## Title

Identification of Systemic Biomarkers in Serum, Urine, and Deoxyribonucleic Acid as Indictors for Environmental Exposures and the Development of Metabolic Outcomes Among Women in the United States



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# Identification of Systemic Biomarkers in Serum, Urine, and Deoxyribonucleic Acid as Indictors for Environmental Exposures and the Development of Metabolic Outcomes Among Women in the United States

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy in Epidemiology

by

Yan Song

2014

## ABSTRACT OF THE DISSERTATION

Identification of Systemic Biomarkers in Serum, Urine, and Deoxyribonucleic Acid as Indictors for Environmental Exposures and the Development of Metabolic Outcomes Among Women in the United States

by

Yan Song

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2014

Professor Simin Liu, Co-Chair

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Type 2 diabetes (T2D) is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and β-cell dysfunction. Excessive body weight is associated with an increased risk of T2D and other major chronic diseases including cardiovascular disease and certain cancers. The current work focuses on the identification of systemic biomarkers in various biological samples, including serum, urine, and DNA, as indicators for environmental risk factors and the development of T2D and obesity in different human populations. Environmental endocrine-disrupting chemicals (EDCs) have been shown to affect the

biosynthesis, secretion, transport, binding action, and metabolism of endogenous sex hormones, and were suggested to be associated with increased risk of developing T2D and obesity. In Chapter 1, we conducted a meta-analysis study to comprehensively assess the role of EDCs in affecting risk of T2D and related metabolic traits. We found that both persistent (including dioxin, polychlorinated biphenyls, and chlorinated pesticides) and non-persistent EDCs (including bisphenol A and phthalates) may affect the risk of developing T2D. To evaluate urinary concentrations of BPA and major phthalate metabolites in relation to prospective weight change, we conducted a prospective analysis in the Nurses' Health Study (NHS) and NHSII in Chapter 2. Our data suggest urinary concentrations of BPA and certain individual phthalate metabolites were associated with modestly greater weight gain in a dose-response fashion. These data are consistent with a potential role of BPA and phthalates in weight gain, although more prospective data are needed to corroborate these observations.

In previous studies, T2D was associated with biomarkers on different molecular pathways. Earlier prospective studies have shown that low birth weight (LBW), an indicator of intrauterine growth restriction, was predictive of higher risk of T2D in adulthood, but the pathways that lead LBW individuals to the onset of T2D are still unclear. In Chapter 3, we assessed the effect of LBW on T2D risk that is explained by potential mediators on different biological pathways using mediation modeling in a case-control study of T2D in the Women's Health Initiative (WHI). We found that the effect of LBW on T2D risk seems mainly mediated by insulin resistance, which is further explained by circulating levels of sex hormone-binding globulin (SHBG), E-selectin, and systolic blood pressure. These prospective data provide quantifiable mechanistic evidence linking LBW to increased risk of T2D whilst presenting risk stratification and intervention in a population at greater risk of developing T2D later in life.

The process of biological aging involves in the pathogenesis process of metabolic disorders including T2D and obesity. It is also necessary to examine the potential predictors and determinants of biological aging. Despite the consistent observations that women outlive men, the mechanisms underlying the relation between sex and longevity have not been fully elucidated. In Chapter 4, we examined the associations of circulating estradiol and testosterone with leukocyte TL in WHI. We found that serum concentration of estradiol was not associated with leukocyte TL in this large sample of postmenopausal women. Total and free testosterone levels were inversely associated with TL in Asian/Pacific Islander women but not in black and Hispanic women. Future studies to replicate our observations are warranted to address potential ethnicity-specific relations.

The dissertation of Yan Song is approved.

Shehnaz K. Hussain

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2014

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## LIST OF ACRONYMS

| | |
|---|---|
| AHEI | Alternate healthy eating index |
| AR | Androgen receptor |
| BMI | Body mass index |
| bp | Base pair |
| BPA | Bisphenol A |
| CI | Confidence interval |
| CV | Coefficient of variation |
| CVD | Cardiovascular disease |
| DDE | Dichlorodiphenyldichloroethylene |
| DDT | Dichlorodiphenyltrichloroethane |
| DEHP | Di-2-ethylhexyl phthalate |
| DNA | Deoxyribonucleic acid |
| EDC | Endocrine disrupting chemicals |
| EPA | Environmental Protection Agency |
| FDR | False discovery rate |
| GLST | Generalized least-squares trend estimation |
| HCB | Hexachlorobenzene |
| HOMA | Homeostatic model assessment |
| HRT | Hormone replacement therapy |
| hsCRP | High-sensitivity C-reactive protein |
| ICAM-1 | Intercellular adhesion molecule 1 |
| ICC | Intraclass coefficient |
| IL-6 | Interleukin 6 |
| LBW | Low birth weight |
| MBP | Monobutyl phthalate |
| MBzP | Monobenzyl phthalate |
| MECPP | Mono(2-ethyl-5-carboxypentyl) phthalate |
| MEHHP | Mono-(2-ethyl-5-hydroxyhexyl) phthalate |
| MEHP | Mono-(2-ethylhexyl) phthalate |
| MEOHP | Mono-(2-ethyl-5-oxohexyl) |
| MEP | Monoethyl phthalate |
| MET | Metabolic equivalent of task |
| MiBP | Mono-isobutyl phthalate |
| NHANES | National Health and Nutrition Examination Survey |
| NHS | Nurses' Health Study |
| NHSII | Nurses' Health Study II |
| OR | Odds ratio |
| PA | Phthalic acid |
| PCB | Polychlorinated biphenyl |
| PCDD | Polychlorinated dibenzo-*p*-dioxin |
| PPARγ | Peroxisome proliferator-activated receptor γ |
| RR | Relative risk |
| SD | Standard deviation |
| SE | Standard error |
| SHBG | Sex hormone-binding globulin |
| T2D | Type 2 diabetes |
| TCDD | Tetrachlorobenzo-*p*-dioxin |
| TG | Triacylglycerol |
| TL | Telomere length |
| TNF-α | Tumor necrosis factor α |
| VCAM-1 | Vascular cell adhesion molecule 1 |
| WHI | Women's Health Initiative |
| WHI-CT | Women's Health Initiative-Clinical Trial |
| WHI-OS | Women's Health Initiative-Observational Study |
| WHR | Waist to hip ratio |
| WMD | Weighted mean difference |

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## PUBLICATIONS

1) **Yan Song**, Nai-Chieh Y. You, Yiqing Song, Mo K Kang, Lifang Hou, Robert Wallace, Charles B Eaton, Lesley F Tinker, and Simin Liu. Intake of Small-to-Medium-Chain Saturated Fatty Acid Is Associated with Peripheral Leukocyte Telomere Length in Postmenopausal Women. Journal of Nutrition. 2013, 143(6) (PMID: 23616516)
2) **Yan Song**, Jorge E. Chavarro, Yin Cao, Weiliang Qiu, Lorelei Mucci, Howard D Sesso, Meir J Stampfer, Edward Giovannucci, Michael Pollak, Simin Liu, and Jing Ma. Whole Milk Intake Is Associated with Prostate Cancer-Specific Mortality among U.S. Male Physicians. Journal of Nutrition. 2013, 143(2) (PMID: 23256145)
3) Liu He, Xun Tang, **Yan Song**, Na Li, Jingrong Li, Zongxin Zhang, Jianjiang Liu, Liping Yu, Haitao Xu, Jianguo Zhang, and Yonghua Hu. Prevalence of Cardiovascular Disease

and Risk Factors in a Rural District of Beijing, China: A Population-Based Survey of 58,308 Residents. BMC Public Health. 2012, 12:34. (PMID: 22248490)

4) Yin Cao, Stacey Kenfield, **Yan Song**, Bernard Rosner, Weiliang Qiu, Howard D Sesso, J Michael Gaziano, and Jing Ma. Cigarette Smoking Cessation, Total and Cause-specific Mortality: A 22-Year Follow-up Study in US Male Physicians. Archives of Internal Medicine. 2011, 171(21). (PMID: 22123811)

5) **Yan Song**, Na Li, Liu He, Xun Tang, Dafang Chen, and Yonghua Hu. A Common Polymorphism of Upstream Transcription Factor 1 Gene Is Associated with Lipid Profile: A Study in Type 2 Diabetes Families. International Journal of Biomedical Sciences. 2009, 5(3). (PMID: 23675152)

6) Feng Wang, Shoulin Wu, **Yan Song**, Xun Tang, Roger Marshall, Mingbin Liang, Yiqun Wu, Xueying Qin, Dafang Chen, and Yonghua Hu. Waist Circumference, Body Mass Index and Waist to Hip Ratio for Prediction of Metabolic Syndrome in Chinese. Nutrition, Metabolism & Cardiovascular Diseases. 2009, 19(8). (PMID: 19188050)

