies that the genetic contribution to complex disease is relatively consistent for variants with small to moderate effects, rather than the case of rare disease in which fewer variants contribute with substantial effects [105].

Detecting and assessing the comprehensive effects of genetic polymorphisms on complex disease has been explored in various ways [106, 107]. One of the most popular ways to overcome this challenge is use of polygenic risk scores (PRS), constructed through the combination of multiple risk variants by weighted or unweighted summation of the number of risk alleles [105, 108, 109]. The utilization of PRS has emerged to investigate the associations between genetic components and complex outcomes such as breast cancer [110-112], prostate cancer [113, 114], blood pressure [115, 116], diabetes [117-120], and mental illness [121-123].

Previous studies that investigated the relationship between PRS and T2D mostly reported positive associations. In Andersson et al. (2013), PRS constructed with 46 variants related to T2D showed positive associations between incident T2D (HR=1.06, 95% CI=1.03-1.08 per risk allele) and decreased β-cell function (b=-1.2, 95% CI=-1.7- -0.8 per risk allele), whereas no associations were noted with insulin sensitivity/resistance indices in a Danish cohort after 5-years follow-up [120]. In Imamura et al. (2013), PRS constructed with 49 susceptibility alleles for T2D were positively associated with T2D (OR=1.13, 95% CI=1.11-1.15 per risk allele) in a Japanese population [124]. In an analysis from the Framingham Offspring Study, PRS with 40 diabetes-related SNPs showed 1.11 times odds of T2D (95% CI=1.03-1.19) per one risk

allele increase [119]. In Qi et al. (2017), authors assessed the associations between PRS from 80 established T2D SNPs and prevalent T2D in HCHS/SOL participants and found a positive association (OR=1.07, 95% CI=1.06-1.09 per risk allele) [117].

E. Associations between POPs and Lipid Profiles

Altered lipid metabolism is involved in the onset of cardiovascular disease [24-27]. Epidemiologic literature suggests that exposure to POPs can result in alterations in serum lipids [39], which is supported by experimental studies in animal models. Bell et al. (1994) found an increase in TG but decreases in total cholesterol, HDL cholesterol, and LDL cholesterol with higher levels of plasma PCBs in female rhesus monkeys [125]. In a study by Lind et al. (2004), PCB 126 exposure in mice was associated with increased serum cholesterol levels, as well as other cardiovascular risk factors such as blood pressure and heart weight [126]. Another study showed altered lipid concentration in mice consuming salmon filets contaminated with OC pesticides, DDE/DDT, and PCBs, which resulted in POPs concentrations in the mice that were similar to those found in fish consuming human populations [33].

Cross sectional epidemiologic studies also suggest that POPs play a role in altered lipid metabolism. In Aminov et al. (2013), OC pesticides, HCB and OXYCHLOR, were associated with lower HDL cholesterol and elevated total lipids, total cholesterol, LDL cholesterol, and TG among residents in Anniston, Alabama. In addition, PCB concentrations were associated with elevated levels of total lipid, total cholesterol, and TG, but not with HDL or LDL cholesterol. In their results, the associations between PCBs and lipid profiles differed by the degree of chlorine substitution on the PCB congeners [35]. In Arrebola et al. (2014), HCB was associated with lower HDL cholesterol and PCBs 138 and 180 showed non-linear relationships with TG and total lipid concentrations, whereas PCB 153 was associated with LDL cholesterol levels among a population in southern Spain [36]. In a study among Canadian Inuit by Singh et al. (2018), PCBs were associated with hypercholesterolemia and elevated levels of TG, total cholesterol,

and LDL cholesterol [40]. Although these studies found somewhat consistent associations of PCBs and OC pesticides with lipid profiles, the findings might be subject to reverse causality bias due to the lipid soluble characteristics of POPs and cross-sectional design [6]. Goncharov et al. (2008) suggested an approach with structural equation modeling (SEM) to evaluate the causal pathway between 101 PCB congeners and 5 OC pesticides, lipid profiles, and heart disease and reported that the association between PCBs and heart disease was mediated by serum lipids [37].

Prospective studies also supported associations between POPs and lipid profiles. Lee et al. (2011) found inverted U-shape associations of PCBs with TG and HDL cholesterol, as well as positive associations between several OC pesticides and TG after an 18-year follow-up among diabetes-free young adult participants in the CARDIA study [38]. In Suarez-Lopez et al. (2019), a summary score of 23 PCBs and 8 OC pesticides were associated with total cholesterol, TG, LDL cholesterol, and cholesterol-HDL ratio in a 23-year follow-up of these CARDIA participants [41]. Penell et al. (2014) conducted a study with an elderly population from Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study and observed strong associations between PCBs 194, 206, 209 and levels of total cholesterol and LDL cholesterol, but not with TG after a 5-year follow-up [39]. In the PIVUS study, authors found inverse relationships between two OC pesticides, hexachlorobenzene (HCB) and trans-nonachlordane (TNONA), and LDL cholesterol. The source of inconsistencies in these two prospective cohorts is not clear but may be related to higher exposure levels in CARDIA, differing exposure mixtures or the very different age ranges of the populations.

Based on the literature, it is likely that some POPs play a role in altered lipid metabolism. However, there are inconsistencies in the literature that require further investigation. For example, PCB congeners or congener groupings as well as individual or grouped OC pesticides associations with lipid profiles differed by study. In addition, dietary fat intake may play a role as an important confounder considering it's a primary source of POPs exposure, however only a

few studies incorporated diet information in the analysis [39, 40]. Finally, most of the existing studies examined non-Hispanic populations. In Aminov et al. (2014), residents in Anniston, AL showed strikingly different levels of POPs by race/ethnicity, comparing African-Americans and Caucasians [127]. In the study, lipid concentrations also differ by race, showing lower TG and higher HDL cholesterol levels in African-Americans compared to Caucasians. The disparity in lipid levels among different racial/ethnic groups has been reported in another study [128]. Considering the racial difference with POPs and lipid levels, it would be worthwhile to conduct a study to assess PCB - lipid associations in Hispanic populations.

F. Thyroid Hormone Regulation and Disruption of Homeostasis

The thyroid is critically involved in the function of the nervous system, cardiovascular system, energy metabolism, and fetal development, and thyroid hormone levels are indicators of the function of the thyroid axis [129-132]. Thyroid hormones include triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH). The most active hormone is T3, which affects almost every tissue in the body, while T4 only has minimal effects. Production of thyroid hormones starts with a release of thyrotropin-releasing hormone (TRH) from the hypothalamus that prompts the anterior pituitary gland to release TSH, and TSH subsequently stimulates the thyroid gland to synthesize T3, 3,3’5’-triiodothyronine (rT3), and T4. The regulation of thyroid hormone production is known as a negative feedback loop, as lower blood concentration of T3 and T4 stimulates the release of TSH, whereas higher concentration of T3 and T4 suppresses TSH production [133]. Thyroid hormones are transported to peripheral tissues bound to proteins such as albumin, thyroxine binding globulin, and transthyretin, with less than 1% of the hormones remaining unbound (free forms of T3 and T4 ; i.e. FT3 and FT4) and biologically active [134]. Predominantly at the tissue level, thyroid hormone activity is controlled by deiodinase enzymes that transform T4 to either biologically active T3 or inactive rT3 [135].

Disrupted thyroid homeostasis has been associated with altered neural differentiation followed by cognitive deficits [136, 137], and metabolic problems such as elevated blood pressure [138] and dyslipidemia [139]. Thyroid hormones affect metabolic pathways involved in energy storage and expenditure through their activities in brain, adipose tissue, liver, pancreas, and skeletal muscle [140-143]. Excess T3 and T4 concentrations, or hyperthyroidism, results in decreased cholesterol levels, fat and weight loss, increased lipolysis, increased glucose absorption, and increased energy expenditure [144-146]. Decreased T3 and T4 levels, or hypothyroidism, can cause increased cholesterol levels, weight gain, decreased lipolysis, reduced glucose absorption, and reduced energy expenditure [146]. In NHANES III, the prevalence of hypothyroidism and hyperthyroidism was 4.6% and 1.3%, respectively [147, 148]. Worldwide, the prevalence of subclinical hypothyroidism ranges from 4% to 8.5%, and the prevalence of subclinical hyperthyroidism is up to 2% [148]. The prevalence of thyroid conditions is different by demographic characteristics. Generally, the prevalence is higher in females than males, increased with age, and higher in non-Hispanic whites and Mexican Americans compared to non-Hispanic blacks [147, 149-151]. In addition, serum TSH levels and prevalence of antithyroid antibodies were higher in females than males, especially in ages > 50 years [147].

The prevalence of thyroid hormone disruption is higher in individuals with T2D. [152-154]. It is well established that thyroid hormones affect insulin secretion. Elevated concentrations of thyroid hormones increase 1) non-oxidative glucose disposal which prompts lactate production, 2) GLUT2 activation on liver, and 3) lipolysis which releases free fatty acids stimulating hepatic glucose output and leads to hyperglycemia, which in turn induces hyperinsulinemia [155, 156]. The sequential activity of excess thyroid hormones can play a role in development of T2D from a subclinical stage, or amplification of hyperglycemia among T2D patients [148]. On the other hand, lower thyroid hormones are involved in decreased glucose

disposal and impaired glucose absorption, which lead to disrupted hepatic glucose release. This mechanism contributes to reduced insulin synthesis and subsequent hypoglycemia [155, 157].

Environmental factors are known to be associated with disrupted thyroid hormones and increased risk of thyroid disease [143, 158-160]. EDCs such as POPs can interfere with the natural thyroid hormones pathways through multiple mechanisms, such as disrupting thyroid hormone binding to carrier proteins, interrupting conversion of T4 to T3 by up-regulating or inhibiting the production of deiodinases, enhancing hepatic metabolism, and playing a role as an thyroid hormone receptor agonist or antagonist [161-164]. In epidemiology studies, the levels of total and free T3 and T4 as well as TSH are commonly studied for correlation with suspected EDCs. From these studies we can infer if the EDC impacts peripheral and/or central hormone levels, but the mechanism through which the EDC acts is not evident. For example, elevated peripheral hormones could be a consequence of perturbed synthesis, transport, activation or metabolism. Less frequently, indicators of thyroid function are investigated, such as the ratio of T3:T4 that may be suggest an impact of the EDC on deiodinase activity or the FT4:TSH ratio that could be altered by a disruption of the negative feedback loop of the HPT axis.

G. Metals and Thyroid Hormones

Previous research on the impact of metals on endogenous thyroid hormones has been mostly focused on heavy metals such as arsenic, cadmium, lead, and mercury [134, 165-167]. Arsenic is a ubiquitous environmental contaminant, with more than 17 million people in the US estimated to be exposed to elevated concentrations of arsenic in their drinking water [168]. Although arsenic concentrations in water in the US are relatively low compared to some other countries, previous studies have reported low to moderate levels of exposure to arsenic can still confer an increased risk of cancer [169-171], preterm birth [172], high blood pressure [173], and diabetes [174-176]. Previous studies evaluated associations between arsenic concentration and thyroid hormone levels [166, 177-179]. Across the studies, arsenic concentration was inversely

associated with FT3 [165, 177, 179]. Interestingly, different directions by sex were noted in the association between arsenic and TSH. A study conducted among policemen in rural and urban areas reported positive associations between arsenic concentration and TSH [177], and the positive direction was supported by a study among males [166]. In a study with data from NHANES 2007-2010, arsenic was inversely associated with TSH levels in men, however the association between arsenic and TSH was positive in females [178].

Cadmium is a heavy metal that has been classified as a known human carcinogen by IARC [180, 181], and as a probable human carcinogen by U.S. Environmental Protection Agency (EPA) [182]. With a long half-life, ranging from 10 to 30 years, cadmium accumulates mostly in liver tissues by binding to metallothionein and transfers to the kidney [180, 183]. Cadmium is measured both in urine and blood, and urinary excreted cadmium reflects relatively long-term exposure, whereas blood cadmium is a better indicator for recent exposure [184]. It is known that females are prone to show higher cadmium levels compared to males due to lack of iron which results in increased absorption of cadmium [185, 186]. Studies of associations between cadmium and thyroid hormone levels presented somewhat inconsistent findings [177, 187-189]. In studies with NHANES 2007-2008 data, blood cadmium was inversely associated with TSH [187], and both urinary and blood cadmium showed positively associations with T3, FT3, T4, and FT4 [187, 189]. Among policemen in rural and urban areas, urinary cadmium was associated with lower FT3 and T4 but elevated TSH [177]. In a study with newborns in Japan, cadmium levels in cord blood was inversely associated with TSH concentration in neonatal blood [188]. Associations between cadmium and cardiovascular disease have also been investigated [190-193], based on observed promotion of atherosclerosis by cadmium [180].

Lead circulates mostly bound to erythrocytes and 90% of lead accumulates in bone, while the remainder stays in soft tissues. Exposure to lead is known to play a role in adverse cognitive function and affects multiple organs such as kidney, liver, and gastrointestinal tract [194, 195]. It is also known that lead is associated with endocrine system disruption through its

effects on adrenal, reproductive and pituitary functions [196]. Although many studies investigated the association between lead concentration and thyroid hormones, the relationships have not been consistent [197]. Observed effects of lead exposure vary by the type of thyroid hormones. In a study investigating associations between blood lead and TSH levels in men, higher lead level was inversely associated with TSH [166]. A birth cohort study in China reported positive associations between lead and FT3 [179], which was consistent with results from NHANES data [187]. In NHANES, a positive association was also observed with lead and T4 [196].

Mercury is a heavy metal which exists in various forms in the environment. For humans, the most common route of exposure is methylmercury from fish, but other foods such as meat and vegetables also can be sources of exposure. [198]. The toxic effects of mercury vary by multiple conditions such as level of exposure, duration of exposure, and age [199]. Major target organs for mercury include central nervous system and kidney, however exposure to mercury can affect various organs including gastrointestinal tract, respiratory system, and thyroid gland [200]. Mercury is also related to adverse neurodevelopmental outcomes due to its high permeability through placenta and blood-brain barrier, and its long-term retention [201, 202]. Predominantly inverse associations have been reported between mercury exposure and various thyroid hormones. Studies with data from NHANES reported inverse associations between mercury and T3, FT3, and T4 in adults [187, 189] and a positive association between elevated mercury concentration and thyroid antibody positivity, a long-term indicator of decreased thyroid hormones [203]. In research with pregnant females, mercury concentration showed negative associations with T3 and FT4 [204, 205]. Research suggests that mercury can be accumulated in thyroid glands with long-term exposure, however the mechanism of action underlying the mercury-thyroid relationship is not fully understood [200].

Fewer studies have assessed associations between other metals and thyroid hormones. Mendy et al. (2012) reported negative associations between thallium and thyroid hormone disruption among US adults from NHANES data [180]. Yorita Christensen (2013) investigated 11 metals in blood and urine from NHANES. In the study, barium and tungsten showed positive associations with FT3 and TSH, respectively, whereas cesium and thallium appeared to have negative associations with TSH and T4, respectively [187]. Cesium was also inversely correlated with FT3 and T3 in a mother-child cohort study in Argentina [206]. In a study with 1644 Chinese females, Guo et al. (2018) reported that manganese and molybdenum were inversely associated with FT4 [165].

The limitations of studies focusing on individual metals discussed previously in this document also applies to investigations of individual metals on thyroid function. Additional investigations on metal mixtures, such as that by Meeker et al are warranted. Meeker et al. (2009) explored associations between 11 metals and TSH levels in males, assessing individual associations of each metal and collective associations by including all 11 metals in a multivariable regression model. Results of analyzing the 11 metals as a mixture, revealed associations of arsenic, copper, and lead with TSH, whereas lead was the only metal that showed a significant association with TSH in single exposure models [166].

H. Methods to Assess Environmental Mixtures on Health Outcomes

One of the challenges in environmental epidemiology is that human beings are exposed to multiple environmental pollutants, and the toxicity of the pollutants can vary by different exposure profiles [207, 208]. Several methods have been proposed to address the effects of multiple exposures on health outcomes, such as environment-wide association studies (EWAS), pairwise correlation comparisons between multiple pollutants [209, 210]. However, these methods still raised issues in analysis of environmental pollutants linked to health outcomes, such as non-linear and non-additive relationships between multiple exposures and outcome,

poor goodness-of-fit of the model due to the higher number of exposures in one model, and correlation and collinearity among the multiple exposures [210, 211].

To overcome the challenges, several approaches have been adopted and developed to address the complex relationships among multiple pollutants [50, 211-213]. Principal component analysis (PCA) is one of the most well-known dimension reduction methods [70], and has been adopted to environmental research to identify metal sources [214, 215] and demonstrate patterns among biomarkers of environmental exposure [216-219]. For example, Neta et al. (2010) adopted PCA to identify pesticide mixtures in cord serum among newborns, to address the correlations among 12 pesticides. In the study, authors identified two predominant components; the first was an organochlorine component, with greater loadings from p,p'-DDE, p,p'-DDT, hexachlorobenzene, and β-hexachlorocyclohexane, and the other component was a chlordane component consisted with trans-nonachlor and oxychlordane [218]. In Pang et al. (2016), nine metals were investigated in two cohort studies and PCA identified that metal clusters varied by each population cohort [219].

Weighted quantile sum (WQS) regression, proposed by Carrico et al. (2014) for estimating the most contributing factor among highly correlated components, has been adopted to environmental research on chemical and metal mixtures in association with health outcomes. WQS estimates a single weighted index, by taking into account the overall exposure to multiple pollutants in the same direction of association, and assesses the contribution of each pollutant to the mixture [50]. In the first stage of WQS, each component is scored into quantiles and combined into an index as following:

$$WQS = \sum_{i=1}^{j} w_i q_i \,,$$

where w_i is the weight for the i th component (out of j components) and q_i is a quantile-transformed component. The weight is estimated from bootstrap samples of the WQS model, by

taking the mean weight. The weights across $w_1 - w_j$ are forced to sum to 1 and have the same direction (directional homogeneity), and the magnitude is the "contribution to outcome" of each component. In the second stage, the weight is fitted into a regression model as following:

$$Y = \beta_0 + \beta_1 WQS + \epsilon_i ,$$

where β_0 is intercept and β_1 is the overall effect of exposure to multiple components.

Weighted quantile sum (WQS) regression approach has been broadly applied in studies with metal and organic chemical mixtures and various health outcomes including cancer, allergy symptoms, and thyroid hormone profiles [210, 220-222]. One of the most recent studies investigated prenatal urinary phthalate metabolite exposure and thyroid hormones and adopted WQS to assess the combined impact of phthalate metabolites, finding inverse associations between the overall phthalate index and thyroxine and thyroid stimulating hormone [222].

It is useful to adopt WQS to evaluate the effects of multiple components as a mixture, however it is not able to assess exposure relationships in different directions (positive or negative) since it assumes directional homogeneity among the components [210]. Quantile g-computation (QGCOMP) was introduced to overcome this challenge, using a generalized version of WQS and relaxing the directional homogeneity assumption [212]. In QGCOMP, the initial steps follow the same steps as WQS, however, when the directional homogeneity assumption is violated, it reclassifies the weights into either positive or negative weights. Those redefined weights represent the proportion of the partial positive or partial negative effects due to a certain component. By relaxing the directional homogeneity assumption and addressing both directions in one model, QGCOMP provides more robust and comprehensive estimates with multiple exposures. Several studies have adopted QGCOMP to evaluate the mixtures effects on health outcomes [220, 223, 224]. In Niehoff et al. (2020), the authors evaluated associations between individual metals and metal mixture with BMI, adopting QGCOMP to

address the metal mixture. In the results, a quantile-increase of metal mixture was associated with higher BMI (β for mixture=0.32, 95% CI=0.00-0.63), while no individual metals presented strong associations [223].

The main strength of WQS and QGCOMP is taking account of effects of multiple components and estimating the effect size of the mixture on the outcome of interest, as well as identifying the largest contributing factor among the multiple exposures. It is particularly useful considering that analysis focusing on a single exposure may be confounded or modified by other exposures, while concurrent modeling of strongly associated exposures may be subject to collinearity impacts. However, certain limitations also exist with those approaches. Both WQS and QGCOMP assume linear relationships between the exposure and outcome, and do not address potential non-linear relationships. Furthermore, the regression models with WQS and QGCOMP approach assume no interaction effects among the correlated exposures [50], therefore adding an interaction term in the model would provide an unreliable weights when the interaction term includes main exposure component [212].

III. GENE-ENVIRONMENT INTERACTION BETWEEN DIABETES-RELATED POLYGENIC RISK SCORES AND PERSISTENT ORGANIC POLLUTANTS ON HYPERGLYCEMIC OUTCOMES IN HISPANIC COMMUNITY HEALTH STUDY / STUDY OF LATINOS

A. Introduction

The rapid increase in diabetes is a global public health concern. In the United States, age-adjusted prevalence of diagnosed diabetes among adults was 8.7% in 2015 (1). Overall, 12% of the Hispanic population had diagnosed diabetes, which was exceeded only in the African-American population [57]. Diagnosis of T2D is based on elevated blood glucose concentration. In the development of T2D, reciprocal activity of insulin sensitivity/resistance and disrupted insulin secretion results in hyperglycemia. Initially, insulin resistance results in abnormal insulin secretion. Subsequently β-cell dysfunction develops due to increased demand on the β-cell from the insulin-mediated glucose disposal, and eventually leads to T2D. Fatty acids and cholesterol may also affect the β-cell failure [60, 61]. Type 2 diabetes (T2D), the most common form of diabetes in adults, has a complex etiology [58]. Standard risk factors for diabetes, such as obesity, sedentary lifestyle and age, are well established [58, 66].

Recent findings have linked environmental pollutants with T2D. Of particular interest are the persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and other organochlorine (OC) pesticides. Persistent organic pollutants (POPs) persist in the environment and accumulate in human and animal adipose tissues, predominantly through contaminated food [6, 11]. Many POPs have half-lives of 10 years or longer in humans, resulting in elevated exposures in older populations. In the National Health and Nutrition Examination Survey (NHANES) 1999-2002 survey cycles, a mixture of POPs was strongly associated with the prevalence of diabetes [5]. However, this study and many others of similar cross-sectional design are potentially subject to reverse causality. To

overcome this challenge, prospective studies have been conducted that support a role of POPs in the etiology of diabetes (11-15). However, challenges remain due to the somewhat inconsistent epidemiologic findings and complexity of correlations among different kinds of POPs. Variations in distributions of POPs mixture across populations and the non-linear relationship between POPs and risk of diabetes are also major issues to be overcome [6].

Genetic susceptibility has also been widely explored for diabetes [19, 91]. Genes such as CAPN10 [92, 93] and TCF7L2 [94], which are involved in glucose metabolism, were identified through linkage studies. In candidate gene studies, genes already known to be involved in glucose metabolism, insulin secretion, insulin receptors, post-receptor signaling, and lipid metabolism were studied and additional genes associated with diabetes were identified, including PPARG, IRS1, IRS2, KCNJ11, WFS-1, and HNF families [95]. In large-scale genome-wide association studies (GWAS), more than 80 susceptibility loci have been identified for diabetes [96-101]. The mechanisms of the gene activity and etiology of diabetes have not been fully discovered, though many of the identified genes are related to beta cell dysfunction or insulin secretion. Interestingly, genes identified in linkage analyses or candidate gene studies that were involved in the insulin signaling pathway were rarely replicated in GWAS [94]. In addition, the weak effect size has remained an issue in establishing the effect of genetic components on diabetes risk. The odds ratios per allele of the identified variants is prone to be smaller than 1.3 [91, 94]. Furthermore, all the risk alleles across the susceptibility loci that have been identified to date only explain about 10% of the heritability of diabetes [94]. Under the circumstances, gene and environment interaction (GxE) studies would expand the understanding of the etiology of diabetes by evaluating the interdependent effects of genetic polymorphisms and environmental chemicals. In spite of emerging interests of the interactive effects between genetic polymorphisms and environmental chemicals, relatively few studies have focused on the effects of gene-environmental pollutant interaction on health outcomes. In regards to diabetes, the vast majority of gene-environment interaction studies have been

performed in light of gene-lifestyle interactions. Among diabetes risk variants, many are likely to be involved in lipid metabolism, insulin resistance, and obesity [19], while POPs have also been linked to disruption of glucose and lipid metabolism and obesity. Thus, investigating the interactive effects of diabetes risk loci and POPs would expand the understanding of the etiology of diabetes. While most of the previous research on gene-environment interactions with POPs have been performed in association with cancer [20-22], a recent study found a synergetic interaction between a SNP on ADIPOQ (rs182052) and β-HCB that increased the risk of T2D in a Chinese population [23].

We investigated the relationships between the SNPs previously reported in association with T2D and serum-levels of POPs concentrations. Interaction effects between susceptible loci and concentration of POPs on diabetes-related traits will be evaluated, as well as main effects of genetic polymorphisms and POPs on diabetes.

B. Methods

1. Data and Study Participants

Study participants were from Hispanic Community Health Study/Study of Latinos (HCHS/SOL). The HCHS/SOL is a multi-center epidemiologic study in Hispanics/Latinos, aiming to evaluate the effects of acculturation on health outcomes and to identify factors that influence the health of the Hispanic/Latino population [225, 226]. The baseline information (Visit 1; V1) was obtained during 2008-2011 with 16,415 multiethnic Hispanic participants aged 18-74 years, in 4 US cities including Chicago, Miami, New York, and San Diego. The first re-examination (Visit 2; V2) of the cohort was performed during 2015-2017.

For this research, baseline blood samples from 2,350 HCHS/SOL participants in the ancillary study “Persistent Organic Pollutants, Endogenous Hormones and Diabetes in Latinos (PI: Persky)” were included. The 2,350 participants of the ancillary study were stratified by baseline (V1) glucose levels, which resulted in 1,175 subjects with prediabetes and 1,175 with

normal glucose levels at baseline (V1), and the participants with prediabetes who transitioned to diabetes during the follow up were oversampled to ensure that approximately half of participants in this category had transitioned.

The 2,350 participants were equally divided by sex. Besides the stratification by baseline glucose and sex, only other inclusion criterion for the ancillary study was returning for 6-year follow-up visit (V2) and provide informed consent. Participants who did not provide consent, had diabetes at V1, aged <45 or >74, no lipid measurements at V1 were excluded from the selection of the ancillary study. In addition, the ancillary study included only one participant per each household.

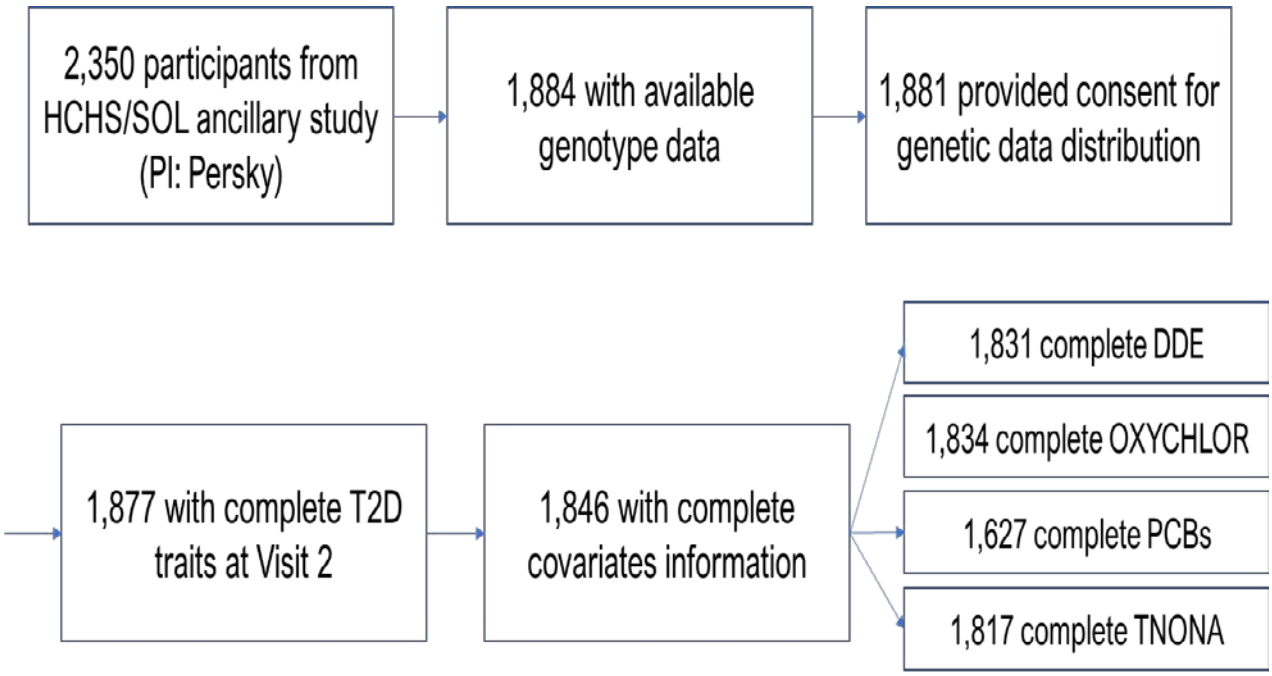

Figure 1. Selection of study participants for Aim 1

Among 2,350 participants, 1,884 had genotype data and 1,881 provided informed consent for genetic data distribution. We excluded participants with missing data for their status of diabetes or prediabetes at V2 (n=4), missing blood samples (n=15), missing demographic or clinical variables which were included analysis (n=16).

For each POP analysis, only participants with complete information of the POP were included. The numbers of participants with complete data for each POP are listed in Table 1. All participants provided written informed consent and the protocol of the study was reviewed by Institutional Review Board at UIC.

2. Assessment of Outcomes

Diabetes was identified if a participant had any of the following traits: fasting glucose ≥126 mg/dL with fasting time > 8 hours, fasting glucose ≥ 200mg/dL with fasting time ≤ 8 hours, glucose ≥ 200 mg/dL with 2-hour oral glucose tolerance test, HbA1c ≥6.5%, or use of antihyperglycemic medication. Prediabetes was defined as fasting glucose 100-125 mg/dL, glucose 140-199 mg/dL with oral glucose tolerance test, or HbA1c 5.7%-6.4%, with no other measurements consistent with diabetes.

In order to assess the effects of genetic polymorphisms and POPs on each etiologic stage of diabetes progression, we set our outcome of interest for status of diabetes into 4 categories: normal glucose or prediabetes (V1) to diabetes (V2), normal glucose (V1) to prediabetes (V2), normal glucose (V1) to hyperglycemia (prediabetes or diabetes at V2), and prediabetes (V1) to diabetes (V2).

We also included HOMA-IR and HOMA-B at V2 as outcomes, in order to assess the effects of genetic and environmental factors on stages of diabetes etiology such as insulin resistance and β-cell dysfunction. Natural log transformed HOMA measurements were used in the statistical models.

3. Measurement of POPs

In the ancillary study, POPs comprising 24 PCBs, 11 BFRs, and 8 OC pesticides were measured on 1.0 ml plasma samples from 2,350 participants. The measurement of POPs was performed at the CDC laboratory (PI: Dr. Sjodin), adopting automatic fortification of the samples with a Gilson 215 liquid handler (Gilson Inc., Middleton, WI). The laboratory methodology for sample processing was described elsewhere [227]. In this analysis, we employed lipid-standardized POPs, and the concentration of each POP was presented as ng/g lipid weight from the formula below [228]:

$$lipid-adjusted\ POP\ (ppb) = \frac{wet-weight\ POP\ (ppt)}{total\ lipid\ (mg/dl)} * 102.6$$

, where total lipid was calculated using the equation suggested in Bernert et at al. (2007) [229]:

$$total\ lipids(mg/dl) = 2.27 * total\ cholesterol + triglyceride + 62.3\ .$$

Levels of POPs below limit of detection (LOD) were replaced by values of the LOD divided by squared root of 2. The LOD varied for each POP and a unique LOD was calculated for each individual sample by the CDC laboratory.

Twenty-seven POPs comprising 24 PCBs and 3 OC pesticides were included in the current study. In the analysis, we used a summary measurement of PCBs (sum PCB) by summing up the 24 individual PCB congeners. Three OC pesticides were analyzed individually. For all POP measurements (sum PCBs and 3 types of OC pesticides), natural log-transformed values were used in the analysis. The types and characteristics of PCBs and OC pesticides included in the analysis were summarized in Table I.

4. Genotyping and Imputation

For genotyping in the HCHS/SOL, an Illumina custom array (15041502 B3, SOL HCHS Custom) was employed, comprising the Illumina Omni 2.5M array (HumanOmni2.5-8v1-1) and selected custom SNPs (~150,000).

The quality control (QC) of genotype data was conducted by the HCHS/SOL Genetic Analysis Center, and resulted in a set of 2,232,944 SNPs that passed the QC. The detailed procedure of QC was described elsewhere [230]. Genotype imputation was conducted using the 1000 Genomes Project phase 1 reference panel, with SHAPEIT2 (v.2.r644) for pre-phasing and IMPUTE2 (v.2.3.0) for imputation [230].

5. Polygenic Risk Score with 118 T2D-Related SNPs

We selected 118 single nucleotide polymorphisms (SNPs) related to T2D from previous genome-wide association studies (GWAS) [100, 231-239]. The SNPs were further subclassified into insulin-resistance related SNPs (18 SNPs) and β-cell dysfunction related SNPs (37 SNPs), based on the potential biological mechanisms of the SNPs [117, 240].

Selected SNPs are listed in Table II. Across the 118 SNPs, we constructed unweighted polygenic risk score (PRS) for each participant summing the dosages of risk alleles. For example, 0, 1, and 2 were assigned to participants with GG, AG, and AA genotypes with rs67156297, respectively, as A is considered as a risk allele for the SNP.

Therefore, theoretical range of the scores would be 0 to 236, since, if a participant had no risk alleles across the 118 SNPs, PRS for the participant was assigned as zero (0 x 118), and 236 (2 x 118) will be the maximum value if a participant had two risk alleles for every SNP. Three types of PRS were constructed, including total PRS (118 SNPs), insulin resistance PRS (18 SNPs), and β-cell dysfunction SNPs (37 SNPs).

6. Statistical Analysis

Descriptive statistics were obtained for participants' characteristics at V1. Participants' basic characteristics such as age, Hispanic/Latino background, education attainment, alcohol and cigarette use, physical activity level, family history of T2D, body mass index (BMI), as well as three PRS (total PRS, insulin resistance PRS, and β-cell dysfunction PRS) were included in descriptive analysis. We also demonstrated the distribution of T2D traits (incident T2D, normal to prediabetes, prediabetes to diabetes, HOMA-IR, and HOMA-B) at V2.

To evaluate the main effects of PRS and POPs on T2D, we separately examined the associations between PRS and T2D traits, and POPs and T2D traits in multivariable regression models. To evaluate potential non-linear relationships between PRS and T2D traits, we used tertiles of each PRS variable and lowest tertile (i.e. smaller number of risk alleles) was used as the reference group. Dose-response relationships between tertiles of PRS and diabetes outcomes at V2 were examined and p-values for trend were obtained from the multivariable models.

In the models for POPs-T2D associations, we categorized the POPs into two groups considering the right-skewed distribution of each POP, specifically one group with POPs less than the 75^{th} percentile (POP < Q4, reference group) and the other group with POPs greater than the 75^{th} percentile (POP ≥ Q4, comparison group). Odds ratio (OR) and 95% confidence intervals (CI) were obtained from logistic regression models for binary outcomes comprising incident T2D, normal to prediabetes, and prediabetes to diabetes. Beta estimates and 95% CI were obtained from linear regression models for HOMA-IR and HOMA-B.

For main effect models, we assessed the exposure-outcome relationships in two different models. In reduced models, we included the first 5 principal components (PCs) for Hispanic/Latino background [117] and study center. The 5 PCs were obtained from the whole genetic data from HCHS/SOL study to cluster the genetic ancestry of study participants [117]. Full models were adjusted for age, sex, first 5 PCs, education (less than high school graduate,

high school graduate, and greater than high school graduate), alcohol (no current use, low level use with less than 7 drinks/week, and high level use with 7+ drinks/week of alcohol) and cigarette use (never smoked, former smoker, and current smoker), physical activity (high, moderate, and low level of activity defined by levels of work and recreational physical activity and total metabolic equivalent values), family history of T2D, BMI (kg/m^2), and study center (Bronx, Chicago, Miami, and San Diego). In the models for POPs and incident T2D, baseline status of glucose levels (i.e. normoglycemic or prediabetic) was added as a covariate. For models with POPs and HOMA measurements at V2, measurements at V1 were included in the models.

Interaction effects between PRS and POPs were also investigated in multivariable linear or logistic regression models. To evaluate the consistency of PRS-T2D associations by different POP levels, we stratified the PRS-T2D models by the two POP groups (POP < and ≥ Q4). In each stratified model, the lowest tertiles of PRS were used as reference group and compared with estimates from the second and third tertiles. We obtained ORs and 95% CI, or beta estimates and 95% CI, and p-values for interaction effects were calculated with likelihood ratio tests for binary outcomes and F-tests for continuous outcomes, respectively. In the interaction analysis, models were adjusted for age, sex, first 5 PCs, education, alcohol and cigarette use, physical activity, family history of T2D, BMI, and study center, as well as baseline diabetes status and HOMA measurements when appropriate.

Subgroup analyses with insulin resistance PRS and β-cell dysfunction PRS were performed in main effect and interaction analyses. To incorporate the survey design of HCHS/SOL study, sampling weights for the ancillary study subgroup were applied for all analyses using PROC SURVEY modules in SAS 9.4 (Cary, NC). We also conducted sensitivity analyses using POPs unadjusted for serum lipids (wet-weight POPs).

TABLE I. CHARACTERISTICS OF SELECTED POPS IN STUDY PARTICIPANTS

Analyte (ng/g lipid)	Full name	Sample N	< LOD (%)	Median (IQR)	min/max
Sum PCB	-	1627	-	127.97 (120.93)	7.04/1865.82
PCB28	2,4,4'-Trichlorobiphenyl	1627	14.87	0.84 (1.00)	0.11/309.10
PCB66	2,3',4,4'-Tetrachlorobiphenyl	1627	31.10	0.50 (0.67)	0.09/140.40
PCB74	2,4,4',5-Tetrachlorobiphenyl	1627	0.06	3.28 (3.61)	0.28/850.30
PCB99	2,2'4,4'5-Pentachlorobiphenyl	1627	0.49	3.38 (3.34)	0.13/91.30
PCB105	2,3,3'4,4'-Pentachlorobiphenyl	1627	9.83	1.20 (1.50)	0.10/48.26
PCB114	2,3,4,4',5-Pentachlorobiphenyl	1627	36.63	0.45 (0.51)	0.05/10.71
PCB118	2,3'4,4'5-Pentachlorobiphenyl	1627	0.06	6.03 (7.36)	0.28/141.80
PCB138158	2,2,3,4,4',5'- and 2,3,3'4,4',6-Hexachlorobiphenyl	1627	0.12	15.18 (15.15)	0.23/223.30
PCB146	2,2',3,4',5,5'-Hexachlorobiphenyl	1627	0.49	3.12 (2.80)	0.13/66.02
PCB153	2,2',4,4',5,5'-Hexachlorobiphenyl	1627	0.00	26.78 (23.84)	1.02/397.50
PCB156	2,3,3',4,4',5-Hexachlorobiphenyl	1627	0.00	3.41 (3.07)	0.13/29.68
PCB157	2,3,3',4,4',5'-Hexachlorobiphenyl	1627	16.23	0.74 (0.72)	0.07/7.50
PCB167	2,3',4,4',5,5',-Hexachlorobiphenyl	1627	11.19	0.92 (0.92)	0.05/13.35
PCB170	2,2',3,3',4,4',5-Heptachlorobiphenyl	1627	0.00	7.79 (6.29)	0.34/60.89
PCB178	2,2',3,3',5,5',6-Heptachlorobiphenyl	1627	4.86	1.51 (1.34)	0.11/22.15
PCB180	2,2',3,4,4',5,5',-Heptachlorobiphenyl	1627	0.00	20.39 (17.84)	0.75/161.90
PCB183	2,2',3,4,4',5',6-Heptachlorobiphenyl	1627	2.27	1.99 (1.77)	0.13/31.33
PCB187	2,2',3,4',5,5',6-Heptachlorobiphenyl	1627	0.06	6.60 (6.18)	0.20/122.00
PCB189	2,3,3',4,4',5,5',-Heptachlorobiphenyl	1627	46.96	0.36 (0.29)	0.07/2.47
PCB194	2,2',3,3',4,4',5,5',-Octachlorobiphenyl	1627	0.37	4.14 (4.20)	0.19/33.95
PCB196203	2,2',3,3',4,4',5',6- and 2,2',3,4,4',5,5',6-Octachlorobiphenyl	1627	0.25	4.06 (4.05)	0.22/46.43
PCB199	2,2',3,3',4,5,6,6',-Octachlorobiphenyl	1627	0.43	4.71 (5.14)	0.13/52.36
PCB206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	1627	1.35	2.34 (2.66)	0.13/32.49
PCB209	Decachlorobiphenyl	1627	0.00	1.48 (1.24)	0.00/38.14
PP-DDE	2,2-Bis(4-chlorophenyl)-1, 1-dichloroethene	1831	0.00	577.81 (1024.25)	4.07 /15060
OXYCHLOR	Oxychlordane	1834	1.90	8.14 (7.83)	0.78/65.47
T-NONA	Trans-Nonachlor	1817	0.28	12.72 (11.69)	1.23/189.00

TABLE II. CHARACTERISTICS OF 118 SELECTED SNPS

Chromosome	SNP	Gene	Position	Risk	Other	RAF
1	rs67156297	ATP8B2	154336716	A	G	0.17
1	rs17106184	FAF1	50909985	G	A	0.93
1	rs3768321	MACF1	40035928	T	G	0.10
1	rs10923931	NOTCH2	120517959	T	G	0.13
1	rs2075423[a]	PROX1	214154719	G	T	0.44
2	rs9309245	ASB3	53397048	G	C	0.34
2	rs243088	BCL11A	60568745	T	A	0.56
2	rs11123406	BCL2L11	111950541	T	C	0.44
2	rs1116357	CCDC85A	57287411	G	A	0.45
2	rs780094[b]	GCKR	27741237	C	T	0.66
2	rs3923113[b]	GRB14	165501849	A	C	0.67
2	rs2943640[b]	IRS1	227093585	C	A	0.80
2	rs7593730[b]	RBMS1	161171454	C	T	0.86
2	rs7560163	RND3/RBM43	151637936	C	G	0.98
2	rs10203174[a]	THADA	43690030	C	T	0.88
2	rs6723108[b]	TMEM163	135479980	T	G	0.98
2	rs2867125	TMEM18	622827	C	T	0.91
3	rs6795735[b]	ADAMTS9	64705365	C	T	0.31
3	rs11717195[a]	ADCY5	123082398	T	C	0.76
3	rs4402960[a]	IGF2BP2	185511687	T	G	0.28
3	rs11927381	IGF2BP2	185508591	C	T	0.32
3	rs6808574	LPP	187740523	C	T	0.72
3	rs1801282[b]	PPARG	12393125	C	G	0.91
3	rs831571[b]	PSMD6	64048297	C	T	0.86
3	rs16861329[a]	ST64GAL1	186666461	C	T	0.78
3	rs7612463[a]	UBE2E2	23336450	C	A	0.89
4	rs60780116	ACSL1	185708807	T	C	0.96
4	rs7674212	CISD2	103988899	G	T	0.49
4	rs6815464[a]	MAEA	1309901	C	G	0.80
4	rs6813195[a]	TMEM154	153520475	C	T	0.62
4	rs2706785	TMEM155	122660250	G	A	0.03
4	rs4458523[a]	WFS1	6289986	G	T	0.66
5	rs459193[b]	ANKRD55	55806751	G	A	0.24
5	rs702634[b]	ARL15	53271420	A	G	0.77
5	rs329122	PHF15	133864599	A	G	0.53
5	rs6878122	ZBED3	76427311	G	A	0.30
6	rs7756992[a]	CDKAL1	20679709	G	A	0.33
6	rs7766070	CDKAL1	20686573	A	C	0.24
6	rs9271774	HLA-DQA1	32594309	C	A	0.20
6	rs2050188	HLA-DRB5	32339897	T	C	0.44
6	rs1535500	KCNK16	39284050	T	G	0.53
6	rs9273401	MHC	32627129	G	A	0.04

TABLE II. CHARACTERISTICS OF 118 SELECTED SNPS (CONTINUED)

Chromosome	SNP	Gene	Position	Risk	Other	RAF
6	rs3132524	POU5F1/TCF19	31136714	C	T	0.79
6	rs622217	SLC22A3	160766770	T	C	0.55
6	rs6918311	SLC35D3	137287702	A	G	0.48
6	rs9502570	SSR1/RREB1	7258617	C	T	0.72
6	rs9470794	ZFAND3	38106844	C	T	0.10
7	rs9648716	BRAF	140612163	T	A	0.22
7	rs17168486[a]	DGKB	14898282	T	C	0.31
7	rs6467136	GCC1	127164958	G	A	0.46
7	rs10278336[a]	GCK	44245363	A	G	0.66
7	rs849135[a]	JAZF1	28196413	G	A	0.60
7	rs13233731[b]	KLF14	130437689	G	A	0.51
7	rs791595[b]	MIR129-LEP	127862802	A	G	0.20
7	rs1182436	MNX1	157027753	C	T	0.78
8	rs516946[a]	ANK1	41519248	C	T	0.75
8	rs12681990	KCNU1	36859186	C	T	0.22
8	rs3802177[a]	SLC30A8	118185025	G	A	0.75
8	rs7845219	TP53INP1	95937502	T	C	0.48
9	rs635634	ABO	136155000	T	C	0.15
9	rs10811661[a]	CDKN2A/B	22134094	T	C	0.86
9	rs1575972	DMRTA1	22301092	T	A	0.93
9	rs7041847[a]	GLIS3	4287466	A	G	0.60
9	rs17584499	PTPRD	8879118	T	C	0.19
9	rs2796441	TLE1	84308948	G	A	0.57
9	rs17791513	TLE4	81905590	A	G	0.88
10	rs11257655[a]	CDC123	12307894	T	C	0.23
10	rs10886471[b]	GRK5	121149403	C	T	0.53
10	rs1111875[a]	HHEX/IDE	94462882	C	T	0.66
10	rs2292626	PLEKHA1	124186714	C	T	0.46
10	rs7903146[a]	TCF7L2	114758349	T	C	0.25
10	rs1802295[a]	VPS26A	70931474	T	C	0.22
10	rs12571751	ZMIZ1	80942631	A	G	0.53
11	rs1552224[a]	ARAP1/CENTD2	72433098	A	C	0.91
11	rs2334499[a]	DUSP8	1696849	T	C	0.42
11	rs1061810	HSD17B12	43877934	A	C	0.42
11	rs3842770[a]	INS-IGF2	2178670	A	G	0.03
11	rs5215[a]	KCNJ11	17408630	C	T	0.33
11	rs163184[a]	KCNQ1	2847069	G	T	0.41
11	rs111669836	MAP3K11	65364385	A	T	0.17
11	rs7107784	MIR4686	2215089	G	A	0.27
11	rs10830963[a]	MTNR1B	92708710	G	C	0.21
12	rs825476	CCDC92	123568456	T	C	0.52
12	rs11063069	CCND2	4374373	G	A	0.17

TABLE II. CHARACTERISTICS OF 118 SELECTED SNPS (CONTINUED)

Chromosome	SNP	Gene	Position	Risk	Other	RAF
12	rs147538848	FAM60A	31466613	A	G	0.00
12	rs2261181[b]	HMGA2	66212318	T	C	0.11
12	rs12427353	HNF1A	121426901	G	C	0.88
12	rs10842994	KLHDC5	27965150	C	T	0.87
12	rs1727313	MPHOSPH9	123640853	G	C	0.93
12	rs7955901[a]	TSPAN8	71433293	C	T	0.88
13	rs576674	KL	33554302	G	A	0.31
13	rs7985179	MIR17HG	91940169	T	A	0.79
13	rs10507349	RNF6	26781528	G	A	0.73
13	rs1359790[a]	SPRY2	80717156	G	A	0.70
14	rs10146997	NRXN3	79945162	G	A	0.23
15	rs2028299[a]	AP3S2	90374257	C	A	0.22
15	rs7163757[a]	C2CD4A	62391608	C	T	0.50
15	rs7178572	HMG20A	77747190	G	A	0.62
15	rs67839313	INAFM2	40619714	C	T	0.16
15	rs12899811[a]	PRC1	91544076	G	A	0.57
15	rs7403531[a]	RASGRP1	38822905	T	C	0.30
15	rs11634397	ZFAND6	80432222	G	A	0.58
16	rs7202877[a]	BCAR1	75247245	T	G	0.89
16	rs2925979	CMIP	81534790	T	C	0.22
16	rs9936385	FTO	53819169	C	T	0.31
16	rs9940149	ITFG3	300641	G	A	0.68
17	rs78761021	GLP2R	9780387	G	A	0.16
17	rs4430796[a]	HNF1B/TCF2	36098040	G	A	0.48
17	rs13342692	SLC16A11	6946287	C	T	0.21
17	rs391300	SRR	2216258	C	T	0.61
17	rs7224685	ZZEF1	4014384	T	G	0.29
18	rs12454712	BCL2	60845884	T	C	0.65
18	rs12970134	MC4R	57884750	A	G	0.16
19	rs10401969	CILP2	19407718	C	T	0.08
19	rs8108269	GIPR	46158513	G	T	0.37
19	rs3786897	PEPD	33893008	A	G	0.67
19	rs157582	TOMM40-APOE	45396219	C	T	0.74
20	rs4812829[a]	HNF4A	42989267	A	G	0.36

a: SNPs known to be related to β-cell dysfunction;
b: SNPs known to be related to insulin resistance.

C. Results

1. Characteristics of Study Participants

Baseline characteristics of study participants are presented in Table III. We also present incidence rates for diabetes at V2 for normoglycemics and prediabetics at V1 (15.9%), prediabetes at V2 for normoglycemics at V1 (50%), hyperglycemia (prediabetic or diabetic) at V2 for normoglycemics at V1 (51.3%), and diabetes at V2 from prediabetics at V1 (25.7%) in Table IV, as well as HOMA measurements.

2. Associations between PRS and Diabetes

Table V presents the associations between total PRS and diabetes outcomes at V2. In the reduced models (Model 1), PRS was associated with increased risk of developing diabetes and prediabetes. Participants in the highest tertile of PRS (T3) showed 1.7 times odds of developing T2D at V2, compared to people in the lowest tertile (T1) of PRS (OR=1.71, 95% CI=1.10-2.67). Similarly, among the normoglycemics at V1, the odds of developing hyperglycemia among participants were 1.83 times higher in people with the highest PRS (T3) compared to the people with the lowest PRS (T1) (OR=1.83, 95% CI=1.07-3.13). Among participants with prediabetes at V1, the odds of developing diabetes were 1.86 times higher in people with the highest PRS compared to the lowest PRS (OR=1.86, 95% CI=1.10-3.15). We observed significant dose-response relationships between total PRS and incident T2D, normoglycemic to hyperglycemic, and prediabetes to diabetes, respectively, which implies additive effects of number of risk alleles on development of hyperglycemia. However, no associations were observed between PRS and the transition from normoglycemic to prediabetes, HOMA-IR, and HOMA-B at V2. In Model 2, we found pattern of associations between PRS and diabetes outcomes that were similar to those seen in Model 1.

TABLE III. BASELINE (V1) CHARACTERISTICS OF 1,846 STUDY PARTICIPANTS

Characteristics		N(%) / mean (SE)
Age, mean (SE)		56.4 (0.4)
Body mass index (BMI), mean (SE)		29.0 (0.2)
Hispanic background	Dominican	193 (9.8)
	Central American	168 (6.7)
	Cuban	337 (28.7)
	Mexican	646 (29.9)
	Puerto Rican	324 (15.9)
	South American	139 (5.0)
	More than one or other heritage	39 (4.1)
Education	Less than HS	712 (41.0)
	HS graduate	413 (22.5)
	Greater than HS	721 (47.2)
Alcohol use	No current use	928 (46.7)
	Low level use	918 (41.3)
	High level use	93 (4.0)
Cigarette use	Never	947 (52.3)
	Former smoker	497 (26.9)
	Current smoker	402 (20.8)
Physical activity level	High	201 (11.2)
	Moderate	835 (49.7)
	Low	810 (49.1)
Family history of T2D	No	1004 (56.7)
	Yes	842 (43.3)
Study Center	Bronx	452 (28.9)
	Chicago	434 (12.0)
	Miami	520 (38.5)
	San Diego	440 (20.6)
Polygenic risk scores (PRS)		
Total PRS, mean (SE)		118.9 (0.3)
Insulin-resistance PRS, mean (SE)		19.9 (0.1)
B-cell dysfunction PRS, mean (SE)		38.8 (0.2)

Tables VI and VII represent the results for insulin resistance PRS and β-cell dysfunction PRS, in association with diabetes outcomes at V2. No associations were observed in relationships between insulin resistance PRS and diabetes at V2. For β-cell dysfunction PRS we only detected significant dose-response relationships among normoglycemic participants with the development of prediabetes and hyperglycemia at V2 (fully adjusted ORs range 2.11 - 2.15).

TABLE IV. DEVELOPMENT OF HYPERGLYCEMIA, HOMA-IR, AND HOMA-B AT V2

Outcomes at V2		N (%)
Normoglycemic or prediabetes to T2D at V2 (total=1,846, male=928, female=918)	No	1355 (84.1)
	Yes	491 (15.9)
Normoglycemic to hyperglycemic at V2 (total=913, male=470, female=443)	No	414 (48.7)
	Yes	499 (51.3)
Normoglycemic to prediabetes at V2 (total=887, male=457, female=430)	No	414 (50.0)
	Yes	473 (50.0)
Prediabetes to diabetes at V2 (total=913, male=470, female=443)	No	468 (51.3)
	Yes	465 (49.7)
HOMA-IR, geomean (SE) (total = 1,846)		2.19 (0.06)
HOMA-B, geomean (SE) (total = 1,846)		106.3 (2.4)

TABLE V. ASSOCIATIONS BETWEEN TOTAL PRS AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	Model 1[a]				Model 2[b]			
		OR / β	95% CI (L)	95% CI (U)	p-trend	OR / β	95% CI (L)	95% CI (U)	p-trend
Normoglycemic or prediabetes to T2D (N=1,846)	T1	ref			0.02	ref			<0.01
	T2	1.36	0.90	2.05		1.35	0.88	2.09	
	T3	1.71	1.10	2.67		1.96	1.22	3.15	
Normoglycemic to hyperglycemic (N=913)	T1	ref			0.02	ref			0.01
	T2	1.43	0.90	2.29		1.39	0.88	2.19	
	T3	1.83	1.07	3.13		1.96	1.17	3.30	
Normoglycemic to prediabetes (N=887)	T1	ref			0.18	ref			0.08
	T2	1.13	0.68	1.87		1.16	0.71	1.89	
	T3	1.43	0.85	2.41		1.61	0.94	2.75	
Prediabetes to diabetes (N=913)	T1	ref			0.02	ref			<0.01
	T2	1.41	0.88	2.25		1.39	0.88	2.19	
	T3	1.86	1.10	3.15		2.00	1.20	3.33	
HOMA-IR (N=1,846)	T1	ref			0.61	ref			0.27
	T2	0.055	-0.067	0.177		0.074	-0.029	0.177	
	T3	0.007	-0.110	0.124		0.059	-0.043	0.160	
HOMA-B (N=1,846)	T1	ref			0.28	ref			0.29
	T2	-0.051	-0.147	0.046		-0.028	-0.111	0.055	
	T3	-0.082	-0.185	0.021		-0.051	-0.145	0.043	

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI.

TABLE VI. ASSOCIATIONS BETWEEN INSULIN RESISTANCE PRS AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	Model 1[a]				Model 2[b]			
		OR / β	95% CI (L)	95% CI (U)	p-trend	OR / β	95% CI (L)	95% CI (U)	p-trend
Normoglycemic or prediabetes to T2D (N=1,846)	T1	ref			0.83	ref			0.86
	T2	1.24	0.79	1.95		1.28	0.79	2.05	
	T3	0.98	0.62	1.55		0.99	0.64	1.54	
Normoglycemic to hyperglycemic (N=913)	T1	ref			0.49	ref			0.28
	T2	1.11	0.67	1.84		1.29	0.78	2.15	
	T3	1.20	0.71	2.03		1.34	0.81	2.21	
Normoglycemic to prediabetes (N=887)	T1	ref			0.45	ref			0.25
	T2	1.11	0.67	1.85		1.31	0.79	2.19	
	T3	1.23	0.72	2.10		1.37	0.82	2.29	
Prediabetes to diabetes (N=913)	T1	ref			0.43	ref			0.50
	T2	1.13	0.67	1.91		1.16	0.68	1.96	
	T3	0.84	0.50	1.43		0.88	0.54	1.44	
HOMA-IR (N=1,846)	T1	ref			0.37	ref			0.20
	T2	0.081	-0.045	0.206		0.070	-0.038	0.178	
	T3	0.055	-0.050	0.160		0.067	-0.028	0.162	
HOMA-B (N=1,846)	T1	ref			0.47	ref			0.35
	T2	0.048	-0.048	0.144		0.032	-0.050	0.113	
	T3	0.041	-0.060	0.141		0.045	-0.047	0.136	

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI.

TABLE VII. ASSOCIATIONS BETWEEN B-CELL DYSFUNCTION PRS AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	Model 1[a]				Model 2[b]			
		OR / β	95% CI (L)	95% CI (U)	p-trend	OR / β	95% CI (L)	95% CI (U)	p-trend
Normoglycemic or prediabetes to T2D (N=1,846)	T1	ref				ref			
	T2	0.99	0.64	1.56	0.69	1.18	0.75	1.86	0.29
	T3	1.09	0.72	1.64		1.25	0.83	1.88	
Normoglycemic to hyperglycemic (N=913)	T1	ref				ref			
	T2	1.49	0.92	2.41	0.02	1.58	0.99	2.52	<0.01
	T3	1.95	1.13	3.38		2.15	1.28	3.62	
Normoglycemic to prediabetes (N=887)	T1	ref				ref			
	T2	1.35	0.83	2.20	0.02	1.43	0.88	2.30	<0.01
	T3	1.94	1.11	3.39		2.11	1.25	3.58	
Prediabetes to diabetes (N=913)	T1	ref				ref			
	T2	0.77	0.44	1.33	0.91	0.87	0.51	1.49	0.81
	T3	0.98	0.59	1.63		1.07	0.65	1.75	
HOMA-IR (N=1,846)	T1	ref				ref			
	T2	-0.046	-0.164	0.072	0.13	0.003	-0.103	0.109	0.56
	T3	-0.090	-0.207	0.027		-0.031	-0.131	0.070	
HOMA-B (N=1,846)	T1	ref				ref			
	T2	-0.043	-0.135	0.048	0.20	-0.027	-0.110	0.056	0.48
	T3	-0.072	-0.182	0.039		-0.035	-0.133	0.063	

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI.

3. Relationships between PRS, Sum PCBs, and Incident Hyperglycemic Outcomes, HOMA-IR and HOMA-B at V2

Table VIII demonstrates the associations between sum PCBs and diabetes outcomes. Generally, people with higher levels of PCB levels at V1 appeared to have higher odds of having diabetes, prediabetes, and hyperglycemia, and ORs were larger for participants who were normoglycemic at V1 than those prediabetic at V1, but associations did not reach significance. Similarly, levels of HOMA-IR and HOMA-B at V2 were not different by PCB levels at V1.

Table IX demonstrates the interactions between total PRS and sum PCBs on diabetes outcomes at V2. For incident T2D, the effects of PRS became greater with elevated PCB levels. In the highest tertiles of total PRS, participants with higher PCB concentrations showed greater OR for developing diabetes, compared to participants with lower PCB concentrations (p-interaction <0.05). A similar pattern was observed for the development of diabetes from prediabetes. No other significant interaction effects were found.

We also found interaction effects between insulin resistance PRS and sum PCBs, with incident T2D (marginal significance with p-interaction=0.06) and prediabetes to diabetes (p-interaction=0.03). Similar to the total PRS, people with higher PCB concentrations had elevated risk of developing diabetes at V2, especially among people in the highest tertiles of insulin resistance PRS. However, we did not observe any significant interaction effects between β-cell dysfunction PRS and sum PCBs. The interaction results with PCBs and insulin resistance PRS, and PCBs with β-cell dysfunction PRS are demonstrated in Tables X and XI.

TABLE VIII. ASSOCIATIONS BETWEEN PCBS AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2 (TOTAL N=1,627)

Outcomes at V2	sum PCBs	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.04	0.63	1.71	0.96	0.58	1.59
Normoglycemic to hyperglycemic	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.32	0.76	2.29	1.47	0.84	2.57
Normoglycemic to prediabetes	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.38	0.79	2.41	1.46	0.83	2.57
Prediabetes to diabetes	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.01	0.58	1.76	0.97	0.57	1.65
HOMA-IR at V2	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	-0.094	-0.219	0.031	-0.030	-0.121	0.062
HOMA-B at V2	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	-0.112	-0.220	-0.005	-0.024	-0.111	0.063

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. In Model 2, model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE IX. INTERACTION EFFECTS BETWEEN TOTAL PRS AND PCBS ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	PCB < 75th Percentile[a]			PCB > 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			<0.05
	T2	1.00	0.73	1.38	1.01	0.58	1.75	
	T3	1.42	1.02	1.98	1.94	1.11	3.40	
Normoglycemic to hyperglycemic	T1	ref			ref			0.32
	T2	0.92	0.65	1.30	0.90	0.49	1.65	
	T3	1.64	1.10	2.46	1.97	0.96	4.04	
Normoglycemic to prediabetes	T1	ref			ref			0.30
	T2	0.92	0.65	1.30	0.91	0.49	1.67	
	T3	1.66	1.10	2.51	2.00	0.96	4.17	
Prediabetes to diabetes	T1	ref			ref			<0.05
	T2	1.02	0.73	1.42	1.06	0.59	1.88	
	T3	1.44	1.01	2.04	1.96	1.09	3.52	
HOMA-IR	T1	ref			ref			0.64
	T2	0.002	-0.149	0.152	-0.005	-0.158	0.147	
	T3	-0.039	-0.177	0.100	-0.046	-0.185	0.093	
HOMA-B	T1	ref			ref			0.53
	T2	0.033	-0.139	0.204	-0.009	-0.178	0.161	
	T3	-0.029	-0.158	0.100	-0.070	-0.195	0.055	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE X. INTERACTION EFFECTS BETWEEN INSULIN RESISTANCE PRS AND PCBS ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	Insulin resistance PRS	PCB < 75th Percentile[a]			PCB ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.06
	T2	1.18	0.83	1.67	1.18	0.63	2.19	
	T3	1.03	0.75	1.41	1.38	0.79	2.40	
Normoglycemic to hyperglycemic	T1	ref			ref			0.34
	T2	1.05	0.74	1.50	1.12	0.61	2.05	
	T3	1.23	0.85	1.78	1.39	0.72	2.68	
Normoglycemic to prediabetes	T1	ref			ref			0.29
	T2	1.07	0.75	1.54	1.11	0.60	2.05	
	T3	1.24	0.85	1.81	1.46	0.75	2.84	
Prediabetes to diabetes	T1	ref			ref			0.03
	T2	1.27	0.88	1.83	1.19	0.62	2.30	
	T3	1.05	0.76	1.47	1.52	0.85	2.73	
HOMA-IR	T1	ref			ref			0.89
	T2	-0.030	-0.167	0.106	-0.032	-0.180	0.117	
	T3	-0.039	-0.192	0.114	-0.040	-0.202	0.121	
HOMA-B	T1	ref			ref			0.81
	T2	0.006	-0.159	0.172	0.005	-0.166	0.175	
	T3	-0.065	-0.177	0.047	-0.067	-0.186	0.052	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE XI. INTERACTION EFFECTS BETWEEN B-CELL DYSFUNCTION PRS AND PCBS ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	β-cell dysfunction PRS	PCB < 75th Percentile[a]			PCB ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.36
	T2	1.01	0.70	1.46	1.16	0.62	2.16	
	T3	1.13	0.80	1.59	1.20	0.66	2.16	
Normoglycemic to hyperglycemic	T1	ref			ref			0.45
	T2	1.04	0.73	1.46	0.99	0.55	1.77	
	T3	1.34	0.89	2.01	1.21	0.58	2.50	
Normoglycemic to prediabetes	T1	ref			ref			0.49
	T2	0.99	0.70	1.41	0.97	0.53	1.76	
	T3	1.35	0.90	2.04	1.22	0.58	2.54	
Prediabetes to diabetes	T1	ref			ref			0.30
	T2	0.90	0.62	1.32	1.11	0.58	2.14	
	T3	1.18	0.82	1.69	1.24	0.66	2.32	
HOMA-IR	T1	ref			ref			0.81
	T2	0.001	-0.149	0.151	-0.008	-0.161	0.144	
	T3	-0.050	-0.197	0.096	-0.059	-0.205	0.086	
HOMA-B	T1	ref			ref			0.26
	T2	0.068	-0.113	0.249	0.061	-0.123	0.245	
	T3	-0.054	-0.168	0.059	-0.061	-0.170	0.048	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

4. Relationships between PRS, DDE, and Incident Hyperglycemic Outcomes, HOMA-IR and HOMA-B at V2

The association between DDE and diabetes outcomes are presented in Table XII. Participants with the highest quartile of DDE concentration showed greater risk of developing diabetes or prediabetes at V2, however no statistically significant associations were found. In our results, HOMA measurements at V2 also did not appear remarkably different by DDE concentrations.

In the interaction analyses between total PRS and DDE, we found similar patterns of the results to the total PRS – PCBs interaction findings. Synergetic interaction effects between DDE and total PRS were noted for incident T2D for both normoglycemic or prediabetic status at V1 and for prediabetics at V1, but not for incident prediabetes or hyperglycemia from normoglycemic status at V1. It is of note that for incident diabetes the ORs among people with highest DDE concentration (≥75th percentile) and highest PRS percentile (T3) appeared greater than the main effects of PRS, which were presented in Table XIII, supporting a synergetic interaction between DDE and PRS on diabetes.

In Tables XIV and XV, we present interaction results by biological mechanism attributed to SNPs. Neither PRS with insulin resistance related SNPs nor β-cell related SNPs showed significant interaction effects with DDE. No stratum-specific associations were observed with DDE and insulin- or β-cell related PRS interactions.

5. Relationships between PRS, OXYCHLOR, and Incident Hyperglycemic Outcomes, HOMA-IR and HOMA-B at V2

The association between OXYCHLOR and diabetes outcomes are presented in Table XVI. There were no associations found between OXYCHLOR and diabetes outcomes at V2, with the estimates indicating null associations.

TABLE XII. ASSOCIATIONS BETWEEN DDE AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2 (TOTAL N=1,831)

Outcomes at V2	DDE	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.39	0.89	2.19	1.30	0.79	2.14
Normoglycemic to hyperglycemic	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.26	0.76	2.09	1.28	0.75	2.19
Normoglycemic to prediabetes	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.32	0.79	2.22	1.33	0.77	2.29
Prediabetes to diabetes	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.49	0.87	2.57	1.44	0.85	2.45
HOMA-IR	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	0.038	-0.087	0.163	-0.008	-0.089	0.074
HOMA-B	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	0.001	-0.102	0.105	-0.013	-0.092	0.066

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. In Model 2, model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements were adjusted for each HOMA measurement at V1.

TABLE XIII. INTERACTION EFFECTS BETWEEN TOTAL PRS AND DDE ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	DDE < 75th Percentile[a]			DDE ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.04
	T2	0.82	0.59	1.13	0.61	0.35	1.08	
	T3	1.58	1.14	2.21	2.38	1.34	4.22	
Normoglycemic to hyperglycemic	T1	ref			ref			0.22
	T2	1.13	0.81	1.57	1.46	0.82	2.61	
	T3	1.19	0.83	1.71	0.87	0.47	1.61	
Normoglycemic to prediabetes	T1	ref			ref			0.26
	T2	1.13	0.81	1.57	1.44	0.81	2.58	
	T3	1.19	0.82	1.71	0.88	0.47	1.64	
Prediabetes to diabetes	T1	ref			ref			0.02
	T2	0.82	0.59	1.15	0.59	0.33	1.09	
	T3	1.55	1.09	2.19	2.48	1.36	4.54	
HOMA-IR	T1	ref			ref			0.20
	T2	0.065	-0.066	0.196	0.044	-0.087	0.175	
	T3	-0.024	-0.168	0.120	-0.045	-0.188	0.098	
HOMA-B	T1	ref			ref			0.69
	T2	-0.005	-0.146	0.135	0.000	-0.131	0.131	
	T3	0.003	-0.135	0.140	0.008	-0.119	0.134	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE XIV. INTERACTION EFFECTS BETWEEN INSULIN RESISTANCE PRS AND DDE ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	Insulin resistance PRS	DDE < 75th Percentile[a]			DDE ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.67
	T2	1.05	0.74	1.47	0.88	0.48	1.61	
	T3	0.86	0.62	1.19	0.86	0.47	1.56	
Normoglycemic to hyperglycemic	T1	ref			ref			0.32
	T2	0.94	0.67	1.32	0.73	0.41	1.30	
	T3	1.14	0.81	1.60	1.12	0.62	2.01	
Normoglycemic to prediabetes	T1	ref			ref			0.30
	T2	0.95	0.68	1.33	0.74	0.41	1.31	
	T3	1.14	0.81	1.61	1.12	0.62	2.01	
Prediabetes to diabetes	T1	ref			ref			0.60
	T2	1.04	0.73	1.49	0.87	0.46	1.63	
	T3	0.86	0.61	1.21	0.85	0.45	1.57	
HOMA-IR	T1	ref			ref			0.42
	T2	-0.011	-0.145	0.122	0.016	-0.132	0.164	
	T3	0.050	-0.078	0.177	0.077	-0.064	0.219	
HOMA-B	T1	ref			ref			0.67
	T2	-0.034	-0.178	0.109	0.007	-0.142	0.155	
	T3	0.036	-0.061	0.132	0.077	-0.026	0.180	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

TABLE XV. INTERACTION EFFECTS BETWEEN B-CELL DYSFUNCTION PRS AND DDE ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	β-cell dysfunction PRS	DDE < 75th Percentile[a]			DDE ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.17
	T2	1.07	0.73	1.57	1.16	0.58	2.31	
	T3	1.10	0.80	1.51	1.30	0.74	2.28	
Normoglycemic to hyperglycemic	T1	ref			ref			0.80
	T2	0.91	0.66	1.27	0.70	0.40	1.23	
	T3	1.54	1.06	2.22	1.72	0.91	3.26	
Normoglycemic to prediabetes	T1	ref			ref			0.87
	T2	0.88	0.63	1.22	0.70	0.40	1.24	
	T3	1.56	1.08	2.26	1.73	0.92	3.26	
Prediabetes to diabetes	T1	ref			ref			0.12
	T2	0.99	0.66	1.49	1.24	0.60	2.57	
	T3	1.13	0.80	1.58	1.27	0.70	2.32	
HOMA-IR	T1	ref			ref			0.90
	T2	-0.018	-0.153	0.116	-0.022	-0.161	0.117	
	T3	0.005	-0.122	0.131	0.001	-0.131	0.133	
HOMA-B	T1	ref			ref			0.51
	T2	-0.042	-0.187	0.104	-0.019	-0.157	0.120	
	T3	-0.037	-0.150	0.077	-0.014	-0.115	0.088	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

In the interaction analysis, no interaction effects nor stratum-specific associations were found for OXCHLOR and total PRS, although we still observed similar patterns to sum PCBs and DDE; the highest PRS tertiles showed elevated risk of having hyperglycemic outcomes at V2, and within the same tertile group, participants with higher OXYCHLOR concentrations had greater risk (Table XVII).

However, we observed significant interactions between OXYCHLOR and insulin resistance PRS. In participants who had normal glucose levels at baseline, people in the highest tertiles of insulin resistance PRS showed 1.38 to 2.17 times odds of developing prediabetes at V2, with greater risk among participants with OXYCHLOR ≥ 75th percentile (OR=1.38, 95% CI=0.96-1.97 for OXYCHLOR < 75 percentile, and OR=2.17, 95% CI=1.14-4.14 for OXYCHLOR ≥ 75 percentile, respectively; p for interaction <0.05).

A similar interaction effect was observed among participants who developed hyperglycemia at V2 (OR=1.36, 95% CI=0.96-1.93 for OXYCHLOR < 75th percentile, and OR=2.12, 95% CI=1.13-3.98 for OXYCHLOR ≥ 75th percentile, respectively; p for interaction <0.05). Another significant interaction effect was found with HOMA-IR at V2. Negative associations were found between insulin resistance PRS and HOMA-IR at V2, although there was no significant stratum-specific association.

We also observed significant interaction effects with OXYCHLOR and β-cell dysfunction PRS, with the same outcomes with OXYCHLOR – insulin resistance PRS interactions; normoglycemic to prediabetes and normoglycemic to hyperglycemia. However, the patterns of the stratum-specific estimates differed, with the highest ORs in participants with OXYCHLOR <75th percentile for normoglycemic to prediabetes (OR=1.29, 95% CI=0.88-1.90). Yet, in the interaction analysis of OXYCHLOR and β-cell dysfunction, there were no significant stratum-specific associations. The results from interaction analyses were displayed in Tables XVIII and XIV.

TABLE XVI. ASSOCIATIONS BETWEEN OXYCHLOR AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2 (TOTAL N=1,834)

Outcomes at V2	OXYCHLOR	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	0.98	0.63	1.53	1.09	0.67	1.78
Normoglycemic to hyperglycemic	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.11	0.66	1.87	1.09	0.64	1.86
Normoglycemic to prediabetes	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.18	0.70	1.99	1.11	0.65	1.90
Prediabetes to diabetes	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.00	0.60	1.67	1.07	0.63	1.81
HOMA-IR at V2	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	-0.028	-0.142	0.086	-0.021	-0.112	0.071
HOMA-B at V2	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	0.005	-0.091	0.101	0.038	-0.039	0.114

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. In Model 2, model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements were adjusted for each HOMA measurement at V1.

TABLE XVII. INTERACTION EFFECTS BETWEEN TOTAL PRS AND OXYCHLOR ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	OXYCHLOR < 75th Percentile[a]			OXYCHLOR ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.75
	T2	0.93	0.69	1.26	0.95	0.57	1.58	
	T3	1.37	0.99	1.90	1.43	0.82	2.48	
Normoglycemic to hyperglycemic	T1	ref			ref			0.79
	T2	0.95	0.68	1.32	0.81	0.44	1.47	
	T3	1.46	0.99	2.15	1.65	0.83	3.31	
Normoglycemic to prediabetes	T1	ref			ref			0.75
	T2	0.95	0.68	1.33	0.81	0.44	1.48	
	T3	1.44	0.97	2.14	1.65	0.81	3.36	
Prediabetes to diabetes	T1	ref			ref			0.80
	T2	0.96	0.70	1.31	1.00	0.58	1.72	
	T3	1.31	0.93	1.86	1.34	0.74	2.42	
HOMA-IR	T1	ref			ref			0.88
	T2	-0.054	-0.198	0.089	-0.020	-0.166	0.126	
	T3	-0.006	-0.142	0.129	0.028	-0.111	0.166	
HOMA-B	T1	ref			ref			0.49
	T2	0.051	-0.098	0.200	0.062	-0.090	0.213	
	T3	0.066	-0.049	0.181	0.077	-0.041	0.195	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE XVIII. INTERACTION EFFECTS BETWEEN INSULIN RESISTANCE PRS AND OXYCHLOR ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	Insulin resistance PRS	OXYCHLOR < 75th Percentile[a]			OXYCHLOR ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.09
	T2	1.03	0.75	1.41	0.77	0.45	1.33	
	T3	0.98	0.73	1.31	1.37	0.83	2.27	
Normoglycemic to hyperglycemic	T1	ref			ref			<0.05
	T2	0.95	0.67	1.35	0.72	0.38	1.37	
	T3	1.36	0.96	1.93	2.12	1.13	3.98	
Normoglycemic to prediabetes	T1	ref			ref			<0.05
	T2	0.95	0.66	1.35	0.71	0.37	1.36	
	T3	1.38	0.96	1.97	2.17	1.14	4.14	
Prediabetes to diabetes	T1	ref			ref			0.11
	T2	1.02	0.73	1.42	0.73	0.41	1.29	
	T3	1.00	0.73	1.36	1.42	0.83	2.42	
HOMA-IR	T1	ref			ref			0.02
	T2	0.081	-0.032	0.195	0.026	-0.096	0.148	
	T3	-0.017	-0.163	0.129	-0.072	-0.222	0.077	
HOMA-B	T1	ref			ref			0.20
	T2	0.096	-0.034	0.225	0.075	-0.056	0.205	
	T3	0.031	-0.077	0.138	0.010	-0.099	0.119	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

TABLE XIX. INTERACTION EFFECTS BETWEEN B-CELL DYSFUNCTION PRS AND OXYCHLOR ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	β-cell dysfunction PRS	OXYCHLOR < 75th Percentile[a]			OXYCHLOR ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.85
	T2	1.09	0.78	1.53	1.35	0.76	2.43	
	T3	1.01	0.75	1.36	0.92	0.56	1.50	
Normoglycemic to hyperglycemic	T1	ref			ref			0.03
	T2	0.99	0.71	1.39	0.95	0.52	1.73	
	T3	1.27	0.86	1.86	0.91	0.46	1.82	
Normoglycemic to prediabetes	T1	ref			ref			0.04
	T2	0.94	0.67	1.33	0.93	0.51	1.71	
	T3	1.29	0.88	1.90	0.92	0.46	1.85	
Prediabetes to diabetes	T1	ref			ref			0.69
	T2	0.97	0.68	1.39	1.30	0.69	2.44	
	T3	1.06	0.76	1.46	0.94	0.55	1.62	
HOMA-IR	T1	ref			ref			0.13
	T2	-0.078	-0.207	0.051	-0.077	-0.195	0.041	
	T3	-0.042	-0.194	0.110	-0.041	-0.183	0.100	
HOMA-B	T1	ref			ref			0.68
	T2	0.083	-0.065	0.231	0.072	-0.069	0.213	
	T3	0.008	-0.110	0.126	-0.003	-0.109	0.103	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

6. Relationships between PRS, TNONA, and Incident Hyperglycemic Outcomes, HOMA-IR and HOMA-B at V2

The associations between TNONA and diabetes at outcomes were demonstrated in Table XX. The highest OR for TNONA ≥ 75th percentile vs. < 75th percentile appeared with normoglycemic to prediabetes, but without statistical significance (OR=1.47, 95% CI=0.87-2.49 in the reduced model, OR=1.46, 95% CI=0.83-2.55 in the full model, respectively). Higher TNONA concentration was associated with lower HOMA-IR, but no significant results were found with and HOMA-B.

We did not find any significant interaction effects between TNONA and total PRS, although the estimates were generally greater in participants with TNONA > 75th percentile compared to participants in TNONA < 75th percentile. We also found increased odds in the highest tertile of total PRS compared to the lowest tertile in most of the binary outcomes, with ORs from 1.40 to 1.91. However, the ORs were significant only among participants with TNONA levels less than the 75th percentile. Table XXI displays the results of interaction effects between total PRS and TNONA on diabetes outcomes at V2.

Table XXII displays the results of interaction effects between insulin resistance PRS and TNONA on diabetes outcomes. Generally, participants in the highest insulin resistance PRS tertile showed greater odds of developing diabetes or prediabetes at V2, and the odds increased in those with TNONA concentrations above the 75th percentile. Significant interactions were observed with incident T2D and prediabetes (V1) to diabetes (V2). Development of prediabetes and hyperglycemia also showed marginal interaction effects between insulin resistance PRS and TNONA concentrations. No remarkable patterns were noted with HOMA measurements. Table XXIII represents the results of interaction effects between β-cell dysfunction PRS and TNONA on diabetes outcomes at V2. We observed no significant stratum-specific associations nor interaction effects.

TABLE XX. ASSOCIATIONS BETWEEN TNONA AND INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR and HOMA-B at V2 (TOTAL N=1,817)

Outcomes at V2	TNONA	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.12	0.73	1.71	0.99	0.61	1.61
Normoglycemic to hyperglycemic	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.38	0.82	2.32	1.42	0.81	2.47
Normoglycemic to prediabetes	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.47	0.87	2.49	1.46	0.83	2.55
Prediabetes to diabetes	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	0.99	0.61	1.59	0.98	0.58	1.65
HOMA-IR at V2	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	0.011	-0.098	0.120	-0.040	-0.127	0.048
HOMA-B at V2	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	0.006	-0.089	0.101	0.022	-0.056	0.100

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. In Model 2, model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements were adjusted for each HOMA measurement at V1.