**PRESENTATIONS**

1) Intake of Small-to-Medium-Chain Saturated Fatty Acid Is Associated with Peripheral Leukocyte Telomere Length in Postmenopausal Women, Society of Epidemiologic Research (SER), Boston, MA, June 20, 2013 (Poster)

2) Association Between Sex Hormones and Leukocyte Telomere Length in Postmenopausal Women, Genomic Analysis Training Program and BWF-IT-MD Symposium, UCLA, Los Angeles, CA, May 23, 2013 (Poster)

3) Association Between Sex Hormones and Leukocyte Telomere Length in Postmenopausal Women, Burroughs Wellcome Fund Programs Unifying Population and Laboratory Based Sciences (PUP) Student Symposium, Emory University, Atlanta, GA, May 15, 2013 (Poster)

4) Lifestyle factors and telomere length in postmenopausal women, 2012 American Heart Association (AHA) Scientific Sessions, Los Angeles, CA, November 6, 2012 (Oral)

5) A systemic review and meta-analysis of hyperinsulinemia in relation to colorectal adenomas and cancer, 2012 World Cancer Congress (WCC), Montréal, Canada, August 29, 2012 (Oral)

6) Intake of dairy products in relation to risk and survival of prostate cancer: a 28-year follow-up study, 2012 World Cancer Congress (WCC), Montréal, Canada, August 28, 2012 (Oral)

7) Unhealthy lifestyle and telomere length in postmenopausal women, Genomic Analysis Training Program and BWF-IT-MD Symposium, UCLA, Los Angeles, CA, May 21, 2012 (Oral)

8) Interrelationship Between Birth Weight, Sex Hormone Biomarkers, and Type 2 Diabetes in Postmenopausal Women, Genomic Analysis Training Program and BWF-IT-MD Symposium, UCLA, Los Angeles, CA, May 31, 2011 (Poster)

## BACKGROUND

In an aging population, the characteristics of metabolism change, and the risks of developing various metabolic disorders also shift[1]. Type 2 diabetes (T2D) is characterized by high blood glucose in the context of insulin resistance and β-cell dysfunction, and is a major cause of heart disease, stroke, kidney failure, nontraumatic lower-limb amputation, and blindness among adults in the US[2]. Excessive body weight is associated with an increased risk of T2D and other major chronic diseases including cardiovascular disease and certain cancers[3]. A recent policy statement from the American Medical Association has officially recognized obesity as a separate disease, not simply a risk factor of other chronic diseases. The epidemic of obesity-diabetes ("diabesity") has become a major challenge in public health, and intensive scientific research on the etiology and predictor of diabesity is warranted[4]. The current work focuses on the identification of systemic biomarkers in various biological samples, including serum, urine, and DNA, as indicators for environmental risk factors and the development of T2D and obesity in different human populations.

Previous work has shown that endogenous sex hormones play critical roles in the development of T2D[5-8]. Endocrine disrupting chemicals (EDCs) are highly heterogeneous and include many man-made industrial solvents and certain byproducts from their production process, which can be broadly categorized into persistent EDCs [e.g. dioxins and polychlorinated biphenyls (PCBs)] and non-persistent EDCs [e.g. bisphenol A (BPA) and phthalates]. Most of the persistent EDCs have been banned decades ago, but they still can be detected in human biological samples due to their long biologic half-lives. In contrast, although non-persistent EDCs such as BPA and phthalates are rapidly degraded in the human body, their exposure is prevalent and continuous due to their widespread use in everyday products, leading to ubiquitous

detection of these chemicals in human blood and urine[9]. Recent work has identified diverse physiological effects attributable to even low doses of exposure to these chemicals, and human studies began to link different EDCs to glucose and insulin metabolisms and the pathogenesis of T2D and obesity[10,11]. Although previous studies have examined the association between EDC exposures and risks of T2D and obesity, the results and conclusions are inconsistent, especially for the non-persistent EDCs. In Chapter 1, we conducted a systematic review and meta-analysis of all available cross-sectional and prospective studies relating different EDCs with risk of T2D and related metabolic traits, expanding the spectrum of EDCs from the most classic EDCs, dioxins, PCBs, and chlorinated pesticides, to the recently accepted non-persistent EDCs, including BPA and phthalates. In Chapter 2, we evaluated urinary concentrations of BPA and major phthalate metabolites in relation to prospective weight change in two cohorts of female nurses in the US.

In previous studies, T2D was associated with biomarkers on different molecular pathways, including insulin resistance[12-14], leptin, cellular aging[15], inflammation[16], endothelial dysfunction[17,18], blood pressure[19], and sex hormones and their binding proteins[20-23]. Data from such studies of biomarkers can be utilized to investigate potential biological pathways of previously established risk factors of T2D whose mechanisms are still not clear. Earlier prospective studies have shown that low birth weight (LBW), an indicator of intrauterine growth restriction, was predictive of higher risk of T2D in adulthood[24]. Impairments in "fetal programming", as reflected by LBW, promote adverse effects on physiology, metabolism, and hormonal function during critical phases of fetal development[25]. However, the pathways that lead LBW individuals to the onset of T2D are still unclear. An improved understanding of the mechanisms through which LBW may influence T2D risk may improve clinical risk

stratification and intervention of the disease. In Chapter 3, we assessed the effect of LBW on T2D risk that is explained by potential mediators on different biological pathways using mediation modeling.

The process of biological aging involves in the pathogenesis process of metabolic disorders including T2D and obesity. It is also necessary to examine the potential predictors and determinants of biological aging. Telomeres are highly conserved regions of repetitive nucleotide sequences (TTAGGG) that protect the ends of chromosomes. Telomere length (TL) decreases over time due to cell division, and may serve as a biomarker for the biological aging process and some age-related complex diseases[26]. Despite the consistent observations that women outlive men, the mechanisms underlying the relation between sex and longevity have not been fully elucidated. Although many theories have been proposed to explain this sex divergence, including oxidative damage and chromosomal complement[27], the role of sex steroids remained untested, especially relating sex hormone concentrations to TL as a marker of biological aging. In Chapter 4, we examined the associations of circulating estradiol and testosterone with leukocyte TL in a multiethnic female population from a nationwide observational cohort in the US to further elucidate the potential roles of sex hormones in biological aging.

# CHAPTER 1. Endocrine-Disrupting Chemicals, Risk of Type 2 Diabetes, and Diabetes-Related Metabolic Traits: A Systematic Review and Meta-analysis

## 1.1 Abstract

**OBJECTIVE**: Elevated blood or urinary concentrations of endocrine-disrupting chemicals (EDCs) may be related to increased type 2 diabetes (T2D) risk. We conducted this study to comprehensively assess the role of EDCs in affecting risk of T2D and related metabolic traits.

**DESIGN**: Systematic review and meta-analysis.

**DATA SOURCES**: Searches of MEDLINE, EMBASE, and hand search for articles published before March 15, 2013.

**STUDY SELECTION CRITERIA**: Both cross-sectional and prospective studies of the association between EDCs [including dioxin, polychlorinated biphenyl (PCB), chlorinated pesticide, bisphenol A (BPA), phthalates] and T2D and related metabolic traits. Three investigators independently extracted information on study design, participant characteristics, EDC types and concentrations, and association measures.

**RESULTS**: We included 39 cross-sectional and seven prospective studies, comprising 51,687 participants from ethnically diverse populations. Serum concentrations of dioxins, PCBs, and chlorinated pesticides were significantly associated with T2D risk; comparing the highest to the lowest concentration category, the pooled relative risks were 1.91 (95% CI, 1.44 to 2.54) for dioxins, 2.39 (95% CI, 1.86 to 3.08) for total PCBs, and 2.30 (95% CI, 1.81 to 2.93) for chlorinated pesticides. Urinary concentrations of BPA and phthalates were significantly associated with T2D risk; comparing the highest to the lowest concentration categories, the

pooled relative risks were 1.49 (95% CI, 1.24 to 1.78) for BPA and 1.64 (95% CI, 1.03 to 2.59) for phthalates. Further, EDC concentrations were associated with indicators of impaired fasting glucose and insulin resistance.

**CONCLUSIONS**: EDCs (both persistent and non-persistent) may affect the risk of developing T2D. There is an urgent need for further investigation of EDCs, especially non-persistent ones, and T2D risk in large prospective studies.