TABLE XXI. INTERACTION EFFECTS BETWEEN TOTAL PRS AND TNONA ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	PRS	TNONA < 75th Percentile[a]			TNONA ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.23
	T2	0.96	0.72	1.28	1.00	0.61	1.63	
	T3	1.40	1.02	1.93	1.64	0.96	2.79	
Normoglycemic to hyperglycemic	T1	ref			ref			0.40
	T2	0.96	0.68	1.35	0.87	0.46	1.64	
	T3	1.53	1.03	2.28	1.85	0.90	3.78	
Normoglycemic to prediabetes	T1	ref			ref			0.33
	T2	0.96	0.68	1.36	0.86	0.46	1.63	
	T3	1.54	1.03	2.29	1.91	0.92	3.94	
Prediabetes to diabetes	T1	ref			ref			0.19
	T2	0.97	0.71	1.31	1.01	0.61	1.69	
	T3	1.36	0.97	1.90	1.62	0.93	2.81	
HOMA-IR	T1	ref			ref			0.53
	T2	-0.109	-0.247	0.028	-0.045	-0.187	0.097	
	T3	-0.024	-0.153	0.106	0.041	-0.094	0.176	
HOMA-B	T1	ref			ref			0.74
	T2	0.025	-0.129	0.179	0.041	-0.119	0.202	
	T3	0.031	-0.076	0.138	0.048	-0.071	0.166	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE XXII. INTERACTION EFFECTS BETWEEN INSULIN RESISTANCE PRS AND TNONA ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	Insulin resistance PRS	TNONA < 75th Percentile[a]			TNONA ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.02
	T2	1.05	0.76	1.45	0.79	0.45	1.41	
	T3	1.00	0.75	1.33	1.53	0.93	2.50	
Normoglycemic to hyperglycemic	T1	ref			ref			0.06
	T2	0.89	0.63	1.27	0.63	0.33	1.18	
	T3	1.45	1.02	2.06	2.34	1.25	4.41	
Normoglycemic to prediabetes	T1	ref			ref			0.05
	T2	0.89	0.62	1.27	0.61	0.32	1.16	
	T3	1.48	1.03	2.11	2.46	1.30	4.68	
Prediabetes to diabetes	T1	ref			ref			0.02
	T2	1.06	0.76	1.49	0.76	0.42	1.39	
	T3	1.01	0.75	1.36	1.63	0.97	2.74	
HOMA-IR	T1	ref			ref			0.08
	T2	0.038	-0.076	0.153	-0.002	-0.125	0.122	
	T3	-0.047	-0.193	0.099	-0.087	-0.237	0.063	
HOMA-B	T1	ref			ref			0.12
	T2	0.089	-0.043	0.221	0.063	-0.075	0.200	
	T3	0.016	-0.089	0.121	-0.011	-0.122	0.100	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE XXIII. INTERACTION EFFECTS BETWEEN BETA-CELL DYSFUNCTION PRS AND TNONA ON INCIDENT HYPERGLYCEMIC OUTCOMES, HOMA-IR AND HOMA-B AT V2

Outcomes at V2	β-cell dysfunction PRS	TNONA < 75th Percentile[a]			TNONA ≥ 75th Percentile[a]			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.63
	T2	1.07	0.76	1.50	1.19	0.68	2.09	
	T3	1.05	0.78	1.42	1.05	0.64	1.72	
Normoglycemic to hyperglycemic	T1	ref			ref			0.15
	T2	1.09	0.77	1.56	1.23	0.65	2.32	
	T3	1.26	0.83	1.89	0.93	0.44	1.95	
Normoglycemic to prediabetes	T1	ref			ref			0.18
	T2	1.05	0.73	1.50	1.22	0.64	2.33	
	T3	1.28	0.85	1.94	0.93	0.44	1.99	
Prediabetes to diabetes	T1	ref			ref			0.55
	T2	0.94	0.66	1.33	1.17	0.65	2.09	
	T3	1.09	0.79	1.51	1.06	0.63	1.79	
HOMA-IR	T1	ref			ref			0.68
	T2	-0.034	-0.167	0.099	-0.058	-0.182	0.065	
	T3	-0.056	-0.198	0.087	-0.080	-0.212	0.052	
HOMA-B	T1	ref			ref			0.34
	T2	0.111	-0.040	0.263	0.078	-0.072	0.228	
	T3	-0.014	-0.124	0.096	-0.047	-0.148	0.054	

a: Models adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

7. Sensitivity Analyses with PRS, Wet POPs, and Incident Hyperglycemic Outcomes, HOMA-IR and HOMA-B at V2

We additionally conducted sensitivity analyses with wet-weight POPs. Associations between each POP and diabetes outcomes at V2, using a dichotomous variable stratified at the 75th percentile of each wet-weight POP concentration, were assessed and there were no remarkable discrepancies from results with lipid-standardized POPs. We also investigated interaction effects between total PRS, insulin resistance PRS, and β-cell dysfunction PRS and each wet POP. The patterns of interaction and stratum-specific estimates were similar with results from lipid-standardized POPs. The results of sensitivity analyses with wet POPs were displayed in the Tables CII – CXVII in Appendix A.

D. Discussion

In this study, we investigated the relationships among PRS constructed with diabetes-related SNPs, POPs, and diabetes-related outcomes after 6 years of follow up in a sub-population of Latinos from HCHS/SOL. Our results demonstrated positive relationships of total and β-cell dysfunction PRS with incident diabetes and prediabetes, but not with HOMA-IR or HOMA-B. No main effects of PCBs and OC pesticides on diabetes related outcomes were observed in our results, which was likely related to dichotomization of POPs exposure to facilitate interaction modeling, however, we found significant interactions between PRS and several POPs on incident hyperglycemic outcomes.

In our results, total PRS constructed with 118 T2D susceptibility loci was associated with increased odds of incident hyperglycemia. These findings are in line with previous studies that investigated associations between genetic risk scores of T2D and diabetes in different ethnic groups [124, 240, 241] as well as the full HCHS/SOL cohort [117]. Our results also indicate that

there are associations between total PRS and subclinical diabetes among people with normal glucose levels at study baseline, which implies the genetic susceptibility of T2D is involved in multiple stages in the development of diabetes including the transition stage (prediabetes) and overt diabetes. It is also intriguing that β-cell dysfunction PRS was more strongly associated with preclinical stages of diabetes rather than overt diabetes in our analyses. In T2D etiology, it is believed that insulin resistance precedes β-cell dysfunction and reduced insulin secretion [59]. However, recent evidence from human studies suggests that hyperresponsiveness and impairment of β-cell function contributes to the development of glucose intolerance, with a subsequent massive decrease of β-cell mass during the later stage of diabetes development [62, 63]. The associations between β-cell dysfunction PRS and incident prediabetes in our study may provide supportive evidence for this new perspective on the role of β-cell function in the etiology of T2D progression.

Our results from main effect analyses of POPs on diabetes displayed no significant associations. Previous prospective studies have shown striking effects of POPs on incident diabetes or prediabetes [7, 10, 12, 86, 90], which is inconsistent with our current analysis. The difference between previous studies and our results may due to different participant characteristics and relatively shorter follow-up time of our study. The prevalence of diabetes is disproportionally higher in Hispanics than other race/ethnicity groups in the US, however, concentrations of PCBs and OC pesticide, except DDE, in our study sample were generally lower than study samples from other studies such as the Coronary Artery Risk Development in Young Adults (CARDIA) and NHANES 2003-2004 [7] (see Table XXIV for comparison of wet-weight POP analytes between studies). The discrepancies in POP concentrations may be due to different race/ethnicity composition across the studies; our study population is composed of participants with various Hispanic backgrounds, whereas CARDIA only consisted of non-Hispanic white and black populations, and NHANES comprises non-Hispanic white, non-

Hispanic black, Hispanics, and other race/ethnic groups. Racial disparity in POPs concentration has been understudied, however, previous research also demonstrated different body burdens of POPs in different race groups [127, 242], and lower POPs levels in Hispanic subjects compared to other race/ethnicity groups [243]. Therefore, though our study design contributes to expanded understanding of the POPs – T2D association in Hispanic population, the effects of POPs in general population may be underestimated in our study. In addition, as shown in Table XXIV, the age groups and years of POPs measurement varied by studies, which is likely to influence the different POPs levels across the study participants of each study.

Categorization of POPs level is another difference between our study and others. We dichotomized the POPs by 75th percentile to compare participants with highest concentrations to the participants with low and moderate POPs levels. However, this is likely to incur loss of information due to dichotomization and to underestimate the magnitude of associations due to the higher exposure levels in the reference group (<75th percentile); therefore our results may be inconsistent with those from other studies and are potentially biased to the null [244]. In addition, non-linear relationships of POPs and outcomes of interest cannot be detected using dichotomized POPs in our analyses.

Although we did not find significant associations between PCBs, OC pesticides and diabetes outcomes in the current analysis, we observed that each POP analyte displayed different patterns of association with diabetes outcomes in terms of the magnitude of effect estimates. Sum PCBs and TNONA showed the largest ORs for normoglycemia to prediabetes or to hyperglycemia, whereas the ORs for prediabetic or normoglycemic to incident T2D and prediabetes to T2D were almost null. In the case of DDE, the ORs were more similar for participants who were normoglycemic and prediabetic at V1 (ranged from 1.28 to 1.44 in the full models).

TABLE XXIV. COMPARISON OF POPs LEVELS ACROSS THE STUDIES

Analytes (pg/g) and Study	Measurement years	Age range (years)	Quartiles			
			Q1	Q2	Q3	Q4
DDE						
HCHS/SOL Ancillary Study	2008-2011	45-74	<1577	1577-3821	3821-7933	>7933
CARDIA controls	1987-1988	20-36	<2153	2154-3312	3313-5731	>5731
NHANES	2003-2004	20-36	<462	463-759	760-1275	>1275
	2003-2004	36-52	<846	847-1444	1445-3035	>3035
OXYCHLOR						
HCHS/SOL Ancillary Study	2008-2011	45-74	<33	33-53	53-87	>87
CARDIA controls	1987-1988	20-36	<110	111-157	158-200	>200
NHANES	2003-2004	20-36	-	<30	31-52	>52
	2003-2004	36-52	<46	47-70	71-108	>108
TNONA						
HCHS/SOL Ancillary Study	2008-2011	45-74	<54	55-84	85-135	>135
CARDIA controls	1987-1988	20-36	<109	110-174	175-250	>250
NHANES	2003-2004	20-36	<27	28-51	52-88	>88
	2003-2004	36-52	<61	62-101	102-169	>169
PCB74						
HCHS/SOL Ancillary Study	2008-2011	45-74	<13	13-21	22-37	>37
CARDIA	1987-1988	20-36	<204	205-349	350-466	>466
NHANES	2003-2004	20-36	<37	38-55	56-89	>89
	2003-2004	36-52	<22	23-31	32-48	>48
PCB153						
HCHS/SOL Ancillary Study	2008-2011	45-74	<106	107-173	174-271	>271
CARDIA controls	1987-1988	20-36	<204	205-349	350-466	>466
NHANES	2003-2004	20-36	<37	38-55	56-89	>89
	2003-2004	36-52	<106	107-155	156-231	>231
PCB178						
HCHS/SOL Ancillary Study	2008-2011	45-74	<5.8	5.9-9.6	9.6-14.9	>14.9
CARDIA controls	1987-1988	20-36	<8	9-15	16-21	>21
NHANES	2003-2004	20-36	<1	2-3	4-5	>5
	2003-2004	36-52	<6	7-9	10-13	>!3
PCB187						
HCHS/SOL Ancillary Study	2008-2011	45-74	<25	26-41	42-63	>63
CARDIA controls	1987-1988	20-36	<44	45-78	79-104	>104
NHANES	2003-2004	20-36	<6	7-11	12-19	>19
	2003-2004	36-52	<23	24-35	36-43	>54

The ORs for OXYCHLOR were also similar across the diabetes outcomes at V2, however the ORs were smaller than those of DDE (ranged from 1.07 to 1.11 in the full models). The pattern of these results remained similar in the analyses with wet-weight PCB, except TNONA; with wet-weight TNONA, the ORs for incident T2D and prediabetes to diabetes were closer to the ORs for normoglycemia to prediabetes and hyperglycemia.

Moreover, it appeared that the effects of polygenic risk of T2D on clinical diabetes indicators were modified by greater exposure to POPs. The significant interaction effects between PRS and POPs were displayed across all four types of POPs in this study. Participants belonging to the highest tertile of PRS were at greater risk of developing subclinical or overt diabetes, compared to participants in lower tertiles. The positive interactions between PRS and POPs suggested higher POPs concentration might elevate the risk among the people with higher genetic risk. Our results are similar to results from a previous study which assessed the interactions between exposure to bisphenol A (BPA) and genetic risk score on T2D [245]. In the study, authors found associations between PRS and T2D, but not with BPA and T2D, and positive interactions existed between PRS and BPA with T2D. While evidence is still insufficient due to the small number of investigations on gene-environment interactions on diabetes risk, these results suggest that relatively higher exposure to endocrine-disrupting chemicals can modify the effects of genetic components, and emphasizes the importance of consideration of the role of environmental pollutants in the pathway between genetics and diabetes development.

Although the interactions between PRS and POPs on hyperglycemic outcomes appeared across all 4 POPs in the study, the patterns of interactions varied by each POP (Table XXV). PRS in total, and stratified by biological function appeared to interact with different POPs. Total PRS showed significant interactions with sum PCBs and DDE. Insulin resistance PRS interacted with sum PCBs, OXYCHLOR, and TNONA. B-cell dysfunction PRS appeared to

interact only with OXYCHLOR. Furthermore, the interactions between PRS and POPs were influential on different hyperglycemic outcomes by their combinations. The interactions of PRS with sum PCBs, DDE, and TNONA were likely to have synergetic effects on incidence of overt diabetes from prediabetes. On the other hand, the PRS interactions with OXYCHLOR were more likely to be associated with development of hyperglycemia from normal glucose status. These results imply that interaction between genetic component and specific environmental chemicals may contribute to progression of diabetes in different stages.

TABLE XXV. SUMMARY OF INTERACTIONS BETWEEN PRS AND POPs ON INCIDENT HYPERGLYCEMIC OUTCOMES AT V2

	sum PCBs	DDE	OXYCHLOR	TNONA
Total PRS	Normoglycemic or prediabetes to T2D Prediabetes to diabetes	Normoglycemic or prediabetes to T2D Prediabetes to diabetes	No interactions	No interactions
Insulin resistance PRS	Prediabetes to diabetes	No interactions	Normoglycemic to hyperglycemic Normoglycemic to prediabetes HOMA-IR	Normoglycemic or prediabetes to T2D Prediabetes to diabetes
β-cell dysfunction PRS	No interactions	No interactions	Normoglycemic to hyperglycemic Normoglycemic to prediabetes	No interactions

The underlying mechanisms of the interaction between genetic components related to T2D and POPs on diabetes pathology are not fully understood. One of the possible mechanisms might be through changes in DNA methylation. DNA methylation is involved in modifying DNA activity and gene transcription without changing genotypes. In previous epidemiologic research, higher POP concentrations were likely to be associated with global DNA methylation levels [246-249]. Results from the studies were not consistent, though a majority of the studies found that elevated POPs levels were associated with DNA hypomethylation [246-248], rather than hypermethylation [249]. Furthermore, several studies demonstrated that global DNA methylation profiles were associated with pathogenesis of diabetes [250, 251]. These studies may provide a key to link the genetic components and POPs with diabetes risk; however, lack of studies with consideration of cell heterogeneity and confounders remained as challenges in this field. Future studies are certainly needed to explore the associations between POPs and DNA methylation to enlighten the mechanism of GxE in association with T2D etiology.

The lack of PRS – POPs interactions on HOMA measurements in our results was expected as no significant main effects were apparent in models of HOMA measurements with either PRS or POPs. However, it is intriguing that even PRS constructed with SNPs known to be biologically related to insulin resistance or β-cell dysfunction did not capture any relationships between genetic components and HOMA-IR or HOMA-B measurements at V2. It may be due to the different genetic ancestry from GWAS to select SNPs for PRS, since most of the GWAS were conducted in non-Hispanic populations [252, 253]. It has been reported that HOMA-IR showed significant heritability in Mexican-Americans [254], which supported a potential role of genetic components in the Hispanic population.

In addition, other factors we did not included in the analysis might mediate or confound the associations between insulin resistance or β-cell dysfunction PRS and HOMA

measurements. Other than glucose or insulin related SNPs, SNPs related to other traits in T2D development may play a role in change of HOMA measurements. For example, a study has reported that PRS constructed with SNPs related to lipid levels were associated with variations in HOMA-IR [255]. Further studies adopting expanded PRS constructed with SNPs associated with T2D pathology may contribute to a more comprehensive understanding of associations between genetic components and T2D etiology including insulin resistance and β-cell dysfunction.

To our knowledge, this is the first study that assessed the interaction between POPs and PRS in association with T2D in a Hispanic population. Most of previous GWAS were conducted in European populations [252, 253], therefore studies adopting PRS in Hispanics are also sparse despite the usefulness of PRS in association with many adverse health outcomes [256]. Efforts have been emerging to generalize the GWAS and results from PRS to other race/ethnic groups besides European populations [256, 257], however, populations with Hispanic/Latino background are still understudied.

In addition, though many studies have investigated the relationships between POPs and T2D, only a few studies assessed the effects of POPs on pathogenic pathways to T2D [38]. Since T2D is a multifactorial disease with a complex etiology, each stage in diabetes progression is likely to share common factors but in different mechanisms. Therefore, it is more important to have clear insight of the factors associated in each stage of diabetes. Our results with prediabetes and hyperglycemia may contribute to extended understanding in transition stages to overt diabetes as well as incident diabetes. Furthermore, studies investigating GxE interactions with environmental pollutants are sparser, and our study is one of the few studies focused on interactions with environmental chemicals and genetic components.

Nonetheless our study has some limitations. First, our study samples were drawn from a subgroup of participants selected for the POPs ancillary study and with genetic information,

therefore potential misclassification may exist. However, as the genotyping was performed independently of the selection of participants for the ancillary study, we believe the potential differentiation between participants with- and without genetic information is nondifferential with respect to the distributions of exposure and outcomes. We further examined the baseline characteristics, distributions of dichotomized POPs, and outcomes at V2 between the two groups in the ancillary study (with- vs. without genetic information), and did not observe notable differences (Appendix A).

Second, we used unweighted PRS in our analysis, which assumed that the effects of variants included in the construction of PRS are identical. Use of weighted PRS by adopting weights from the GWAS literature can reflect the different effect size of each SNP, however, a challenge remains because most of the GWAS studies have been performed in European and Asian populations and adopting the effect sizes from these populations may incur more bias in the study of a Hispanic population. Nevertheless, the associations between unweighted PRS and T2D in our analysis are consistent with other studies assessed T2D PRS and diabetes relationships.

Last but not least, we investigated the effects of individual or summed POPs, namely sum PCBs, p'p-DDE, OXYCHLOR, and TNONA. Since human beings are exposed to multiple chemicals and those chemicals are correlated with each other due to their common exposure pathways, future studies assessing the effect of POPs mixtures will contribute to deeper understanding in the effect of POPs on diabetes. Statistical considerations in exploring mixtures in GxE studies also remained as a challenge in this area.

In conclusion, in this prospective study we found associations between the PRS constructed with SNPs related to T2D and β-cell dysfunction and development of diabetes and prediabetes among Hispanics in a longitudinal setting. Exposure to POPs modified the effects of polygenic risk of T2D on the diabetes related outcomes. Our results support the effects of

genetic components on diabetes, and the importance of more research on GxE especially with environmental pollutants. We anticipate our results will contribute to understanding the role of genetic components and POPs, along with their interactions in T2D etiology among Hispanic/Latino population.

IV. ASSOCIATIONS BETWEEN PERSISTENT ORGANIC POLLUTANTS AND LIPID PROFILES IN HISPANIC COMMUNITY HEALTH STUDY / STUDY OF LATINOS

A. Introduction

Altered lipid metabolism has been considered a risk factor for development of cardiovascular disease [24-27]. It has been also thought that dyslipidemia is involved in development of T2D through disruption of β-cell function and insulin secretion [28-30], although debates are arising from genetic studies using Mendelian randomization that reported no association of low HDL cholesterol and high TG with T2D [31, 32]. Epidemiologic literature suggests that exposure to persistent organic pollutants (POPs) can result in alterations in serum lipids [39]. POPs are a group of halogenated toxic chemicals including aldrin, chlordane, 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), dichloro-diphenyl-trichloroethane (DDT), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), mirex, dioxins and furans. Although many countries have banned production and import of POPs, some countries in Central or South America had longer duration of POPs use, and therefore, populations with the origin in those countries may have higher levels of POPs in the body, particularly OC pesticides [67, 68].

Persistent organic pollutants (POPs) may influence human health through multiple mechanisms, including altering lipid metabolism, disrupting glucose transport and the insulin signaling pathway, and impacting synthesis, metabolism, transport and action of steroid and thyroid hormones [6, 34], therefore it is plausible that POPs play a role in development of metabolic diseases. However, epidemiologic research with POPs and metabolic disorders is complex due to the lipophilic characteristics of POPs. Since POPs are lipid-soluble and accumulate in lipid-containing tissues, higher lipid concentration in body may play a role as a confounder, masking associations between POPs and health outcomes [6, 88]. On the other

hand, since POPs are known to disrupt normal mechanisms in metabolic and endocrine systems, elevated lipid levels might be a result in higher exposure to POPs [38]. Therefore, the association between POPs and lipid profiles was challenging to evaluate in cross-sectional studies that incur potential reverse causality [6].

Results from cross-sectional studies reported that POPs contributed to altered lipid synthesis, including higher total- and LDL cholesterol, and triglycerides, as well as lower HDL cholesterol [34-37]. A few prospective studies also supported associations between POPs and lipid profiles [38, 39, 41]. However, there are inconsistencies in the literature that require further investigation. For example, the PCB congeners or congener groupings as well as individual or grouped OC pesticides associated with lipid profiles differed by study population and study design. In addition, the role of factors such as dietary fat consumption and use of lipid lowering medication in association with POPs – lipid relationship have not been vigorously assessed in previous studies. Dietary fat intake may play a role as an important confounder considering it is a primary source of POPs exposure in the general population and it also affects lipid synthesis, however only a few studies have incorporated diet information in the analysis [39, 40]. Taking medications for lipid control may affect the associations between POPs and lipid by inducing bias in the POPs and/or serum lipid measurements, but excluding persons using lipid medications may attenuate associations. Yet studies that evaluated the impact of lipid medication use are sparse. Finally, due to lack of studies that have assessed both cross-sectional and longitudinal associations in the same population, the impact of reverse-causality on cross sectional findings is unclear.

In this study, we aimed to investigate the associations between POPs concentrations and lipid profiles both in cross-sectional and longitudinal settings. We also assessed the role of lipid lowering medication in the longitudinal analysis of our study.

B. Methods

1. Study Design and Participants

Study participants were from Hispanic Community Health Study/Study of Latinos (HCHS/SOL). The HCHS/SOL is a multi-center epidemiologic study in Hispanics/Latinos, aiming to evaluate the effects of acculturation on health outcomes and to identify factors influence the health of Hispanic/Latino population [225, 226]. The baseline information (Visit 1; V1) was obtained during 2008-2011 with participants aged 18-74 years, and annual follow-up interviews were conducted. The first re-examination (Visit 2; V2) of the cohort was performed during 2015-2017. For this research, data from 2,350 HCHS/SOL participants in the ancillary study "Persistent Organic Pollutants, Endogenous Hormones and Diabetes in Latinos (PI: Persky)" were included. The 2,350 participants of the ancillary study are consisted with 1,175 subjects with prediabetes and 1,175 with normal glucose levels at baseline (V1) who returned for the first follow-up examination (V2). As mentioned in Aim 1, the participants in the ancillary study were oversampled for people in transitioning from prediabetes to diabetes.

Out of 2,350 total participants in the ancillary study, we excluded participants whose full lipid profiles were not available either at V1 (n=46) or V2 (n=40). We excluded participants who did not have complete covariate information (n=106). We also excluded participants who did not have complete POPs information. Finally, for the purpose of the study to look at the development of dyslipidemia, we restricted eligible subjects to participants not on lipid lowering medication at V1 (excluded n=253; 232 (91.7%) on statins, 21 (8.3%) on fibric/nicotinic acids), to avoid potential bias to the lipid concentrations, and potentially POPs concentrations, from medication use. Figure 2 displays the procedure of participants selection.

All participants provided written informed consent and the protocol of the study was reviewed by Institutional Review Board at UIC.

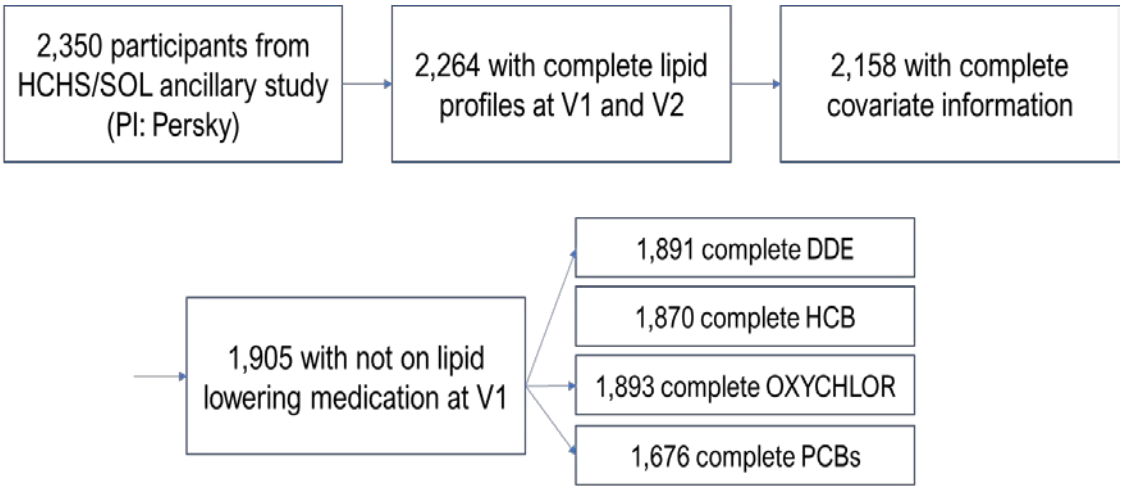

Figure 2. Selection of study participants for Aim 2

2. <u>Measurement of Lipid Profiles and POPs</u>

The HCHS/SOL participants provided approximately 58-76 mL of blood samples per each visit. Fasting blood samples were taken, and processed for laboratory assays at the University of Minnesota Medical Center, stored at -70°C as frozen specimens before being shipped from each study site. Lipid profiles included in the study comprised total cholesterol (mg/dl), HDL-cholesterol (mg/dl), LDL cholesterol (mg/dl), and triglycerides (mg/dl) from participants' blood samples at V1 and V2. HDL-cholesterol in serum was measured using a magnesium/dextran sulfate method (Roche Diagnostics, Indianapolis, IN). Serum triglycerides were measured using a glycerol blanking enzymatic method (Roche Modular P chemistry analyzer; Roche Diagnostics). LDL-cholesterol was calculated by the formula below [258]:

$$LDL\ cholesterol = total\ cholesterol - HDL\ cholesterol - TG/5$$

Twenty-four PCBs and 3 OC pesticides measured in the HCHS/SOL ancillary study were included in this analysis. In the ancillary study, POPs were measured on 1.0 ml plasma samples. The measurement of POPs was performed at the CDC laboratory (PI: Dr. Sjodin), adopting automatic fortification of the samples with a Gilson 215 liquid handler (Gilson Inc,; Middleton, WI).

The laboratory methodology for sample processing was described elsewhere [227]. In this analysis, we primarily employed wet-weight POPs (pg/g) and lipid-standardized POPs (ng/g lipid) as supplemental analysis, calculated from the formula below [228]:

$$lipid-adjusted\ POP\ (ppb) = \frac{wet-weight\ POP\ (ppt)}{total\ lipid\ (mg/dl)} * 102.6$$

, where total lipid was calculated using the equation suggested in Bernert et at al. (2007) [229]:

$$total\ lipids(mg/dl) = 2.27 * total\ cholesterol + triglyceride + 62.3\ .$$

Levels of POPs below limit of detection (LOD) were replaced by values of each LOD divided by squared root of 2. In the analysis, we used a summary measurement of PCBs (sum PCBs) by summing up all the individual PCB measurements. Three OC pesticides were modelled individually. The characteristics of PCB and OC pesticides congener included in the analysis are presented in Table XXVI.

3. Covariates

Participants' age, sex, Hispanic background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one heritages or others), education attainment, alcohol and cigarette use, physical activity level, lipid lowering medication use at V2,

dietary scores, acculturation scores, and study center (Bronx, Chicago, Miami, or San Diego) were obtained from self-report by HCHS/SOL participants.

Education attainment was classified into three groups including less than high school, high school graduate, and greater than high school. Alcohol use was classified into no current use, low-level use (less than 7 drinks per week), and high-level use (7 or more drinks per week). Cigarette smoking was categorized into 3 groups as never smoker, former smoker, and current smoker. Physical activity level was classified into 3 categories (high, moderate, and low) based on intensity of activities in work, leisure time, and transportation, along with total metabolic equivalent (MET) values.

Participants were asked about lipid lowering medication use in past 30 days before each visit, and any use of medications for antihyperlipidemia was coded as lipid medication use. Acculturation scores were measured based on participants' nativity, resident years in the US, and language spoken at home. In HCHS/SOL, participants' diet pattern was assessed using Alternative Healthy Eating Index 2010 (AHEI-2010) [259] with scores from 11 food and nutrition groups, each with a range from worst (scored as zero) to best (scored as 10).

We included scores for red/processed meat consumption (servings/day), trans fat intake (% energy), and polyunsaturated fatty acids intake (% energy) in current analyses. As clinical characteristics of study participants, we included BMI and change of BMI from V1 to V2. We also included baseline glucose status (normoglycemic or prediabetes), as well as change of glucose status from V1 to V2. The change of glucose status was measured as a binary variable. If participants' baseline glucose status was maintained or improved at V2 (normoglycemic both at V1 and V2, prediabetes both at V1 and V2, or prediabetes at V1 and normoglycemic at V2), the variable was coded as 0, whereas worsen glucose status (normoglycemic at V1 and developing prediabetes or overt diabetes at V2, or prediabetes at V1 and developing overt diabetes at V2) was coded as 1.

4. Statistical Analysis

Descriptive statistics were obtained for study participants' characteristics, main exposures, main outcomes of the study. Age, BMI, change of BMI, AHEI scores for dietary fat consumption, acculturation scores, and each component of lipid profiles were treated as continuous variables.

Categorical variables comprised Hispanic background, education, alcohol and cigarette use, physical activity level, lipid lowering medication, baseline glucose status, change of glucose status, and study center. For POPs, we used quintiles of sum PCBs, DDE, HCB, and OXYCHLOR to assess non-linear associations between each POP and lipid profiles.

Associations between each POP and lipid profiles were evaluated using linear regression models for individual POPs and lipid components, and beta estimates were obtained as well as 95% confidence intervals (CIs). We constructed a stepwise investigation with multiple models to assess the associations of POPs – lipid profiles adjusting for other contributing factors. In Model 1, we only included participants' age, sex, Hispanic background, baseline lipids, and study center as covariates.

We additionally adjusted Model 2 for acculturation scores, dietary fat consumption (intake of red and processed meat, trans fat, and PUFA), education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status. In Model 3, we additionally controlled lipid lowering medication use at V2 along with all the covariates included in Model 2.

We primarily investigated the associations between wet-weight POPs and lipid profiles and performed the same analyses with lipid-standardized POPs as a sensitivity analysis. Interactions between POPs and other important covariates such as sex, lipid medication use at V2, baseline glucose status were assessed by adding product terms in the regression models.

We performed the same analysis in two different study settings; cross-sectional analysis with lipid profiles at V1 as outcomes, and longitudinal analysis with lipid profiles at V2 as the outcomes. In the models for longitudinal analysis with V2 lipid concentrations, we included

baseline lipid levels, BMI changes from V1 to V2, and glucose status changes from V1 and V2. For longitudinal analyses, we additionally performed a sensitivity analysis for wet-weight POPs with all POPS included in the study, and lipid profiles by excluding participants on lipid-lowering medication at V2.

To address potential concern with controlling baseline lipid levels for the models with lipid-standardized POPs, we also performed another sensitivity analysis with same models but using the change of lipid levels (lipid at V2 – lipid at V1) as outcomes. All analysis was done using PROC SURVEYREG modules in SAS 9.4 (Cary, NC), applying survey sampling weights for the ancillary study population.

C. Results

1. Characteristics of Study Participants

To demonstrate the basic characteristics of study participants, we obtained the number and proportion for categorical variables (participants' sex, Hispanic background, alcohol and cigarette use, physical activity, use of lipid lowering medication, and study center) and mean with standard error for continuous variables (age, BMI, dietary scores, and acculturation scores), respectively.

Because we did not find any significant interactions between POPs and sex, lipid lowering medication use at V2, and baseline glucose status on outcomes, we did not perform stratified analysis with any of the covariates and presented results with whole study samples. Table XXVII displays the baseline characteristics of study participants.

Table XXVIII presents characteristics of lipid profiles including total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG at V1 and V2, respectively. Briefly, the mean levels of each lipid component were decreased at V2 compared to the levels at V1.

TABLE XXVI. CHARACTERISTICS OF POPS INCLUDED IN AIM 2

Analyte (pg/g wet weight)	Full name	Sample N	< LOD (%)	Median (IQR)	min/max
Sum PCBs	-	1676	-	805.9 (490.5-1244.3)	46.25/16990
PCB28	2,4,4'-Trichlorobiphenyl	1676	17.20	5.31 (2.94-9.28)	1.13/1823.0
PCB66	2,3',4,4'-Tetrachlorobiphenyl	1676	33.10	3.24 (1.62-5.64)	0.92/828.1
PCB74	2,4,4',5-Tetrachlorobiphenyl	1676	0.18	20.93 (12.76-36.13)	1.13/5017.0
PCB99	2,2'4,4'5-Pentachlorobiphenyl	1676	0.48	21.06 (12.40-35.88)	0.92/957.4
PCB105	2,3,3'4,4'-Pentachlorobiphenyl	1676	10.86	7.59 (4.20-14.54)	0.99/284.7
PCB114	2,3,4,4',5-Pentachlorobiphenyl	1676	41.29	2.81 (1.55-4.89)	0.71/63.2
PCB118	2,3'4,4'5-Pentachlorobiphenyl	1676	0.06	38.7 (22.0-69.72)	1.56/1214.0
PCB138158	2,2,3,4,4',5'- and 2,3,3'4,4',6-Hexachlorobiphenyl	1676	0.12	92.24 (57.85-158.26)	1.41/2352.0
PCB146	2,2',3,4',5,5'-Hexachlorobiphenyl	1676	0.66	19.71 (11.85-30.74)	1.13/695.4
PCB153	2,2',4,4',5,5'-Hexachlorobiphenyl	1676	0.00	170.19 (103.74-269.05)	4.99/4187.0
PCB156	2,3,3',4,4',5-Hexachlorobiphenyl	1676	0.66	20.79 (12.47-32.34)	1.13/312.7
PCB157	2,3,3',4,4',5'-Hexachlorobiphenyl	1676	19.51	4.67 (2.78-7.58)	0.78/79.0
PCB167	2,3',4,4',5,5',-Hexachlorobiphenyl	1676	13.19	5.66 (3.43-9.44)	0.99/134.1
PCB170	2,2',3,3',4,4',5-Heptachlorobiphenyl	1676	0.06	48.15 (29.38-73.10)	1.13/641.4
PCB178	2,2',3,3',5,5',6-Heptachlorobiphenyl	1676	5.85	9.30 (5.59-14.38)	0.92/233.3
PCB180	2,2',3,4,4',5,5',-Heptachlorobiphenyl	1676	0.00	127.73 (76.12-193.69)	5.15/1705.0
PCB183	2,2',3,4,4',5',6-Heptachlorobiphenyl	1676	2.74	12.85 (19.81-7.77)	0.92/330.0
PCB187	2,2',3,4',5,5',6-Heptachlorobiphenyl	1676	0.30	40.61 (24.66-62.78)	1.13/1285.0
PCB189	2,3,3',4,4',5,5',-Heptachlorobiphenyl	1676	50.84	2.21 (1.48-3.42)	0.71/26.0
PCB194	2,2',3,3',4,4',5,5',-Octachlorobiphenyl	1676	0.48	26.83 (15.22-41.41)	1.13/357.6
PCB196203	2,2',3,3',4,4',5',6- and 2,2',3,4,4',5,5',6-Octachlorobiphenyl	1676	0.36	25.52 (14.87-42.04)	1.27/489.0
PCB199	2,2',3,3',4,5,6,6',-Octachlorobiphenyl	1676	0.48	28.74 (15.60-48.32)	1.13/551.5
PCB206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	1676	1.67	14.69 (8.33-25.82)	1.06/342.3
PCB209	Decachlorobiphenyl	1676	6.09	8.70 (4.98-20.61)	1.06/313.0
PP-DDE	2,2-Bis(4-chlorophenyl)-1, 1-dichloroethene	1891	0.00	3821.7 (1544.7-8051.8)	23.8/119900
HCB	Hexachlorobenzene	1870	0.00	69.10 (49.1-116.1)	11.9/10120
OXYCHLOR	Oxychlordane	1893	2.22	51.1 (32.1-85.7)	5.6/537.0

TABLE XXVII. CHARACTERISTICS OF 1,905 STUDY PARTICIPANTS AT V1

Participants characteristics (Total N=1,905)		N (%)
Age, mean (SE)		55.7 (0.4)
Sex	Male	941 (46.3)
	Female	964 (53.7)
Body mass index (BMI), mean (SE)		28.9 (0.2)
Hispanic background	Central American	190 (7.3)
	Cuban	320 (27.0)
	Dominican	172 (8.5)
	Mexican	745 (33.7)
	Puerto Rican	295 (14.2)
	South American	140 (4.9)
	More than one or other heritage	43 (4.3)
Education	Less than HS	727 (36.5)
	HS graduate	427 (19.5)
	Greater than HS	751 (44.0)
Alcohol use	No current use	936 (52.2)
	Low level use	880 (43.8)
	High level use	89 (4.0)
Cigarette use	Never	1009 (53.5)
	Former smoker	505 (26.3)
	Current smoker	391 (20.2)
Physical activity level	High	223 (9.6)
	Moderate	864 (46.6)
	Low	818 (43.9)
Lipid lowering medication use at V2	No	1957 (83.1)
	Yes	308 (16.9)
Red/processed meat consumption; AHEI-2010, mean (SE)		3.8 (0.08)
Trans fat consumption: AHEI-2010, mean (SE)		8.2 (0.02)
Polyunsaturated fat consumption: AHEI-2010, mean (SE)		5.5 (0.03)
Acculturation score, mean (SE)		1.79 (0.05)
Study Center	Bronx	404 (25.3)
	Chicago	467 (12.7)
	Miami	510 (37.1)
	San Diego	524 (25.0)

Table XXVIII. LEVELS OF LIPID COMPONENTS IN STUDY PARTICIPANTS, MEAN (SE)

Lipid profiles	N	mean (SE)
At V1		
Total cholesterol (mg/dL)	1905	213.4 (1.66)
HDL cholesterol (mg/dL)	1905	51.7 (0.51)
LDL cholesterol (mg/dL)	1905	135.7 (1.50)
Triglycerides (mg/dL)	1905	130.1 (2.62)
At V2		
Total cholesterol (mg/dL)	1905	200.0 (1.28)
HDL cholesterol (mg/dL)	1905	53.1 (0.62)
LDL cholesterol (mg/dL)	1905	123.2 (1.18)
Triglycerides (mg/dL)	1905	118.8 (2.2)

2. Associations between Sum PCBs (V1) and Lipid Profiles at V2

Table XXIX displays the associations between baseline sum PCBs and total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG at V2. We found non-linear negative associations between sum PCBs and HDL-cholesterol in Models 1 and 2, before controlling lipid lowering medication at V2. Participants in the 2nd and 5th quintiles of sum PCBs appeared to have lower HDL cholesterol compared to the participants in the lowest quintile (β=-2.93, 95% CI=-5.36 - -0.50 for Q2 vs. Q1 in Model 2; β=-3.10, 95% CI=-5.70 - -0.50 for Q5 vs. Q1 in Model 2, respectively). However, the associations were attenuated in Model 3, which further adjusted for medication use at V2, in both quintiles. We observed a borderline association between sum PCBs and elevated TG only in Model 1 (β=11.41, 95% CI=-0.99 – 23.81 for Q5 vs. Q1). No associations were found between sum PCBs and total cholesterol and LDL cholesterol.

TABLE XXIX. ASSOCIATIONS BETWEEN BASELINE (V1) WET-WEIGHT SUM PCBS AND LIPIDS AT V2 (TOTAL N=1,676)

Lipids at V2	Quintiles of sum PCBs	MODEL 1[a]			MODEL 2[b]			MODEL 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-1.26	-6.82	4.30	-1.47	-7.17	4.23	-1.82	-9.58	5.94
	Q3 vs. Q1	-3.56	-9.52	2.41	-3.28	-9.27	2.71	-4.51	-12.11	3.10
	Q4 vs. Q1	0.65	-6.71	8.00	0.39	-6.91	7.68	-0.98	-9.24	7.28
	Q5 vs. Q1	-6.55	-14.49	1.40	-6.38	-14.32	1.56	-7.85	-17.42	1.71
HDL	Q2 vs. Q1	-2.67	-5.15	-0.19	-2.93	-5.36	-0.50	-1.55	-4.64	1.53
	Q3 vs. Q1	-1.64	-4.06	0.78	-2.06	-4.52	0.40	-0.23	-2.84	2.39
	Q4 vs. Q1	-2.14	-4.61	0.34	-1.56	-3.96	0.84	0.08	-2.82	2.99
	Q5 vs. Q1	-3.54	-6.20	-0.89	-3.10	-5.70	-0.50	-1.50	-4.70	1.70
LDL	Q2 vs. Q1	0.81	-4.48	6.11	1.44	-3.77	6.65	-0.72	-7.54	6.10
	Q3 vs. Q1	-2.03	-7.38	3.32	-0.90	-6.28	4.47	-2.96	-9.90	3.98
	Q4 vs. Q1	2.34	-4.49	9.18	2.70	-4.04	9.43	1.06	-6.43	8.54
	Q5 vs. Q1	-5.50	-12.67	1.67	-4.46	-11.50	2.58	-6.67	-15.09	1.75
TG	Q2 vs. Q1	2.45	-6.14	11.03	0.73	-7.76	9.23	0.25	-9.74	10.2
	Q3 vs. Q1	0.03	-9.90	9.96	-1.20	-11.08	8.69	-7.78	-18.52	2.96
	Q4 vs. Q1	5.35	-5.91	16.61	0.53	-10.17	11.2	-4.48	-16.75	7.80
	Q5 vs. Q1	11.41	-0.99	23.81	5.98	-5.72	17.6	-0.19	-13.70	13.3

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and change of glucose status change at V2;
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

3. Associations between DDE (V1) and Lipid Profiles at V2

Table XXX displays the associations between baseline DDE and lipid profiles at V2. Across all the lipid components including total cholesterol, HDL cholesterol, LDL cholesterol, and TG, we did not find any associations of levels of DDE and lipid profiles in the study participants.

4. Associations between Baseline HCB (V1) and Lipid Profiles at V2

Table XXXI presents the associations between baseline HCB levels and lipid profiles at V2. The strongest association was found with TG, which showed 12.68 mg/dl increase of TG levels among participants in the 3rd quintile of HCB, compared to the participants in the lowest quintile (β=12.68, 95% CI=1.41-23.94).

We also found an inverse association in the highest quintile of HCB and HDL cholesterol in Model 1, showing that participants with highest HCB levels (Q5) had -2.92 mg/dl lower HDL cholesterol on average, compared to the participants with lowest HCB levels (Q1).

Other quintile categories (Q2-Q4) of HCB also showed marginally significant associations between HDL cholesterol and suggested a possible linear relationship between baseline HCB concentration and decreased HDL cholesterol levels at V2 (p-trend=0.06). However, the significant associations from the most parsimonious model disappeared in Models 2 and 3, after adjusted for other covariates and lipid-lowering medication use at V2. The directions for association of baseline HCB with total cholesterol and LDL cholesterol were likely to be mixed by quintile categories. However, no statistically significant associations were found between HCB and total cholesterol, and LDL cholesterol.

TABLE XXX. ASSOCIATIONS BETWEEN BASELINE (V1) WET-WEIGHT DDE AND LIPIDS AT V2 (TOTAL N=1,891)

Lipids at V2	Quintiles of DDE	MODEL 1[a]			MODEL 2[b]			MODEL 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-1.07	-6.78	4.65	-1.57	-7.29	4.14	3.05	-3.37	9.46
	Q3 vs. Q1	2.05	-4.02	8.12	0.27	-5.83	6.38	2.53	-4.65	9.71
	Q4 vs. Q1	-4.31	-11.4	2.82	-4.82	-12.0	2.36	-4.24	-12.3	3.86
	Q5 vs. Q1	1.83	-5.78	9.44	1.29	-6.43	9.01	2.33	-6.16	10.81
HDL	Q2 vs. Q1	-1.73	-4.18	0.71	-1.14	-3.51	1.22	0.06	-2.79	2.91
	Q3 vs. Q1	0.68	-1.69	3.06	1.90	-0.42	4.22	2.14	-0.83	5.11
	Q4 vs. Q1	-0.77	-3.50	1.97	0.14	-2.39	2.66	0.89	-2.23	4.01
	Q5 vs. Q1	-2.10	-4.98	0.78	-0.88	-3.53	1.77	-0.50	-3.83	2.83
LDL	Q2 vs. Q1	0.22	-5.22	5.66	-0.72	-6.11	4.67	2.82	-3.51	9.15
	Q3 vs. Q1	1.04	-5.01	7.09	-1.22	-7.25	4.82	0.95	-6.03	7.94
	Q4 vs. Q1	-2.79	-9.86	4.28	-4.35	-11.3	2.65	-3.64	-11.3	4.10
	Q5 vs. Q1	3.00	-4.63	10.6	1.47	-5.91	8.86	2.21	-5.99	10.41
TG	Q2 vs. Q1	2.51	-6.66	11.6	1.00	-7.78	9.77	2.30	-6.68	11.28
	Q3 vs. Q1	6.98	-3.10	17.0	3.54	-6.25	13.34	4.61	-6.37	15.60
	Q4 vs. Q1	1.86	-9.58	13.3	0.26	-10.4	10.94	-1.30	-12.6	10.07
	Q5 vs. Q1	10.92	-1.46	23.2	7.81	-4.31	19.93	10.81	-2.42	24.03

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and change of glucose status change at V2;
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

TABLE XXXI. ASSOCIATIONS BETWEEN WET-WEIGHT HCB (V1) AND LIPIDS AT V2 (TOTAL N=1,870)

Lipids at V2	Quintiles of HCB	MODEL 1[a]			MODEL 2[b]			MODEL 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	3.26	-2.62	9.15	2.95	-2.64	8.53	0.69	-5.99	7.36
	Q3 vs. Q1	3.21	-3.34	9.76	2.21	-4.10	8.53	1.02	-6.30	8.33
	Q4 vs. Q1	-5.55	-12.82	1.73	-5.28	-12.39	1.83	-6.37	-14.25	1.50
	Q5 vs. Q1	-1.93	-10.91	7.05	-1.62	-10.49	7.24	-5.29	-15.24	4.66
HDL	Q2 vs. Q1	-1.93	-4.16	0.29	-1.42	-3.54	0.70	-0.79	-3.37	1.79
	Q3 vs. Q1	-2.28	-4.82	0.26	-1.08	-3.41	1.25	0.04	-2.77	2.86
	Q4 vs. Q1	-2.26	-4.74	0.22	-0.33	-2.53	1.86	0.48	-2.34	3.31
	Q5 vs. Q1	-2.92	-5.62	-0.22	-0.79	-3.35	1.76	-0.09	-3.29	3.11
LDL	Q2 vs. Q1	5.21	-0.57	10.98	4.69	-0.69	10.06	2.63	-3.79	9.04
	Q3 vs. Q1	2.35	-3.95	8.65	1.13	-4.90	7.15	-0.12	-7.23	6.99
	Q4 vs. Q1	-4.23	-11.73	3.28	-4.92	-11.92	2.07	-5.31	-13.07	2.46
	Q5 vs. Q1	1.59	-6.94	10.11	0.61	-7.68	8.89	-1.66	-11.14	7.83
TG	Q2 vs. Q1	2.45	-6.18	11.07	0.67	-7.52	8.86	-2.74	-12.20	6.72
	Q3 vs. Q1	12.68	1.41	23.94	8.48	-1.88	18.84	3.68	-7.70	15.06
	Q4 vs. Q1	8.37	-1.86	18.59	1.81	-7.99	11.61	-5.12	-16.49	6.24
	Q5 vs. Q1	6.14	-7.42	19.70	-1.58	-14.80	11.64	-8.94	-24.30	6.42

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and change of glucose status change at V2;
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

5. Associations between Baseline OXYCHLOR (V1) and Lipid Profiles at V2

Table XXXII displays the associations between baseline wet-weights OXYCHLOR and lipid profiles at V2. While no other lipid component at V2 showed associations with baseline levels of OXYCHLOR, we found an inverse association between HDL cholesterol and the 2nd quintile of OXYCHLOR before controlling lipid-lowering medication use at V2 (β= -2.26, 95% CI=-4.39 - -0.12 for Model 1; β= -4.15, 95% CI=-8.07- -0.23 for Model 2, respectively). The association was attenuated in Model 3, after adjustment for use of lipid-lowering medication use at V2.

6. Sensitivity Analyses with Participants Not on Lipid Lowering Medication

We investigated the associations between each baseline wet-weight POP and lipid profiles at V2 in a subgroup of the study population. In this sensitivity analyses, we restricted analytic study participants by further excluding participants on lipid lowering medication at V2. In the results of sensitivity analyses, sum PCBs showed inverse associations with total cholesterol (Q3) and HDL cholesterol (Q4,Q5) after adjustment for all covariates.

In the results, participants with higher quintiles of HCB levels (Q4 and Q5) at baseline of the study had lower levels of HDL cholesterol at V2 in a reduced model with fewer covariates in the model, but the association was attenuated in the full model.

No remarkable associations with DDE and OXYCHLOR were noted, except that 2nd quintile of OXYCHLOR showed an inverse association with HDL cholesterol which was attenuated in Model 2. Tables XXXIII – XXXVI present the results from the sensitivity analyses.

TABLE XXXII. ASSOCIATIONS BETWEEN WET-WEIGHT OXYCHLOR (V1) AND LIPIDS AT V2 (TOTAL N=1,893)

Lipids at V2	Quintiles of OXYCHLOR	MODEL 1[a]			MODEL 2[b]			MODEL 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-2.52	-7.39	2.35	-3.86	-8.69	0.96	-3.68	-10.05	2.70
	Q3 vs. Q1	0.86	-5.20	6.92	0.29	-5.71	6.29	-1.37	-8.38	5.63
	Q4 vs. Q1	-1.34	-7.92	5.23	-1.10	-7.58	5.38	-3.72	-10.60	3.16
	Q5 vs. Q1	-5.16	-11.9	1.53	-4.53	-11.24	2.17	-4.15	-11.87	3.57
HDL	Q2 vs. Q1	-2.26	-4.39	-0.12	-4.15	-8.07	-0.23	-1.69	-4.32	0.94
	Q3 vs. Q1	-0.87	-2.92	1.18	-2.58	-6.41	1.24	1.03	-1.50	3.56
	Q4 vs. Q1	-0.71	-2.98	1.56	-3.40	-7.57	0.77	1.50	-1.14	4.13
	Q5 vs. Q1	-2.24	-4.64	0.17	-3.74	-7.78	0.29	-0.03	-2.96	2.89
LDL	Q2 vs. Q1	0.48	-4.34	5.30	-0.75	-5.40	3.91	-0.08	-6.21	6.04
	Q3 vs. Q1	1.37	-4.62	7.37	0.97	-4.87	6.80	-0.88	-7.87	6.10
	Q4 vs. Q1	-0.48	-6.87	5.92	-0.16	-6.49	6.18	-2.05	-8.97	4.86
	Q5 vs. Q1	-3.00	-9.51	3.51	-2.32	-8.65	4.00	-2.33	-9.98	5.32
TG	Q2 vs. Q1	1.24	-7.68	10.2	-1.63	-10.1	6.86	-4.09	-14.0	5.86
	Q3 vs. Q1	7.17	-3.61	17.94	3.66	-6.31	13.6	-0.08	-11.8	11.6
	Q4 vs. Q1	8.06	-3.70	19.8	3.47	-7.23	14.1	-4.60	-16.7	7.54
	Q5 vs. Q1	7.40	-5.27	20.0	2.87	-8.60	14.3	-1.55	-14.3	11.3

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and change of glucose status change at V2.
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

TABLE XXXIII. ASSOCIATIONS BETWEEN WET-WEIGHT PCBs (V1) AND LIPIDS V2, EXCLUDING PARTICIPANTS ON MEDICATION AT V1 AND V2

Lipids at V2	Quintiles of sum PCBs	MODEL 1[a]			MODEL 2[b]		
		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-4.72	-10.06	0.62	-4.45	-9.82	0.92
	Q3 vs. Q1	-6.82	-12.57	-1.07	-6.18	-11.90	-0.45
	Q4 vs. Q1	0.70	-6.26	7.66	0.33	-6.49	7.15
	Q5 vs. Q1	-5.66	-13.43	2.12	-5.27	-13.06	2.51
HDL	Q2 vs. Q1	-3.68	-6.19	-1.16	-3.81	-6.23	-1.39
	Q3 vs. Q1	-1.99	-4.58	0.61	-2.42	-5.01	0.16
	Q4 vs. Q1	-2.79	-5.57	-0.02	-2.00	-6.23	-1.39
	Q5 vs. Q1	-3.33	-6.21	-0.44	-3.18	-5.01	0.16
LDL	Q2 vs. Q1	-0.43	-5.26	4.40	0.29	-4.43	5.00
	Q3 vs. Q1	4.55	-8.78	1.32	-2.58	-7.58	2.41
	Q4 vs. Q1	-2.77	-1.82	10.92	4.53	-1.63	10.70
	Q5 vs. Q1	-2.77	-9.45	3.91	-1.84	-8.39	4.72
TG	Q2 vs. Q1	-0.40	-9.34	8.55	-1.81	-10.77	7.14
	Q3 vs. Q1	-1.57	-11.97	8.84	-2.09	-12.58	8.40
	Q4 vs. Q1	2.52	-9.91	14.94	-2.42	-14.21	9.36
	Q5 vs. Q1	6.08	-6.89	19.06	2.93	-9.60	15.47

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.

TABLE XXXIV. ASSOCIATIONS BETWEEN WET-WEIGHT DDE (V1) AND LIPIDS V2, EXCLUDING PARTICIPANTS ON MEDICATION AT V1 AND V2

Lipids at V2	Quintiles of DDE	MODEL 1[a]			MODEL 2[b]		
		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-1.58	-7.61	4.46	-2.63	-8.66	3.41
	Q3 vs. Q1	3.05	-2.97	9.07	1.31	-4.85	7.46
	Q4 vs. Q1	1.56	-5.84	8.96	0.96	-6.55	8.48
	Q5 vs. Q1	4.08	-3.71	11.86	3.52	-4.30	11.34
HDL	Q2 vs. Q1	-1.76	-4.24	0.73	-1.36	-3.77	1.06
	Q3 vs. Q1	1.29	-1.10	3.68	2.40	0.05	4.76
	Q4 vs. Q1	-0.57	-3.34	2.21	0.23	-2.34	2.80
	Q5 vs. Q1	-2.52	-5.47	0.44	-1.31	-4.09	1.46
LDL	Q2 vs. Q1	-0.25	-5.71	5.21	-1.48	-6.89	3.93
	Q3 vs. Q1	1.63	-3.82	7.09	-0.59	-6.18	4.99
	Q4 vs. Q1	2.80	-3.72	9.32	1.26	-5.29	7.82
	Q5 vs. Q1	5.54	-1.68	12.75	4.09	-2.93	11.10
TG	Q2 vs. Q1	2.45	-7.45	12.35	1.23	-8.26	10.71
	Q3 vs. Q1	5.73	-5.01	16.46	2.52	-8.06	13.11
	Q4 vs. Q1	1.08	-11.23	13.40	-0.16	-11.91	11.58
	Q5 vs. Q1	10.29	-2.81	23.39	7.00	-6.00	19.99

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.

b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.

TABLE XXXV. ASSOCIATIONS BETWEEN WET-WEIGHT HCB (V1) AND LIPIDS V2, EXCLUDING PARTICIPANTS ON MEDICATION AT V1 AND V2

Lipids at V2	Quintiles of HCB	MODEL 1[a]			MODEL 2[b]		
		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	2.15	-3.61	7.91	2.49	-3.02	8.00
	Q3 vs. Q1	3.28	-3.50	10.06	2.52	-3.99	9.02
	Q4 vs. Q1	-2.51	-9.78	4.75	-2.43	-9.72	4.87
	Q5 vs. Q1	3.17	-5.01	11.34	2.76	-5.37	10.89
HDL	Q2 vs. Q1	-2.11	-4.44	0.21	-1.80	-3.95	0.36
	Q3 vs. Q1	-1.20	-3.80	1.40	-0.26	-2.63	2.12
	Q4 vs. Q1	-2.76	-5.48	-0.04	-0.99	-3.33	1.34
	Q5 vs. Q1	-3.48	-6.49	-0.46	-1.74	-4.53	1.05
LDL	Q2 vs. Q1	4.07	-1.25	9.40	4.14	-0.84	9.13
	Q3 vs. Q1	2.37	-3.86	8.59	1.23	-4.72	7.18
	Q4 vs. Q1	-0.66	-7.24	5.93	-1.68	-8.12	4.76
	Q5 vs. Q1	6.97	-0.44	14.38	5.15	-2.15	12.45
TG	Q2 vs. Q1	2.40	-6.67	11.47	1.54	-7.11	10.19
	Q3 vs. Q1	7.53	-4.57	19.63	4.51	-6.75	15.77
	Q4 vs. Q1	9.37	-2.07	20.81	4.73	-6.18	15.63
	Q5 vs. Q1	7.72	-7.10	22.55	2.88	-11.69	17.45

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.

TABLE XXXVI. ASSOCIATIONS BETWEEN WET-WEIGHT OXYCHLOR (V1) AND LIPIDS V2, EXCLUDING PARTICIPANTS ON MEDICATION AT V1 AND V2

Lipids at V2	Quintiles of OXYCHLOR	MODEL 1[a]			MODEL 2[b]		
		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-2.66	-7.35	2.02	-3.08	-7.69	1.53
	Q3 vs. Q1	1.06	-4.85	6.97	1.08	-4.83	6.98
	Q4 vs. Q1	-0.58	-7.02	5.87	0.18	-6.07	6.43
	Q5 vs. Q1	-4.75	-11.31	1.81	-3.62	-10.33	3.09
HDL	Q2 vs. Q1	-2.21	-4.34	-0.08	-1.53	-3.64	0.58
	Q3 vs. Q1	-0.50	-2.54	1.54	0.13	-1.83	2.10
	Q4 vs. Q1	-0.35	-2.79	2.10	0.36	-1.98	2.71
	Q5 vs. Q1	-1.90	-4.41	0.61	-1.39	-3.78	1.00
LDL	Q2 vs. Q1	-0.50	-4.69	3.70	-1.08	-5.10	2.94
	Q3 vs. Q1	1.08	-4.26	6.43	1.04	-4.23	6.31
	Q4 vs. Q1	-0.08	-5.97	5.82	0.42	-5.21	6.04
	Q5 vs. Q1	-2.23	-8.11	3.66	-1.16	-7.15	4.82
TG	Q2 vs. Q1	0.05	-9.11	9.22	-2.37	-11.39	6.66
	Q3 vs. Q1	3.87	-6.85	14.59	1.15	-9.02	11.33
	Q4 vs. Q1	5.37	-7.11	17.84	2.21	-9.52	13.94
	Q5 vs. Q1	2.44	-11.28	16.16	-0.36	-13.14	12.42

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.

b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.

7. Results with Lipid-Standardized POPs

We further assessed the associations between POPs at V1 and lipid profiles at V2 using levels of lipid-standardized POPs. The associations of lipid-standardized DDE and OXYCHLOR with lipid profiles were not remarkably deviated from the results with wet-weights DDE and OXYCHLOR. We found associations between lipid-standardized sum PCBs and decreased levels of total cholesterol and LDL cholesterol, which were not apparent with wet-weights sum PCBs. The associations were sustained in Model 3, after controlling lipid lowering medication use at V2. Participants in the highest quintile of sum PCB had almost 10 mg/dl lower total cholesterol and LDL cholesterol compared to the participants in the lowest quintile (β=-9.71, 95% CI=-19.27 - -0.16 for Model 3 with total cholesterol; β=-9.77, 95% CI=-18.25 - -1.28 for Model 3 with LDL cholesterol, respectively). Similar patterns were observed in the associations between lipid-standardized HCB at V1 and lipid profiles at V2. Participants in the highest quintile of HCB showed lower levels of cholesterol compared to the participants in the lowest quintile (β=-11.98, 95% CI=-20.92 - -3.04 for Model 3). In addition, participants in the 4th quintile of HCB had lower levels of LDL cholesterol compared to the participants in the lowest quintile (β=-8.31, 95% CI= -15.86 - -0.76 for Model 3). The results from lipid-standardized POPs are displayed in Tables XXXVII - XL.

The results from sensitivity analyses examining the associations of lipid-standardized POPs with changes of lipid levels (V2 lipid – V1 lipid) were not notably different from the main results using V2 lipids as an outcome and V1 lipids as a covariate.

TABLE XXXVII. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED PCBS (V1) AND LIPIDS AT V2

Lipids at V2	Quintiles of sum PCBs	Model 1[a]			Model 2[b]			Model 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-6.42	-11.99	-0.86	-7.20	-12.92	-1.47	-9.08	-16.90	-1.26
	Q3 vs. Q1	-4.42	-11.01	2.17	-5.19	-11.71	1.33	-5.54	-13.82	2.75
	Q4 vs. Q1	-3.72	-10.90	3.47	-4.15	-11.02	2.72	-8.06	-16.17	0.05
	Q5 vs. Q1	-5.02	-13.10	3.06	-6.33	-14.37	1.71	-9.71	-19.27	-0.16
HDL	Q2 vs. Q1	-2.63	-5.25	0.00	-2.09	-4.68	0.50	0.12	-2.98	3.21
	Q3 vs. Q1	-0.86	-3.01	1.29	-0.31	-2.38	1.75	1.57	-0.98	4.12
	Q4 vs. Q1	-2.17	-4.76	0.41	-0.94	-3.38	1.49	-0.14	-3.04	2.75
	Q5 vs. Q1	-2.45	-5.15	0.25	-1.50	-4.08	1.07	-0.15	-3.20	2.90
LDL	Q2 vs. Q1	-5.70	-11.34	-0.05	-5.86	-11.38	-0.34	-9.14	-16.39	-1.90
	Q3 vs. Q1	-3.22	-9.35	2.91	-3.16	-9.26	2.93	-5.46	-12.99	2.08
	Q4 vs. Q1	-3.59	-10.20	3.02	-3.58	-9.92	2.75	-7.54	-14.91	-0.16
	Q5 vs. Q1	-5.34	-12.58	1.90	-5.49	-12.65	1.66	-9.77	-18.25	-1.28
TG	Q2 vs. Q1	4.06	-6.42	14.5	-0.21	-9.97	9.55	-5.71	-16.84	5.42
	Q3 vs. Q1	-1.04	-11.31	9.23	-5.07	-14.60	4.45	-8.30	-20.21	3.60
	Q4 vs. Q1	6.97	-4.75	18.6	0.85	-9.39	11.0	-2.46	-14.29	9.37
	Q5 vs. Q1	7.05	-5.22	19.3	0.39	-10.92	11.7	-3.62	-17.29	10.0

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

TABLE XXXVIII. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED DDE (V1) AND LIPIDS AT V2

Lipids at V2	Quintiles of DDE	Model 1[a]			Model 2[b]			Model 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-1.43	-7.39	4.53	-1.84	-7.71	4.03	-0.52	-7.07	6.02
	Q3 vs. Q1	3.33	-3.10	9.76	2.19	-4.41	8.79	3.32	-4.17	10.81
	Q4 vs. Q1	-3.22	-10.48	4.05	-4.10	-11.45	3.25	-5.38	-13.63	2.88
	Q5 vs. Q1	1.24	-6.29	8.77	0.88	-6.65	8.42	0.01	-8.29	8.32
HDL	Q2 vs. Q1	-1.18	-3.69	1.32	-0.74	-3.12	1.64	0.12	-2.79	3.04
	Q3 vs. Q1	0.96	-1.47	3.39	2.13	-0.18	4.44	2.37	-0.58	5.31
	Q4 vs. Q1	-0.52	-3.21	2.17	0.34	-2.15	2.83	0.75	-2.29	3.80
	Q5 vs. Q1	-1.75	-4.66	1.15	-0.66	-3.34	2.02	-0.33	-3.65	3.00
LDL	Q2 vs. Q1	-0.31	-5.96	5.34	-0.99	-6.54	4.55	0.07	-6.47	6.62
	Q3 vs. Q1	2.04	-4.55	8.63	0.36	-6.28	7.00	1.81	-5.71	9.32
	Q4 vs. Q1	-2.07	-8.73	4.59	-3.71	-10.33	2.92	-4.55	-12.20	3.09
	Q5 vs. Q1	2.64	-4.84	10.12	1.35	-5.87	8.56	0.34	-7.70	8.38
TG	Q2 vs. Q1	0.88	-8.86	10.62	-0.55	-9.52	8.42	-1.60	-11.03	7.84
	Q3 vs. Q1	5.66	-4.42	15.73	2.58	-7.18	12.34	1.72	-8.80	12.24
	Q4 vs. Q1	1.23	-10.45	12.91	-0.96	-12.01	10.09	-2.79	-14.16	8.58
	Q5 vs. Q1	7.45	-4.56	19.47	4.56	-7.16	16.28	6.74	-6.20	19.69

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

TABLE XXXIX. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED HCB (V1) AND LIPIDS AT V2

Lipids at V2	Quintiles of HCB	Model 1[a]			Model 2[b]			Model 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	2.87	-3.30	9.05	2.11	-3.75	7.97	-2.10	-8.83	4.62
	Q3 vs. Q1	1.57	-5.19	8.33	0.78	-5.76	7.33	-3.49	-11.00	4.01
	Q4 vs. Q1	-4.59	-11.9	2.78	-5.13	-12.05	1.79	-9.32	-16.82	-1.82
	Q5 vs. Q1	-4.46	-12.6	3.70	-5.32	-13.21	2.56	-11.98	-20.92	-3.04
HDL	Q2 vs. Q1	-1.54	-3.98	0.90	-0.61	-2.93	1.71	-0.62	-3.29	2.05
	Q3 vs. Q1	-1.05	-3.39	1.29	0.20	-1.94	2.33	0.86	-1.83	3.55
	Q4 vs. Q1	-1.80	-4.34	0.74	0.56	-1.68	2.79	0.61	-2.12	3.33
	Q5 vs. Q1	-2.14	-4.94	0.66	0.27	-2.40	2.94	0.53	-2.85	3.92
LDL	Q2 vs. Q1	3.12	-2.90	9.13	2.55	-2.99	8.08	-1.37	-7.89	5.14
	Q3 vs. Q1	1.12	-5.10	7.34	0.25	-5.80	6.29	-3.61	-10.91	3.69
	Q4 vs. Q1	-4.50	-11.8	2.87	-5.72	-12.55	1.11	-8.31	-15.86	-0.76
	Q5 vs. Q1	-1.89	-9.73	5.94	-3.55	-11.13	4.04	-8.89	-17.79	0.01
TG	Q2 vs. Q1	6.45	-4.54	17.43	1.45	-9.11	12.01	0.99	-11.12	13.09
	Q3 vs. Q1	5.80	-4.99	16.59	1.18	-8.93	11.30	-3.29	-15.01	8.43
	Q4 vs. Q1	6.38	-4.36	17.12	-2.15	-12.24	7.94	-9.38	-21.15	2.39
	Q5 vs. Q1	0.33	-13.1	13.84	-9.33	-22.13	3.48	-13.76	-29.60	2.09

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

TABLE XL. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED OXYCHLOR (V1) AND LIPIDS AT V2

Lipids at V2	Quintiles of OXYCHLOR	Model 1[a]			Model 2[b]			Model 3[c]		
		β	95% CI		β	95% CI		β	95% CI	
Total cholesterol	Q2 vs. Q1	-4.14	-9.15	0.87	-4.41	-9.49	0.68	-4.64	-11.37	2.09
	Q3 vs. Q1	-0.88	-7.39	5.64	-0.98	-7.35	5.39	-0.89	-8.38	6.60
	Q4 vs. Q1	-4.96	-10.89	0.97	-4.00	-9.87	1.88	-4.60	-11.45	2.25
	Q5 vs. Q1	-2.79	-9.62	4.04	-2.29	-9.18	4.60	-4.61	-12.61	3.39
HDL	Q2 vs. Q1	-0.88	-3.19	1.43	-0.28	-2.53	1.97	0.85	-2.05	3.75
	Q3 vs. Q1	0.21	-2.24	2.66	0.62	-1.83	3.07	2.03	-1.08	5.14
	Q4 vs. Q1	0.37	-2.17	2.92	1.26	-1.32	3.84	2.80	-0.46	6.05
	Q5 vs. Q1	-0.73	-3.54	2.08	0.12	-2.64	2.88	1.62	-1.90	5.14
LDL	Q2 vs. Q1	-3.51	-8.59	1.56	-3.85	-8.78	1.08	-4.88	-11.25	1.48
	Q3 vs. Q1	-0.90	-7.11	5.30	-1.11	-7.02	4.80	-1.41	-8.29	5.47
	Q4 vs. Q1	-4.48	-10.30	1.34	-3.64	-9.36	2.08	-4.63	-11.21	1.95
	Q5 vs. Q1	-2.99	-9.64	3.67	-2.35	-8.79	4.09	-4.88	-12.24	2.49
TG	Q2 vs. Q1	3.68	-6.47	13.83	0.52	-8.85	9.88	-0.07	-11.07	10.93
	Q3 vs. Q1	4.40	-6.04	14.84	2.99	-6.45	12.43	-0.58	-12.08	10.92
	Q4 vs. Q1	1.04	-8.54	10.62	-3.11	-12.17	5.96	-7.45	-18.21	3.31
	Q5 vs. Q1	8.21	-2.69	19.11	3.59	-6.31	13.49	-1.14	-12.46	10.18

a: adjusted for age, gender, Hispanic background, baseline lipids, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, change of BMI, baseline glucose status (normoglycemic or prediabetes), and glucose status change at V2.
c: additionally adjusted for lipid lowering medication use at V2 with Model 2 covariates.

8. Results from Cross-Sectional Analysis

We further examined the relationships between POPs (V1) and lipid profiles at V1 as cross-sectional analyses. The associations between sum PCBs (V1) and lipid profiles at V1 are presented in Tables XLI and XLII for wet-weights and lipid-standardized concentrations, respectively. In the wet-weights sum PCBs, strong dose-response relationships appeared with all 4 lipid components. Increased lipid levels were observed as elevated PCB concentrations. The trends were maintained both in Models 1 and 2, with- and without confounder adjustment. With lipid-standardized PCBs, the direction of association was changed, and non-significant inverse relationships were found between sum PCBs and total cholesterol, LDL, and TG for each stratum. No significant dose-response relationships were observed.

In Table XLIII, the associations between baseline wet-weights DDE and lipid profiles at V1 show similar trend with the associations between baseline sum PCBs and lipids. Positive relationships between wet-weights DDE and total cholesterol, LDL cholesterol, TG were observed, and a negative relationship was found with HDL cholesterol. There were striking dose-response relationships between wet-weights DDE and across the 4 lipid components, except the DDE-HDL in Model 2. However, with lipid-standardized DDE, no association was found with lipid profiles at V1 (Table XLIV).

Table XLV and XLVI display the associations of wet-weight and lipid-standardized HCBs with lipid profiles at V1, respectively. We also found positive relationships between wet-weights HCB (V1) and total cholesterol, LDL cholesterol, and TG with strong dose-response relationships. We observed non-monotonic negative relationships between lipid-standardized HCB and TG.

The associations between OXYCHLOR (V1) and lipid profiles at V1 demonstrated similar patterns with other POPs (V1) – lipids (V1) relationships. Positive associations with

strong dose-response relationships were appeared with wet-weights OXYCHLOR and total cholesterol, LDL cholesterol, and TG. Inverse non-monotonic associations were found with wet-weights OXYCHLOR and HDL cholesterol. No significant associations were observed with lipid-standardized OXYCHLOR and lipid profiles at V1 (Tables XLVII and XLVIII).

TABLE XLI. ASSOCIATIONS BETWEEN WET-WEIGHT PCBs (V1) AND LIPIDS at V1

Lipids at V2	Quintiles of sum PCBs	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	10.66	2.57	18.76	<.001	9.66	1.52	17.80	<.001
	Q3 vs. Q1	9.10	0.62	17.59		9.18	0.63	17.72	
	Q4 vs. Q1	22.28	13.15	31.41		20.87	11.95	29.79	
	Q5 vs. Q1	33.50	23.07	43.93		32.52	22.28	42.75	
HDL	Q2 vs. Q1	0.77	-2.44	3.99	<.001	-0.09	-3.34	3.16	<.001
	Q3 vs. Q1	0.95	-2.39	4.28		0.25	-3.12	3.61	
	Q4 vs. Q1	-0.32	-3.71	3.08		-1.52	-4.93	1.90	
	Q5 vs. Q1	2.54	-1.02	6.10		1.22	-2.30	4.75	
LDL	Q2 vs. Q1	7.44	0.57	14.32	<.001	7.13	0.02	14.25	<.001
	Q3 vs. Q1	4.68	-2.32	11.69		5.09	-2.08	12.25	
	Q4 vs. Q1	13.25	5.60	20.89		12.65	5.12	20.17	
	Q5 vs. Q1	22.58	13.33	31.82		22.30	13.22	31.37	
TG	Q2 vs. Q1	12.32	1.02	23.61	<.001	13.16	1.60	24.73	<.001
	Q3 vs. Q1	17.13	3.93	30.33		19.02	6.79	31.24	
	Q4 vs. Q1	46.88	31.83	61.93		48.88	34.57	63.19	
	Q5 vs. Q1	42.09	26.85	57.33		45.22	30.35	60.09	

a: adjusted for age, gender, Hispanic background, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLII. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED PCBS (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of sum PCBs	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	5.69	-2.69	14.07	0.30	4.07	-3.94	12.09	0.17
	Q3 vs. Q1	-8.24	-16.39	-0.08		-9.42	-17.55	-1.29	
	Q4 vs. Q1	-1.76	-10.48	6.95		-3.49	-12.26	5.28	
	Q5 vs. Q1	-3.47	-13.78	6.84		-5.32	-15.46	4.82	
HDL	Q2 vs. Q1	2.77	-0.64	6.19	0.17	1.89	-1.43	5.20	0.63
	Q3 vs. Q1	-0.73	-3.87	2.42		-2.03	-5.17	1.11	
	Q4 vs. Q1	1.60	-2.03	5.23		0.39	-3.17	3.95	
	Q5 vs. Q1	3.44	-0.29	7.16		1.44	-2.18	5.06	
LDL	Q2 vs. Q1	3.39	-3.46	10.24	0.15	2.47	-4.39	9.32	0.10
	Q3 vs. Q1	-7.50	-14.71	-0.29		-7.86	-14.96	-0.76	
	Q4 vs. Q1	-3.52	-10.84	3.79		-4.61	-11.98	2.77	
	Q5 vs. Q1	-4.89	-14.22	4.44		-5.83	-14.85	3.20	
TG	Q2 vs. Q1	-2.34	-17.40	12.73	0.35	-1.37	-15.09	12.35	0.76
	Q3 vs. Q1	-0.06	-17.12	16.99		2.38	-13.84	18.59	
	Q4 vs. Q1	0.80	-15.67	17.27		3.71	-11.74	19.17	
	Q5 vs. Q1	-9.95	-27.79	7.88		-4.53	-22.14	13.07	

a: adjusted for age, gender, Hispanic background, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLIII. ASSOCIATIONS BETWEEN WET-WEIGHT DDE (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of DDE	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	9.29	0.28	18.30	0.00	10.14	1.34	18.94	0.01
	Q3 vs. Q1	11.24	1.09	21.40		8.25	-1.28	17.77	
	Q4 vs. Q1	16.28	5.66	26.91		15.11	4.89	25.33	
	Q5 vs. Q1	15.15	4.28	26.01		13.79	3.35	24.24	
HDL	Q2 vs. Q1	-0.06	-3.36	3.23	0.05	0.30	-2.69	3.29	0.15
	Q3 vs. Q1	-2.68	-6.03	0.67		-2.89	-6.15	0.38	
	Q4 vs. Q1	-2.80	-6.82	1.22		-1.65	-5.51	2.21	
	Q5 vs. Q1	-3.05	-6.75	0.64		-2.11	-5.50	1.28	
LDL	Q2 vs. Q1	5.77	-1.99	13.53	0.00	6.25	-1.13	13.63	0.01
	Q3 vs. Q1	7.68	-0.37	15.73		5.30	-2.43	13.03	
	Q4 vs. Q1	13.07	4.12	22.02		11.54	3.00	20.07	
	Q5 vs. Q1	11.34	2.36	20.33		9.72	0.89	18.54	
TG	Q2 vs. Q1	17.94	4.03	31.86	<.001	17.95	4.07	31.82	0.00
	Q3 vs. Q1	30.93	11.74	50.13		28.80	11.85	45.74	
	Q4 vs. Q1	29.87	14.92	44.82		25.94	11.16	40.72	
	Q5 vs. Q1	33.97	17.70	50.24		30.66	15.10	46.22	

a: adjusted for age, gender, Hispanic background, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLIV. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED DDE (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of DDE	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	0.10	-9.41	9.60	0.34	0.04	-9.09	9.17	0.15
	Q3 vs. Q1	2.73	-7.49	12.96		-0.45	-10.37	9.47	
	Q4 vs. Q1	0.59	-10.21	11.38		-1.48	-11.83	8.86	
	Q5 vs. Q1	-5.55	-16.77	5.68		-7.63	-18.55	3.29	
HDL	Q2 vs. Q1	-0.44	-3.82	2.94	0.05	-0.12	-3.11	2.87	0.18
	Q3 vs. Q1	-2.08	-6.04	1.88		-2.09	-5.82	1.63	
	Q4 vs. Q1	-2.47	-5.78	0.84		-1.59	-4.76	1.58	
	Q5 vs. Q1	-3.01	-6.62	0.59		-1.83	-5.13	1.46	
LDL	Q2 vs. Q1	-1.70	-9.67	6.26	0.77	-1.69	-9.22	5.84	0.66
	Q3 vs. Q1	2.88	-5.29	11.05		0.50	-7.57	8.56	
	Q4 vs. Q1	1.96	-7.36	11.27		-0.12	-8.96	8.72	
	Q5 vs. Q1	-3.18	-12.41	6.06		-5.24	-14.38	3.91	
TG	Q2 vs. Q1	11.1	-7.56	29.85	0.24	9.20	-7.49	25.89	0.36
	Q3 vs. Q1	9.36	-5.77	24.49		5.38	-8.84	19.60	
	Q4 vs. Q1	5.48	-10.00	20.96		1.02	-14.47	16.51	
	Q5 vs. Q1	2.86	-13.72	19.45		-3.15	-18.93	12.63	

a: adjusted for age, gender, Hispanic background, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLV. ASSOCIATIONS BETWEEN WET-WEIGHT HCB (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of HCB	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	11.78	4.05	19.51	<.001	12.17	4.57	19.77	<.001
	Q3 vs. Q1	22.44	15.03	29.85		22.69	15.64	29.74	
	Q4 vs. Q1	38.46	27.98	48.94		39.44	30.23	48.66	
	Q5 vs. Q1	40.48	29.20	51.76		38.47	27.30	49.64	
HDL	Q2 vs. Q1	-0.93	-3.71	1.84	0.33	-0.99	-3.80	1.82	0.67
	Q3 vs. Q1	2.20	-0.76	5.16		1.56	-1.35	4.46	
	Q4 vs. Q1	-0.85	-4.25	2.54		-0.39	-3.69	2.90	
	Q5 vs. Q1	-3.67	-7.47	0.13		-2.34	-6.26	1.57	
LDL	Q2 vs. Q1	8.69	2.23	15.16	<.001	9.23	2.80	15.66	<.001
	Q3 vs. Q1	15.36	8.93	21.78		16.03	9.93	22.14	
	Q4 vs. Q1	30.10	20.62	39.58		30.51	22.24	38.79	
	Q5 vs. Q1	30.66	21.16	40.17		28.76	19.35	38.18	
TG	Q2 vs. Q1	19.90	5.55	34.25	<.001	19.47	5.52	33.42	<.001
	Q3 vs. Q1	24.56	11.72	37.40		25.63	11.30	39.97	
	Q4 vs. Q1	46.18	30.94	61.43		46.73	31.31	62.15	
	Q5 vs. Q1	67.15	44.52	89.78		59.98	39.54	80.41	

a: adjusted for age, gender, Hispanic background, and study center.
b: additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLVI. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED HCB (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of HCB	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	-2.85	-10.46	4.76	0.62	-2.37	-10.13	5.38	0.44
	Q3 vs. Q1	-8.11	-16.76	0.54		-6.10	-14.95	2.75	
	Q4 vs. Q1	0.20	-12.01	12.42		-0.01	-10.85	10.83	
	Q5 vs. Q1	-4.02	-15.54	7.49		-6.48	-17.27	4.32	
HDL	Q2 vs. Q1	0.11	-2.67	2.89	1.00	-0.23	-3.03	2.57	0.82
	Q3 vs. Q1	0.77	-2.11	3.66		0.17	-2.71	3.05	
	Q4 vs. Q1	1.13	-2.38	4.64		1.17	-2.39	4.73	
	Q5 vs. Q1	-1.50	-5.39	2.39		-0.63	-4.74	3.48	
LDL	Q2 vs. Q1	0.01	-6.40	6.42	0.87	0.75	-5.83	7.33	0.65
	Q3 vs. Q1	-4.61	-11.76	2.53		-2.66	-10.00	4.68	
	Q4 vs. Q1	2.51	-8.75	13.77		2.06	-7.78	11.89	
	Q5 vs. Q1	-1.98	-11.71	7.76		-4.19	-13.39	5.00	
TG	Q2 vs. Q1	-15.22	-29.23	-1.22	0.28	-14.83	-28.47	-1.20	0.15
	Q3 vs. Q1	-21.44	-36.46	-6.42		-18.16	-33.27	-3.05	
	Q4 vs. Q1	-17.07	-32.64	-1.49		-16.12	-30.58	-1.67	
	Q5 vs. Q1	-3.08	-26.83	20.67		-8.66	-28.41	11.09	

a: adjusted for age, gender, Hispanic background, and study center.
b:additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLVII. ASSOCIATIONS BETWEEN WET-WEIGHT OXYCHLOR (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of OXYCHLOR	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	0.40	-7.46	8.27	<.001	2.08	-5.79	9.95	<.001
	Q3 vs. Q1	10.97	2.70	19.24		12.13	3.79	20.47	
	Q4 vs. Q1	19.50	11.04	27.97		21.00	12.33	29.67	
	Q5 vs. Q1	30.48	20.41	40.56		33.00	22.76	43.24	
HDL	Q2 vs. Q1	-2.41	-5.45	0.64	0.13	-2.63	-5.52	0.27	0.12
	Q3 vs. Q1	-2.15	-5.21	0.91		-2.54	-5.38	0.31	
	Q4 vs. Q1	-3.59	-6.85	-0.34		-3.74	-6.92	-0.57	
	Q5 vs. Q1	-2.44	-5.81	0.93		-2.40	-5.47	0.67	
LDL	Q2 vs. Q1	3.17	-3.35	9.68	<.001	4.62	-2.00	11.25	<.001
	Q3 vs. Q1	10.42	3.56	17.28		11.65	4.81	18.49	
	Q4 vs. Q1	15.16	8.14	22.18		16.97	9.90	24.03	
	Q5 vs. Q1	23.43	14.80	32.07		25.63	16.91	34.36	
TG	Q2 vs. Q1	-1.99	-21.58	17.60	<.001	0.18	-17.72	18.08	<.001
	Q3 vs. Q1	13.25	-6.60	33.09		14.82	-4.44	34.08	
	Q4 vs. Q1	39.46	19.29	59.62		38.69	19.08	58.29	
	Q5 vs. Q1	47.36	26.91	67.81		48.78	27.83	69.73	

a: adjusted for age, gender, Hispanic background, and study center.
b:additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

TABLE XLVIII. ASSOCIATIONS BETWEEN LIPID-STANDARDIZED OXYCHLOR (V1) AND LIPIDS AT V1

Lipids at V2	Quintiles of OXYCHLOR	Model 1[a]				Model 2[b]			
		β	95% CI		p-trend	β	95% CI		p-trend
Total cholesterol	Q2 vs. Q1	3.35	-4.61	11.31	0.89	4.02	-3.82	11.85	0.84
	Q3 vs. Q1	-4.64	-12.48	3.21		-3.92	-11.94	4.10	
	Q4 vs. Q1	-1.11	-9.36	7.14		-0.18	-8.70	8.35	
	Q5 vs. Q1	1.10	-8.70	10.90		2.98	-6.69	12.65	
HDL	Q2 vs. Q1	-0.94	-3.89	2.01	0.25	-0.67	-3.47	2.13	0.23
	Q3 vs. Q1	-2.44	-5.59	0.70		-2.46	-5.55	0.63	
	Q4 vs. Q1	-2.42	-5.67	0.83		-2.23	-5.58	1.12	
	Q5 vs. Q1	-1.58	-5.05	1.89		-1.73	-5.10	1.65	
LDL	Q2 vs. Q1	5.24	-1.68	12.16	0.86	5.72	-1.18	12.63	0.84
	Q3 vs. Q1	-2.24	-8.65	4.17		-1.42	-8.17	5.34	
	Q4 vs. Q1	0.38	-6.57	7.32		1.64	-5.65	8.93	
	Q5 vs. Q1	1.16	-7.62	9.93		2.77	-6.05	11.60	
TG	Q2 vs. Q1	-5.06	-23.73	13.62	0.25	-5.50	-22.06	11.07	0.23
	Q3 vs. Q1	-0.05	-19.41	19.30		-0.49	-18.47	17.49	
	Q4 vs. Q1	4.49	-13.89	22.86		1.94	-16.62	20.50	
	Q5 vs. Q1	7.45	-11.20	26.10		9.59	-9.14	28.32	

a: adjusted for age, gender, Hispanic background, and study center.
b:additionally adjusted for acculturation scores, red- and processed meat consumption, PUFA, trans-fat consumption, education, alcohol and cigarette use, physical activity, BMI, and baseline glucose status (normoglycemic or prediabetes).