## 1.2 Introduction

Type 2 diabetes (T2D) is a major cause of heart disease, stroke, kidney failure, nontraumatic lower-limb amputation, and new cases of blindness among adults in the United States[2]. Increasing evidence has shown that endogenous sex hormones and sex hormone-binding globulin (SHBG) play critical roles in the development of T2D[5-8]. Environmental endocrine-disrupting chemicals (EDCs) have been shown to affect the biosynthesis, secretion, transport, binding action, and metabolism of endogenous hormones including sex steroid hormones and their binding protein[28]. Although earlier research of EDC exposures mainly focused on the toxic effects on reproductive and developmental parameters[28], recent work has identified diverse physiological effects attributable to even low doses of exposure. In the 1990s, human studies began to link different EDCs to glucose and insulin metabolisms and the pathogenesis of T2D[10].

EDCs are highly heterogeneous and include numerous man-made industrial solvents and certain byproducts from their production process [e.g. polychlorinated biphenyls (PCBs) and dioxins (polychlorinated dibenzo-*p*-dioxins/dibenzofurans, PCDD/F)], plastic-associated compounds [e.g. bisphenol A (BPA) and phthalates], chlorinated pesticides [e.g. dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), oxychlordane, and mirex], and certain pharmaceutical agents (e.g. diethylstilbestrol). EDCs can be broadly classified into two main categoties: persistent EDCs (e.g. dioxins and PCBs) and non-persistent EDCs. Although dioxins and PCBs have been banned since the 1970s, they still can be detected in humans, wildlife, and marine animals to date because of their long biologic half-lives. In contrast, “non-persistent” EDCs such as BPA and phthalates are rapidly degraded in the human body. Despite their short biologic half-lives, BPA and phthalates exposure is prevalent and continuous due to their widespread use in everyday products, leading to consistent detection of

these EDCs in human blood and urine[9].

Although previous studies have examined the association between EDC expousures and T2D risks, the results and conclusions are inconsistent, especially for the non-persistent EDCs. A recent narrative review summarized human, animal, and mechmistic studies of the association between different types of environmental factors, including certein EDCs, and risk of diabetes[29]. Wu and colleagues reported significant associations of total PCBs, and one of the chlorinated pesticides, hexachlorobenzene (HCB), with risk of diabetes in a recent meta-analysis[30]. Nonetheless, studies that provide systematic and quantitative summary regarding the association between a broader range of EDCs and T2D risk are still lacking. To comprehensively assess the relations between EDCs and risk of T2D, we conducted a systematic review and meta-analysis of all available cross-sectional and prospective studies relating different EDCs with risk of T2D and related metabolic traits (including fasting blood glucose and insulin, postprandial blood glucose and insulin, and insulin resistance), expanding the spectrum of EDCs from the most classic EDCs, dioxins, PCBs, and chlorinated pesticides, to the recently accepted non-persistent EDCs, including BPA and phthalates.

## 1.3 Methods

### 1.3.1 Data sources and searches

This review followed a predefined protocol and was registered at http://www.crd.york.ac.uk/PROSPERO as CRD42013003930. We conducted a comprehensive search of MEDLINE and EMBASE for cross-sectional and prospective studies examining the association of EDCs in relation to T2D. We included the EDCs that have been listed in the Endocrine Society Scientific Statement[28] and have population studies available. Specifically, we

used the keywords endocrine disruptor, dioxin, polychlorinated biphenyl, polybrominated biphenyl, pesticide, bisphenol A, phthalate, diabetes, glucose, insulin, and insulin resistance (see Appendix for exact search strategy). We restricted the publishing time to before March 15, 2013. We did not make restrictions on language of the articles. We also did a hand search from the references of retrieved articles.

### 1.3.2 Study selection

Screening of retrieved articles was conducted in parallel by two investigators independently. In the first round of screening (n=6551), 6443 articles were excluded for at least one of the following reasons: nonhuman studies (1579 articles, including chemistry, animal, cell line, and isolated tissue studies), reviews /editorials /guidelines /letters /comments (93 articles), and studies with irrelevant exposures or outcomes (4771 articles) (Figure 1.1). In the second round of screening of the full texts retrieved (n=108), 62 articles were excluded for at least one of the following reasons: updated data available from other studies for the same population (e.g. multiples studies using the same NHANES population) or duplicate publications (21 articles), no variance data (four articles), no proper association measures for synthesis (17 articles), no serum/urine measurements of EDCs (nine articles), mortality studies (eight articles), and studies in children (three article). Our search strategy and inclusion/exclusion criteria resulted in a total of 46 articles being included in the current meta-analysis.

### 1.3.3 Data extraction and quality assessment

Using a standardized data extraction form, three independent investigators extracted and tabulated all data. Discrepancies were resolved via referencing the original article and via group discussions. Information extracted included lead author, journal, publication year, country or region, population, sex, average age, average BMI, study design, sample size, length of follow-

up (if prospective), diagnostic criteria of T2D, number of T2D cases, EDC concentrations, relative risks (RR) of T2D comparing various levels of EDCs, mean and standard deviation (SD, derived if needed) of diabetes-related metabolic traits comparing various levels of EDCs, ranges of highest and lowest concentration categories, and covariates adjusted (if any) in the statistical models.

### 1.3.4 Data synthesis and analysis

The primary summary measure of association was the RRs of T2D comparing the highest to the lowest categories of serum or urinary EDC concentrations. For the studies using pg/mL (or pg/g wet weight) as the unit for lipophilic EDCs, we approximated the lipid-standardized concentration (ng/g lipid) by dividing by a constant of 6.33 (which is based on an average of 6.33 mg of total lipid per 1 mL of blood as shown by Silverstone et al.[31]). For the studies that did not provide RRs for total dioxins, PCBs, chlorinated pesticides, and phthalates but reported RRs for individual PCDD/F or PCB congeners, pesticides, or phthalate metabolites, we used the average of ln(RR) and the average of standard errors (SE) of ln(RR) to calculate the combined RR estimates for each individual study, under the assumption that there is a common effect size among these individual components. The US Environmental Protection Agency (EPA) has categorized 12 PCB congeners as "dioxin-like" because their toxicity and structural features are similar to 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD)[32]. In our analysis for PCBs, we also examined the specific associations of these dioxin-like PCBs with T2D. For RRs reported from multiple regression models, we used estimates from the models with the most covariates adjusted. Reported RRs in each study were pooled using DerSimonian and Laird random-effects models[33]. *P* values for sex and race differences were calculated using two-sample *z* tests. For PCB153 and DDE, which are the most frequently studied individual PCB congener and chlorinated pesticide,

as well as BPA, we estimated dose-response association using a two-stage generalized least-squares trend estimation (GLST)[34] that calculates study-specific slopes first and then pools the slopes using a random-effects model. For sensitivity analyses, we excluded studies with a broad exposure range in the reference group and studies using another population as the reference group, because these studies may miss the potential effect at low-dose levels. For diabetes-related metabolic traits, studies reported the association between EDC concentrations and the continuous traits in different forms: means and SEs in different categories, mean difference between categories, RR after categorization of the continuous traits, correlation coefficients, and regression coefficients. We transformed all other indicators of association into mean difference between the highest and lowest EDC concentration categories[35]. Mean difference from each individual study was pooled via random-effects models[33] to obtain the overall weighted mean difference (WMD). To identify and quantify between-study heterogeneity, we calculated *P* values for Cochran's *Q*, the ratio of true heterogeneity to total variance $I^2$ (%), and variance of true effect size $T^2$. The $I^2$ estimates of 25, 50, and 75 indicated low, medium, and high heterogeneity, respectively. To assess the presence of publication bias, we used both the Egger's test and the funnel plots where ln(RR)s were plotted against their corresponding SEs. We also used cumulative meta-analysis with studies sorted in the sequence from most precise to least precise as well as the trim-and-fill method[36] to assess the impact of studies with less precision on the RR estimates and to estimate and adjust for the numbers and outcomes of missing studies. All analyses were conducted using STATA 12.1 (StataCorp, College Station, TX), $P<0.05$ was considered statistically significant ($P<0.10$ was considered statistically significant for tests for heterogeneity and Egger's test for publication bias).

## 1.4 Results

The descriptive characteristics of participants included in cross-sectional and prospective studies are presented in Table 1.1, and the biologic half-lives of some EDCs are listed in Table 1.2.