D. Discussion

In this study, we examined the associations between sum PCBs, DDE, HCB, and OXYCHLOR concentrations at V1 and serum lipid profiles at V2, including total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG in a Hispanic population. Our results from cross-sectional associations showed strong associations between wet-weight POPs and elevated total cholesterol, LDL cholesterol, and TG, but generally not HDL cholesterol. With lipid-standardized POPs, cross sectional associations were attenuated, although an inverse association between lipid-standardized HCB and TG was evident in quintiles 2-4. Our results with longitudinal associations of lipids at V2, adjusting for V1 lipids, showed inverse relationships between HDL cholesterol and wet-weights sum PCBs, HCB, and OXYCHLOR, but only in reduced models. In the sensitivity analysis excluding participants on lipid lowering medication at V2, sum PCBs were associated with lower HDL cholesterol levels. In the longitudinal analyses with lipid-standardized POPs, total cholesterol and LDL cholesterol were inversely associated with sum PCBs and HCB. We did not observe any associations between wet-weight or lipid-standardized DDE and lipid profiles at V2.

The strong cross-sectional associations between wet-weight POPs and total cholesterol, LDL cholesterol, and TG found in our study are consistent with results from previous studies that investigated wet-weight POPs and serum lipids [35-37]. However, we did not observe any associations between POPs and HDL cholesterol in our cross-sectional analysis, whereas other cross-sectional studies reported inverse associations [35, 36]. Our results with longitudinal associations between PCBs, HCB, and OXYCHLOR and lower levels of HDL cholesterol are in line with other studies with prospective design [38, 39, 41]. Biological mechanisms underlying the relationships between POPs and adverse changes in lipid profiles are not fully understood yet, however evidence suggests that POPs may participate in lipid homeostasis disorders through epigenetic mechanisms with genes involving lipid homeostasis regulation, such as

insulin-induced gene-1 (Insig-1) and lipin-1 (Lpin1) [34], which is also supported by an in-vitro study demonstrating that exposure to POPs contributed to increased lipid content by interfering with the receptor and signaling transduction of leptin sensitivity [260]. Abnormal lipid concentrations are well-known risk factors for cardiovascular disease (CVD) [26, 261], and we anticipate our results may contribute to understanding of associations between POPs and lipid profiles as well as, indirectly, the role of POPs in the development of CVD.

We observed decreased levels of total cholesterol and TG with elevated concentration of some lipid adjusted POPs in longitudinal analyses, which is not consistent with the majority of results from other studies. However, studies which assessed non-linear relationships detected inverse associations between POPs and elevated cholesterol and /or triglyceride levels at least in some strata [38, 41], or negative associations in models of continuous cholesterol and/or triglyceride measurements [36, 39]. These unexpected associations were often ignored due to the lack of significance of results or unrevealed plausible mechanisms. It is also challenging to compare the unexpected direction of associations because different composition of participants across studies. It is known that the burden of POPs may vary by race/ethnicity [127, 243]. In addition, body burden of POPs may differ by the geographic or age distribution of the study population. Furthermore, since almost all humans are likely to be exposed to at least a minimal dose of POPs, it is not possible to have clean controls as a reference group, which can induce heterogeneity among studies. More studies with repeated measurements in large populations are necessary for further understanding in this problem.

In the longitudinal analyses, we assessed the associations between POPs and lipid profiles with- and without participants using lipid lowering medication using sequential models and sensitivity analyses, which overcome the limitations of previous studies with lack of consideration for lipid lowering treatment in association with POPs and lipid profiles [38, 41]. With the sequential modeling, we observed the attenuation of POPs effects on HDL cholesterol

levels when controlling for lipid medication use, which suggested that medication use played a role in the associations between wet-weight POPs and levels of HDL cholesterol, either as a confounder or by introducing bias in the POPs and/or lipid measurements. Although we did not find statistical evidence of interaction between POPs and lipid lowering medication use (not shown), the attenuated associations in the final models imply that lipid lowering medication modified the effects of POPs on decreased levels of HDL cholesterol in population with moderate (Q2) or excessive (Q5) exposure to POPs. The potential effect modification by lipid lowering medication, but without statistical significance, has been also reported in a different cohort study [39]. The potential effect modification of lipid lowering medication needs to be investigated further with more information on antilipidemic medication subtypes.

In analysis with wet-weight POPs, the results between cross-sectional and longitudinal analyses were substantially different in our study. While PCBs, OXYCHLOR, and HCB showed associations with elevated total cholesterol, LDL cholesterol, and TG in cross-sectional analyses (Tables XLIV-LIV), but no associations with HDL, the three POPs demonstrated some associations only with quintiles 2 and 4 of HDL cholesterol in longitudinal analyses. Although the findings from cross-sectional analyses were generally consistent with other studies, the different pattern of associations in our side-by-side cross sectional and longitudinal analyses supports the necessity of longitudinal studies to elucidate the long-term effects of POPs on lipid metabolism. Indeed, our findings suggest that reverse causality may be biasing our cross-sectional estimates. Furthermore, the associations might be exaggerated or underestimated depending the timing of biomarker measurement and time window of exposure to POPs [262]. Therefore, to capture the change of lipid profiles during the lifespan, repeated measurement of POPs and lipids with more follow-up time also would be needed.

We investigated the associations between different types of POPs and lipid profiles. Our results, which showed the three types of wet-weight POPs – sum PCBs, HCB, and OXYCHLOR – were all inversely associated with HDL cholesterol in the reduced models. Furthermore, the associations with sum PCBs and OXYCHLOR demonstrated similar nonmonotonic dose responses in those models, showing strong associations in 2nd and 5th quintiles rather than the middle range of the exposures. The similarity between associations with different POP congeners may be a result of either confounding by uncontrolled POPs or the collective effects of POPs, which suggests the necessity of investigating the effects of different POPs as a mixture. Human beings are exposed to multiple environmental pollutants simultaneously, and analysis of environmental mixtures is a growing field of environmental epidemiology [46]. Future studies adopting advanced methods for chemical mixture analysis are needed to expand the understanding of the relationships between POPs and lipid profiles.

We investigated the associations between POPs and lipid profiles using either wet-weight or lipid-standardized POPs. Due to the lipid soluble characteristics of POPs, standardizing or adjusting POPs for lipid concentrations has been considered necessary to account for potential confounding from variability in lipid levels among participants and related to feeding and fasting. [43, 263]. However, the use of lipid-standardized POPs versus wet-weight POPs in epidemiologic studies, particularly those related to cardiometabolic outcomes, is controversial because POPs also could contribute to altered lipid synthesis, and in this case, using lipid standardized POPs would be overcontrolling and mask the true relationship between POPs and the cardiometabolic outcome [38, 264]. In line with this, a simulation study presented less biased results with wet-weight POPs compared to lipid-standardized POPs [265], and recent studies tend to present the results from both lipid-standardized and wet-weight POPs and showed strongest associations with wet-weight POPs, which is consistent with our results [39,

41]. Future research should emphasize more sophisticated analysis methodology, such as structural equation modeling, to address this complex issue.

The strengths of our study came from the prospective study design of HCHS/SOL, which enabled us to explore both cross-sectional and longitudinal associations between POPs and lipid profiles. Since POPs are lipid soluble and stored in adipose tissues once absorbed in the body, elevated lipid concentration may influence circulating POPs concentrations, and adjustment for serum lipids has been routinely used in statistical analysis of POPs exposures [228, 244]. On the other hand, POPs are known to alter the lipid metabolism in animal models [6]. Therefore, it is critically important to assess the possibility of reverse causality in the association between POPs and lipid profiles in cross sectional studies. To our knowledge, this is the first study that has adopted a side-by-side assessment with cross-sectional and longitudinal study designs. In addition, the substantial number of study participants also provided advantages for statistical modeling without concern for lack of statistical power. Finally, our study may contribute to the understanding of effects of environmental chemicals on altered lipid concentrations in an Hispanic population, which is a population at high risk of metabolic diseases but still understudied [266].

Our study has several limitations of note. Although it was likely that lipid lowering medication use played a role in the association between wet-weight POPs and HDL cholesterol levels, the information on the lipid lowering medication use was limited in our data in terms of the length of medication use and types of lipid-lowering medications. We controlled for lipid lowering medication use reported at participants' second visit (V2), which may not be sufficient to capture the variability from different durations of lipid lowering medication use by participants. In addition, potential effects from other drugs were not considered in current study. Another limitation is lack of more specific dietary information. Despite the importance of diet information with respect to source of POPs exposure [267] and changes in lipid profiles [268, 269], we were

not able to utilize the itemized dietary information such as diary product consumption or fish intake, which might induce residual confounding in our study. However, we cannot rule out the possibility that body burdens of POPs may play a role as a mediator in association with contaminated food consumption and altered lipid profiles, and in this case controlling for dietary variables would be over-adjustment for the model [36]. Future study is needed to elucidate the relationships between diet, POPs, and lipid profiles.

In conclusion, we assessed the cross-sectional and longitudinal associations between POPs and lipid profiles in Hispanic population. Our findings suggest effects of POPs on altered lipid metabolism but predominantly limited to the cross-sectional models that may be biased by reverse causality. In longitudinal models, POPs may have some impact on decreased HDL cholesterol, however further investigation is needed with more information on lipid lowering medication and dietary information for more comprehensive understanding of the relationship.

V. ASSOCIATIONS OF EXPOSURE TO METAL AND METAL MIXTURES WITH THYROID HORMONES

A. Introduction

The thyroid is critically involved in the functions of nervous system, metabolism and development [129], and the function of thyroid is measured by levels of thyroid hormones; thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). Most of the circulating T3 and T4 are bound to proteins such as albumin, globulin, and transthyretin, and only less than 1% of the hormones remained unbound (free forms of T3 and T4 ; i.e. FT3 and FT4) and biologically active [134]. Disrupted thyroid homeostasis has been known to be associated with altered neural differentiation followed by cognitive deficits [136, 137], and metabolic problems such as elevated blood pressure [138] and dyslipidemia [139]. The role of heavy metals, such as lead, mercury and cadmium, in thyroid hormone disruption has been investigated, however, the results in human populations are sparse for some metals and yet inconclusive [134, 165-167]. For example, in a study among policemen, higher urinary cadmium levels were associated with lower free T3 (FT3) and free T4 (FT4) but elevated TSH levels [270], whereas results from the National Health and Nutrition Examination Survey (NHANES) showed positive associations between urinary cadmium concentrations and FT3, T3, and T4, but no associations with TSH [134, 189]. According to the results from previous studies, the directions of association between metal and thyroid hormone levels also vary by type of metal. Results from a study investigating the effects of multiple metal exposure and thyroid hormone levels showed inconsistent directions between single metals and TSH; higher levels of cadmium, chromium, selenium, thallium, and zinc were associated with elevated TSH levels, while negative associations were shown with copper and lead [166].

Since human beings are often exposed to multiple metals simultaneously, it is important to assess the effects of metal mixtures on health outcomes rather than individual effects of single metal [46-48]. Evaluating the effects of simultaneous exposure to multiple metals is complicated by challenges such as non-additive and non-linear relationships between mixtures and health outcomes and multicollinearity among metals [49]. To overcome these problems, various approaches have been proposed including weighted quantile sum regression (WQS) [50], principal component analysis (PCA), Bayesian kernel machine regression (BKMR) [51], and most recently, quantile g-computation (QGCOMP) [52].

Among those methods, WQS has been used in many studies to evaluate the effects of multiple components as a mixture [210, 220-222], however it is not able to assess associations of exposures in different directions (positive or negative) since it assumes directional homogeneity among the components [210]. Quantile g-computation (QGCOMP) was introduced to overcome this challenge, and employs a generalized version of WQS relaxing the directional homogeneity assumption, and ,therefore, enables assessment of effects of exposures with opposite directions simultaneously [212]. Several studies have adopted QGCOMP to evaluate the associations between environmental mixtures on health outcomes such as allergy symptoms, body mass index (BMI), and changes in thyroid hormone [220, 223, 224].

Considering the inconclusive associations between metals and thyroid hormones, it is necessary to understand the combined effects of various metals; however, studies adopted these methods with metals and thyroid hormones are still meager. In this study, we aimed to utilize both traditional (linear regression) and advanced statistical methods to investigate the associations between single metal exposure, metal mixtures, and thyroid hormones.

B. Methods

1. Data and Study Participants

Study participants are from the National Health and Nutrition Examination Survey (NHANES). NHANES, conducted by Centers for Disease Control and Prevention (CDC; Atlanta, GA) to investigate the health and nutritional status of adults and children in the US, is a nationwide study with the complex multistage and stratified cluster design. The components of NHANES include questionnaires to obtain demographic, dietary, socioeconomic, health and medical information of study participants, and examinations comprising dental, medical, and physiological measurements, as well as laboratory tests for biological parameters and environmental exposures.

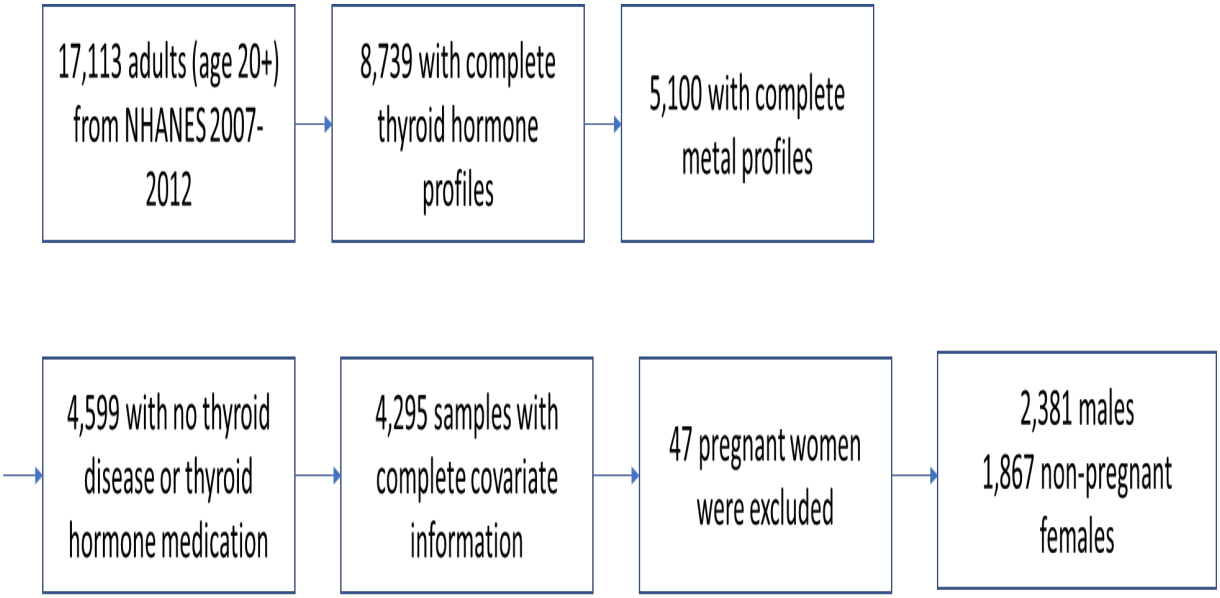

Figure 3. Selection of study participants for Aim 3

For the purpose of the study, we selected NHANES data from 2007 to 2013 in order to include cycles with thyroid profiles. We restricted the study participants to individuals aged 20 or older at the time of participation. Among the 17,113 adults, eligibility criteria for the analytic sample was: 1) complete thyroid hormone profiles (N=8,374 excluded), 2) complete metal profiles from 12 metals used in the study (N=3,639 excluded), 3) no self-reported thyroid disease or thyroid hormone medication use (levothyroxine, liothyronine, methimazole, propylthiouracil, and thyroid desiccated extract) at the point of survey (N=497 excluded for self-reported thyroid disease, 84 were on thyroid hormone medication, 80 had thyroid disease and were taking thyroid hormone medication simultaneously), 4) complete covariate information (N=304 excluded), and 5) not pregnant at the time of survey (N=47 excluded). As a result, the final analytic sample included 2,381 males and 1,867 non-pregnant females. Figure 3 represents the process for compiling the final analytic data. The NCHS Institutional Review Board approved the NHANES protocol, and written informed consent was provided by all study participants.

2. Measurements of Metals

We included 12 metals and metalloids measured through NHANES 2007-2012. Nine metals and metalloids (arsenic, barium, cobalt, cesium, molybdenum, antimony, thallium, tungsten, and uranium) were measured from urine, and 3 metals (cadmium, lead, and mercury) were obtained from participants' blood samples. To account for urine dilution, creatinine-adjusted concentrations were used for urinary metals, calculated by dividing the metal concentration by creatinine concentration. Urinary inorganic arsenic was calculated by subtracting urinary arsenobetaine levels from urinary total arsenic measurements. For metal concentrations below limit of detection (LOD), values were imputed by dividing the LOD by square root of 2. All metals were natural log transformed after examining the normality of each metal distribution and quartiles of each metal were used to assess linear or non-linear

relationships with thyroid hormones. The ranges of quartiles of each metal and metalloid were presented in Table CXVIII.

3. Measurement of Thyroid Hormones

We included serum levels of five measurements of thyroid hormones in the analysis, including triiodothyronine (T3), free T3 (FT3), thyroxine (T4), free T4 (FT4), and thyroid stimulating hormones (TSH). We also included T3:T4 ratio and FT4:TSH ratio as the outcomes in the analyses as indicators of thyroid function, specifically deiodinase activity and the negative feedback of the HPT axis, respectively.

We performed natural log transformation with all thyroid hormones and thyroid hormone ratios. For T3:T4 ratio and FT4:TSH ratio, the gravimetric units were transformed to the international system units for each hormone, then calculated ratios were natural log transformed.

4. Covariates

We included *a priori* covariates in the statistical analysis, including age, race/ethnicity, education attainment, smoking status, serum cotinine, menopausal status (for females only), hormone medication besides thyroid hormones (including adrenal cortical steroids, sex hormones, growth hormones, prolactin inhibitors, calcitonin, somatostatin and somatostatin analogs, selective estrogen receptor modulators, aromatase inhibitors, synthetic ovulation stimulants, and calcimimetics), body mass index (BMI), and study cycle.

The covariates were selected considering their relationships with metal concentration and thyroid hormones. Age, serum cotinine, and BMI were included as continuous scales, whereas all the other covariates were included as categorical variables.

5. Statistical Analysis

All analyses were stratified by sex *a priori* considering potential sex-related differences in the impact of environmental factors on endogenous hormone synthesis, action and metabolism [271, 272]. Descriptive analyses included exploring pairwise correlations and distributions of the 12 metals, as well as participant characteristics. To compare the participant characteristics by sex, Student's t-tests were used for continuous variables and Chi-square tests were used for categorical variables.

To investigate the relationships between metals and thyroid hormones, a multi-step procedure was used. In the first step, we assessed single exposure associations with linear regression models for each thyroid hormone. Each thyroid hormone model included one metal as main exposure, adjusted for all covariates as below:

$$Y_{thyroid\ hormone} = \beta_0 + \beta_1\ Metal_j + \ \beta_{2\ldots k}\ Covariates + \epsilon_i,$$

where β_0 denotes the model intercept, $Metal_j$ is the *j* th metal among the twelve-metal included in the analysis. Beta coefficients with 95% CI and p-values for linear trend (p-trend) were obtained.

A metal was included in the second step if the metal has either a significant dose-response relationship determined by p-trend <0.05, or a non-linear relationship determined by at least one significant quartile determined by p-value <0.05. In the second step, each thyroid hormone model included multiple metals from the first step, to assess the relationship of each metal with thyroid hormones under the existence of other metals as below:

$$Y_{thyroid\ hormone} = \beta_0 + \beta_1\ Metal_1 + \ \beta_2\ Metal_2 + \cdots + \ \beta_k\ Metal_k + \ \beta_{k+1}\ Covariates + \epsilon_i,$$

where *k* is the number of significant metals from the first step. Similar to the previous step, beta coefficients with 95% CI and p-trend were obtained from linear regression models.

We further examined associations of metal mixture and thyroid hormones, as well as relative contributions of each metal in a mixture. Metals that showed monotonic dose-response relationships (p-trend <0.05) in the second step were included in this last stage. We adopted quantile-based g-computation (QGCOMP) in this step to assess the relative contribution of different metals in the mixture. Quantile-based g-computation (QGCOMP) is a generalized version of weighted quantile sum (WQS) regression [212], which is used to assess the associations between mixtures and health outcomes. In both WQS regression and QGCOMP, correlated exposures (e.g. metals) are combined into one index to enable estimation of associations between a mixture and outcome [50, 212, 222]. In WQS regression, a weighted linear index is calculated by grouping multiple metals into ordinal quantile variables. Then empirical weights for each metal are obtained from bootstrap samples, and fitted in a regression model.

In QGCOMP, which follows same initial steps of WQS, the continuous variables for metal are transformed into the quantized variables, and a linear model is fitted as below:

$$Y_{thyroid\ hormone} = \beta_0 + \sum_{j=1}^{p} \beta_j\ Metal_j^q + \beta_{p+1\ldots p+k}\ Covariates + \epsilon_i,$$

where $Metal_j^q$ is a quantized version of $Metal_j$. At this point we assume directional homogeneity, and when the directional homogeneity assumption is violated, the weights are redefined as either positive or negative weights which represent the proportion in each direction. We used R package '*QGCOMP*' for analysis for QGCOMP [212]. All other analysis was performed using SAS version 9.4 (Cary, NC).

C. Results

1. Correlations among 12 Metals Included in the Study

Pearson's correlation coefficients with pairwise comparison of 12 metals included in the study are presented in Figure 4. While the significance is striking with small p-values, the most of the magnitudes of correlation are low to moderate. The greatest correlation coefficient was found between cesium and thallium (r = 0.57, p <0.01), followed by barium and cobalt (r=0.43, p<0.01) in males. In females, the top two pairs with highest correlation were consistent with those in males, with slightly different magnitude (r=0.61, p<0.01 for cesium and thallium, r=0.38, p<0.01 for barium and cobalt, respectively).

A. Males

	Arsenic	Barium	Cobalt	Cesium	Molybdenum	Antimony	Thallium	Tungsten	Uranuium	Mercury	Cadmium	Lead
Arsenic	1.00	0.03	0.04	0.17	0.15	-0.02	0.16	0.04	0.06	0.42	0.05	0.16
Barium		1.00	0.43	0.27	0.09	0.12	0.18	0.11	0.18	-0.01	0.04	0.04
Cobalt			1.00	0.35	0.23	0.18	0.20	0.18	0.19	-0.06	0.18	0.16
Cesium				1.00	0.20	0.07	0.57	0.05	0.06	0.21	0.04	0.12
Molybdenum					1.00	0.10	0.16	0.35	0.07	0.05	-0.04	-0.01
Antimony						1.00	0.07	0.23	0.24	-0.09	0.10	0.14
Thallium							1.00	0.03	0.03	0.23	-0.05	0.00
Tungsten								1.00	0.30	-0.08	0.01	0.01
Uranuium									1.00	-0.08	0.14	0.16
Mercury										1.00	0.01	0.10
Cadmium											1.00	0.37
Lead												1.00

B. Females

	Arsenic	Barium	Cobalt	Cesium	Molybdenum	Antimony	Thallium	Tungsten	Uranuium	Mercury	Cadmium	Lead
Arsenic	1.00	-0.02	0.01	0.16	0.15	-0.09	0.15	-0.01	-0.01	0.38	0.00	0.12
Barium		1.00	0.38	0.30	0.10	0.22	0.25	0.15	0.21	0.03	0.06	0.08
Cobalt			1.00	0.26	0.18	0.11	0.19	0.11	0.08	-0.05	0.16	0.03
Cesium				1.00	0.23	0.17	0.61	0.07	0.19	0.20	0.05	0.17
Molybdenum					1.00	0.11	0.18	0.36	0.10	0.04	-0.08	-0.03
Antimony						1.00	0.14	0.24	0.30	-0.02	0.04	0.10
Thallium							1.00	0.05	0.09	0.24	0.01	0.04
Tungsten								1.00	0.31	-0.03	-0.01	0.01
Uranuium									1.00	-0.02	0.12	0.19
Mercury										1.00	0.01	0.14
Cadmium											1.00	0.35
Lead												1.00

p <0.01 p<0.05 NS

Figure 4. Correlation among 12 metals in males and females, from NHANES 2007-2012 dataset

2. Distribution of Analytes and Participant Characteristics

Given the skewness of the analytes, we calculated geometric means and standard errors of each analyte, stratified by sex. Briefly, all the analytes had significantly different geometric means by sex, except TSH and FT4:TSH ratio (Table XLIX).

TABLE XLIX. DISTRIBUTIONS OF THYROID HORMONES BY SEX

Analytes	Male (N=2381)		Female (N=1867)		P-value
	Geometric Means	SE	Geometric Means	SE	
Total T3 (T3, ng/dL)	113.427	0.446	111.986	0.518	0.035
Free T3 (FT3, pg/mL)	3.236	0.008	3.072	0.009	<.001
Total T4 (T4, ug/dL)	7.622	0.030	8.008	0.035	<.001
Free T4 (FT4, ng/dL)	0.794	0.003	0.782	0.003	0.003
Thyroid stimulating hormones (TSH, uIU/mL)	1.510	0.019	1.519	0.023	0.768
T3 :T4 ratio (SI unit)	0.067	0.000	0.072	0.000	<.001
FT4:TSH ratio (SI unit)	0.526	0.007	0.515	0.008	0.315
Arsenic (ug/L)	5.643	0.113	6.061	0.141	0.020
Barium (ug/L)	1.113	0.022	1.434	0.031	<.001
Cobalt (ug/L)	0.270	0.003	0.439	0.007	<.001
Cesium (ug/L)	3.855	0.039	4.762	0.055	<.001
Molybdenum (ug/L)	38.442	0.509	44.554	0.665	<.001
Antimony (ug/L)	0.054	0.001	0.064	0.001	<.001
Thallium (ug/L)	0.132	0.001	0.168	0.002	<.001
Tungsten (ug/L)	0.072	0.001	0.083	0.002	<.001
Uranium (ug/L)	0.006	0.000	0.008	0.000	<.001
Mercury (ug/L)	1.000	0.021	0.890	0.019	<.001
Cadmium (ug/L)	0.349	0.006	0.402	0.007	<.001
Lead (ug/dL)	1.566	0.021	1.076	0.016	<.001

Table L demonstrates the descriptive characteristics of study participants. Males tended to have lower BMI, higher serum cotinine, higher proportion of smokers, lower non-thyroid hormone medication use than females. The distributions of race/ethnicity and education attainment were also different by sex (p<0.017 for race/ethnicity, p<0.001 for education, respectively).

TABLE L. CHARACTERISTICS OF STUDY PARTICIPANTS, BY SEX

Variables		Male (N=2381)		Female (N=1867)		p
		Mean or N	SD or %	Mean or N	SD or %	
Age	Mean (SD)	48.9	17.6	48.4	17.4	0.358
Body mass index (kg/m2)	Mean (SD)	28.5	5.8	29.2	7.3	0.002
Serum cotinine (ng/mL)	Mean (SD)	71.2	149.9	43.1	103.8	<.001
Race/ethnicity, N (%)	NH-White	1039	43.6	795	42.6	0.017
	NH-Black	514	21.6	406	21.8	
	Hispanic	600	25.2	529	28.3	
	Others	228	9.6	137	7.3	
Education, N (%)	Less than HS	680	28.6	529	28.3	<.001
	HS graduate	572	24.0	376	20.1	
	Some college or more	614	25.8	575	30.8	
	Graduate or above	515	21.6	387	20.7	
Smoking, N(%)	Never	1809	76.0	1508	80.8	<.001
	Ever	572	24.0	359	19.2	
Non-thyroid hormone medication, N (%)	No	2358	99.0	1811	97.0	<.001
	Yes	23	1.0	56	3.0	
Postmenopausal (female only), N (%)				920	49.3	N/A

3. Associations between Metals and T3

Tables LI and LII demonstrate the sex-specific associations between single metals and T3 from linear regression models. Arsenic and tungsten showed inverse dose-response relationships with T3 both in males in females (p-inverse trend=0.021 in males and 0.002 in females for arsenic, respectively; p-inverse trend=0.01 in males and 0.002 in females for

tungsten, respectively). Uranium showed a dose-response relationship in males (p-inverse trend=0.011), but in females it showed a non-linear relationship with an inverted U-shape. On the other hand, molybdenum had a negative dose-response relationship in females (p-inverse trend=0.037), but showed a non-monotonic association with T3 in males. Cesium and lead also had non-linear relationships with T3 in males, with an inverted U-shape. In females, barium, cadmium, thallium, uranium demonstrated non-linear relationships with T3.

From the first step, we selected metals with significant dose-response or non-linear relationships. In males, arsenic, cesium, lead, molybdenum, tungsten, and uranium were simultaneously assessed in the second step. In females, arsenic, barium, cadmium, molybdenum, thallium, tungsten, and uranium were included in the second step. Tables LIII and LIV demonstrate the results from linear regression multi-metal models. In males, the linear trends of arsenic and tungsten remained significant, adjusting for other metals with non-linear relationships (p-inverse trend=0.006 for arsenic, p-inverse trend=0.043 for tungsten, respectively). The non-monotonic relationships with lead, molybdenum, and uranium remained significant, whereas the association was attenuated for cesium. In females, the dose-response relationships with arsenic and tungsten remained significant adjusting for other metals (p-inverse trend=0.001 for arsenic, p-inverse trend=0.018 for tungsten, respectively). Interestingly, cadmium and thallium demonstrated dose-response relationships in this step, whereas the two metals showed non-monotonic associations in the first step (p-inverse trend=0.036 for cadmium, and p-positive trend=0.013 for thallium, respectively). While barium and uranium still demonstrated non-linear relationships in this stage, the associations between molybdenum and T3 were attenuated. In multi-metal models, arsenic and tungsten showed significant dose-response relationships in both sexes.

Lastly, we investigated the effects of metals showing linear relationships with T3 as a mixture. For males, arsenic and tungsten were assessed simultaneously as a mixture, and for females, arsenic, cadmium, thallium, and tungsten were included in this step.

TABLE LI. SINGLE METAL ASSOCIATIONS WITH T3, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.010	-0.030	0.011	0.357	0.021
	Q3 vs. Q1	-0.020	-0.041	0.001	0.061	
	Q4 vs. Q1	-0.024	-0.046	-0.002	0.035	
Antimony	Q2 vs. Q1	-0.016	-0.036	0.004	0.109	0.955
	Q3 vs. Q1	0.006	-0.015	0.026	0.571	
	Q4 vs. Q1	-0.006	-0.027	0.015	0.558	
Barium	Q2 vs. Q1	0.006	-0.014	0.026	0.533	0.837
	Q3 vs. Q1	-0.004	-0.025	0.017	0.683	
	Q4 vs. Q1	0.006	-0.016	0.028	0.577	
Cadmium	Q2 vs. Q1	-0.003	-0.023	0.017	0.755	0.676
	Q3 vs. Q1	-0.005	-0.027	0.018	0.699	
	Q4 vs. Q1	-0.005	-0.034	0.023	0.712	
Cobalt	Q2 vs. Q1	0.008	-0.011	0.026	0.432	0.460
	Q3 vs. Q1	-0.001	-0.022	0.019	0.895	
	Q4 vs. Q1	-0.009	-0.034	0.015	0.455	
Cesium	Q2 vs. Q1	0.014	-0.006	0.033	0.163	0.114
	Q3 vs. Q1	0.021	0.000	0.042	0.049	
	Q4 vs. Q1	0.015	-0.008	0.039	0.202	
Lead	Q2 vs. Q1	0.022	-0.002	0.046	0.074	0.261
	Q3 vs. Q1	0.025	0.001	0.049	0.044	
	Q4 vs. Q1	0.019	-0.007	0.045	0.147	
Mercury	Q2 vs. Q1	0.010	-0.011	0.031	0.330	0.753
	Q3 vs. Q1	-0.004	-0.026	0.017	0.689	
	Q4 vs. Q1	0.001	-0.020	0.023	0.909	
Molybdenum	Q2 vs. Q1	0.011	-0.009	0.031	0.273	0.860
	Q3 vs. Q1	0.021	0.000	0.041	0.045	
	Q4 vs. Q1	-0.008	-0.029	0.014	0.472	
Thallium	Q2 vs. Q1	0.015	-0.004	0.034	0.131	0.074
	Q3 vs. Q1	0.018	-0.003	0.039	0.089	
	Q4 vs. Q1	0.019	-0.004	0.041	0.102	
Tungsten	Q2 vs. Q1	0.004	-0.016	0.024	0.702	0.010
	Q3 vs. Q1	0.001	-0.020	0.021	0.962	
	Q4 vs. Q1	-0.030	-0.051	-0.009	0.005	
Uranium	Q2 vs. Q1	-0.010	-0.030	0.010	0.319	0.011
	Q3 vs. Q1	-0.025	-0.045	-0.004	0.020	
	Q4 vs. Q1	-0.024	-0.045	-0.003	0.027	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LII. SINGLE METAL ASSOCIATIONS WITH T3, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.016	-0.042	0.010	0.224	0.002
	Q3 vs. Q1	-0.028	-0.054	-0.002	0.034	
	Q4 vs. Q1	-0.040	-0.067	-0.014	0.003	
Antimony	Q2 vs. Q1	0.022	-0.005	0.048	0.117	0.691
	Q3 vs. Q1	0.013	-0.014	0.039	0.343	
	Q4 vs. Q1	0.001	-0.025	0.027	0.938	
Barium	Q2 vs. Q1	0.023	-0.003	0.050	0.086	0.203
	Q3 vs. Q1	0.027	0.001	0.053	0.046	
	Q4 vs. Q1	0.020	-0.007	0.046	0.141	
Cadmium	Q2 vs. Q1	-0.023	-0.049	0.004	0.096	0.052
	Q3 vs. Q1	-0.022	-0.049	0.005	0.107	
	Q4 vs. Q1	-0.036	-0.070	-0.002	0.036	
Cobalt	Q2 vs. Q1	0.016	-0.016	0.048	0.335	0.292
	Q3 vs. Q1	-0.001	-0.031	0.030	0.973	
	Q4 vs. Q1	-0.005	-0.035	0.024	0.733	
Cesium	Q2 vs. Q1	0.005	-0.024	0.033	0.751	0.888
	Q3 vs. Q1	-0.001	-0.029	0.027	0.958	
	Q4 vs. Q1	0.004	-0.024	0.032	0.792	
Lead	Q2 vs. Q1	0.003	-0.021	0.026	0.811	0.128
	Q3 vs. Q1	0.020	-0.007	0.047	0.145	
	Q4 vs. Q1	0.019	-0.012	0.050	0.234	
Mercury	Q2 vs. Q1	-0.014	-0.038	0.011	0.268	0.122
	Q3 vs. Q1	-0.008	-0.033	0.016	0.513	
	Q4 vs. Q1	-0.025	-0.051	0.002	0.071	
Molybdenum	Q2 vs. Q1	0.021	-0.005	0.047	0.113	0.037
	Q3 vs. Q1	0.002	-0.024	0.028	0.879	
	Q4 vs. Q1	-0.019	-0.045	0.007	0.145	
Thallium	Q2 vs. Q1	0.014	-0.015	0.042	0.348	0.067
	Q3 vs. Q1	0.029	0.002	0.056	0.037	
	Q4 vs. Q1	0.024	-0.003	0.050	0.084	
Tungsten	Q2 vs. Q1	-0.017	-0.042	0.009	0.210	0.002
	Q3 vs. Q1	-0.024	-0.049	0.002	0.069	
	Q4 vs. Q1	-0.040	-0.065	-0.015	0.002	
Uranium	Q2 vs. Q1	-0.029	-0.056	-0.002	0.033	0.113
	Q3 vs. Q1	-0.009	-0.035	0.018	0.524	
	Q4 vs. Q1	-0.031	-0.058	-0.004	0.023	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LIII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND T3, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.011	-0.045	-0.002	0.295	0.006
	Q3 vs. Q1	-0.024	-0.045	-0.002	0.031	
	Q4 vs. Q1	-0.030	-0.053	-0.007	0.010	
Cesium	Q2 vs. Q1	0.009	-0.011	0.029	0.366	0.251
	Q3 vs. Q1	0.018	-0.004	0.041	0.114	
	Q4 vs. Q1	0.012	-0.015	0.039	0.375	
Lead	Q2 vs. Q1	0.021	-0.003	0.045	0.086	0.133
	Q3 vs. Q1	0.028	0.004	0.052	0.024	
	Q4 vs. Q1	0.024	-0.002	0.049	0.074	
Molybdenum	Q2 vs. Q1	0.010	-0.010	0.030	0.339	0.520
	Q3 vs. Q1	0.022	0.001	0.043	0.039	
	Q4 vs. Q1	-0.002	-0.025	0.021	0.872	
Tungsten	Q2 vs. Q1	0.006	-0.015	0.026	0.588	0.043
	Q3 vs. Q1	0.003	-0.018	0.025	0.751	
	Q4 vs. Q1	-0.022	-0.045	0.000	0.054	
Uranium	Q2 vs. Q1	-0.009	-0.029	0.011	0.357	0.070
	Q3 vs. Q1	-0.022	-0.043	-0.001	0.042	
	Q4 vs. Q1	-0.016	-0.038	0.006	0.150	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LIV. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND T3, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.018	-0.044	0.008	0.169	0.001
	Q3 vs. Q1	-0.031	-0.057	-0.005	0.021	
	Q4 vs. Q1	-0.043	-0.070	-0.016	0.002	
Barium	Q2 vs. Q1	0.022	-0.005	0.049	0.109	0.115
	Q3 vs. Q1	0.029	0.002	0.055	0.036	
	Q4 vs. Q1	0.025	-0.002	0.052	0.072	
Cadmium	Q2 vs. Q1	-0.022	-0.049	0.004	0.102	0.036
	Q3 vs. Q1	-0.024	-0.050	0.003	0.082	
	Q4 vs. Q1	-0.035	-0.069	-0.001	0.043	
Molybdenum	Q2 vs. Q1	0.022	-0.004	0.048	0.096	0.281
	Q3 vs. Q1	0.006	-0.021	0.033	0.655	
	Q4 vs. Q1	-0.008	-0.036	0.020	0.560	
Thallium	Q2 vs. Q1	0.010	-0.019	0.038	0.492	0.013
	Q3 vs. Q1	0.028	0.001	0.056	0.043	
	Q4 vs. Q1	0.028	0.000	0.055	0.052	
Tungsten	Q2 vs. Q1	-0.016	-0.042	0.011	0.245	0.018
	Q3 vs. Q1	-0.020	-0.046	0.007	0.141	
	Q4 vs. Q1	-0.032	-0.060	-0.004	0.023	
Uranium	Q2 vs. Q1	-0.029	-0.055	-0.002	0.035	0.320
	Q3 vs. Q1	-0.005	-0.031	0.022	0.719	
	Q4 vs. Q1	-0.023	-0.050	0.005	0.112	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

Tables LV and LVI, and Figure 5 represent the results from this step; the beta estimate for each metal was obtained from linear regression models, and the beta estimates for mixtures were obtained from QGCOMP. In males, the mixture consisting of arsenic and tungsten showed a negative association with T3 (b=-0.016, 95% CI=-0.025 - -0.006). Both arsenic and tungsten contributed to the inverse relationship with T3, and arsenic appeared to have a slightly larger weight than tungsten. In females, the mixture of arsenic, cadmium, thallium, and tungsten also showed an overall negative relationship with T3 (b=-0.024, 95% CI=-0.041 - -0.008). However, thallium had a positive association, whereas the other three metals contributed in a negative direction, in order of tungsten, arsenic, and cadmium.