### 1.4.1 Dioxins

The body of literature concerning dioxins included 12,546 participants from 11 cross-sectional studies. Seven studies had diabetes as an outcome of interest and seven studies included diabetes-related metabolic traits (Figure 1.2). Medians of serum dioxin concentrations [as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent] ranged from 4.0 to 20.5 pg/g lipid. Serum concentrations of dioxins (including different PCDD/F congeners) were significantly associated with higher T2D risk. Participants in the highest dioxin concentration categories (TCDD >2.0 to >5.2 pg/g lipid) had a 91% higher risk of T2D (RR=1.91; 95% CI, 1.44 to 2.54) than those in the lowest categories (TCDD ≤1.0 to ≤2.8 pg/g lipid). No significant between-study heterogeneity was detected (*P* for Cochran's $Q$=0.77; $I^2$=0), nor was there any significant publication bias (Egger's *P*=0.12). For a sensitivity analysis, we excluded two studies with broad exposure range in reference group[37,38], and the RR did not change materially (RR=1.86; 95% CI, 1.35 to 2.57). In the analysis of metabolic traits (Table 1.4), dioxin concentrations were associated with higher fasting glucose (WMD=3.96 mg/dL; 95% CI, 1.23 to 6.70 mg/dL; comparing the highest concentration categories to the lowest) and higher 2-h post-challenge glucose (WMD=4.09 mg/dL; 95% CI, 0.78 to 7.40 mg/dL; comparing the highest concentration categories to the lowest). Association with 2-h post-challenge insulin was only available in one study; the mean difference was 229 μIU/mL (95% CI, 70 to 388 μIU/mL), comparing the highest concentration categories to the lowest.

### 1.4.2 Polychlorinated biphenyls (PCBs)

The body of PCB studies included 21,703 participants from 20 cross-sectional populations and 4681 from seven prospective cohorts (with follow-up periods of 5 to 25 years). There were 22 studies that had T2D as an outcome and six studied diabetes-related metabolic traits. Medians of serum PCB concentrations (as PCB153) ranged from 82.8 to 807.5 ng/g lipid. In both cross-sectional and prospective studies, serum PCB concentrations (including different PCB congeners) were significantly associated with higher risk of T2D. The pooled RR of T2D comparing highest (PCB153 >104 to >1,348 ng/g lipid) and lowest (PCB153 ≤60 to ≤455 ng/g lipid) categories of PCB concentration was 2.39 (95% CI, 1.86 to 3.08, Figure 1.3A). There were some significant between-study heterogeneities among studies of PCBs with T2D (*P* for Cochran's $Q$=0.018; $I^2$=43.4). Subgroup analyses were further conducted to assess the potential sources of these heterogeneities (Table 1.3), which indicated differences by sex and race (*P*=0.08 and 0.032). In particular, comparing the highest concentration categories to the lowest, the RR in women (2.65; 95% CI, 1.57 to 4.48) was significantly greater than that in men (1.73; 95% CI, 0.80 to 3.75). The effect size in whites (RR=1.94; 95% CI, 1.43 to 2.62) was also significantly lower than that in non-whites (RR=2.91; 95% CI, 1.60 to 5.30), comparing the highest to the lowest concentration categories. The effect size of cross-sectional studies was significantly higher than the effect size from prospective studies (*P*<0.001). Association in prospective studies with <10 y follow-up did not differ significantly from association in those with longer follow-up time. Dose-response analysis of PCB153 showed an RR of 1.18 per 100 ng/g lipid increase (95% CI, 1.07 to 1.30). Although no publication bias for the studies of PCBs was detected (*P* from Egger's test = 0.51), for a sensitivity analysis, we used the trim-and-fill method and calculated a corrected RR of 2.33 (95% CI, 1.81 to 3.00), comparing the highest to the lowest concentration

categories (Figure 1.4). The cumulative forest plot also indicated that the estimates remained stable when several small studies with less precision were excluded (Figure 1.5). For another sensitivity analysis, we excluded three studies with broad exposure range[38-40] and one study using different population as reference group[41], and the RR did not change materially (RR=2.30; 95% CI, 1.75 to 3.02). For metabolic traits (Table 1.4), PCB concentrations were associated with higher fasting glucose (WMD=3.27 mg/dL; 95% CI, 1.87 to 4.67 mg/dL; comparing the highest concentration categories to the lowest).

Besides the studies of PCBs, we also have identified three studies of PBBs – a group of structurally similar chemicals, which were also categorized as EDCs[42-44]. Two studies reported RR in both sexes combined (RR=1.9; 95% CI, 0.9 to 4.0[43] and RR=1.8; 95% CI, 0.6 to 5.8[42]). Another study[44] reported association of PBBs in opposite direction for men and women (RR=0.50; 95% CI, 0.23 to 1.12 for men; RR=1.52; 95% CI, 0.74 to 1.12 for women).

### 1.4.3 Chlorinated pesticides

The body of chlorinated pesticides studies included 13,998 participants from 16 cross-sectional populations and 2549 from five prospective cohorts (with follow-up periods of 5 to 18 years). There were 17 studies that had T2D as an outcome and four studied diabetes-related metabolic traits. Medians of serum chlorinated pesticide concentrations (as DDE) ranged from 112.8 to 1817 ng/g lipid. In both cross-sectional and prospective studies, serum chlorinated pesticides (including different types of pesticides) concentrations were significantly associated with higher risk of T2D. The pooled RR of T2D comparing highest (DDE >545 to >9258 ng/g lipid) and lowest (DDE ≤112 to ≤3602 ng/g lipid) categories of chlorinated pesticides concentration for all cross-sectional and prospective studies was 2.30 (95% CI, 1.81 to 2.93). The majority of the studies studied chlorinated pesticides individually. All individual pesticides

were associated with higher risk of T2D (pooled RR ranging from 1.55 to 2.88, data not shown). Dose-response analysis of DDE showed an RR of 1.18 per 1000 ng/g lipid increase (95% CI, 1.08 to 1.28). The between-study heterogeneity was not statistically significant (*P* for Cochran's *Q*=0.34; $I^2$=9.5). When all of the aforementioned data were stratified by sex (Table 1.3), concentrations of chlorinated pesticides were significantly associated with T2D in women (RR=2.54; 95% CI, 1.25 to 5.14; comparing the highest concentration categories to the lowest). In contrast, there were not enough data available to support similar association in men (RR=7.32; 95% CI, 0.92 to 58.41; comparing the highest concentration categories to the lowest). Similar to that in the analysis for PCBs, the association was stronger in non-whites (RR=2.64; 95% CI, 1.56 to 4.49) than in whites (RR=1.95; 95% CI, 1.40 to 2.71), comparing the highest concentration categories to the lowest. Association in prospective studies with <10 y follow-up did not differ significantly from association in those with longer follow-up time. Moderate publication bias was detected (*P* from Egger's test=0.06). Using trim-and-fill method (Figure 1.7), we obtained a corrected RR of 2.00 (95% CI, 1.51 to 2.66) comparing the highest concentration categories to the lowest. The estimates did not change materially after excluding studies with small sample sizes (Figure 1.8). For a sensitivity analysis, we excluded two studies with broad exposure range[39,40], and the RR did not change materially (RR=2.22; 95% CI, 1.72 to 2.85). For metabolic traits (Table 1.4), higher concentrations of chlorinated pesticides were associated with higher fasting glucose, 2-h post-challenge glucose, fasting insulin, 2-h post-challenge insulin, and HOMA-IR, albeit not statistically significant.

### 1.4.4 Non-persistent EDCs

The body of BPA studies included 14,550 participants from seven cross-sectional populations. Four studies had T2D as an outcome and five studied diabetes-related metabolic

traits. Median urinary concentration of BPA ranged from 0.81 to 9.40 ng/mL. The pooled RR (Figure 1.9) of T2D comparing the highest BPA concentrations (>1.43 to >4.20 ng/mL) to the lowest (≤0.47 to ≤1.36 ng/mL) was 1.49 (95% CI, 1.24 to 1.78). Dose-response analysis of BPA showed an RR of 1.11 per 1 ng/mL increase (95% CI, 1.05 to 1.16). No significant publication bias was detected. Higher BPA concentrations were also significantly associated with higher HOMA-IR (WMD=0.80; 95% CI, 0.36 to 1.25), and non-significantly associated with higher fasting glucose (WMD=1.19 mg/dL; 95% CI, –0.28 to 2.66 mg/dL).

The body of phthalate studies included 5825 participants from four cross-sectional studies. Two studies had T2D as an outcome and four studied diabetes-related traits. Medians of urinary phthalate concentrations (as MEP) were around 11.6 ng/mL. The pooled RR (Figure 1.10) of T2D comparing the highest (MEP >17.5 ng/mL) and lowest (MEP≤7.2 ng/mL) total urinary phthalate metabolites concentrations was 1.64 (95% CI, 1.03 to 2.59). No significant publication bias was detected. Among all phthalate metabolites, MEP and monoisobutyl phthalate (MiBP) have been included in more than one study with RR comparing the highest concentration categories to the lowest. Higher concentrations of both MEP and MiBP (which have been included in multiple studies) were associated with higher risk of T2D (MEP: RR=1.39; 95% CI, 0.55 to 3.48; MiBP: RR=1.90; 95% CI, 1.17 to 3.09; comparing the highest concentration categories to the lowest). The relation between phthalate concentrations and fasting glucose was reported by only one study, while three studies investigated the association between phthalate concentrations and HOMA-IR. Comparing the highest concentration categories to the lowest, the mean difference of all metabolites for fasting glucose was 0.78 mg/dL (95% CI, –0.40 to 1.96 mg/dL), and the pooled WMD of all metabolites for HOMA-IR was 0.42 (95% CI, –0.16 to 0.99).