TABLE LV. ASSOCIATIONS BETWEEN METAL MIXTURES AND T3, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.009	-0.029	0.012	0.391	0.028
	Q3 vs. Q1	-0.018	-0.039	0.003	0.092	
	Q4 vs. Q1	-0.023	-0.045	-0.001	0.040	
Tungsten	Q2 vs. Q1	0.004	-0.016	0.023	0.723	0.013
	Q3 vs. Q1	0.001	-0.019	0.022	0.898	
	Q4 vs. Q1	-0.029	-0.050	-0.008	0.007	
Mixture (from QGCOMP)		*-0.016*	*-0.025*	*-0.006*	*0.001*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LVI. ASSOCIATIONS BETWEEN METAL MIXTURES AND T3, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.018	-0.044	0.008	0.173	0.001
	Q3 vs. Q1	-0.031	-0.057	-0.005	0.019	
	Q4 vs. Q1	-0.044	-0.071	-0.017	0.001	
Cadmium	Q2 vs. Q1	-0.022	-0.049	0.004	0.102	0.036
	Q3 vs. Q1	-0.023	-0.050	0.004	0.093	
	Q4 vs. Q1	-0.037	-0.070	-0.003	0.034	
Thallium	Q2 vs. Q1	0.015	-0.013	0.044	0.292	0.008
	Q3 vs. Q1	0.034	0.007	0.061	0.015	
	Q4 vs. Q1	0.034	0.007	0.061	0.015	
Tungsten	Q2 vs. Q1	-0.015	-0.041	0.010	0.243	0.002
	Q3 vs. Q1	-0.023	-0.049	0.002	0.070	
	Q4 vs. Q1	-0.040	-0.065	-0.015	0.002	
Mixture (from QGCOMP)		*-0.024*	*-0.041*	*-0.008*	*0.002*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

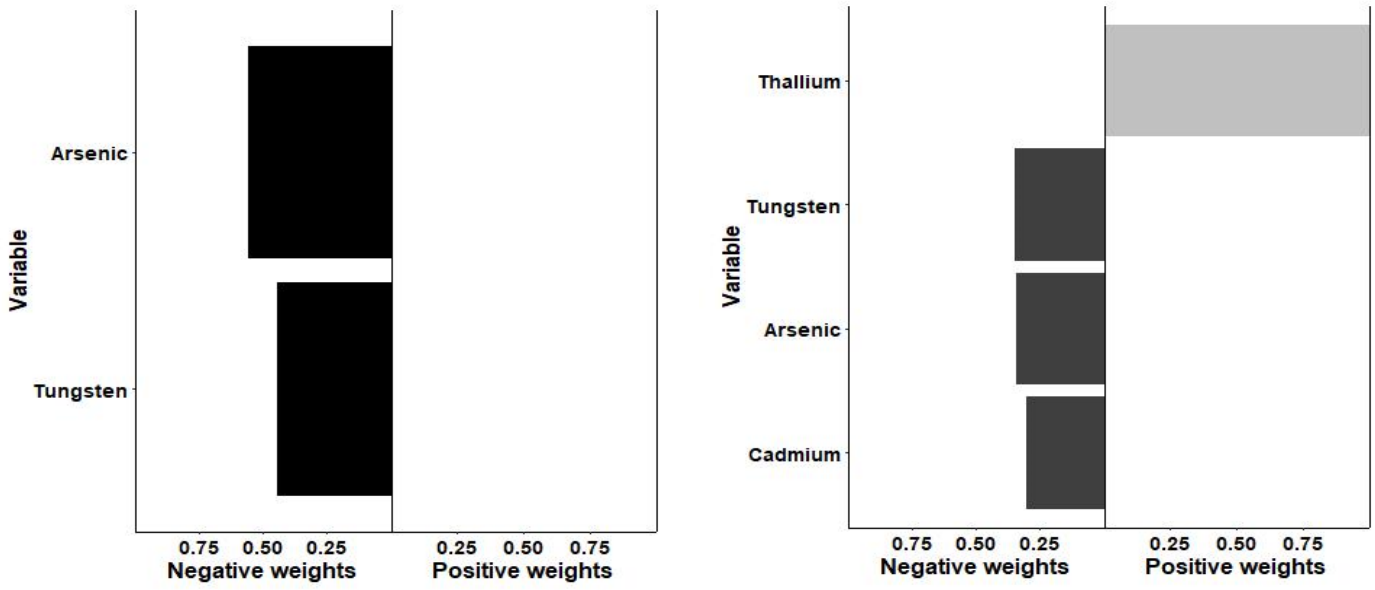

Figure 5. The directional contribution of metals to T3. Left: males; Right: females

4. Associations between Metals and FT3

Tables LVII and LVIII demonstrate the results of associations between each metal and FT3. In males, arsenic (p-inverse trend=0.027), antimony (p-inverse trend=0.047), barium (p-positive trend=0.022), cobalt (p-inverse trend=0.043), lead (p-positive trend=0.004), molybdenum (p-inverse trend=0.001), and tungsten (p-inverse trend=0.001) showed dose-response relationships with FT3. Among the other metals, we did not find any non-linear relationships. In female, arsenic (p-inverse trend=0.042), antimony (p- inverse trend=0.039), tungsten (p-inverse trend=0.015), and uranium (p-inverse trend=0.035) showed dose-response relationships. Thallium had a non-linear relationship with FT3 in females.

Results from the second step with FT3 are represented in Tables LIX and LX. Adjusting for metals which appeared significant in the first step simultaneously, arsenic, barium, cobalt, lead, and tungsten still showed linear relationships with FT3 levels in male. It appeared that arsenic, cobalt, tungsten had negative associations with FT3, whereas barium and lead showed dose-response relationships in a positive direction. Antimony and molybdenum showed linear relationships in the previous stage, however, the two metals were likely to have non-linear relationships after adjusting for other significant metals. Among the metals that showed dose-response relationships with FT3 in females, only arsenic remained significant in multi-metal models. Thallium, which demonstrated a non-linear association in the first step, had a linear relationship in this stage (p-inverse trend=0.017), though the effect size of Q3 and Q4 were similar (beta=0.026 for Q3 vs. Q1, beta=0.022 for Q4 vs. Q1).

In the last stage, we assessed the metals as a mixture and investigated the relationships with FT3. For males, arsenic, barium, cobalt, lead, and tungsten were assessed simultaneously as a mixture. Only arsenic and thallium were included in this step for females (Tables LXI and LXII). Both in males and females, the mixtures consisting of the contributing metals did not present significant associations with FT3.

TABLE LVII. SINGLE METAL ASSOCIATIONS WITH FT3, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.000	-0.011	0.012	0.993	0.027
	Q3 vs. Q1	-0.005	-0.016	0.007	0.447	
	Q4 vs. Q1	-0.014	-0.026	-0.001	0.032	
Antimony	Q2 vs. Q1	-0.011	-0.022	-0.001	0.041	0.047
	Q3 vs. Q1	0.004	-0.007	0.016	0.478	
	Q4 vs. Q1	-0.018	-0.030	-0.007	0.002	
Barium	Q2 vs. Q1	0.015	0.004	0.027	0.007	0.022
	Q3 vs. Q1	0.006	-0.006	0.017	0.348	
	Q4 vs. Q1	0.018	0.006	0.030	0.003	
Cadmium	Q2 vs. Q1	0.001	-0.010	0.012	0.848	0.740
	Q3 vs. Q1	-0.004	-0.017	0.009	0.526	
	Q4 vs. Q1	-0.001	-0.017	0.015	0.935	
Cobalt	Q2 vs. Q1	-0.005	-0.015	0.006	0.396	0.043
	Q3 vs. Q1	-0.010	-0.021	0.002	0.100	
	Q4 vs. Q1	-0.012	-0.026	0.002	0.084	
Cesium	Q2 vs. Q1	0.002	-0.009	0.013	0.764	0.609
	Q3 vs. Q1	0.008	-0.004	0.020	0.179	
	Q4 vs. Q1	0.000	-0.013	0.013	0.953	
Lead	Q2 vs. Q1	0.011	-0.003	0.024	0.118	0.004
	Q3 vs. Q1	0.017	0.004	0.031	0.012	
	Q4 vs. Q1	0.021	0.007	0.035	0.004	
Mercury	Q2 vs. Q1	0.011	-0.001	0.022	0.068	0.714
	Q3 vs. Q1	-0.002	-0.014	0.010	0.692	
	Q4 vs. Q1	0.002	-0.010	0.014	0.736	
Molybdenum	Q2 vs. Q1	-0.011	-0.022	0.000	0.056	0.001
	Q3 vs. Q1	0.002	-0.009	0.013	0.738	
	Q4 vs. Q1	-0.027	-0.039	-0.016	<.0001	
Thallium	Q2 vs. Q1	-0.007	-0.018	0.004	0.214	0.739
	Q3 vs. Q1	0.002	-0.009	0.014	0.698	
	Q4 vs. Q1	0.000	-0.013	0.012	0.949	
Tungsten	Q2 vs. Q1	-0.003	-0.014	0.008	0.579	0.001
	Q3 vs. Q1	-0.007	-0.018	0.005	0.237	
	Q4 vs. Q1	-0.020	-0.031	-0.008	0.001	
Uranium	Q2 vs. Q1	-0.009	-0.020	0.002	0.101	0.057
	Q3 vs. Q1	-0.010	-0.022	0.001	0.078	
	Q4 vs. Q1	-0.011	-0.023	0.001	0.061	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LVIII. SINGLE METAL ASSOCIATIONS WITH FT3, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.002	-0.014	0.018	0.791	0.042
	Q3 vs. Q1	-0.005	-0.021	0.011	0.555	
	Q4 vs. Q1	-0.015	-0.031	0.001	0.070	
Antimony	Q2 vs. Q1	0.007	-0.010	0.023	0.423	0.039
	Q3 vs. Q1	-0.002	-0.018	0.014	0.767	
	Q4 vs. Q1	-0.013	-0.028	0.003	0.120	
Barium	Q2 vs. Q1	0.012	-0.005	0.028	0.164	0.182
	Q3 vs. Q1	0.015	-0.002	0.031	0.075	
	Q4 vs. Q1	0.012	-0.004	0.028	0.148	
Cadmium	Q2 vs. Q1	-0.003	-0.020	0.013	0.693	0.918
	Q3 vs. Q1	0.000	-0.017	0.016	0.970	
	Q4 vs. Q1	-0.003	-0.024	0.018	0.791	
Cobalt	Q2 vs. Q1	0.019	-0.001	0.038	0.063	0.918
	Q3 vs. Q1	0.010	-0.009	0.028	0.300	
	Q4 vs. Q1	0.007	-0.011	0.025	0.421	
Cesium	Q2 vs. Q1	-0.003	-0.020	0.014	0.739	0.663
	Q3 vs. Q1	-0.005	-0.022	0.012	0.563	
	Q4 vs. Q1	-0.004	-0.021	0.013	0.656	
Lead	Q2 vs. Q1	0.002	-0.013	0.016	0.804	0.115
	Q3 vs. Q1	0.011	-0.006	0.028	0.202	
	Q4 vs. Q1	0.013	-0.006	0.032	0.180	
Mercury	Q2 vs. Q1	-0.003	-0.018	0.012	0.722	0.107
	Q3 vs. Q1	-0.009	-0.024	0.006	0.233	
	Q4 vs. Q1	-0.012	-0.028	0.004	0.153	
Molybdenum	Q2 vs. Q1	0.010	-0.006	0.026	0.236	0.350
	Q3 vs. Q1	0.004	-0.012	0.020	0.588	
	Q4 vs. Q1	-0.005	-0.021	0.011	0.559	
Thallium	Q2 vs. Q1	0.016	-0.001	0.034	0.069	0.101
	Q3 vs. Q1	0.023	0.007	0.040	0.006	
	Q4 vs. Q1	0.015	-0.001	0.032	0.065	
Tungsten	Q2 vs. Q1	0.007	-0.009	0.022	0.421	0.015
	Q3 vs. Q1	-0.012	-0.028	0.003	0.116	
	Q4 vs. Q1	-0.013	-0.029	0.002	0.087	
Uranium	Q2 vs. Q1	-0.005	-0.021	0.012	0.577	0.035
	Q3 vs. Q1	-0.006	-0.022	0.010	0.469	
	Q4 vs. Q1	-0.018	-0.034	-0.001	0.036	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LIX. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND FT3, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.001	-0.013	0.010	0.841	0.017
	Q3 vs. Q1	-0.005	-0.017	0.006	0.378	
	Q4 vs. Q1	-0.015	-0.027	-0.002	0.021	
Antimony	Q2 vs. Q1	-0.011	-0.021	0.000	0.058	0.232
	Q3 vs. Q1	0.008	-0.004	0.019	0.184	
	Q4 vs. Q1	-0.014	-0.026	-0.002	0.020	
Barium	Q2 vs. Q1	0.018	0.007	0.029	0.002	0.001
	Q3 vs. Q1	0.011	-0.002	0.023	0.085	
	Q4 vs. Q1	0.028	0.015	0.041	<.0001	
Cobalt	Q2 vs. Q1	-0.007	-0.017	0.004	0.227	0.018
	Q3 vs. Q1	-0.013	-0.026	-0.001	0.035	
	Q4 vs. Q1	-0.017	-0.032	-0.002	0.023	
Lead	Q2 vs. Q1	0.009	-0.004	0.022	0.187	0.004
	Q3 vs. Q1	0.018	0.005	0.032	0.008	
	Q4 vs. Q1	0.021	0.007	0.036	0.003	
Molybdenum	Q2 vs. Q1	-0.010	-0.021	0.001	0.088	0.074
	Q3 vs. Q1	0.005	-0.006	0.017	0.381	
	Q4 vs. Q1	-0.020	-0.032	-0.007	0.002	
Tungsten	Q2 vs. Q1	0.000	-0.011	0.011	0.991	0.044
	Q3 vs. Q1	-0.001	-0.013	0.011	0.855	
	Q4 vs. Q1	-0.012	-0.024	0.000	0.057	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LX. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND FT3, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.001	-0.016	0.015	0.931	0.029
	Q3 vs. Q1	-0.007	-0.023	0.009	0.362	
	Q4 vs. Q1	-0.017	-0.033	0.000	0.047	
Antimony	Q2 vs. Q1	0.008	-0.009	0.024	0.367	0.151
	Q3 vs. Q1	0.000	-0.016	0.016	0.989	
	Q4 vs. Q1	-0.009	-0.025	0.008	0.314	
Thallium	Q2 vs. Q1	0.017	0.000	0.035	0.049	0.017
	Q3 vs. Q1	0.026	0.009	0.042	0.003	
	Q4 vs. Q1	0.022	0.005	0.039	0.010	
Tungsten	Q2 vs. Q1	0.010	-0.006	0.026	0.230	0.096
	Q3 vs. Q1	-0.007	-0.023	0.009	0.381	
	Q4 vs. Q1	-0.008	-0.024	0.008	0.352	
Uranium	Q2 vs. Q1	-0.003	-0.019	0.014	0.735	0.246
	Q3 vs. Q1	-0.001	-0.018	0.015	0.870	
	Q4 vs. Q1	-0.010	-0.027	0.008	0.284	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LXI. ASSOCIATIONS BETWEEN METAL MIXTURES AND FT3, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.0014	-0.0128	0.01	0.8122	0.009
	Q3 vs. Q1	-0.0053	-0.017	0.0064	0.3734	
	Q4 vs. Q1	-0.0166	-0.0289	-0.0042	0.0087	
Barium	Q2 vs. Q1	0.0179	0.0067	0.0291	0.0017	0.001
	Q3 vs. Q1	0.0111	-0.001	0.0232	0.0724	
	Q4 vs. Q1	0.0271	0.014	0.0402	<.0001	
Cobalt	Q2 vs. Q1	-0.0077	-0.0183	0.003	0.1584	0.005
	Q3 vs. Q1	-0.0154	-0.0278	-0.0031	0.0139	
	Q4 vs. Q1	-0.0185	-0.0331	-0.0038	0.0134	
Lead	Q2 vs. Q1	0.0102	-0.0031	0.0236	0.1335	0.003
	Q3 vs. Q1	0.0179	0.0045	0.0314	0.009	
	Q4 vs. Q1	0.0216	0.0073	0.0359	0.0031	
Tungsten	Q2 vs. Q1	-0.0028	-0.0137	0.0082	0.6228	0.003
	Q3 vs. Q1	-0.0054	-0.0167	0.0059	0.3486	
	Q4 vs. Q1	-0.0183	-0.03	-0.0066	0.0021	
Mixture (from QGCOMP)		*-0.016*	*-0.010*	*0.005*	*0.568*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LXII. ASSOCIATIONS BETWEEN METAL MIXTURES AND FT3, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.0006	-0.0151	0.0164	0.9386	0.015
	Q3 vs. Q1	-0.0075	-0.0235	0.0085	0.361	
	Q4 vs. Q1	-0.0181	-0.0346	-0.0015	0.0321	
Thallium	Q2 vs. Q1	0.0172	-0.0002	0.0346	0.0521	0.035
	Q3 vs. Q1	0.0248	0.0082	0.0415	0.0035	
	Q4 vs. Q1	0.0194	0.0028	0.036	0.0221	
Mixture (from QGCOMP)		*-0.001*	*-0.007*	*0.006*	*0.835*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

5. Associations between Metals and T4

Tables LXIII and LXIV demonstrate the results of associations between each metal and T4. In males, arsenic and barium showed linear inverse relationships with T4, although the linearity in barium was likely to be led by the larger decrease of T4 levels in third quartile (beta for Q2 vs. Q1=-0.027, Q3 vs. Q1=-0.052, and Q4 vs. Q1=-0.032, respectively). Among the other metals, cobalt, cesium, and mercury appeared to have non-linear relationships with T4 in males. More metals showed dose-response inverse relationships in females than in males, including arsenic , barium , cesium , mercury , thallium, and tungsten. It is notable that cesium and mercury showed non-linear relationships in males. Uranium showed a non-linear association with T4 in females.

Tables LXV and LXVI demonstrate the results for multi-metal models with T4 levels. For males, arsenic (inverse), barium (inverse), and cesium (positive) remained significant after adjusting for other metals. For females, only arsenic and tungsten retained their dose-response inverse relationships with T4.

In the last stage, we assessed the associations between metal mixtures for the metals that showed dose-response relationships in the second stage with T4. The results are presented in Tables LXVII, LXVIII, and Figure 6. Arsenic, barium, and cesium were assessed as a mixture in males, and showed a negative association with T4 (beta for mixture=-0.019, 95% CI=-0.031 - 0.008). In the mixture, cesium contributed in positive direction while arsenic and barium in a negative direction. The effects of arsenic and barium are almost identical. In female, arsenic and tungsten were assessed as a mixture and showed a negative association with T4 (beta for mixture=-0.028, 95% CI=-0.039- -0.018).

TABLE LXIII. SINGLE METAL ASSOCIATIONS WITH T4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.013	-0.034	0.009	0.255	<.001
	Q3 vs. Q1	-0.028	-0.050	-0.005	0.015	
	Q4 vs. Q1	-0.044	-0.067	-0.020	0.000	
Antimony	Q2 vs. Q1	-0.011	-0.032	0.010	0.309	0.197
	Q3 vs. Q1	0.004	-0.018	0.026	0.722	
	Q4 vs. Q1	-0.021	-0.043	0.001	0.064	
Barium	Q2 vs. Q1	-0.027	-0.048	-0.005	0.014	<.001
	Q3 vs. Q1	-0.052	-0.074	-0.030	<.0001	
	Q4 vs. Q1	-0.032	-0.055	-0.009	0.006	
Cadmium	Q2 vs. Q1	-0.014	-0.036	0.007	0.191	0.984
	Q3 vs. Q1	-0.007	-0.031	0.018	0.594	
	Q4 vs. Q1	0.003	-0.028	0.034	0.849	
Cobalt	Q2 vs. Q1	-0.014	-0.033	0.006	0.181	0.055
	Q3 vs. Q1	-0.023	-0.045	-0.001	0.037	
	Q4 vs. Q1	-0.019	-0.045	0.008	0.163	
Cesium	Q2 vs. Q1	-0.007	-0.028	0.014	0.509	0.069
	Q3 vs. Q1	0.024	0.002	0.047	0.034	
	Q4 vs. Q1	0.013	-0.012	0.038	0.298	
Lead	Q2 vs. Q1	0.006	-0.019	0.032	0.622	0.102
	Q3 vs. Q1	-0.021	-0.046	0.005	0.116	
	Q4 vs. Q1	-0.014	-0.041	0.013	0.313	
Mercury	Q2 vs. Q1	0.008	-0.015	0.030	0.505	0.084
	Q3 vs. Q1	-0.023	-0.046	-0.001	0.045	
	Q4 vs. Q1	-0.011	-0.034	0.012	0.335	
Molybdenum	Q2 vs. Q1	0.010	-0.011	0.031	0.365	0.252
	Q3 vs. Q1	0.011	-0.011	0.033	0.314	
	Q4 vs. Q1	-0.017	-0.040	0.006	0.146	
Thallium	Q2 vs. Q1	-0.001	-0.021	0.020	0.962	0.403
	Q3 vs. Q1	-0.002	-0.024	0.020	0.862	
	Q4 vs. Q1	-0.011	-0.035	0.013	0.360	
Tungsten	Q2 vs. Q1	0.003	-0.018	0.024	0.793	0.172
	Q3 vs. Q1	-0.001	-0.023	0.021	0.930	
	Q4 vs. Q1	-0.016	-0.038	0.006	0.159	
Uranium	Q2 vs. Q1	-0.002	-0.023	0.019	0.864	0.355
	Q3 vs. Q1	-0.019	-0.041	0.003	0.092	
	Q4 vs. Q1	-0.005	-0.027	0.017	0.656	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LXIV. SINGLE METAL ASSOCIATIONS WITH T4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.023	-0.047	0.002	0.068	<.001
	Q3 vs. Q1	-0.040	-0.065	-0.015	0.001	
	Q4 vs. Q1	-0.059	-0.085	-0.034	<.0001	
Antimony	Q2 vs. Q1	0.005	-0.021	0.031	0.698	0.954
	Q3 vs. Q1	0.019	-0.006	0.044	0.137	
	Q4 vs. Q1	-0.003	-0.028	0.022	0.823	
Barium	Q2 vs. Q1	-0.011	-0.037	0.014	0.381	0.022
	Q3 vs. Q1	-0.006	-0.031	0.020	0.664	
	Q4 vs. Q1	-0.031	-0.057	-0.006	0.014	
Cadmium	Q2 vs. Q1	-0.006	-0.031	0.020	0.670	0.454
	Q3 vs. Q1	-0.004	-0.030	0.022	0.762	
	Q4 vs. Q1	0.016	-0.016	0.048	0.329	
Cobalt	Q2 vs. Q1	-0.017	-0.048	0.014	0.274	0.120
	Q3 vs. Q1	-0.019	-0.048	0.010	0.198	
	Q4 vs. Q1	-0.024	-0.052	0.004	0.089	
Cesium	Q2 vs. Q1	-0.011	-0.038	0.016	0.419	0.013
	Q3 vs. Q1	-0.028	-0.055	-0.002	0.037	
	Q4 vs. Q1	-0.031	-0.058	-0.005	0.022	
Lead	Q2 vs. Q1	0.000	-0.022	0.023	0.994	0.199
	Q3 vs. Q1	0.001	-0.025	0.027	0.924	
	Q4 vs. Q1	0.023	-0.007	0.052	0.137	
Mercury	Q2 vs. Q1	-0.016	-0.040	0.007	0.177	0.010
	Q3 vs. Q1	-0.027	-0.050	-0.003	0.027	
	Q4 vs. Q1	-0.031	-0.056	-0.006	0.017	
Molybdenum	Q2 vs. Q1	0.012	-0.013	0.037	0.353	0.053
	Q3 vs. Q1	-0.010	-0.035	0.015	0.414	
	Q4 vs. Q1	-0.017	-0.042	0.008	0.185	
Thallium	Q2 vs. Q1	-0.028	-0.055	-0.001	0.041	0.035
	Q3 vs. Q1	-0.015	-0.041	0.011	0.269	
	Q4 vs. Q1	-0.034	-0.060	-0.009	0.008	
Tungsten	Q2 vs. Q1	-0.007	-0.032	0.018	0.574	0.003
	Q3 vs. Q1	-0.032	-0.056	-0.008	0.010	
	Q4 vs. Q1	-0.031	-0.055	-0.007	0.012	
Uranium	Q2 vs. Q1	-0.021	-0.046	0.005	0.111	0.082
	Q3 vs. Q1	-0.027	-0.052	-0.002	0.036	
	Q4 vs. Q1	-0.024	-0.049	0.002	0.067	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LXV. ADUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND T4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.010	-0.032	0.012	0.374	<0.001
	Q3 vs. Q1	-0.027	-0.050	-0.004	0.020	
	Q4 vs. Q1	-0.043	-0.068	-0.018	0.001	
Barium	Q2 vs. Q1	-0.027	-0.048	-0.006	0.013	0.002
	Q3 vs. Q1	-0.050	-0.073	-0.027	<.0001	
	Q4 vs. Q1	-0.030	-0.055	-0.005	0.019	
Cobalt	Q2 vs. Q1	-0.005	-0.025	0.015	0.622	0.280
	Q3 vs. Q1	-0.015	-0.039	0.009	0.208	
	Q4 vs. Q1	-0.011	-0.039	0.017	0.456	
Cesium	Q2 vs. Q1	0.000	-0.020	0.021	0.977	<0.001
	Q3 vs. Q1	0.038	0.015	0.061	0.001	
	Q4 vs. Q1	0.033	0.008	0.059	0.011	
Mercury	Q2 vs. Q1	0.010	-0.012	0.033	0.362	0.394
	Q3 vs. Q1	-0.019	-0.042	0.005	0.119	
	Q4 vs. Q1	0.000	-0.025	0.025	0.989	

Models were adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LXVI. ADUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND T4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.020	-0.045	0.005	0.108	<.001
	Q3 vs. Q1	-0.033	-0.059	-0.007	0.012	
	Q4 vs. Q1	-0.049	-0.077	-0.021	0.001	
Barium	Q2 vs. Q1	-0.009	-0.034	0.017	0.516	0.180
	Q3 vs. Q1	0.004	-0.022	0.029	0.773	
	Q4 vs. Q1	-0.021	-0.047	0.006	0.122	
Cesium	Q2 vs. Q1	-0.005	-0.033	0.022	0.713	0.438
	Q3 vs. Q1	-0.017	-0.045	0.012	0.258	
	Q4 vs. Q1	-0.013	-0.044	0.019	0.434	
Mercury	Q2 vs. Q1	-0.010	-0.033	0.014	0.420	0.439
	Q3 vs. Q1	-0.014	-0.039	0.010	0.246	
	Q4 vs. Q1	-0.009	-0.037	0.019	0.525	
Thallium	Q2 vs. Q1	-0.022	-0.050	0.006	0.118	0.802
	Q3 vs. Q1	0.000	-0.028	0.027	0.979	
	Q4 vs. Q1	-0.011	-0.041	0.018	0.453	
Tungsten	Q2 vs. Q1	-0.004	-0.029	0.021	0.749	0.020
	Q3 vs. Q1	-0.029	-0.053	-0.004	0.023	
	Q4 vs. Q1	-0.025	-0.050	0.000	0.049	
Uranium	Q2 vs. Q1	-0.017	-0.043	0.008	0.183	0.602
	Q3 vs. Q1	-0.018	-0.044	0.007	0.159	
	Q4 vs. Q1	-0.009	-0.035	0.018	0.521	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXVII. ASSOCIATIONS BETWEEN METAL MIXTURES AND T4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.010	-0.032	0.012	0.359	<.001
	Q3 vs. Q1	-0.029	-0.052	-0.007	0.010	
	Q4 vs. Q1	-0.045	-0.068	-0.021	0.000	
Barium	Q2 vs. Q1	-0.029	-0.050	-0.008	0.007	<.001
	Q3 vs. Q1	-0.053	-0.076	-0.031	<.0001	
	Q4 vs. Q1	-0.035	-0.058	-0.011	0.004	
Cesium	Q2 vs. Q1	-0.001	-0.021	0.020	0.943	0.001
	Q3 vs. Q1	0.036	0.013	0.058	0.002	
	Q4 vs. Q1	0.031	0.005	0.056	0.018	
Mixture (from QGCOMP)		*-0.019*	*-0.031*	*-0.008*	*<.001*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

TABLE LXVIII. ASSOCIATIONS BETWEEN METAL MIXTURES AND T4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.024	-0.048	0.001	0.056	<.001
	Q3 vs. Q1	-0.040	-0.064	-0.015	0.002	
	Q4 vs. Q1	-0.058	-0.083	-0.032	<.0001	
Tungsten	Q2 vs. Q1	-0.006	-0.031	0.019	0.640	0.007
	Q3 vs. Q1	-0.030	-0.054	-0.006	0.016	
	Q4 vs. Q1	-0.027	-0.051	-0.004	0.024	
Mixture (from QGCOMP)		*-0.028*	*-0.039*	*-0.018*	*<.001*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle

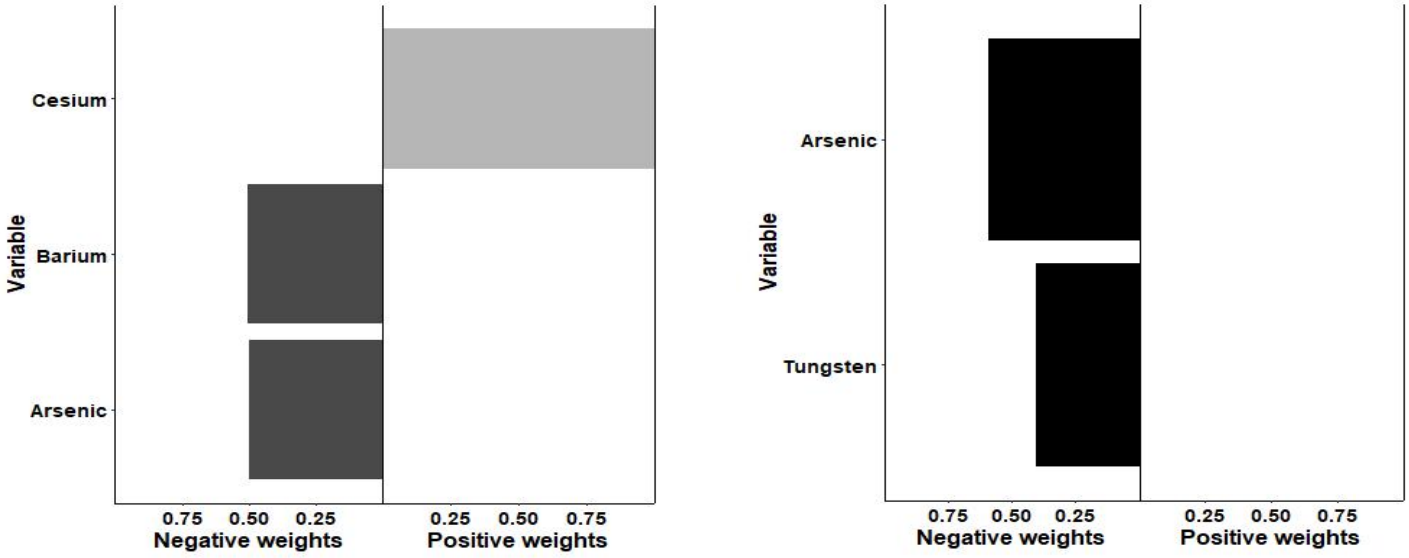

Figure 6. The directional contribution of metals to T4. Left: males; Right: females

6. Associations between Metals and FT4

We investigated the association between each metal and FT4 levels using linear regression models. The results of single associations between metal exposure and levels of FT4 were presented in the Tables LXIX and LXX. Barium (inverse), cadmium (positive), thallium (inverse), and tungsten (inverse) showed dose-response relationships with FT4 levels, and cobalt showed a non-linear association in males.

In females, barium also showed a dose-response inverse relationship, however, cadmium and tungsten appeared to have non-linear relationships in female. Unlike in males, lead showed a strong linear relationship in females.

We assessed the adjusted associations between multiple metals and FT4 levels in the multivariable regression models, using the metals showed dose-response or non-linear relationships between FT4 in the first step. Tables LXXI and LXXII demonstrate the results of

the adjusted associations. The statistical trend was significant for the relationship between barium and FT4 in males, however it was likely led by the trend between participants belonged Q3 and Q4 of barium concentrations, which showed the strongest associations. Cadmium showed a positive linear association, whereas thallium and tungsten showed negative associations with FT4 in males.

The patterns of association with barium and cadmium were consistent with single metal models in females; after co-adjustment for other selected metals int this step, barium showed a non-monotonic negative association, and cadmium showed a positive association with FT4. The second quartile of tungsten retained a significant positive association with FT4, however, the association between cadmium and FT4 was attenuated in females.

In the mixtures analysis for metals and T4 using QGCOMP, the metals showing linear associations with FT4 in the second stage were assessed to address their relative contributions and the effects of mixture itself. For males, four metals comprising barium, cadmium, thallium, and tungsten were included in this stage. In the linear regression model with the four metals, every metal remained significant in terms of their linear trend.

As a mixture, those four metals had a negative relationship with FT4 in males (beta for mixture=-0.015, 95% CI=-0.028- -0.001). For females, only barium and lead were included and assessed as a mixture, however the result was not significant (beta for mixture=0.002, 95% CI=-0.008- 0 012). The results from the mixtures analysis were presented in the Tables LXXIII and LXXIV, and Figure 7 demonstrate the relative contribution of each metal in the metal mixture in males and females, respectively.

TABLE LXIX. SINGLE METAL ASSOCIATIONS WITH FT4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.009	-0.010	0.028	0.354	0.786
	Q3 vs. Q1	0.002	-0.018	0.021	0.855	
	Q4 vs. Q1	-0.001	-0.022	0.020	0.932	
Antimony	Q2 vs. Q1	-0.012	-0.030	0.007	0.211	0.831
	Q3 vs. Q1	0.012	-0.007	0.031	0.211	
	Q4 vs. Q1	-0.011	-0.030	0.009	0.283	
Barium	Q2 vs. Q1	-0.012	-0.031	0.007	0.207	0.001
	Q3 vs. Q1	-0.035	-0.054	-0.016	0.000	
	Q4 vs. Q1	-0.026	-0.046	-0.006	0.011	
Cadmium	Q2 vs. Q1	0.017	-0.002	0.036	0.074	0.002
	Q3 vs. Q1	0.019	-0.003	0.040	0.083	
	Q4 vs. Q1	0.045	0.018	0.072	0.001	
Cobalt	Q2 vs. Q1	-0.012	-0.029	0.006	0.191	0.128
	Q3 vs. Q1	-0.022	-0.041	-0.002	0.027	
	Q4 vs. Q1	-0.009	-0.032	0.014	0.425	
Cesium	Q2 vs. Q1	-0.011	-0.029	0.008	0.253	0.822
	Q3 vs. Q1	-0.004	-0.023	0.016	0.731	
	Q4 vs. Q1	-0.004	-0.026	0.018	0.719	
Lead	Q2 vs. Q1	0.012	-0.010	0.035	0.283	0.854
	Q3 vs. Q1	-0.006	-0.028	0.017	0.608	
	Q4 vs. Q1	0.005	-0.019	0.029	0.704	
Mercury	Q2 vs. Q1	0.006	-0.014	0.025	0.572	0.101
	Q3 vs. Q1	-0.017	-0.037	0.003	0.094	
	Q4 vs. Q1	-0.010	-0.030	0.010	0.315	
Molybdenum	Q2 vs. Q1	0.013	-0.005	0.032	0.158	0.584
	Q3 vs. Q1	0.008	-0.011	0.027	0.420	
	Q4 vs. Q1	-0.006	-0.026	0.014	0.580	
Thallium	Q2 vs. Q1	-0.024	-0.042	-0.006	0.010	0.001
	Q3 vs. Q1	-0.029	-0.048	-0.010	0.003	
	Q4 vs. Q1	-0.031	-0.052	-0.010	0.003	
Tungsten	Q2 vs. Q1	-0.009	-0.027	0.010	0.343	<.001
	Q3 vs. Q1	-0.016	-0.035	0.003	0.105	
	Q4 vs. Q1	-0.034	-0.053	-0.014	0.001	
Uranium	Q2 vs. Q1	-0.004	-0.023	0.014	0.649	0.923
	Q3 vs. Q1	-0.013	-0.033	0.006	0.175	
	Q4 vs. Q1	0.003	-0.017	0.022	0.785	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXX. SINGLE METAL ASSOCIATIONS WITH FT4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.009	-0.031	0.012	0.395	0.087
	Q3 vs. Q1	-0.011	-0.033	0.011	0.307	
	Q4 vs. Q1	-0.020	-0.042	0.002	0.079	
Antimony	Q2 vs. Q1	-0.002	-0.025	0.021	0.856	0.505
	Q3 vs. Q1	0.002	-0.020	0.024	0.860	
	Q4 vs. Q1	0.006	-0.016	0.028	0.607	
Barium	Q2 vs. Q1	-0.028	-0.051	-0.006	0.014	0.005
	Q3 vs. Q1	-0.027	-0.050	-0.005	0.016	
	Q4 vs. Q1	-0.036	-0.058	-0.014	0.002	
Cadmium	Q2 vs. Q1	0.013	-0.010	0.035	0.262	0.060
	Q3 vs. Q1	0.012	-0.010	0.035	0.288	
	Q4 vs. Q1	0.031	0.003	0.060	0.031	
Cobalt	Q2 vs. Q1	0.009	-0.018	0.036	0.525	0.167
	Q3 vs. Q1	0.000	-0.025	0.026	0.993	
	Q4 vs. Q1	-0.010	-0.034	0.015	0.453	
Cesium	Q2 vs. Q1	-0.007	-0.031	0.017	0.562	0.058
	Q3 vs. Q1	-0.010	-0.033	0.014	0.415	
	Q4 vs. Q1	-0.022	-0.046	0.001	0.066	
Lead	Q2 vs. Q1	0.019	-0.001	0.039	0.060	<.001
	Q3 vs. Q1	0.025	0.002	0.048	0.034	
	Q4 vs. Q1	0.049	0.023	0.075	0.000	
Mercury	Q2 vs. Q1	-0.001	-0.022	0.019	0.891	0.316
	Q3 vs. Q1	-0.014	-0.035	0.006	0.173	
	Q4 vs. Q1	-0.007	-0.029	0.016	0.546	
Molybdenum	Q2 vs. Q1	-0.002	-0.024	0.020	0.858	0.648
	Q3 vs. Q1	-0.002	-0.024	0.020	0.885	
	Q4 vs. Q1	0.005	-0.017	0.027	0.681	
Thallium	Q2 vs. Q1	-0.019	-0.043	0.004	0.111	0.181
	Q3 vs. Q1	-0.002	-0.025	0.021	0.873	
	Q4 vs. Q1	-0.022	-0.044	0.001	0.059	
Tungsten	Q2 vs. Q1	0.027	0.005	0.049	0.015	0.302
	Q3 vs. Q1	-0.007	-0.028	0.014	0.521	
	Q4 vs. Q1	0.000	-0.021	0.022	0.969	
Uranium	Q2 vs. Q1	-0.002	-0.024	0.021	0.871	0.499
	Q3 vs. Q1	-0.005	-0.027	0.017	0.636	
	Q4 vs. Q1	0.008	-0.014	0.031	0.480	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXI. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND FT4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	-0.010	-0.029	0.009	0.291	0.012
	Q3 vs. Q1	-0.032	-0.052	-0.011	0.002	
	Q4 vs. Q1	-0.021	-0.043	0.001	0.061	
Cadmium	Q2 vs. Q1	0.018	0.000	0.037	0.054	0.002
	Q3 vs. Q1	0.019	-0.002	0.040	0.081	
	Q4 vs. Q1	0.044	0.017	0.071	0.001	
Cobalt	Q2 vs. Q1	-0.001	-0.019	0.017	0.899	0.999
	Q3 vs. Q1	-0.007	-0.028	0.014	0.497	
	Q4 vs. Q1	0.004	-0.021	0.029	0.748	
Thallium	Q2 vs. Q1	-0.022	-0.039	-0.004	0.019	0.004
	Q3 vs. Q1	-0.027	-0.046	-0.008	0.007	
	Q4 vs. Q1	-0.028	-0.049	-0.007	0.008	
Tungsten	Q2 vs. Q1	-0.007	-0.026	0.011	0.428	0.024
	Q3 vs. Q1	-0.014	-0.033	0.005	0.160	
	Q4 vs. Q1	-0.031	-0.050	-0.011	0.002	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND FT4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	-0.032	-0.055	-0.010	0.005	0.003
	Q3 vs. Q1	-0.030	-0.052	-0.008	0.008	
	Q4 vs. Q1	-0.040	-0.062	-0.018	0.000	
Cadmium	Q2 vs. Q1	0.013	-0.009	0.035	0.258	0.180
	Q3 vs. Q1	0.010	-0.012	0.033	0.364	
	Q4 vs. Q1	0.024	-0.005	0.053	0.101	
Lead	Q2 vs. Q1	0.018	-0.002	0.037	0.079	<.001
	Q3 vs. Q1	0.025	0.002	0.048	0.034	
	Q4 vs. Q1	0.047	0.021	0.073	0.000	
Tungsten	Q2 vs. Q1	0.028	0.006	0.050	0.012	0.506
	Q3 vs. Q1	-0.005	-0.026	0.017	0.673	
	Q4 vs. Q1	0.003	-0.018	0.024	0.777	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXIII. ASSOCIATIONS BETWEEN METAL MIXTURES AND FT4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	-0.011	-0.029	0.008	0.260	0.007
	Q3 vs. Q1	-0.033	-0.052	-0.013	0.001	
	Q4 vs. Q1	-0.022	-0.042	-0.002	0.033	
Cadmium	Q2 vs. Q1	0.018	0.000	0.037	0.055	0.002
	Q3 vs. Q1	0.019	-0.002	0.040	0.079	
	Q4 vs. Q1	0.045	0.018	0.071	0.001	
Thallium	Q2 vs. Q1	-0.022	-0.040	-0.004	0.017	0.004
	Q3 vs. Q1	-0.027	-0.046	-0.008	0.006	
	Q4 vs. Q1	-0.028	-0.049	-0.008	0.008	
Tungsten	Q2 vs. Q1	-0.008	-0.026	0.011	0.417	0.003
	Q3 vs. Q1	-0.014	-0.033	0.005	0.159	
	Q4 vs. Q1	-0.031	-0.050	-0.011	0.002	
Mixture (from QGCOMP)		*-0.015*	*-0.028*	*-0.001*	*0.030*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXIV. ASSOCIATIONS BETWEEN METAL MIXTURES AND FT4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	-0.031	-0.053	-0.008	0.007	0.002
	Q3 vs. Q1	-0.030	-0.052	-0.008	0.009	
	Q4 vs. Q1	-0.039	-0.061	-0.017	0.001	
Lead	Q2 vs. Q1	0.020	0.000	0.040	0.046	<.001
	Q3 vs. Q1	0.028	0.005	0.051	0.016	
	Q4 vs. Q1	0.052	0.026	0.078	<.0001	
Mixture (from QGCOMP)		*0.002*	*-0.008*	*0.012*	*0.732*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

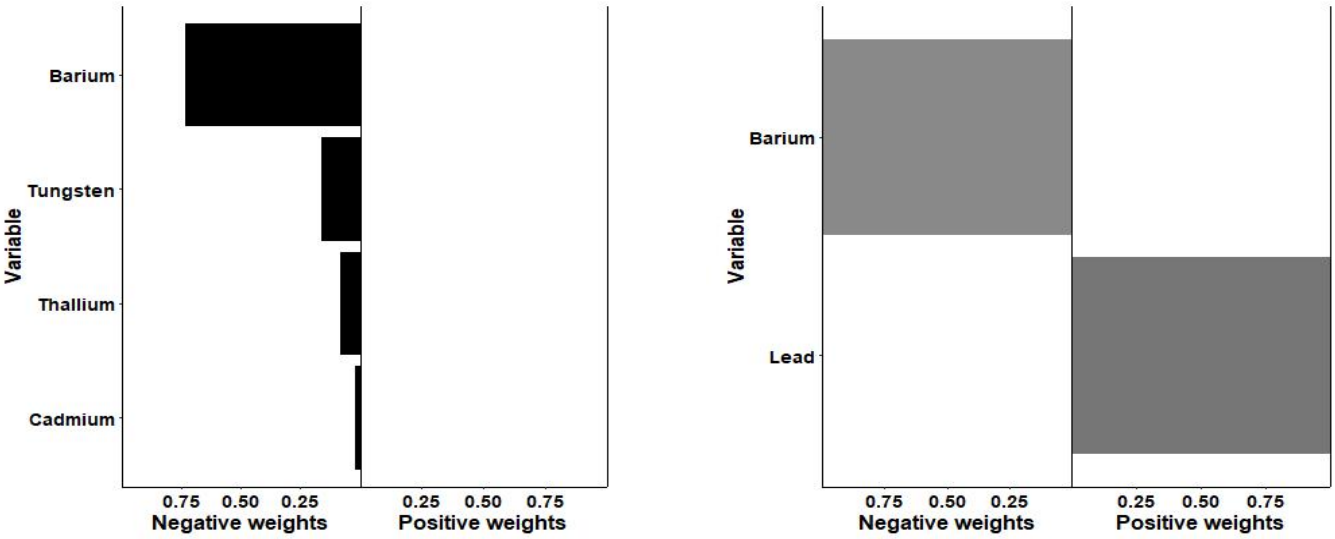

Figure 7. The directional contribution of metals to FT4. Left: males; Right: females

7. Associations between Metals and TSH

We investigated the association between each metal and TSH levels using linear regression models (Tables LXXV and LXXVI). In males, cesium is the only metal that showed a dose-response relationship (p-trend=0.038). Tungsten and uranium had non-linear associations with TSH in males. Among females, arsenic showed a positive linear relationship and barium showed a negative association with TSH. Exposure to cobalt had a negative non-linear association with TSH in females, with a pattern of inverted U-shape. We assessed the associations between multiple metals and TSH levels, including metals showing associations in single metal analysis (Tables LXXVII and LXXVIII). In both sexes, the patterns of association between metals and TSH were similar in the adjusted analysis compared to the single metal analysis, with cesium having a significant inverse association in males and arsenic having a positive association in females. However, the significance of the negative linear trend of barium among females was attenuated.