## 1.5 Discussion

In this meta-analysis of 39 cross-sectional and seven prospective studies of diverse populations, we observed significant associations of serum concentrations of persistent EDCs (dioxins, PCBs, and chlorinated pesticides) and urinary concentrations of non-persistent EDCs (BPA and phthalates) with T2D risk. Significant associations between EDCs and diabetes-related metabolic traits, including increased fasting glucose, 2-h post-challenge glucose, fasting insulin, and HOMA-IR, were also observed. Further, sex-specific associations were also identified in that high serum concentrations of PCBs were weakly associated with T2D in men but more strongly associated with T2D in women. A larger sex dimorphism was suggested in one study of PBB, which indicated that higher blood PBB concentration was positively associated with T2D risk in women and inversely associated with T2D risk in men[44].

To our knowledge, this is the first meta-analysis to report significant associations of a full spectrum of EDCs as listed in the Endocrine Society Scientific Statement[28] including both persistent and non-persistent EDCs with T2D risk. In a recent meta-analysis of prospective studies, Wu and colleagues also reported significant associations of total PCBs (OR=1.70; 95% CI, 1.28 to 2.27), and one of the chlorinated pesticide, hexachlorobenzene (HCB, OR=2.00; 95% CI, 1.13 to 3.53) with risk of T2D[30]. The effect sizes are similar to the results from our analysis. For the current meta-analysis, we expanded the spectrum of EDCs from the most classic EDC, dioxins, to the recently accepted non-persistent EDCs, BPA and phthalates. In addition, given the limited numbers of prospective studies, we included both cross-sectional and prospective to comprehensively evaluate the existing evidence for the association between EDCs and T2D. We

also assessed the association between these EDCs and diabetes-related traits, in order to examine the effects of EDCs on glucose metabolism quantitatively.

Previous animal studies have demonstrated that exposure to persistent EDCs, BPA, and phthalates alter blood glucose and insulin homeostasis[45-47]. Persistent EDCs, including dioxins, PCBs, and chlorinated pesticides, may impair glucose metabolism by affecting the expression of insulin-like growth factor 1 and its binding protein, by reducing cellular glucose uptake, or by direct binding and activation of the aryl hydrocarbon receptor pathway[48-50]. The activation of aryl hydrocarbon receptor by persistent EDCs, especially dioxin-like EDCs, may antagonize peroxisome proliferator-activated receptor γ (PPARγ) function, thus suggesting that these EDCs may affect the risk of T2D through antagonism of PPARγ functions[50]. While initially considered a weak estrogen, BPA is equipotent with estradiol in its ability to stimulate rapid response via estrogen receptors[51], inducing physiological responses at concentrations as low as 0.1 nmol/L (0.023 ng/mL)[52]. At such environmentally relevant low doses, BPA has also been shown to suppress adiponectin release from adipocyte, stimulate accumulation of triacylglycerol (TG) in adipocytes and hepatocytes, and up-regulate genes involved in lipid metabolism, adipocyte differentiation, and inflammation, some pathways that are beyond the classic sex hormone receptors-mediated systems[53-55]. Recent studies have also shown that di-2-ethylhexyl phthalate (DEHP) and BPA can influence the metabolism and local bioavailability of endogenous androgens and estrogens[56,57].

The sex-specific differences in the PCB-T2D relation are consistent with our previous observation in prospective cohorts of men and women linking sex-steroid to T2D risk[5,7,8]. Endogenous sex hormones (testosterone and estradiol) and their binding protein (SHBG) affect the risk of developing T2D differently in men and women. Specifically, higher testosterone

levels were associated with increased risk of T2D in women but not in men, whereas SHBG appeared more protective in women than in men. While the exact mechanism responsible for the sex-specific relation remains to be determined, EDCs (as exogenous sex hormone mimetics) may also perturb estrogen and/or androgen signaling and alter metabolic regulatory mechanisms in a sex-dependent manner[58].

Asides from the EDCs included in this systematic review, other chemicals (e.g. parabens, triclosan/triclocarban, and oxybenzone) found in cosmetics and personal-care products have also been shown to have endocrine-disrupting effects[59-61]. However there are as yet no studies that have directly investigated their health effects on T2D risk in humans.

Several limitations need to be considered when interpreting our findings. First, because all the studies included are observational and the majority are cross-sectional, potential selection biases and confounding may exist. Also, although the statistical tests for heterogeneity were not significant, there appeared to be considerable heterogeneity of the studies included, in terms of study design and characteristics of population. To the extent possible, we have identified association measures that were from the model with the most comprehensive adjustment of confounders in the original studies. Findings in subgroup analyses were consistent across different groups. Consistency from the total and sex-specific associations due to the same EDCs in prospective studies provided further assurance toward limiting the potential impact due to residual confounding. Moreover, LaKind et al. suggested that using cross-sectional datasets like NHANES to draw conclusions about non-persistent EDCs and chronic diseases may be inappropriate[62]. Thus, future prospective studies are needed to establish a causal association between non-persistent EDCs and risk of diabetes.

Second, we extracted the association measures as RR or WMD comparing the highest to the lowest concentration categories in each study, despite the fact that all studies used different categorizations of EDC concentrations (e.g. in different quantiles or other categorizations). The median exposure levels were in similar ranges across populations except for several studies for occupational exposures. Because each study included different subgroups/types of the EDCs, we provided the reference concentration levels for the highest and lowest concentration categories according to the corresponding concentrations of the most frequently studied congeners or types (TCDD for dioxins, PCB153 for PCBs, DDE for chlorinated pesticides, and MEP for phthalates). This is a reasonable approach given that the concentrations of individual congeners or types are highly correlated with the total concentration levels according to the data from these studies[63]. It is important to note that the association between EDCs and diabetes may be nonlinear. However, in the existing population studies on this association, few studies detected a significant nonlinear pattern of association, and most studies modeled the association under linear assumption. Thus, in order to combine the existing evidence, we also assumed the relationships are linear, or at least monotone. However, this may miss the potential nonlinear/non-monotonic relationships, and future appropriately designed large prospective studies and close re-examination of existing datasets are important.

Third, since humans are very likely to be exposed to a cocktail of pollutants (especially for persistent EDCs such as dioxins and PCBs), it is rather difficult, if not impossible, to separate effects of individual pollutants. On the other hand, it is reasonable to examine joint effects by categories of pollutants that belong to the same category and are usually present concomitantly as shown in all the studies included.

Fourth, because of the lack of prospective studies concerning dioxins, BPA, and phthalates and T2D risk, we only included cross-sectional studies in the analysis. Future studies are clearly needed to help establish causality concerning these EDCs in large prospective cohorts. We have identified four studies investigating the association between urinary concentration of BPA and T2D using data from NHANES[64-67]. We did not include all of these studies in the analysis since this would introduce bias by giving more weight to the results from the same NHANES dataset. We chose the study by Shankar et al.[66] because it covers the whole time range of all the other studies (2003-2008), and reported RR by quartiles, which can be synthesized into our meta-analysis. When stratified by NHANES cycles, the three other studies reported significant association between urinary BPA concentration and T2D in the 2003-2004 cycle (RR=1.39; 95% CI, 1.21 to 1.60[64], 1.41; 95% CI, 1.21 to 1.64[65], and 1.23; 95% CI, 1.07 to 1.42[67] by one SD increase of BPA), whereas no significant association was detected in the 2005-2006 and 2007-2008 cycles (for 2005-2006 cycle: RR=1.07; 95% CI, 0.85 to 1.34[65] and 1.05; 95% CI, 0.94 to 1.18[67]; for 2007-2008 cycle: RR=1.06; 95% CI, 0.91 to 1.23[67]). Moreover, for the study of Ning et al.[68], although the RR comparing the highest with the lowest quartiles of urinary BPA concentration was significant, they did not detect a significant linear trend of the association and reach a conclusion of no association. Although the pooled RR for BPA in the current analysis is significant, all the aforementioned uncertainties need to be taken into consideration while explaining the results, and these uncertainties necessitate future larger prospective studies to confirm or refute this association.

Fifth, publication biases were possible for studies of chlorinated pesticides, although the main effect estimates remained stable in our sensitivity analyses excluding smaller studies. Additionally, studies that did not provide sufficient information for association measures

synthesis in this meta-analysis (e.g. no data on variance[69], association measures on log scale[70-74], and no blood/urine measurement of EDCs[75]) also supported a positive association between serum/urine concentrations of EDCs and T2D risk.

In conclusion, our systematic review of 46 studies comprising 51,687 individuals from different populations (including white, black, Hispanic, Asian, and Native American) indicates that serum concentrations of persistent EDCs and urinary concentrations of non-persistent EDCs may significantly affect T2D risk, and that the magnitudes of associations with PCBs appear sex-dependent (stronger in women than in men). Our findings emphasize the importance of environmental factors in the etiology of T2D. Moreover, these findings highlight the importance of investigating the sex-specific risk of T2D according to different EDCs. As the existing studies regarding the association between non-persistent EDCs and risk of T2D are all cross-sectional, large and high-quality prospective studies that comprehensively assess concentrations of these EDCs are urgently needed to clarify the role of these environmental factors in the ongoing T2D epidemics in human populations.

## 1.6 Tables and Figures

**Table 1.1 Characteristics of 39 cross-sectional and 7 prospective studies of environmental endocrine disruptors and type 2 diabetes risk and related metabolic traits.**

| Source | Country or region | Study population | Sample size | Mean age | Men, % | Ascertainment of diabetes | Diabetes cases | EDCs studied and median concentrations* | Metabolic traits studied |
|---|---|---|---|---|---|---|---|---|---|
| **Cross-sectional studies** | | | | | | | | | |
| Calvert et al., 1999[37] | US | Plant workers | 541 | 56 | 94 | Self-report /FPG | 44 | Dioxin ($Cl_4$, 7 pg/g lipid) ‡,§ | FPG |
| Longnecker et al., 2000[76] | US | Veterans of Operation Ranch Hand | 1197 | 53 | 100 | Self-report /post-challenge | 169 | Dioxin ($Cl_4$, 4.0 pg/g lipid) | FPG, 2hG, FI |
| Cranmer et al., 2000[77] | US | Healthy subjects living near a plant in Arkansas | 69 | 51 | 41 | | | Dioxin ($Cl_4$, median N/A) | FPG, 2hG, FI, 2hI |
| Fierens et al., 2003[78] | Belgium | Subjects living around iron plant /waste dumping site /waste incinerator /referent area | 257 | 52 | 45 | Self-report, type 2 | 9 | Dioxin (total, 25.9 pg TEQ/g lipid)†,‡<br>PCB (12 congeners, 410.8 ng/g lipid) †,‡ | |
| Lee et al., 2006[79] | US | NHANES 1999-2002 | 2016 | 49† | 45 | FPB /history | 217 | Dioxin ($Cl_7$, 49.3 ng/g lipid; $Cl_8$, 418.5 ng/g lipid) †<br>PCB (PCB153 48.5 ng/g lipid) †<br>Chlorinated pesticides (O, 20.3 ng/g lipid; DDE, 504.5 ng/g lipid; TN, 28.7 ng/g lipid) † | |
| Chen et al., 2006[80] | Taiwan | Subjects living near municipal waste incinerators | 1034 | 46† | 51 | Self-reported | 29 | Dioxin (total, 16.7 pg TEQ/g lipid) †,‡ | FPG |
| Everett et al., 2007[81] | US | NHANES 1999-2002 | 1830 | -- | -- | Self-report /HbA1c | -- | Dioxin ($Cl_6$, 70.6 pg/g lipid) †<br>PCB (PCB126, 57.6 pg/g lipid) †<br>Chlorinated pesticides (DDT, 23.7 ng/g lipid) † | |
| Uemura et al., 2008[38] | Japan | Residents from 25 prefectures in Japan | 1374 | 44† | 46 | Self-report /HbA1c | 65 | Dioxin (total, 12.0 pg TEQ/g lipid)<br>PCB (12 congeners, 7.6 pg TEQ/g lipid) | HbA1c |
| Chang et al., 2010[82] | Taiwan | Residents living near contaminated area | 1234 | -- | 52 | | | Dioxin (total, 20.5 pg TEQ/g lipid) | FPG, HOMA-IR, HOMA-β |

| Source | Country or region | Study population | Sample size | Mean age | Men, % | Ascertainment of diabetes | Diabetes cases | EDCs studied and median concentrations* | Metabolic traits studied |
|---|---|---|---|---|---|---|---|---|---|
| Everett et al., 2012[83] | US | NHANES 1999-2004 | 2245 | -- | -- | Self-report /HbA1c | 284 | Dioxin ($Cl_6$, 0.3 pg/g serum; $Cl_7$, 0.4 pg/g serum; $Cl_8$, 2.9 pg/g serum; $Cl_5$, 0.05 pg/g serum) ‡<br>PCB (4 congeners; PCB126, 0.2 pg/g serum; PCB169, 0.2 pg/g serum; PCB118, 90 pg/g serum; PCB156, 56 pg/g serum) ‡ | |
| Longnecker et al., 2001[84] | US | Pregnant women | 2245 | 24 | 0 | Clinically recruited | 44 | PCB (congeners not specified, 2.8 μg/L) † | |
| Rylander et al., 2005[85] | Sweden | Fishermen and their wives | 380 | 61 | 52 | Self-report | 22 | PCB (PCB153, 307.9 ng/g lipid) †<br>Chlorinated pesticides (DDE, 606.9 ng/g lipid) † | |
| Codru et al., 2007[86] | US | Native American | 352 | 49 | 38 | FPG /medication | 71 | PCB (101 congeners, 579.8 ng/g lipid)<br>Chlorinated pesticides (M, 12.4 ng/g lipid; DDE, 349.5 ng/g lipid; HCB, 11.1 ng/g lipid) | |
| Rignell-Hydbom et al., 2007[87] | Sweden | Fishermen's wives | 543 | 49 | 0 | Self-report | 15 | PCB (PCB153, 82.8 ng/g lipid) †<br>Chlorinated pesticides (DDE, 142.8 ng/g lipid) † | |
| Jorgensen et al., 2008[88] | Denmark | Greenland Inuit | 692 | 49 | 44 | FPG /OGTT | 71 | PCB (13 congeners; PCB153, 807.5 ng/g lipid; PCB138, 360.0 ng/g lipid; PCB180, 465.0 ng/g lipid) †<br>Chlorinated pesticides (M, 32.5 ng/g lipid; HCB, 370.0 ng/g lipid; HCH, 36.0 ng/g lipid; O, 257.5 ng/g lipid; TN, 525 ng/g lipid; DDT, 31 ng/g lipid; DDE, 1500.0 ng/g lipid) † | FPG, 2hG, FI, 2hI |
| Chen et al., 2008[89] | Taiwan | Pregnant women from polluted area | 40 | 28 | 0 | | | Dioxin (total, 9.7 pg TEQ/g lipid) †<br>PCB (12 congeners, 5.1 pg TEQ/g lipid) † | FI, HOMA-IR |
| Philibert et al., 2009[39] | Canada | First Nation population | 101 | 45 | 49 | Self-report | 25 | PCB (8 congeners, 742.6 ng/g lipid)<br>Chlorinated pesticides (DDE, 536.5 ng/g lipid) | |
| Langer et al., 2009[90] | Slovak | Subjects from polluted area | 2046 | 21-75 | 41 | | | PCB (15 congeners, 1123 ng/g lipid) † | FPG, FI |