TABLE LXXV. SINGLE METAL ASSOCIATIONS WITH TSH, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.034	-0.103	0.034	0.328	0.492
	Q3 vs. Q1	0.009	-0.062	0.079	0.807	
	Q4 vs. Q1	-0.044	-0.118	0.031	0.250	
Antimony	Q2 vs. Q1	-0.035	-0.101	0.032	0.306	0.383
	Q3 vs. Q1	-0.026	-0.095	0.043	0.459	
	Q4 vs. Q1	-0.034	-0.104	0.036	0.344	
Barium	Q2 vs. Q1	-0.023	-0.090	0.044	0.495	0.629
	Q3 vs. Q1	0.008	-0.062	0.078	0.825	
	Q4 vs. Q1	-0.030	-0.103	0.043	0.417	
Cadmium	Q2 vs. Q1	0.023	-0.045	0.091	0.509	0.367
	Q3 vs. Q1	-0.028	-0.105	0.048	0.470	
	Q4 vs. Q1	-0.038	-0.135	0.058	0.437	
Cobalt	Q2 vs. Q1	-0.011	-0.074	0.051	0.725	0.439
	Q3 vs. Q1	-0.038	-0.108	0.031	0.279	
	Q4 vs. Q1	-0.016	-0.099	0.067	0.704	
Cesium	Q2 vs. Q1	-0.019	-0.084	0.046	0.564	0.038
	Q3 vs. Q1	-0.062	-0.133	0.009	0.087	
	Q4 vs. Q1	-0.072	-0.150	0.007	0.073	
Lead	Q2 vs. Q1	0.004	-0.077	0.085	0.915	0.257
	Q3 vs. Q1	0.008	-0.074	0.089	0.855	
	Q4 vs. Q1	0.046	-0.040	0.132	0.298	
Mercury	Q2 vs. Q1	-0.045	-0.115	0.025	0.210	0.603
	Q3 vs. Q1	-0.059	-0.130	0.013	0.109	
	Q4 vs. Q1	-0.017	-0.089	0.056	0.654	
Molybdenum	Q2 vs. Q1	-0.047	-0.114	0.021	0.174	0.237
	Q3 vs. Q1	-0.066	-0.134	0.003	0.060	
	Q4 vs. Q1	-0.034	-0.106	0.038	0.350	
Thallium	Q2 vs. Q1	0.048	-0.016	0.113	0.144	0.266
	Q3 vs. Q1	0.010	-0.059	0.079	0.770	
	Q4 vs. Q1	-0.043	-0.118	0.032	0.259	
Tungsten	Q2 vs. Q1	0.013	-0.053	0.080	0.693	0.062
	Q3 vs. Q1	-0.008	-0.076	0.061	0.828	
	Q4 vs. Q1	0.082	0.011	0.152	0.023	
Uranium	Q2 vs. Q1	0.080	0.013	0.146	0.019	0.402
	Q3 vs. Q1	0.048	-0.022	0.117	0.180	
	Q4 vs. Q1	0.037	-0.033	0.107	0.298	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXVI. SINGLE METAL ASSOCIATIONS WITH TSH, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.108	0.024	0.192	0.012	0.020
	Q3 vs. Q1	0.108	0.024	0.193	0.012	
	Q4 vs. Q1	0.113	0.027	0.200	0.010	
Antimony	Q2 vs. Q1	0.011	-0.077	0.099	0.805	0.974
	Q3 vs. Q1	-0.029	-0.114	0.056	0.503	
	Q4 vs. Q1	0.012	-0.072	0.097	0.774	
Barium	Q2 vs. Q1	-0.082	-0.169	0.004	0.063	0.030
	Q3 vs. Q1	-0.081	-0.167	0.005	0.065	
	Q4 vs. Q1	-0.106	-0.192	-0.020	0.015	
Cadmium	Q2 vs. Q1	-0.059	-0.146	0.028	0.185	0.200
	Q3 vs. Q1	-0.079	-0.166	0.009	0.077	
	Q4 vs. Q1	-0.058	-0.168	0.053	0.305	
Cobalt	Q2 vs. Q1	-0.126	-0.230	-0.021	0.018	0.130
	Q3 vs. Q1	-0.052	-0.151	0.046	0.300	
	Q4 vs. Q1	-0.112	-0.207	-0.016	0.022	
Cesium	Q2 vs. Q1	-0.040	-0.132	0.053	0.401	0.320
	Q3 vs. Q1	-0.058	-0.149	0.032	0.208	
	Q4 vs. Q1	-0.050	-0.141	0.042	0.291	
Lead	Q2 vs. Q1	-0.010	-0.087	0.067	0.795	0.777
	Q3 vs. Q1	0.027	-0.062	0.116	0.551	
	Q4 vs. Q1	-0.035	-0.136	0.067	0.501	
Mercury	Q2 vs. Q1	-0.004	-0.083	0.076	0.931	0.842
	Q3 vs. Q1	0.039	-0.042	0.119	0.347	
	Q4 vs. Q1	-0.009	-0.096	0.078	0.846	
Molybdenum	Q2 vs. Q1	-0.001	-0.086	0.084	0.983	0.977
	Q3 vs. Q1	0.001	-0.084	0.086	0.982	
	Q4 vs. Q1	0.001	-0.084	0.086	0.989	
Thallium	Q2 vs. Q1	0.054	-0.039	0.146	0.255	0.174
	Q3 vs. Q1	0.017	-0.071	0.106	0.705	
	Q4 vs. Q1	-0.034	-0.121	0.053	0.445	
Tungsten	Q2 vs. Q1	-0.082	-0.167	0.003	0.057	0.548
	Q3 vs. Q1	-0.032	-0.115	0.051	0.447	
	Q4 vs. Q1	-0.046	-0.128	0.036	0.268	
Uranium	Q2 vs. Q1	0.040	-0.047	0.127	0.372	0.561
	Q3 vs. Q1	0.055	-0.031	0.140	0.210	
	Q4 vs. Q1	0.028	-0.059	0.115	0.533	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXVII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND TSH, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Cesium	Q2 vs. Q1	-0.019	-0.084	0.046	0.563	0.033
	Q3 vs. Q1	-0.060	-0.131	0.010	0.094	
	Q4 vs. Q1	-0.076	-0.155	0.002	0.055	
Tungsten	Q2 vs. Q1	0.007	-0.060	0.074	0.835	0.082
	Q3 vs. Q1	-0.009	-0.078	0.060	0.801	
	Q4 vs. Q1	0.076	0.004	0.149	0.040	
Uranium	Q2 vs. Q1	0.074	0.007	0.141	0.029	0.660
	Q3 vs. Q1	0.040	-0.031	0.110	0.269	
	Q4 vs. Q1	0.024	-0.048	0.096	0.515	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXVIII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND TSH, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.110	0.026	0.194	0.010	0.012
	Q3 vs. Q1	0.116	0.031	0.201	0.007	
	Q4 vs. Q1	0.119	0.032	0.206	0.007	
Barium	Q2 vs. Q1	-0.081	-0.169	0.007	0.071	0.061
	Q3 vs. Q1	-0.077	-0.166	0.012	0.090	
	Q4 vs. Q1	-0.102	-0.193	-0.010	0.030	
Cobalt	Q2 vs. Q1	-0.108	-0.213	-0.002	0.045	0.403
	Q3 vs. Q1	-0.034	-0.137	0.069	0.517	
	Q4 vs. Q1	-0.082	-0.184	0.020	0.116	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

8. Associations between Metals and T3:T4 Ratio

In Tables LXXIX and LXXX, we present the association between each metal and T3:T4 ratio from linear regression models. In males, three metals showed positive linear associations with the T3:T4 ratio; barium, lead, and thallium. Barium and thallium also showed positive associations in females. Cesium also showed a positive dose-response relationship and cadmium had a negative linear relationship with T3:T4 ratio among females. We assessed the associations between multiple metals and T3:T4 ratio, only including metals with significant associations in the single metal analysis. In the second step with T3:T4 ratio, barium, lead, and thallium were included for males, and barium cadmium, cesium, and thallium were included for females.

In males, barium and lead retained their significant positive associations with T3:T4 ratio. In females, barium and thallium still showed significant positive associations and cadmium a significant negative association with T3:T4 ratio. With the metals with a linear trend in the second step, the associations between metal mixtures and T3:T4 ratios were investigated. A mixture consisting of barium and lead showed a positive association with the T3:T4 ratio in males, with greater weights from barium (beta for mixture=0.026, 95% CI=0.014-0.037). No significant mixture effect was observed in females.

9. Associations between Metals and FT4:TSH Ratio

Tables LXXXV and LXXXVI demonstrate the results of the association between each metal and the FT4:TSH ratio from linear regression. In males, tungsten showed a negative association with significant linear trend (p-trend=0.010), and the second quartile of uranium showed a decreased FT4:TSH ratio compared to the first quartile (beta=-0.084, 95% CI=-0.156 - -0.012). Among females, arsenic showed a negative dose-response relationship with the FT4:TSH ratio. In addition, cobalt and tungsten showed a non-linear association in females.

TABLE LXXIX. SINGLE METAL ASSOCIATIONS WITH T3:T4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.003	-0.020	0.026	0.801	0.120
	Q3 vs. Q1	0.008	-0.016	0.031	0.534	
	Q4 vs. Q1	0.020	-0.005	0.045	0.122	
Antimony	Q2 vs. Q1	-0.005	-0.028	0.017	0.644	0.207
	Q3 vs. Q1	0.002	-0.021	0.025	0.867	
	Q4 vs. Q1	0.015	-0.009	0.039	0.221	
Barium	Q2 vs. Q1	0.033	0.010	0.055	0.004	0.001
	Q3 vs. Q1	0.048	0.024	0.071	<.0001	
	Q4 vs. Q1	0.038	0.014	0.063	0.002	
Cadmium	Q2 vs. Q1	0.011	-0.012	0.034	0.342	0.725
	Q3 vs. Q1	0.002	-0.024	0.028	0.874	
	Q4 vs. Q1	-0.008	-0.041	0.024	0.613	
Cobalt	Q2 vs. Q1	0.021	0.000	0.042	0.051	0.251
	Q3 vs. Q1	0.022	-0.001	0.045	0.066	
	Q4 vs. Q1	0.009	-0.019	0.037	0.517	
Cesium	Q2 vs. Q1	0.021	-0.001	0.043	0.064	0.756
	Q3 vs. Q1	-0.003	-0.027	0.021	0.802	
	Q4 vs. Q1	0.002	-0.024	0.028	0.879	
Lead	Q2 vs. Q1	0.016	-0.012	0.043	0.263	0.011
	Q3 vs. Q1	0.045	0.018	0.073	0.001	
	Q4 vs. Q1	0.033	0.004	0.062	0.025	
Mercury	Q2 vs. Q1	0.003	-0.021	0.026	0.812	0.179
	Q3 vs. Q1	0.019	-0.005	0.043	0.128	
	Q4 vs. Q1	0.013	-0.012	0.037	0.314	
Molybdenum	Q2 vs. Q1	0.001	-0.021	0.024	0.905	0.358
	Q3 vs. Q1	0.010	-0.013	0.033	0.410	
	Q4 vs. Q1	0.009	-0.015	0.033	0.467	
Thallium	Q2 vs. Q1	0.015	-0.006	0.037	0.167	0.018
	Q3 vs. Q1	0.020	-0.003	0.043	0.095	
	Q4 vs. Q1	0.030	0.005	0.055	0.021	
Tungsten	Q2 vs. Q1	0.001	-0.021	0.024	0.927	0.318
	Q3 vs. Q1	0.002	-0.022	0.025	0.901	
	Q4 vs. Q1	-0.014	-0.038	0.010	0.257	
Uranium	Q2 vs. Q1	-0.008	-0.031	0.014	0.472	0.167
	Q3 vs. Q1	-0.006	-0.029	0.018	0.636	
	Q4 vs. Q1	-0.019	-0.042	0.005	0.125	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXX. SINGLE METAL ASSOCIATIONS WITH T3:T4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.007	-0.020	0.034	0.624	0.171
	Q3 vs. Q1	0.012	-0.015	0.040	0.389	
	Q4 vs. Q1	0.019	-0.009	0.047	0.186	
Antimony	Q2 vs. Q1	0.016	-0.012	0.045	0.258	0.746
	Q3 vs. Q1	-0.006	-0.034	0.021	0.656	
	Q4 vs. Q1	0.004	-0.024	0.031	0.783	
Barium	Q2 vs. Q1	0.035	0.007	0.063	0.016	0.001
	Q3 vs. Q1	0.033	0.005	0.060	0.022	
	Q4 vs. Q1	0.051	0.023	0.079	0.000	
Cadmium	Q2 vs. Q1	-0.017	-0.045	0.011	0.235	0.012
	Q3 vs. Q1	-0.018	-0.046	0.010	0.211	
	Q4 vs. Q1	-0.052	-0.088	-0.017	0.004	
Cobalt	Q2 vs. Q1	0.033	-0.001	0.067	0.058	0.685
	Q3 vs. Q1	0.019	-0.014	0.051	0.258	
	Q4 vs. Q1	0.019	-0.012	0.050	0.225	
Cesium	Q2 vs. Q1	0.016	-0.014	0.046	0.303	0.017
	Q3 vs. Q1	0.027	-0.002	0.057	0.068	
	Q4 vs. Q1	0.035	0.005	0.065	0.020	
Lead	Q2 vs. Q1	0.003	-0.022	0.028	0.826	0.778
	Q3 vs. Q1	0.019	-0.010	0.048	0.197	
	Q4 vs. Q1	-0.004	-0.037	0.029	0.828	
Mercury	Q2 vs. Q1	0.002	-0.024	0.028	0.865	0.387
	Q3 vs. Q1	0.018	-0.008	0.045	0.168	
	Q4 vs. Q1	0.007	-0.022	0.035	0.652	
Molybdenum	Q2 vs. Q1	0.009	-0.018	0.037	0.511	0.824
	Q3 vs. Q1	0.012	-0.015	0.040	0.379	
	Q4 vs. Q1	-0.003	-0.030	0.025	0.859	
Thallium	Q2 vs. Q1	0.042	0.012	0.072	0.006	<.001
	Q3 vs. Q1	0.044	0.015	0.072	0.003	
	Q4 vs. Q1	0.058	0.030	0.086	<.0001	
Tungsten	Q2 vs. Q1	-0.010	-0.037	0.018	0.499	0.779
	Q3 vs. Q1	0.008	-0.019	0.035	0.541	
	Q4 vs. Q1	-0.009	-0.036	0.017	0.490	
Uranium	Q2 vs. Q1	-0.008	-0.036	0.020	0.569	0.942
	Q3 vs. Q1	0.018	-0.010	0.046	0.197	
	Q4 vs. Q1	-0.007	-0.035	0.021	0.621	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXI. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND T3:T4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	0.032	0.010	0.055	0.005	0.003
	Q3 vs. Q1	0.045	0.021	0.068	0.000	
	Q4 vs. Q1	0.034	0.010	0.059	0.007	
Lead	Q2 vs. Q1	0.013	-0.014	0.040	0.338	0.028
	Q3 vs. Q1	0.043	0.016	0.070	0.002	
	Q4 vs. Q1	0.028	-0.001	0.057	0.058	
Thallium	Q2 vs. Q1	0.013	-0.008	0.035	0.231	0.066
	Q3 vs. Q1	0.016	-0.008	0.039	0.186	
	Q4 vs. Q1	0.024	-0.002	0.049	0.066	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND T3:T4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	0.030	0.001	0.058	0.042	0.009
	Q3 vs. Q1	0.024	-0.004	0.052	0.097	
	Q4 vs. Q1	0.043	0.014	0.071	0.003	
Cadmium	Q2 vs. Q1	-0.019	-0.047	0.010	0.198	0.007
	Q3 vs. Q1	-0.020	-0.049	0.008	0.160	
	Q4 vs. Q1	-0.055	-0.091	-0.020	0.002	
Cesium	Q2 vs. Q1	0.002	-0.029	0.032	0.905	0.743
	Q3 vs. Q1	0.006	-0.026	0.037	0.733	
	Q4 vs. Q1	0.005	-0.029	0.040	0.767	
Thallium	Q2 vs. Q1	0.038	0.008	0.069	0.015	0.011
	Q3 vs. Q1	0.035	0.005	0.066	0.025	
	Q4 vs. Q1	0.047	0.015	0.080	0.004	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXIII. ASSOCIATIONS BETWEEN METAL MIXTURE AND T3:T4, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	0.033	0.011	0.056	0.004	0.001
	Q3 vs. Q1	0.047	0.023	0.070	<.0001	
	Q4 vs. Q1	0.037	0.013	0.061	0.003	
Lead	Q2 vs. Q1	0.014	-0.013	0.041	0.316	0.021
	Q3 vs. Q1	0.044	0.017	0.071	0.002	
	Q4 vs. Q1	0.029	0.000	0.058	0.047	
Mixture (from QGCOMP)		*0.026*	*0.014*	*0.037*	*<0.001*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXIV. ASSOCIATIONS BETWEEN METAL MIXTURE AND T3:T4, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Barium	Q2 vs. Q1	0.030	0.001	0.058	0.040	0.007
	Q3 vs. Q1	0.024	-0.004	0.052	0.092	
	Q4 vs. Q1	0.043	0.015	0.071	0.003	
Cadmium	Q2 vs. Q1	-0.019	-0.047	0.010	0.195	0.007
	Q3 vs. Q1	-0.020	-0.049	0.008	0.160	
	Q4 vs. Q1	-0.055	-0.091	-0.019	0.002	
Thallium	Q2 vs. Q1	0.039	0.009	0.069	0.011	0.002
	Q3 vs. Q1	0.037	0.008	0.066	0.012	
	Q4 vs. Q1	0.050	0.021	0.079	0.001	
Mixture (from QGCOMP)		*0.006*	*-0.008*	*0.021*	*0.409*	

a: adjusted for significant metals from the previous stage, along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

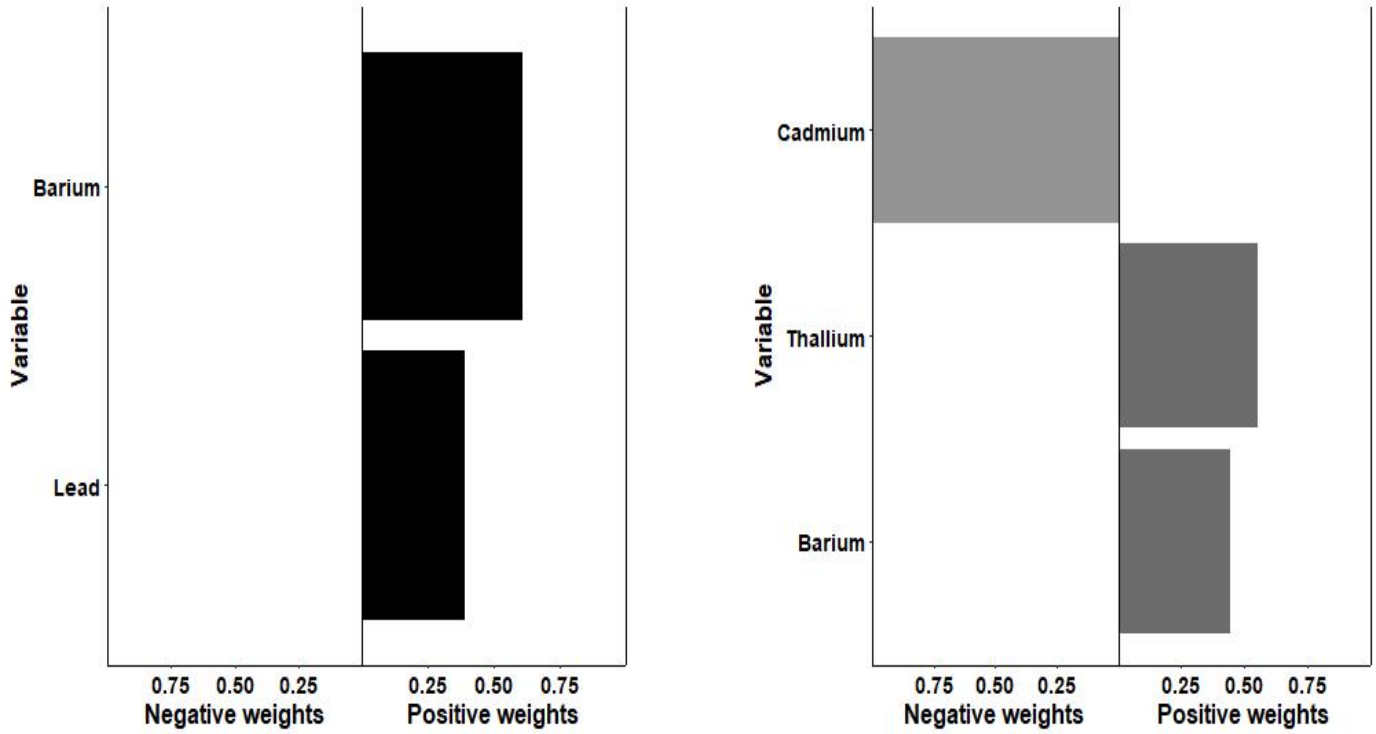

Figure 8. The directional contribution of metals to T3:T4. Left: males; Right: females

We assessed the associations between multiple metals and the FT4:TSH ratio, only including metals with significant associations in the single metal analysis. Tungsten and uranium were included in the analysis among males, while arsenic, cobalt, and tungsten were included for the analyses among females. Tables LXXXVII and LXXXVIII represent the results, which demonstrate consistent patterns and directions from the single metal analysis in both sexes.

For FT4:TSH ratio, a mixtures analysis was not performed due to the lack of more than one metal in each sex with a linear relationship with the ratio in the second stage.

TABLE LXXXV. SINGLE META ASSOCIATIONS WITH FT4:TSH, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	0.043	-0.031	0.118	0.255	0.574
	Q3 vs. Q1	-0.007	-0.083	0.070	0.859	
	Q4 vs. Q1	0.043	-0.038	0.123	0.300	
Antimony	Q2 vs. Q1	0.023	-0.049	0.095	0.534	0.454
	Q3 vs. Q1	0.038	-0.037	0.113	0.317	
	Q4 vs. Q1	0.023	-0.053	0.100	0.551	
Barium	Q2 vs. Q1	0.011	-0.061	0.084	0.760	0.709
	Q3 vs. Q1	-0.043	-0.119	0.033	0.267	
	Q4 vs. Q1	0.004	-0.075	0.083	0.921	
Cadmium	Q2 vs. Q1	-0.006	-0.079	0.068	0.881	0.107
	Q3 vs. Q1	0.047	-0.036	0.131	0.268	
	Q4 vs. Q1	0.083	-0.022	0.188	0.120	
Cobalt	Q2 vs. Q1	0.000	-0.069	0.068	0.992	0.746
	Q3 vs. Q1	0.017	-0.059	0.092	0.666	
	Q4 vs. Q1	0.007	-0.084	0.097	0.884	
Cesium	Q2 vs. Q1	0.009	-0.062	0.079	0.811	0.065
	Q3 vs. Q1	0.059	-0.019	0.136	0.137	
	Q4 vs. Q1	0.068	-0.018	0.153	0.120	
Lead	Q2 vs. Q1	0.008	-0.080	0.096	0.861	0.276
	Q3 vs. Q1	-0.014	-0.102	0.075	0.764	
	Q4 vs. Q1	-0.041	-0.135	0.053	0.390	
Mercury	Q2 vs. Q1	0.050	-0.026	0.126	0.195	0.953
	Q3 vs. Q1	0.042	-0.036	0.119	0.296	
	Q4 vs. Q1	0.006	-0.073	0.085	0.877	
Molybdenum	Q2 vs. Q1	0.060	-0.013	0.133	0.107	0.343
	Q3 vs. Q1	0.073	-0.001	0.147	0.053	
	Q4 vs. Q1	0.029	-0.050	0.107	0.472	
Thallium	Q2 vs. Q1	-0.072	-0.142	-0.002	0.045	0.834
	Q3 vs. Q1	-0.039	-0.114	0.036	0.307	
	Q4 vs. Q1	0.012	-0.069	0.093	0.772	
Tungsten	Q2 vs. Q1	-0.022	-0.095	0.050	0.545	0.010
	Q3 vs. Q1	-0.008	-0.082	0.066	0.830	
	Q4 vs. Q1	-0.115	-0.192	-0.039	0.003	
Uranium	Q2 vs. Q1	-0.084	-0.156	-0.012	0.023	0.426
	Q3 vs. Q1	-0.061	-0.136	0.015	0.114	
	Q4 vs. Q1	-0.035	-0.111	0.042	0.375	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXVI. SINGLE META ASSOCIATIONS WITH FT4:TSH, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.117	-0.207	-0.027	0.011	0.010
	Q3 vs. Q1	-0.120	-0.211	-0.029	0.010	
	Q4 vs. Q1	-0.133	-0.226	-0.040	0.005	
Antimony	Q2 vs. Q1	-0.013	-0.108	0.081	0.785	0.897
	Q3 vs. Q1	0.031	-0.061	0.123	0.506	
	Q4 vs. Q1	-0.007	-0.098	0.084	0.886	
Barium	Q2 vs. Q1	0.054	-0.039	0.148	0.256	0.181
	Q3 vs. Q1	0.054	-0.039	0.147	0.256	
	Q4 vs. Q1	0.070	-0.022	0.163	0.137	
Cadmium	Q2 vs. Q1	0.072	-0.022	0.165	0.134	0.101
	Q3 vs. Q1	0.091	-0.003	0.185	0.058	
	Q4 vs. Q1	0.089	-0.030	0.208	0.142	
Cobalt	Q2 vs. Q1	0.134	0.022	0.247	0.019	0.283
	Q3 vs. Q1	0.052	-0.054	0.159	0.335	
	Q4 vs. Q1	0.102	-0.001	0.205	0.052	
Cesium	Q2 vs. Q1	0.032	-0.067	0.132	0.522	0.638
	Q3 vs. Q1	0.049	-0.049	0.146	0.330	
	Q4 vs. Q1	0.027	-0.072	0.126	0.589	
Lead	Q2 vs. Q1	0.029	-0.054	0.112	0.490	0.264
	Q3 vs. Q1	-0.002	-0.098	0.093	0.961	
	Q4 vs. Q1	0.084	-0.025	0.193	0.132	
Mercury	Q2 vs. Q1	0.002	-0.084	0.088	0.962	0.671
	Q3 vs. Q1	-0.053	-0.140	0.034	0.230	
	Q4 vs. Q1	0.002	-0.092	0.095	0.971	
Molybdenum	Q2 vs. Q1	-0.001	-0.093	0.091	0.982	0.934
	Q3 vs. Q1	-0.003	-0.094	0.089	0.956	
	Q4 vs. Q1	0.004	-0.088	0.096	0.932	
Thallium	Q2 vs. Q1	-0.073	-0.173	0.027	0.150	0.346
	Q3 vs. Q1	-0.019	-0.114	0.076	0.697	
	Q4 vs. Q1	0.012	-0.081	0.106	0.797	
Tungsten	Q2 vs. Q1	0.109	0.018	0.200	0.019	0.756
	Q3 vs. Q1	0.025	-0.064	0.114	0.580	
	Q4 vs. Q1	0.047	-0.041	0.135	0.299	
Uranium	Q2 vs. Q1	-0.042	-0.135	0.052	0.385	0.706
	Q3 vs. Q1	-0.060	-0.152	0.032	0.201	
	Q4 vs. Q1	-0.020	-0.113	0.074	0.682	

a: adjusted for age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXVII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND FT4:TSH, IN MALES

Parameter		Estimate[a]	95% CI		p	p-trend
Tungsten	Q2 vs. Q1	-0.016	-0.089	0.057	0.666	0.014
	Q3 vs. Q1	-0.006	-0.082	0.069	0.867	
	Q4 vs. Q1	-0.111	-0.190	-0.032	0.006	
Uranium	Q2 vs. Q1	-0.073	-0.146	-0.001	0.049	0.896
	Q3 vs. Q1	-0.045	-0.122	0.031	0.247	
	Q4 vs. Q1	-0.009	-0.088	0.069	0.817	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

TABLE LXXXVIII. ADJUSTED ASSOCIATIONS BETWEEN CONTRIBUTING METALS AND FT4:TSH, IN FEMALES

Parameter		Estimate[a]	95% CI		p	p-trend
Arsenic	Q2 vs. Q1	-0.117	-0.208	-0.027	0.011	0.008
	Q3 vs. Q1	-0.122	-0.213	-0.031	0.009	
	Q4 vs. Q1	-0.134	-0.228	-0.041	0.005	
Cobalt	Q2 vs. Q1	0.133	0.021	0.245	0.020	0.234
	Q3 vs. Q1	0.061	-0.046	0.168	0.262	
	Q4 vs. Q1	0.108	0.004	0.211	0.041	
Tungsten	Q2 vs. Q1	0.110	0.019	0.200	0.018	0.712
	Q3 vs. Q1	0.025	-0.064	0.114	0.587	
	Q4 vs. Q1	0.046	-0.042	0.134	0.306	

a: adjusted for 12 metals along with age, race, education, BMI, smoking status, serum cotinine, hormone therapy, and study cycle.

D. Discussion

In this study, we investigated the associations of 12 metals and thyroid hormone profiles in NHANES 2007-2012 data. We addressed the relationships of thyroid hormones with multiple metals as a mixture, as well as with each individual metal. To our knowledge, this is the first paper that addressed the effect size of metal mixture on thyroid hormones in NHANES data, assessing multiple metals with sex-stratified analysis. The results from our analysis are summarized in Figure 9.

MALES

Hormone	T3			FT3			T4			FT4			TSH			T3:T4			FT4:TSH		
Stage	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Arsenic	M	M	M	M	M	M	M	M	M												
Antimony				M	NM																
Barium				M	M	M	M	M	M	M	M	M				M	M	M			
Cadmium										M	M	M									
Cobalt				M	M	M	NM			NM											
Cesium	NM						NM	M	M				M	M							
Lead	NM	NM		M	M	M										M	M	M			
Mercury							NM														
Molybdenum	NM	NM		M	NM																
Thallium										M	M	M				M					
Tungsten	M	M	M	M	M	M				M	M	M	NM	NM					M	M	
Uranium	M	NM											NM	NM					NM	NM	
Mixture P			<0.01			0.57			<0.01			0.03			NA			<0.01			NA

FEMALES

Hormone	T3			FT3			T4			FT4			TSH			T3:T4			FT4:TSH		
Stage	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Arsenic	M	M	M	M	M	M	M	M	M				M	M					M	M	
Antimony				M																	
Barium	NM	NM					M			M	M	M	M	NM		M	M	M			
Cadmium	NM	M	M							NM						M	M	M			
Cobalt													NM	NM					NM	NM	
Cesium							M														
Lead										M	M	M				M					
Mercury							M														
Molybdenum	M																				
Thallium	NM	M	M	NM	M	M	M									M	M	M			
Tungsten	M	M	M	M			M	M	M	NM	NM								NM	NM	
Uranium	NM	NM		M			NM														
Mixture P			<0.01			0.84			<0.01			0.73			NA			0.41			NA

Stage 1=individual metal model; Stage 2=control for other significant metals in stage 1; Stage 3=Qgcomp mixture model

M=monotonic dose response; NM: non-monotonic dose response

green=positive association orange=negative association

Figure 9. Summary of results from three stages assessing associations between single and multiple associations between metals and thyroid hormones.

In our results, the numbers and types of involved metals varied by each thyroid hormone. Nevertheless, some metals appeared to be associated with thyroid hormones more consistently than others. Arsenic was inversely associated with T3, FT3, T4 in males and females, and positively associated with TSH and negatively associated with FT4:TSH ratio in females. The inverse associations between arsenic and thyroid hormones are consistent with previous studies [165, 178, 179]. In a study by Jain, arsenic was inversely associated with TT4 in males and females [178]. In Ciarrocca's study and Guo's study, arsenic showed negative associations with T3, FT3, and FT4 [165, 177]. The mechanism of action of arsenic to disrupt thyroid hormones has not been widely investigated in humans. Although animal models confirmed the toxicity of arsenic on thyroid endocrine system, varying results across different animals do not consistently explain the arsenic mechanisms on thyroid hormones [273]. One potential explanation was suggested in a study by Davey et al., in which arsenic at low concentrations altered the response of thyroid hormone receptor (TR) elements and TR-mediated gene expressions, which disrupting binding of T3 to the receptors [274]. Interrupted binding of T3 to the receptors also may result in changes with T3:T4 ratio, however, we did not observe significant associations between arsenic and T3:T4 ratio in our analyses.

Cadmium was another heavy metal that was associated with various thyroid hormones. Our result with cadmium and FT4 is consistent with the direction of association from Chen's study in NHANES [189], however the directions with T3 and FT3 were inconsistent, possibly due to the sex-stratification analysis in our study. In addition, while a couple of previous studies reported negative associations between blood cadmium and TSH levels [187, 188], we did not observe significant relationships in our analysis. The discrepancies between our analysis and other studies from NHANES data may due to the different range of study cycles, and sex-stratified analysis in our study.

It is interesting that contribution of cadmium to thyroid hormones was notably dependent on sex. In our study, cadmium had inverse relationships with T3 and T3:T4 ratio in females, but

a positive association with FT4 in males. The difference may due to higher level of cadmium in females compared to males. Previous literatures have shown that females are prone to show higher cadmium concentrations than males, mainly because females are more likely to be iron depleted which is associated increased absorption of cadmium in intestine and therefore higher cadmium levels in body [185, 186]. Despite many animal studies the mechanism involved in cadmium disruption of thyroid hormones remains unclear [275-277].

Among trace metals, barium, thallium, and tungsten also impacted multiple thyroid hormones in our study. Our results for T4 and FT4 with barium are consistent with a previous study that investigated the associations between 11 individual metals and thyroid hormones in NHANES data by Yorita-Christensen, as well as the relationship of thallium with FT4, and tungsten with T3 and T4 [187]. The results for thallium and thyroid hormones in this study are also in line with the results from NHANES analysis by Mendy et al. [180]. However, research about those metals in association with thyroid hormones are sparse, which suggests a need for further research.

Our results emphasize the necessity to conduct sex-stratified analysis in research with metals and thyroid hormones (use reference from methods here too). Many metals showed different associations by sex. In addition, sex-stratified analysis is more reasonable considering the distribution of metals differed by sex. In this study, we used sex-specific quartiles of each metal in order to detect potential non-linear or non-monotonic relationships between metals and thyroid hormones, and, given the different mean values of the metal concentrations by sex, it is likely that the ranges of quartiles differ by sex. Although there are a limited number of studies with sex-stratified analyses in this field, inconsistent metal-thyroid hormone associations by sex have been found in previous research. For example, studies in males showed positive associations between arsenic and TSH [166, 177], whereas Jain found a negative association in females [178].

We adopted a multiple stage procedure to address the associations between metal mixtures and thyroid hormone levels. Starting with single metal association analysis, we identified individual metals with significant linear or non-linear associations with thyroid hormones, and assessed if these associations remained significant in multi-metal models. Finally, we explored the consistency of our findings for metals with linear associations with hormones using a mixtures approach with QGCOMP. We observed changes in the patterns of association across these steps for several metals and thyroid hormones. For example, molybdenum showed a significant inverse association with T3 among females in single metal association analysis; however, the effect was attenuated, after control for metals such as arsenic and cadmium, however the direction of association was consistent across the stages of analysis. Our findings suggest that focusing on a single metal using traditional regression methods may exaggerate the effects of the metal on health outcomes, since we observed the attenuation of numerous associations with control for confounding by other metals (e.g. barium, cesium, mercury, and thallium in the second stage of T4 analysis among females).

The key message of our observations is in line with a study by Meeker et al. (2009), which investigated the associations between multiple metals and TSH in males [166]. In the study, they adopted a two-stage approach with multivariable regression models; first, single association analysis was conducted for each individual metal, and second, final regression model with multiple metals was constructed. As a result, arsenic, copper, and lead appeared to be associated with TSH level in the final model, while arsenic and copper were significant association in the initial stage [166]. Although the impactful metals in their study are not consistent with results from the current investigation, both studies imply the necessity of research in metal mixtures in association with thyroid hormones. In addition, our analysis extended theirs by incorporating QGCOMP as a confirmatory methodology to assess the effect magnitude and significance of the mixture.