| Source | Country or region | Study population | Sample size | Mean age | Men, % | Ascertainment of diabetes | Diabetes cases | EDCs studied and median concentrations* | Metabolic traits studied |
|---|---|---|---|---|---|---|---|---|---|
| Ukropec et al., 2010[91] | Slovak | Recruited by physicians from the heavily polluted districts | 2047 | 21-75 | 41 | FPG /2-hPG | 296 | PCB (15 congeners, 1122.5 ng/g lipid) †<br>Chlorinated pesticides (HCB, 668.5 ng/g lipid; DDE, 1817.0 ng/g lipid; DDT, 49.5 ng/g lipid; HCH, 46.5 ng/g lipid) † | |
| Airaksinen et al., 2011[92] | Finland | Birth cohort in Helsinki | 1988 | 62 | 46 | FPG /2-hPG /medication | 308 | PCB (PCB153, 290 ng/g lipid)<br>Chlorinated pesticides (O, 11 ng/g lipid; TN, 28 ng/g lipid; DDE, 470 ng/g lipid) | |
| Dirinck et al., 2011[93] | Belgium | Obese subjects visiting a weight management clinic (BMI≥30), and controls (BMI18-25) | 144 | 40 | 51 | | | PCB (4 congeners, 13 ng/g lipid)<br>Chlorinated pesticides (DDE, 205 ng/g lipid; β-HCH, 19 ng/g lipid) | FPG, 2hG, FI, 2hI, HOMA-IR |
| Silverstone et al., 2012[31] | US | Anniston Community Health Survey | 603 | 54 | 27 | Self-report /FPG | 207 | PCB (35 congeners, 3.18 ng/g serum) †,‡<br>Chlorinated pesticides (DDE, median N/A) | |
| Gasull et al., 2012[94] | Spain | Catalan Health Interview Survey in Catalonia | 684 | 44 | 41 | Self-report /FPG | 143 | PCB (7 congeners; PCB153, 564 pg/mL; PCB118, 99 pg/mL; PCB138, 389 pg/mL; PCB180, 492 ph/mL) ‡<br>Chlorinated pesticides (HCB, 1068 pg/mL; HCH, 615 pg/mL; DDT, 148 pg/mL; DDE, 2703 pg/mL) ‡ | |
| Lim et al., 2008[43] | US | NHANES 2003-2004 | 1367 | 50 | 47 | FPG/random glucose/medication | 156 | PBB (PBB153, 3.1 ng/g lipid) † | |
| Cox et al., 2007[95] | US | Hispanic Health and Nutrition Survey (HNANES) 1982-1984 | 1303 | 41† | 41 | Self-report | 89 | Chlorinated pesticides (HCB, 1.34 ng/g serum; TN, 1.52 ng/g serum; DDT, 3.22 ng/g serum; DDE, 9.00 ng/g serum; HCH, 1.70 ng/g serum; O, 1.22 ng/g serum; D, 1.50 ng/g serum) | |
| Lee et al., 2007[96] | US | NHANES 1999-2002 | 749 | 48 | 46 | | | Dioxin (PCDD/F, median N/A)<br>PCB (9 congeners, median N/A)<br>Chlorinated pesticides (O, TN, DDT, HCH, median N/A) | HOMA-IR |

| Source | Country or region | Study population | Sample size | Mean age | Men, % | Ascertainment of diabetes | Diabetes cases | EDCs studied and median concentrations* | Metabolic traits studied |
|---|---|---|---|---|---|---|---|---|---|
| Son et al., 2010[97] | South Korea | Community-based health survey in Uljin County | 80 | 56 | 53 | FPG /medication | 40 | Chlorinated pesticides (O, 9.7 ng/g lipid; TN, 25.8 ng/g lipid; HE, 9.6 ng/g lipid; HCB, 21.3 ng/g lipid; HCH, 51.0 ng/g lipid; M, 3.2 ng/g lipid; DDE, 514.2 ng/g lipid; DDD, 6.2 ng/g lipid; DDT, 29.0 ng/g lipid) †,‡ | |
| Park et al., 2010[98] | South Korea | Community-based survey in Uljin county | 100 | 57 | 28 | | | Chlorinated pesticides (HCH, 53.8 ng/g lipid; HCB, 21.1 ng/g lipid; O, 8.8 ng/g lipid; TN, 26.5 ng/g lipid; HE, 10.6 ng/g lipid; DDE, 453.7 ng/g lipid; DDT, 21.7 ng/g lipid) †,‡ | HOMA-IR |
| Arrebola et al., 2013[99] | Spain | Non-cancer surgery patients | 386 | 52 | 51 | Self-report and FPG | 34 | Chlorinated pesticides (DDE, 159.5 ng/g lipid) | |
| Lang et al., 2008[64] | US | NHANES 2003-2004 | 1455 | 43† | 48 | Self-report | 136 | BPA, 4.60 ng/mL†,‡ | FPG, FI, HOMA-IR, HOMA-β |
| Ning et al., 2011[68] | China | Residents in Shanghai | 3423 | 59 | 40 | Self-report | 1087 | BPA, 0.81 ng/mL | FPG, 2hG, FI |
| Shankar et al., 2011[66] | US | NHANES 2003-2008 | 3967 | 45 | 47 | Self-report | 467 | BPA, 3.93 ng/mL†,‡ | |
| Li et al., 2011[100] | China | Patients with PCOS | 89 | 27 | 0 | | | BPA, 9.40 ng/mL† | HOMA-IR |
| Wang et al., 2012[101] | China | Residents in Shanghai | 3390 | 61 | 40 | | | BPA, 0.81 ng/mL | FPG, FI, HOMA-IR |
| Kim et al., 2012[102] | South Korea | Population-based survey | 1210 | 54† | 42 | Self-report | 99 | BPA, 2.1 ng/mL†,‡ | |
| Stahlhut et al., 2007[103] | US | NHANES 1999-2002 | 1443 | 48† | 100 | | | Phthalates (MBP, 21.2 μg/g creatinine; MBzP, 14.2 μg/g creatinine; MEHP, 3.8 μg/g creatinine; MEP, 188.1 μg/g creatinine; MEHHP, 19.6 μg/g creatinine; MEOHP, 13.2 μg/g creatinine) | HOMA-IR |
| Lind et al., 2012[104] | Sweden | Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) | 1016 | 70 | 50 | FPG /history | 119 | Phthalates (MEHP, 4.5 ng/mL; MEP, 11.6 ng/mL; MiBP, 13.5 ng/mL; MMP, 1.5 ng/mL) | HOMA-IR |

| Source | Country or region | Study population | Sample size | Mean age | Men, % | Ascertainment of diabetes | Diabetes cases | EDCs studied and median concentrations* | Metabolic traits studied |
|---|---|---|---|---|---|---|---|---|---|
| James-Todd et al., 2012[105] | US | NHANES 2001-2008 | 2350 | 48† | 0 | Self-report | 215 | Phthalates [MEP, 164.8 ng/mL; MnBP, 17.7 ng/mL; MiBP, 3.7 ng/mL; MBzP, 9.7 ng/mL; MCPP, 2.0 ng/mL; total DEHP (MEHP, MEHHP, and MEOHP), 11.1 ng/mL]‡ | FPG, HOMA-IR, HbA1c |
| Olsen et al., 2012[106] | Sweden | Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) | 1016 | 70 | 50 | FPG /history | | BPA, median value not given<br>Phthalates (MEHP, MEP, MiBP, MMP, median value not given) | FPG |
| **Prospective studies** | | | | | | | | | |
| Vasiliu et al., 2006[44] | US | Michigan PBB Cohort (25 y follow-up) | 1384 | 40† | 50 | Self-report | 180 | PCB (congener not specified, 3.0 μg/L) †<br>PBB (PBB153, 7.0 μg/L) † | |
| Wang et al., 2008[41] | Taiwan | Victims in the Yucheng registry (24 y follow-up) | 748 | 55 | 41 | Self-report | -- | PCB (congener not specified, 1.67 μg/L)# | |
| Turyk et al., 2009[107] | US | Great Lakes sport fish consumers (8.4 y follow-up) | 471 | 47 | 59 | Self-report | 36 | PCB (18 congeners, 2.46 ng/g serum; PCB118, 0.16 ng/g serum) †,‡<br>Chlorinated pesticides (DDE, 3.4 ng/g serum) †,‡ | |
| Rignell-Hydbom et al., 2009[40] | Sweden | Women's Health in the Lund Area (WHILA) (7-11 y follow-up) | 78¶ | 58 | 0 | Register, type 2 | 39 | PCB (PCB153, 1230.0 pg/mL) †<br>Chlorinated pesticides (DDE, 3250.0 pg/mL) † | |
| Lee et al., 2010[42] | US | CARDIA Cohort (18 y follow-up) | 180 | 27 | 38 | Medication | 90 | PCB (35 congeners; PCB74, 99 pg/g serum; PCB153, 350 pg/g serum; PCB178, 16 pg/g serum; PCB187, 79 pg/g serum)<br>PBB (PBB153, 17 pg/g serum)<br>Chlorinated pesticides (O, 158 pg/g serum; TN, 175 pg/g serum; HCH, 109 pg/g serum; DDE, 3313 pg/g serum; M, 22 pg/g serum) | |
| Lee et al., 2011[108] | Sweden | Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) (5 y follow-up) | 725 | 70 | 48 | FPG/medication, type 2 | 36 | PCB (14 congeners; PCB153, 1424.0 pg/mL; PCB138, 831.5 pg/mL; PCB180, 1172.5 pg/mL) †<br>Chlorinated pesticides (DDE, 1895.5 pg/mL; TN, 142.0 pg/mL; HCB, 254.5 pg/mL) † | |

| Source | Country or region | Study population | Sample size | Mean age | Men, % | Ascertainment of diabetes | Diabetes cases | EDCs studied and median concentrations* | Metabolic traits studied |
|---|---|---|---|---|---|---|---|---|---|
| Wu et al., 2013[30] | US | Nurses' Health Study—Breast Cancer Study (18 y follow-up) | 673 | 59 | 0 | Self-reported | 24 | PCB (22 congeners; PCB118, 65.8 ng/g lipid; PCB138, 94.5 ng/g lipid; PCB153, 104.5 ng/g lipid; PCB180, 74.5 ng/g lipid) †,‡ Chlorinated pesticides (DDT, 53.4 ng/g lipid; DDE, 774.9 ng/g lipid; HCB, 29.7 ng/g lipid) †,‡ | |
| Wu et al., 2013[30] | US | Nurses' Health Study—Non-Hodgkin Lymphoma Study (18 y follow-up) | 422 | 59 | 0 | Self-reported | 24 | PCB (56 congeners; PCB118, 48.6 ng/g lipid; PCB138, 63.8 ng/g lipid; PCB153, 106.8 ng/g lipid; PCB180, 71.5 ng/g lipid) †,‡ Chlorinated pesticides (DDT, 44.1 ng/g lipid; DDE, 987.0 ng/g lipid; HCB, 37.0 ng/g lipid) †,‡ | |