Our study has several limitations. First, the associations between metals and thyroid hormones addressed in the study was assessed in cross-sectional study design, therefore a potential issue of temporality exists. Furthermore, the effects of environmental pollutants on health outcomes, not only for the metals and thyroid hormones, might vary by the timing and duration of exposure [262], however in this study we were not able to assess more detailed information for metal exposure. Second, as addressing metals as a mixture, we assume the combined effects induced by multiple metals are in linear relationships with thyroid hormones. However, the reciprocal effects may be in non-linear or non-additive patterns, which we were not able to capture using the methods we adopted in this study. Bobb et al. proposed a method, Bayesian kernel machine regression (BKMR), to address the non-linear and non-additive relationships [51]. However, in BKMR estimations it is assumed that the metals besides the main exposure are fixed at the 25% or 75% percentile, which still poses a limitation. Finally, by adopting QGCOMP as a complementary method to linear regression, we were not able to apply the complex survey design of NHANES, as a limitation of the R package. The complex survey design and sample weights are unique features of NHANES which generate estimates representative of the general population in the U.S. Due to this limitation, the results of this study should be interpreted as results from convenience sample, rather than a representative sample from NHANES.

In conclusion, we demonstrated that multiple metals are associated with various thyroid hormones, and the pattern of association can vary by metal, hormone and sex. It is important to assess the associations between multiple metals and thyroid hormone profiles because the effects of metals might be reinforced or attenuated after adjusting for other metal exposures. Adopting methods such as QGCOMP to evaluate the metals as a mixture may be helpful to confirm the findings from the linear regression models, by accommodating the reciprocal actions among various metals.

VI. CONCLUSIONS

In this study, we investigated the associations between endocrine-disrupting environmental pollutants, specifically POPs and metals, and altered endocrine traits in a Hispanic population from HCHS/SOL and a sample of US adults. Our work adopted various epidemiologic methods to explore the relationships of environmental risk factors with health outcomes. First, to evaluate the effects of environmental pollutants in relation with other risk factors, we assessed longitudinal individual associations between PRS and POPs with hyperglycemic outcomes as well as the interactive effects between PRS and POPs on these outcomes. Second, to gain a more complete understanding of the challenge of reverse causality in environmental epidemiology, we explored the associations between POPs and lipid profiles in parallel using cross-sectional and longitudinal study designs. Lastly, to evaluate the collective effects of environmental pollutants, we investigated associations between metals and thyroid hormone profiles by assessing the effects of various metals as a single component as well as a metal mixture.

In our first study, we found interaction effects between higher exposure to POPs and PRS constructed with T2D SNPs on hyperglycemic outcomes. Interestingly, we observed the GxE effects without significant main effects of POPs on overt diabetes, prediabetes, or HOMA measurements, which suggested higher exposure to POPs might modify the effects of genetic components. Growing research on the impacts of environmental chemical exposures on epigenetic changes may provide a key to explain the mechanisms of action in the interactive relationship between POPs and genetic polymorphisms.

Furthermore, our findings, which showed the greater risk of developing adverse conditions among populations with similar genetic risks but elevated concentration of POPs, suggested the importance of managing and monitoring modifiable risk factors, and provides justification for exploring targeted public health interventions. Despite the unmodifiable risk

profile of genetic polymorphisms, its effect on health may be modifiable by interventions to minimize exposure to environmental risk factors, such as advisories for personal dietary modifications, and policy-based regulations to decrease human exposure to man-made chemicals or metals.

In our second study, we explored cross-sectional and longitudinal associations between POPs and lipid profiles in effort to clarify long-standing concerns about reverse causality bias in studies of POPs and lipid concentrations. We observed associations of PCBs and OC pesticides with total cholesterol, LDL cholesterol, and TG in cross-sectional analyses, whereas non-monotonic associations were found only with HDL cholesterol in longitudinal analyses. Our results from longitudinal analysis supported the biological plausibility of POPs adversely impacting lipid HDL cholesterol. In addition, the discrepancies in our findings between cross-sectional and longitudinal relationships of POPs and lipid profiles emphasize the necessity of longitudinal studies in this area of research to understand the effects of environmental risk factors.

Although our study showed associations between POPs and lipid profiles, it is limited by a lack of information on important covariates such as detailed classification of lipid lowering medications used by participants and food items, which could be a source of POPs exposure as well as a risk factor for altered lipid profiles. Future investigations of environmental pollutants and the endocrine system should consider the interrelationship among variables in the study design phase, especially considering the complex and multifactorial pathway underlying the associations between exposure and outcome. Advanced analytical methodology may be needed to fully explore these multifactorial pathways.

Lastly, we extended the traditional epidemiological approach of assessing effects of a single exposure to investigating the combined impact of multiple exposures. In our third study with metal mixtures, we explored the single- and multiple associations of metals with thyroid hormone profiles. Under different modeling scenarios, we observed changes of the associations

not only in the magnitude and significance, but also with respect to the shape of the dose response (monotonic versus non-monotonic). In addition, with the utilization of the advanced mixtures statistical methodology, we estimated effects of metals as a mixture on thyroid hormones. We anticipate our findings will provide further understanding of the relationships between metal mixtures and thyroid hormones; however, at the same time it should be noted that additional relationships between metals or metals and thyroid hormones may exist that were not detected with our methodology, such as interactions and non-monotonic associations of outcome and mixture.

There has been growing consensus of the necessity to study multiple risk factors simultaneously, and the number of risk factors to be considered in environmental epidemiology is increasing substantially with the development of exposome methodology to identify targeted and untargeted exposures in human populations. We anticipate that applying analytical methods to incorporate multiple environmental pollutants in epidemiological investigations will contribute to further understanding in the impacts of environmental factors on morbidity and mortality, as well as to establishing evidence for developing interventions and guidance in the area of public health.

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APPENDICES

APPENDIX A

TABLE LXXXIX. COMPARISON OF BASELINE CHARACTERISTICS BETWEEN PARTICIPANTS WITH- AND WITHOUT GENETIC INFORMATION IN THE ANCILLARY STUDY OF HCHS/SOL

Characteristics		With SNP (N=1,849)	w/o SNP (N=444)	P-value
Age, mean (SE)		56.4 (0.4)	56.6 (0.6)	0.277
Body mass index (BMI), mean (SE)		29.0 (0.2)	29.0 (0.4)	0.887
Hispanic background	Dominican	194 (9.9)	32 (7.3)	<0.01
	Central American	168 (6.7)	48 (8.4)	
	Cuban	337 (28.6)	39 (17.2)	
	Mexican	647 (29.9)	216 (45.4)	
	Puerto Rican	324 (15.9)	68 (14.6)	
	South American	140 (5.0)	33 (4.5)	
	More than one or other heritage	39 (4.0)	8 (2.6)	
Education	Less than HS	713 (37.1)	186 (16.5)	0.517
	HS graduate	414 (19.7)	90 (16.5)	
	Greater than HS	722 (43.1)	168 (43.3)	
Alcohol use	No current use	920 (54.6)	244 (56.7)	0.861
	Low level use	836 (41.4)	182 (39.4)	
	High level use	93 (4.0)	18 (3.9)	
Cigarette use	Never	949 (52.3)	262 (59.0)	0.205
	Former smoker	498 (26.9)	106 (22.1)	
	Current smoker	402 (20.8)	76 (18.9)	
Physical activity level	High	202 (9.3)	50 (9.2)	0.391
	Moderate	836 (45.7)	184 (40.6)	
	Low	811 (45.0)	210 (50.1)	
Family history of T2D	No	1007 (56.8)	239 (53.5)	0.421
	Yes	842 (43.2)	205 (46.5)	
Study Center	Bronx	454 (29.0)	77 (21.0)	<0.01
	Chicago	435 (12.0)	123 (14.0)	
	Miami	520 (38.5)	77 (25.2)	
	San Diego	440 (20.5)	167 (39.7)	

APPENDIX A (CONTINUED)

TABLE XC. COMPARISON OF POPS DISTRIBUTION BY 75TH PERCENTILE BETWEEN PARTICIPANTS WITH- AND WITHOUT GENETIC INFORMATION

POPs	With SNP	W/O SNP	p-value
PCB < 75th percentile	1215 (66.3)	299 (68.9)	0.56
PCB > 75th percentile	414 (33.7)	91 (31.1)	
DDE < 75th percentile	1387 (72.9)	321 (73.5)	0.86
DDE > 75th percentile	22.6 (27.2)	122 (26.5)	
OXYCHLOR < 75th percentile	1375 (70.5)	335 (70.9)	0.92
OXYCHLOR > 75th percentile	462 (29.5)	107 (29.1)	
TNONA < 75th percentile	1363 (68.8)	333 (69/4)	0.88
TNONA > 75th percentile	457 (31.2)	107 (30.6)	

TABLE XCI. COMPARISON OF T2D TRAITS BETWEEN PARTICIPANTS WITH- AND WITHOUT GENETIC INFORMATION

Outcomes at V2		with genetic info	w/o genetic info	P-value
Normoglycemic or prediabetes to T2D	Yes	491 (15.9)	103 (22.3)	0.118
Normoglycemic to hyperglycemic	Yes	499 (51.3)	115 (48.3)	0.555
Normoglycemic to prediabetes	Yes	473 (50.0)	110 (47.4)	0.616
Prediabetes to diabetes	Yes	465 (25.7)	98 (35.3)	0.110
HOMA-IR, geomean (SE)		3.00 (0.07)	3.04 (0.17)	0.858
HOMA-B, geomean (SE)		121.4 (2.5)	116.9 (5.1)	0.433

APPENDIX A (CONTINUED)

TABLE XCII. ASSOCIATIONS BETWEEN WET-WEIGHT PCB AND HYPERGLYCEMIC OUTCOMES AT V2

T2D Outcomes	wet PCB	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.26	0.79	2.01	1.18	0.72	1.93
Normoglycemic to hyperglycemic	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.64	0.93	2.87	1.85	1.04	3.30
Normoglycemic to prediabetes	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.66	0.94	2.95	1.76	0.98	3.15
Prediabetes to diabetes	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	1.11	0.66	1.89	1.11	0.66	1.88
HOMA-IR at V2	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	-0.098	-0.202	0.005	0.016	-0.078	0.110
HOMA-B at V2	PCB <75th percentile	ref			ref		
	PCB ≥75th percentile	-0.049	-0.175	0.078	-0.019	-0.099	0.061

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. In Model 2, model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

APPENDIX A (CONTINUED)

TABLE XCIII. INTERACTION EFFECTS BETWEEN TOTAL PRS AND WET-WEIGHT PCB ON HYPERGLYCEMIC OUTCOMES

Outcomes at V2	PRS	wet PCB < 75 Percentile[a]			wet PCB > 75 Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.06
	T2	0.99	0.73	1.35	1.02	0.60	1.73	
	T3	1.39	1.00	1.92	1.82	1.07	3.11	
Normoglycemic to hyperglycemic	T1	ref			ref			0.32
	T2	0.90	0.62	1.29	0.84	0.44	1.62	
	T3	1.69	1.11	2.59	2.10	0.97	4.57	
Normoglycemic to prediabetes	T1	ref			ref			0.32
	T2	0.89	0.62	1.28	0.82	0.42	1.60	
	T3	1.71	1.11	2.64	2.15	0.97	4.79	
Prediabetes to diabetes	T1	ref			ref			0.06
	T2	0.99	0.72	1.37	1.01	0.58	1.75	
	T3	1.41	0.99	1.99	1.87	1.06	3.29	
HOMA-IR	T1	ref			ref			0.86
	T2	0.024	-0.134	0.181	0.034	-0.125	0.193	
	T3	0.012	-0.136	0.160	0.022	-0.125	0.169	
HOMA-B	T1	ref			ref			0.55
	T2	0.030	-0.135	0.194	-0.003	-0.162	0.156	
	T3	-0.020	-0.156	0.116	-0.052	-0.180	0.075	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

APPENDIX A (CONTINUED)

TABLE XCIV. INTERACTION EFFECTS BETWEEN INSULIN RESISTANCE PRS AND WET-WEIGHT PCB ON HYPERGLYCEMIC OUTCOMES

Outcomes at V2	Insulin resistance PRS	wet PCB < 75 Percentile[a]			wet PCB > 75 Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.02
	T2	1.18	0.86	1.63	1.10	0.63	1.93	
	T3	1.04	0.77	1.40	1.52	0.92	2.52	
Normoglycemic to hyperglycemic	T1	ref			ref			0.41
	T2	0.97	0.66	1.41	0.88	0.45	1.72	
	T3	1.28	0.88	1.88	1.55	0.77	3.09	
Normoglycemic to prediabetes	T1	ref			ref			0.43
	T2	1.00	0.68	1.46	0.90	0.46	1.77	
	T3	1.28	0.86	1.90	1.54	0.76	3.15	
Prediabetes to diabetes	T1	ref			ref			0.02
	T2	1.28	0.91	1.80	1.16	0.64	2.09	
	T3	1.06	0.77	1.45	1.63	0.96	2.79	
HOMA-IR	T1	ref			ref			0.83
	T2	0.030	-0.114	0.173	0.028	-0.128	0.184	
	T3	0.009	-0.142	0.160	0.007	-0.154	0.169	
HOMA-B	T1	ref			ref			0.94
	T2	-0.008	-0.165	0.148	0.002	-0.158	0.162	
	T3	-0.055	-0.167	0.056	-0.045	-0.163	0.073	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

APPENDIX A (CONTINUED)

TABLE XCV. INTERACTION EFFECTS BETWEEN B-CELL PRS AND WET-WEIGHT PCB ON HYPERGLYCEMIC OUTCOMES

Outcomes at V2	β-cell dysfunction PRS	wet PCB < 75 Percentile[a]			wet PCB > 75 Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.55
	T2	1.01	0.70	1.44	1.21	0.68	2.18	
	T3	1.11	0.79	1.56	1.09	0.62	1.93	
Normoglycemic to hyperglycemic	T1	ref			ref			0.66
	T2	1.06	0.73	1.54	1.05	0.54	2.04	
	T3	1.34	0.88	2.03	1.25	0.58	2.66	
Normoglycemic to prediabetes	T1	ref			ref			0.69
	T2	1.00	0.69	1.47	0.98	0.50	1.94	
	T3	1.36	0.89	2.08	1.28	0.59	2.77	
Prediabetes to diabetes	T1	ref			ref			0.48
	T2	0.89	0.62	1.29	1.12	0.60	2.08	
	T3	1.17	0.81	1.67	1.16	0.63	2.13	
HOMA-IR	T1	ref			ref			0.96
	T2	0.005	-0.149	0.159	0.026	-0.129	0.182	
	T3	0.019	-0.134	0.172	0.041	-0.110	0.191	
HOMA-B	T1	ref			ref			0.27
	T2	0.045	-0.128	0.218	0.063	-0.110	0.235	
	T3	-0.028	-0.145	0.089	-0.011	-0.118	0.097	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

APPENDIX A (CONTINUED)

TABLE XCVI. ASSOCIATIONS BETWEEN WET-WEIGHT DDE AND HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	wet DDE	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.48	0.95	2.31	1.42	0.87	2.31
Normoglycemic to hyperglycemic	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.58	0.91	2.72	1.71	0.99	2.95
Normoglycemic to prediabetes	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.66	0.96	2.88	1.76	1.01	3.06
Prediabetes to diabetes	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	1.58	0.93	2.71	1.52	0.91	2.56
HOMA-IR at V2	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	0.085	-0.035	0.204	0.026	-0.056	0.107
HOMA-B at V2	DDE <75th Percentile	ref			ref		
	DDE ≥75th Percentile	0.053	-0.051	0.156	0.031	-0.050	0.112

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI

APPENDIX A (CONTINUED)

TABLE XCVII. INTERACTION EFFECTS BETWEEN TOTAL PRS AND WET-WEIGHT DDE ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	PRS	DDE < 75th Percentile[a]			DDE > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.17
	T2	0.83	0.60	1.13	0.63	0.36	1.10	
	T3	1.50	1.08	2.07	2.02	1.16	3.52	
Normoglycemic to hyperglycemic	T1	ref			ref			0.88
	T2	1.06	0.76	1.48	1.27	0.69	2.32	
	T3	1.37	0.93	2.02	1.28	0.64	2.58	
Normoglycemic to prediabetes	T1	ref			ref			0.82
	T2	1.06	0.76	1.49	1.26	0.68	2.32	
	T3	1.36	0.92	2.02	1.30	0.64	2.65	
Prediabetes to diabetes	T1	ref			ref			0.14
	T2	0.83	0.60	1.16	0.61	0.34	1.10	
	T3	1.45	1.03	2.04	2.04	1.14	3.67	
HOMA-IR	T1	ref			ref			0.13
	T2	0.108	-0.017	0.232	0.077	-0.046	0.200	
	T3	0.008	-0.136	0.152	-0.023	-0.165	0.119	
HOMA-B	T1	ref			ref			0.09
	T2	0.120	-0.032	0.273	0.087	-0.068	0.242	
	T3	0.043	-0.080	0.166	0.010	-0.113	0.133	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

APPENDIX A (CONTINUED)

TABLE XCVIII. INTERACTION EFFECTS BETWEEN INSULIN-RESISTANCE PRS AND WET-WEIGHT DDE ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	Insulin resistance PRS	DDE < 75th Percentile[a]			DDE > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.30
	T2	1.02	0.73	1.43	0.81	0.45	1.47	
	T3	0.83	0.60	1.13	0.78	0.44	1.37	
Normoglycemic to hyperglycemic	T1	ref			ref			0.87
	T2	0.98	0.70	1.38	0.82	0.46	1.48	
	T3	1.16	0.81	1.65	1.23	0.66	2.30	
Normoglycemic to prediabetes	T1	ref			ref			0.88
	T2	0.98	0.69	1.39	0.81	0.45	1.47	
	T3	1.17	0.82	1.68	1.25	0.67	2.37	
Prediabetes to diabetes	T1	ref			ref			0.26
	T2	1.01	0.71	1.44	0.76	0.41	1.43	
	T3	0.83	0.60	1.15	0.77	0.43	1.39	
HOMA-IR	T1	ref			ref			0.58
	T2	0.009	-0.116	0.134	0.046	-0.093	0.184	
	T3	0.087	-0.043	0.217	0.123	-0.018	0.265	
HOMA-B	T1	ref			ref			0.16
	T2	0.060	-0.093	0.212	0.082	-0.077	0.242	
	T3	0.082	-0.005	0.169	0.105	0.002	0.208	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

APPENDIX A (CONTINUED)

TABLE XCIX. INTERACTION EFFECTS BETWEEN B-CELL PRS AND WET-WEIGHT DDE ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	β-cell dysfunction PRS	DDE < 75th Percentile[a]			DDE > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.35
	T2	1.03	0.71	1.51	1.03	0.52	2.03	
	T3	1.08	0.79	1.48	1.24	0.70	2.18	
Normoglycemic to hyperglycemic	T1	ref			ref			0.17
	T2	0.83	0.60	1.15	0.52	0.30	0.89	
	T3	1.86	1.29	2.67	3.05	1.61	5.79	
Normoglycemic to prediabetes	T1	ref			ref			0.15
	T2	0.80	0.57	1.11	0.52	0.30	0.90	
	T3	1.89	1.30	2.74	3.06	1.59	5.89	
Prediabetes to diabetes	T1	ref			ref			0.35
	T2	0.95	0.64	1.42	1.10	0.54	2.25	
	T3	1.10	0.78	1.54	1.17	0.65	2.13	
HOMA-IR	T1	ref			ref			0.81
	T2	0.038	-0.092	0.168	0.022	-0.110	0.155	
	T3	0.038	-0.091	0.167	0.023	-0.110	0.155	
HOMA-B	T1	ref			ref			0.55
	T2	0.068	-0.091	0.227	0.062	-0.102	0.225	
	T3	0.016	-0.084	0.116	0.010	-0.093	0.112	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

APPENDIX A (CONTINUED)

TABLE C. ASSOCIATIONS BETWEEN WET-WEIGHT OXYCHLOR AND HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	wet OXYCHLOR	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.21	0.79	1.85	1.22	0.78	1.92
Normoglycemic to hyperglycemic	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.18	0.69	2.01	1.21	0.70	2.09
Normoglycemic to prediabetes	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.25	0.73	2.14	1.23	0.71	2.13
Prediabetes to diabetes	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	1.15	0.71	1.89	1.24	0.76	2.01
HOMA-IR at V2	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	0.046	-0.065	0.156	0.019	-0.065	0.103
HOMA-B at V2	OXYCHLOR <75th percentile	ref			ref		
	OXYCHLOR ≥75th percentile	0.040	-0.049	0.129	0.042	-0.030	0.115

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI

APPENDIX A (CONTINUED)

TABLE CI. INTERACTION EFFECTS BETWEEN TOTAL PRS AND WET-WEIGHT OXYCHLOR ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	PRS	OXYCHLOR < 75th Percentile[a]			OXYCHLOR > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.41
	T2	0.95	0.71	1.27	1.01	0.62	1.64	
	T3	1.39	1.02	1.90	1.53	0.91	2.56	
Normoglycemic to hyperglycemic	T1	ref			ref			0.58
	T2	0.94	0.66	1.33	0.83	0.44	1.55	
	T3	1.51	1.02	2.24	1.77	0.86	3.63	
Normoglycemic to prediabetes	T1	ref			ref			0.56
	T2	0.94	0.67	1.34	0.83	0.44	1.57	
	T3	1.49	1.00	2.24	1.76	0.84	3.68	
Prediabetes to diabetes	T1	ref			ref			0.48
	T2	0.97	0.72	1.32	1.07	0.64	1.78	
	T3	1.33	0.95	1.85	1.42	0.82	2.47	
HOMA-IR	T1	ref			ref			0.86
	T2	-0.020	-0.161	0.120	0.020	-0.127	0.167	
	T3	0.035	-0.090	0.161	0.076	-0.055	0.207	
HOMA-B	T1	ref			ref			0.52
	T2	0.055	-0.088	0.197	0.063	-0.081	0.208	
	T3	0.067	-0.042	0.177	0.076	-0.034	0.186	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

APPENDIX A (CONTINUED)

TABLE CII. INTERACTION EFFECTS BETWEEN INSULIN-RESISTANCE PRS AND WET-WEIGHT OXYCHLOR ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	Insulin resistance PRS	OXYCHLOR < 75th Percentile[a]			OXYCHLOR > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.11
	T2	1.12	0.82	1.53	1.05	0.62	1.77	
	T3	0.94	0.71	1.25	1.21	0.74	1.96	
Normoglycemic to hyperglycemic	T1	ref			ref			0.15
	T2	0.95	0.66	1.37	0.74	0.38	1.42	
	T3	1.32	0.92	1.89	1.88	0.98	3.61	
Normoglycemic to prediabetes	T1	ref			ref			0.34
	T2	0.94	0.65	1.36	0.70	0.36	1.39	
	T3	1.35	0.94	1.94	1.97	1.02	3.81	
Prediabetes to diabetes	T1	ref			ref			0.11
	T2	1.11	0.80	1.54	0.96	0.55	1.68	
	T3	0.95	0.71	1.29	1.26	0.76	2.11	
HOMA-IR	T1	ref			ref			0.04
	T2	0.089	-0.023	0.201	0.060	-0.056	0.176	
	T3	0.044	-0.104	0.193	0.015	-0.132	0.162	
HOMA-B	T1	ref			ref			0.26
	T2	0.086	-0.036	0.209	0.073	-0.052	0.197	
	T3	0.042	-0.064	0.148	0.029	-0.078	0.135	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

APPENDIX A (CONTINUED)

TABLE CIII. INTERACTION EFFECTS BETWEEN B-CELL PRS AND WET-WEIGHT OXYCHLOR ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	β-cell dysfunction PRS	OXYCHLOR < 75th Percentile[a]			OXYCHLOR > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.50
	T2	1.02	0.73	1.43	1.07	0.60	1.88	
	T3	1.09	0.81	1.47	1.15	0.70	1.90	
Normoglycemic to hyperglycemic	T1	ref			ref			0.11
	T2	0.98	0.70	1.39	0.92	0.50	1.70	
	T3	1.29	0.87	1.92	1.02	0.50	2.09	
Normoglycemic to prediabetes	T1	ref			ref			0.12
	T2	0.95	0.67	1.34	0.93	0.50	1.73	
	T3	1.31	0.87	1.95	1.01	0.49	2.10	
Prediabetes to diabetes	T1	ref			ref			0.51
	T2	0.90	0.64	1.28	1.06	0.58	1.94	
	T3	1.12	0.81	1.56	1.13	0.65	1.95	
HOMA-IR	T1	ref			ref			0.50
	T2	0.010	-0.121	0.140	-0.008	-0.135	0.119	
	T3	0.001	-0.141	0.142	-0.017	-0.153	0.119	
HOMA-B	T1	ref			ref			0.62
	T2	0.091	-0.052	0.235	0.077	-0.060	0.215	
	T3	0.013	-0.100	0.126	-0.001	-0.103	0.100	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

APPENDIX A (CONTINUED)

TABLE CIV. ASSOCIATIONS BETWEEN WET-WEIGHT TNONA AND HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	wet TNONA	Model 1[a]			Model 2[b]		
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)
Normoglycemic or prediabetes to T2D	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.47	0.97	2.23	1.46	0.88	2.42
Normoglycemic to hyperglycemic	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.42	0.83	2.43	1.43	0.82	2.48
Normoglycemic to prediabetes	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.42	0.82	2.46	1.38	0.78	2.43
Prediabetes to diabetes	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	1.20	0.74	1.94	1.32	0.81	2.17
HOMA-IR at V2	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	0.061	-0.047	0.168	0.008	-0.079	0.096
HOMA-B at V2	TNONA <75th Percentile	ref			ref		
	TNONA ≥75th Percentile	0.020	-0.069	0.109	0.011	-0.061	0.082

a: adjusted for first 5 PCs for Hispanic background and study center.
b: Model 1 + additionally adjusted for age, sex, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. In Model 2, model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements were adjusted for each HOMA measurement at V1.

TABLE CV. INTERACTION EFFECTS BETWEEN TOTAL PRS AND WET-WEIGHT TNONA ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	PRS	TNONA < 75th Percentile[a]			TNONA > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.23
	T2	0.92	0.69	1.24	0.87	0.53	1.44	
	T3	1.42	1.04	1.94	1.73	1.04	2.88	
Normoglycemic to hyperglycemic	T1	ref			ref			0.52
	T2	0.95	0.66	1.36	0.84	0.44	1.63	
	T3	1.55	1.01	2.38	1.85	0.84	4.10	
Normoglycemic to prediabetes	T1	ref			ref			0.31
	T2	0.96	0.67	1.37	0.87	0.44	1.69	
	T3	1.58	1.02	2.44	2.00	0.90	4.44	
Prediabetes to diabetes	T1	ref			ref			<0.05
	T2	0.96	0.71	1.29	0.96	0.59	1.58	
	T3	1.41	1.02	1.94	1.88	1.12	3.14	
HOMA-IR	T1	ref			ref			0.16
	T2	-0.089	-0.223	0.045	-0.024	-0.156	0.108	
	T3	0.007	-0.127	0.141	0.072	-0.060	0.205	
HOMA-B	T1	ref			ref			0.79
	T2	-0.026	-0.176	0.124	0.009	-0.144	0.161	
	T3	0.024	-0.083	0.132	0.059	-0.053	0.171	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1

APPENDIX A (CONTINUED)

TABLE CVI. INTERACTION EFFECTS BETWEEN INSULIN-RESISTANCE PRS AND WET-WEIGHT TNONA ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	Insulin resistance PRS	TNONA < 75th Percentile[a]			TNONA > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.44
	T2	1.06	0.77	1.47	0.84	0.48	1.47	
	T3	0.93	0.69	1.24	1.13	0.68	1.86	
Normoglycemic to hyperglycemic	T1	ref			ref			0.18
	T2	0.92	0.63	1.32	0.68	0.35	1.33	
	T3	1.38	0.95	1.98	1.99	1.03	3.87	
Normoglycemic to prediabetes	T1	ref			ref			0.09
	T2	0.92	0.63	1.34	0.69	0.35	1.36	
	T3	1.42	0.98	2.07	2.20	1.12	4.32	
Prediabetes to diabetes	T1	ref			ref			0.11
	T2	1.10	0.79	1.53	0.88	0.50	1.55	
	T3	0.96	0.71	1.30	1.30	0.79	2.15	
HOMA-IR	T1	ref			ref			0.38
	T2	0.049	-0.071	0.168	0.033	-0.106	0.171	
	T3	0.005	-0.128	0.137	-0.011	-0.161	0.138	
HOMA-B	T1	ref			ref			0.29
	T2	0.053	-0.075	0.181	0.042	-0.090	0.173	
	T3	0.012	-0.090	0.114	0.000	-0.107	0.108	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

TABLE CVII. INTERACTION EFFECTS BETWEEN B-CELL PRS AND WET-WEIGHT TNONA ON HYPERGLYCEMIC OUTCOMES AT V2

Outcomes at V2	β-cell dysfunction PRS	TNONA < 75th Percentile[a]			TNONA > 75th Percentile			P-interaction
		OR / β	95% CI (L)	95% CI (U)	OR / β	95% CI (L)	95% CI (U)	
Normoglycemic or prediabetes to T2D	T1	ref			ref			0.18
	T2	1.07	0.76	1.50	1.30	0.74	2.28	
	T3	1.10	0.82	1.47	1.17	0.74	1.86	
Normoglycemic to hyperglycemic	T1	ref			ref			0.25
	T2	1.08	0.75	1.55	1.17	0.61	2.25	
	T3	1.28	0.83	1.97	1.00	0.45	2.22	
Normoglycemic to prediabetes	T1	ref			ref			0.28
	T2	1.02	0.71	1.48	1.11	0.57	2.16	
	T3	1.31	0.84	2.04	1.03	0.46	2.32	
Prediabetes to diabetes	T1	ref			ref			0.17
	T2	0.92	0.66	1.30	1.14	0.64	2.01	
	T3	1.16	0.85	1.58	1.26	0.77	2.05	
HOMA-IR	T1	ref			ref			0.06
	T2	-0.086	-0.221	0.048	-0.067	-0.201	0.066	
	T3	-0.004	-0.138	0.130	0.015	-0.118	0.148	
HOMA-B	T1	ref			ref			0.97
	T2	0.034	-0.124	0.191	0.032	-0.123	0.187	
	T3	-0.024	-0.130	0.082	-0.026	-0.124	0.073	

a: adjusted for age, sex, first 5 PCs for Hispanic background, study center, education, alcohol consumption, smoking status, physical activity, family history of diabetes, and BMI. Model with T2D was adjusted for baseline status of diabetes, and models with HOMA measurements at V2 were adjusted for each HOMA measurement at V1.

APPENDIX A (CONTINUED)

TABLE CVIII. RANGES OF EACH METAL AND METALLOID (LOG-TRANSFORMED) FROM NHANES 2007-2012 DATASET

Metals	IQR	Median	Minimum	Maximum
Arsenic	1.035	1.730	-4.605	6.185
Antimony	0.790	-2.908	-4.802	1.256
Barium	1.224	0.226	-3.919	4.826
Cadmium	1.020	-1.109	-2.207	2.175
Cesium	0.660	1.421	-0.547	4.714
Cobalt	0.791	-1.166	-3.712	3.219
Lead	0.899	0.262	-1.715	3.517
Mercury	1.299	-0.117	-2.207	3.928
Molybdenum	0.798	3.709	0.016	6.367
Thallium	0.686	-1.932	-4.070	0.582
Tungsten	1.059	-2.628	-5.199	2.279
Uranium	1.096	-5.065	-7.409	0.306

APPENDIX B

Approval Notice

Continuing Review

July 11, 2019

Victoria W. Persky, MD, MPH

Epidemiology and Biostatistics

Phone: (312) 996-4783 / Fax: (312) 996-0064

RE: **Protocol # 2015-0908**
Persistent Organic Pollutants, Endogenous Hormones and Diabetes in Latinos

Dear Dr. Persky:

Your application was reviewed and approved by the Expedited review process on July 11, 2019. You may now continue your research. You may now continue your research.

Please note the following information about your approved research protocol:

Please note that as per the revised Federal Regulations (2018 Common Rule) and OPRS policies your research no longer requires a Continuing Review; therefore, the approved documents are stamped only with an approval date. Although your research no longer requires a Continuing Review, you will receive annual reminder notices regarding your investigator responsibilities (i.e., submission of amendments, final reports, and prompt reports), and will be asked to complete an Institutional Status Report which will be sent to you via email every 3 years. If you fail to submit an Institutional Status Report, your research study will be administratively closed by the IRB. For more information regarding Continuing Review and Administrative Closure of Research visit: http://research.uic.edu/node/735.

Protocol Approval Date: July 11, 2019 - July 10, 2020

Approved Subject Enrollment #: 2350

Performance Sites: UIC, University of North Carolina-Chapel Hill, University of Minnesota, Albert Einstein College of Medicine, NY, University of Miami, San Diego State University , School of Public Health, Centers for Disease Control and Prevention

APPENDIX B (CONTINUED)

Sponsor: NIEHS

Institutional Proposal (IP) #: 00011824
Grant/Contract No: R01 ES025159-01A1
Grant/Contract Title: Persistent Organic Pollutants, Endogenous Hormones and Diabetes in Latinos

Research Protocol(s):

a) Persistent Organic Pollutants, Endogenous Hormones and Diabetes in Latinos, Version 8, 5-16-19.

Documents that require an approval stamp or separate signature can be accessed via OPRS Live. The documents will be located in the specific protocol workspace. You must access and use only the approved documents to recruit and enroll subjects into this research project.

Informed Consent(s):

a) All subjects have consented for use of specimens and data in parent HCHS/SOL study
b) Female - Notification Letter 1 of Abnormal Hormone Report (Spanish), Version 1, 06/06/2019
c) Female - Notification Letter 1 of Abnormal Hormone Report (English), Version 1, 06/06/2019
d) Female - Notification Letter 2 of Abnormal Hormone Report (English), Version 1, 06/06/2019
e) Notification Letter of Abnormal Hormone Report (Spanish), Version 3, 04/19/2019
f) Male - Notification Letter 1 of Abnormal Hormone Report (English), Version 1, 06/06/2019
g) Male - Notification Letter 1 of Abnormal Hormone Report (Spanish), Version 1, 06/06/2019
h) Notification Letter of Abnormal Hormone Report (English), Version 3, 04/19/2019
i) Female - Notification Letter 2 of Abnormal Hormone Report (Spanish), Version 1, 06/06/2019

Additional Determinations for Research Involving Minors:

These determinations have not been made for this study since it has not been approved for enrollment of minors.

Your research continues to meet the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific categories:

Protocol reviewed under expedited review procedures [45 CFR 46.110 and/or 21 CFR 56.110] Category: 5

APPENDIX B (CONTINUED)

Please remember to:

→ Use your **research protocol number** (2015-0908) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with the policies of the UIC Human Subjects Protection Program (HSPP) and the guidance ***Investigator Responsibilities.***

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further conditions, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the OPRS office at (312) 996-1711 or me at (312) 996-0548. Please send any correspondence about this protocol to OPRS via OPRS Live.

Sincerely,

Brandi L. Drumgole, B.S.
Assistant Director, IRB # 3
Office for the Protection of Research Subjects

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923
OVCR Administration, M/C 672

APPENDIX B (CONTINUED)

Approval Notice

Amendment – Expedited Review

UIC Amendment # 14

January 7, 2020

Victoria W. Persky, MD, MPH

Epidemiology and Biostatistics

Phone: (312) 996-4783 / Fax: (312) 996-0064

RE: **Protocol # 2015-0908**
"Persistent Organic Pollutants, Endogenous Hormones and Diabetes in Latinos"

Please note, the following personnel are required to have the **CITI Information Privacy and Security (IPS) – Basic Course training completed** prior to any forthcoming amendments/continuing review(s): **Kyeezu Kim, Robert Sargis, Noel Chavez, Terry Unterman** and **Kelly O'Shea** (lapsed training).

For further information, please visit the OPRS website: https://research.uic.edu/human-subjects-irbs/education-training/.

Dear Dr. Persky:

Your application was reviewed and approved on January 6, 2020. The amendment to your research may now be implemented.

Please note the following information about your approved amendment:

Amendment Approval Date: January 6, 2020

Amendment:

Summary: UIC Amendment #14, dated 01/02/2020 and accepted via OPRS Live on 01/02/2020, includes the following:

(1) Christine Kotek and Chibuzor Abasilim to the study as key research personnel (data analysis).

Please be sure to:

APPENDIX B (CONTINUED)

→ Use your research protocol number (2015-0908) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with the policies of the UIC Human Subjects Protection Program (HSPP) and the guidance ***Investigator Responsibilities***.

Please note that the IRB has the right to ask further questions, seek additional information, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the OPRS at (312) 996-1711 or me at (312) 355-3949. Please send any correspondence about this protocol to OPRS via OPRS Live.

Sincerely,

Eddie Mendoza
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923

APPENDIX B (CONTINUED)

Notice of Determination

Activity Does Not Represent Human Subjects Research

March 14, 2020

20200282-131022-1

Mary Ellen Turyk, PhD

Epidemiology and Biostatistics

Phone: (312) 355-4673 / Fax: (312) 996-0064

RE: **Protocol # 2020-0282**
"Associations between metal mixtures and thyroid hormone profiles – results from NHANES 2007-2012 study"

Sponsor: None

Dear Dr. Turyk:

The UIC Office for the Protection of Research Subjects received your Determination application and has determined that this activity **DOES NOT meet the definition of human subject research** as defined by 45 CFR 46.102(e)/ 21 CFR 50.3(g) and 21 CFR 56.102(e).

Specifically, this research will involve a secondary analysis of de-identified data obtained from the National Health and Nutrition Examination Survey (NHANES) 2007-2012.

You may conduct your activity without further submission to the IRB.

Please note:

- If this activity is used in conjunction with any other research involving human subjects, prospective IRB approval or a Claim of Exemption is required.
- If this activity is altered in such a manner that may result in the activity representing human subject research, a NEW Determination application must be submitted.

cc: Ronald C. Hershow, Epidemiology and Biostatistics

VITA

NAME: Kyeezu Kim

EDUCATION

Ph.D., School of Public Health, University of Illinois – Chicago	2020
M.P.H., Graduate School of Public Health, Seoul National University, (Seoul, Korea)	2010
B.A., Seoul National University, (Seoul, Korea)	2007

WORK EXPERIENCE

Northwestern University, Feinberg School of Medicine / Research Intern

Jun. 2019 – Aug. 2019

University of Illinois – Chicago, School of Public Health / Research Assistant

Jan. 2018 – Jul. 2020

Institute for Health Research and Policy, University of Illinois at Chicago / Research Assistant

Mar. 2017 – Dec. 2018

University of Illinois Hospital & Health Sciences System / Research Assistant

Sep. 2016 – Feb. 2017

University of Colorado, Denver – Anschutz Medical Campus / Research Intern

Aug. 2014 – May. 2016

Seoul National University, College of Medicine / Research Associate

Aug. 2014 – May. 2016

National Cancer Center (Gyeonggi-do, Korea) / Research Associate

Feb. 2011 – Dec. 2013

PEER-REVIEW PAPER PUBLICATIONS

Pre-diagnostic Carbohydrate Intake and Treatment Failure after Radical Prostatectomy for Early-Stage Prostate Cancer (2019): Kyeezu Kim, Angela Kong, Robert C Flanigan, Marcus L Quek, Courtney MP Hollowell, Patricia P Vidal, Jefferey Branch, Leslie A Dean, Virgilia Macias, Andre A Kajadacsy-Balla, Marian L Fitzgibbon, Daisy Cintron, Li Liu, Vincent L Freeman. *Cancer Causes & Control*, Volume 30, Issue 3, 271-279

Colorectal Cancer Susceptibility Loci and Influence on Survival (2018): Nan Song, Kyeezu Kim, Aesun Shin, Ji Won Park, Hee Jin Chang, Jiajun Shi, Qiuyin Cai, Dae Yong Kim, Wei Zheng, Jae Hwan Oh. *Genes, Chromosomes, and Cancer,* Volume 57, Issue 12, 630-637

Association between CASR Polymorphisms, Calcium Intake, and Colorectal Cancer Risk. (2013): Kyee-Zu Kim, Aesun Shin, Jeongseon Kim, Ji Won Park, Sung Chan Park, Hyo Seong Choi, and Jae Hwan Oh. *PloS One* Volume 8, Issue 3, e59628

Polymorphisms in Adiposity-Related Genes are Associated with Age at Menarche and Menopause in Breast Cancer Patients and Healthy Women. (2012): Kyee-Zu Kim, Aesun Shin, Yeon-Su Lee, Sook-Young Kim, Yeonju Kim, and Eun-Sook Lee. *Human Reproduction*, Volume 27, Issue 7, pp 2193-2200

The Beneficial Effect of Leisure-Time Physical Activity on Bone Mineral Density in Pre-and Postmenopausal Women. (2012): Kyee-Zu Kim, Aesun Shin, Jeonghee Lee, Seung-Kwon Myung, and Jeongseon Kim. *Calcified Tissue International,* Volume 91, Issue 3, pp 178-185

Directed Causal Network Construction Using Linkage Analysis with Metabolic Syndrome-Related Expression Quantitative Traits. (2012): Kyee-Zu Kim, Jin-Young Min, Geun-Yong Kwon, Joohon Sung, and Sung-il Cho. *Genomics and Informatics*, Volume 9, Issue 4, pp 143-151

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TEACHING EXPERIENCE

Epidemiologic Computing / Fall 2016 – Teaching Assistant (Instructor: Dr. Garth Rauscher)

Analytic Research Methods (Cohort B) / Fall 2017 – Teaching Assistant (Instructor: Drs. Saria Awadalla and Sara Baghikar)

Analytic Research Methods (Part 2) / Spring 2018 – Teaching Assistant (Instructor: Drs. Jerrilyn Cambron and Brittany Lapin)