*Serum concentrations for dioxins, PCBs, and chlorinated pesticides; urinary concentrations for BPA and phthalates.
†Derived from data presented in original articles via weighted averaging.
‡Reported mean instead of median.
§Concentration in reference population, rather than the occupationally exposed workers.
||Median not reported and cannot be derived. Numbers shown are 75$^{th}$ percentiles.
¶Participants with the longest follow-up time in the original study.
#Concentration in reference population, rather than the PCB-poisoning victims.
Abbreviations: A, aldrin; AC, α-chlordane; BPA, bisphenol A; $Cl_4$: 2,3,7,8-tetrachlorodibenzo-p-dioxin; $Cl_5$: 2,3,4,7,8-pentachlorodibenzofuran; $Cl_6$: 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin $Cl_7$: 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin; $Cl_8$: 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin; CN, cis-nonachlor; D, dieldrin; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DEHP, di-2-ethylhexyl phthalate; FI, fasting insulin; FPG, fasting plasma glucose; GC, γ-chlordane; HCB, hexachlorobenzene; HCH, β-hexachlorocyclohexane; HE, heptachlor epoxide; HOMA-IR, homeostatic model assessment-insulin resistance; HOMA-β, homeostatic model assessment-β cell function; M, mirex; MBzP, monobenzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; MnBP, mono-n-butyl phthalate; O, oxychlordane; PBB, polybrominated biphenyls; PCB, polychlorinated biphenyls; TEQ, dioxin toxic equivalent (concentrations of dioxins and dioxin-like PCBs expressed as equivalent concentrations of 2,3,7,8-TCDD); TN, trans-nonachlor; 2hG, 2-h post-challenge glucose; 2hI, 2-h post-challenge insulin.

**Table 1.2 Biologic half-lives of some known endocrine-disrupting chemicals (EDCs).**

| Category | Biologic half-life examples |
|---|---|
| **Persistent EDCs*** | |
| Dioxins | 7.1 years (2,3,7,8-tetrachlorodibenzodioxin, TCDD)[109] |
| Polychlorinated biphenyls (PCB) | 2.6 years (Aroclor 1242)<br>4.8 years (Aroclor 1254)[110] |
| Polybrominated biphenyls (PBB) | 10.8 years[111] |
| Chlorinated pesticides | 2.5 years (*p*,*p*'-dichlorodiphenyldichloroethylene, DDE)[112]<br>5.7 years (dichlorodiphenyltrichloroethane, DDT)[113]<br>Several months (Mirex)[114]<br>9-12 months (Aldrin)[115] |
| Perfluorinated compounds (PFC) | 3.8 years (perfluorooctanoic acid, PFOA)[116] |
| **Non-Persistent EDCs†** | |
| Bisphenol A (BPA) | 43 hours[117] |
| Phthalates | 2 hours (phase I) + 5 hours (phase II) (di-2-ethylhexyl phthalate, DEHP)[118] |
| Triclosan | 0.45 hours (phase I) + 2.42 hours (phase II)[119] |

*Persistent EDCs: EDCs with low water solubility and high lipid solubility, leading to their bioaccumulation in adipose tissue. Biologic half-lives of persistent EDCs are several years or above.
†Non-persistent EDCs: EDCs with high water solubility, leading to their fast excretion from human body. Biologic half-lives of non-persistent EDCs are usually within several hours.

**Table 1.3 Analysis of sources of heterogeneity among studies assessing the associations between polychlorinated biphenyls and chlorinated pesticides and type 2 diabetes risk.**

| | Polychlorinated biphenyls (PCB)* | | | Chlorinated pesticides† | | |
|---|---|---|---|---|---|---|
| | N of subjects (N of studies) | Relative risk (95% CI) | *P* for difference‡ | N of subjects (N of studies) | Relative risk (95% CI) | *P* for difference‡ |
| Overall random-effects | 21,530 (21) | 2.39 (1.86 to 3.08) | | 12,341 (17) | 2.30 (1.81 to 2.93) | |
| Study design | | | | | | |
| Cross-sectional | 15,482 (13) | 2.90 (2.14 to 3.92) | <0.001 | 9792 (11) | 2.28 (1.73 to 3.02) | 0.36 |
| Prospective | 4681 (8) | 1.65 (1.16 to 2.34) | | 2549 (6) | 2.43 (1.39 to 4.25) | |
| Follow-up <10 y | 1274 (3) | 2.21 (1.03 to 4.75) | 0.93 | 1274 (3) | 4.82 (2.15 to 10.77) | 1.00 |
| Follow-up >10 y | 3407 (5) | 1.50 (1.01 to 2.25) | | 1275 (3) | 1.47 (0.76 to 2.84) | |
| Sex§ | | | | | | |
| Male | 2339 (5) | 1.73 (0.80 to 3.75) | 0.08 | 279 (1) | 7.32 (0.92 to 58.41) | 0.97 |
| Female | 6301 (9) | 2.65 (1.57 to 4.48) | | 1365 (4) | 2.54 (1.25 to 5.14) | |
| Race‖ | | | | | | |
| White | 6250 (7) | 1.94 (1.43 to 2.62) | 0.032 | 5908 (6) | 1.95 (1.40 to 2.71) | 0.07 |
| Non-white | 3267 (5) | 2.91 (1.60 to 5.30) | | 2528 (5) | 2.64 (1.56 to 4.49) | |

*RR comparing highest (corresponding to PCB153 >104 to >1348 ng/g lipid) to the lowest (corresponding to PCB153 ≤60 to ≤455 ng/g lipid) PCB concentration categories.

†RR comparing highest (corresponding to DDE >545 to >9258 ng/g lipid) to the lowest (corresponding to DDE ≤112 to ≤3602 ng/g lipid) chlorinated pesticide concentration categories.

‡*P* values were calculated using two-sample *z* tests.

§Restricted to the studies which provided sex-specific associations.

‖Restricted to the studies of only whites or only non-whites. For the studies did not provide data on race distributions, they studies from European countries were deemed as all whites and the Asian countries were deemed as all non-whites.

Abbreviations: DDE, dichlorodiphenyldichloroethylene; PCB, polychlorinated biphenyls.

**Table 1.4 Random effect pooled mean differences of diabetes-related metabolic traits comparing the highest to the lowest endocrine disrupting chemical concentration categories.**

| EDC | Fasting Glucose (mg/dL) | | 2-h Glucose (mg/dL) | | Fasting Insulin (μIU/mL) | | 2-h Insulin (μIU/mL) | | HOMA-IR* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N of subjects (N of studies) | Mean difference (95% CI) | N of subjects (N of studies) | Mean difference (95% CI) | N of subjects (N of studies) | Mean difference (95% CI) | N of subjects (N of studies) | Mean difference (95% CI) | N of subjects (N of studies) | Mean difference (95% CI) |
| **Persistent** | | | | | | | | | | |
| Dioxin† | 4075 (5) | 3.96 (1.23 to 6.70) | 1266 (2) | 4.09 (0.78 to 7.40) | 1343 (3) | 1.69 (-0.57 to 3.95) | 69 (1) | 229.00 (70.24 to 387.76) | 2023 (3) | 0.46 (-0.16 to 1.09) |
| PCB‡ | 2882 (3) | 3.27 (1.87 to 4.67) | 836 (2) | 0.72 (-7.44 to 8.87) | 2882 (3) | -0.48 (-2.06 to 1.09) | 836 (2) | -17.56 (-59.06 to 23.93) | 933 (3) | -2.05 (-4.65 to 0.56) |
| Chlorinated Pesticides§ | 836 (2) | 0.81 (-3.31 to 4.93) | 836 (2) | 8.91 (-2.10 to 19.92) | 836 (2) | 0.30 (-0.65 to 1.25) | 836 (2) | 5.93 (-35.74 to 47.60) | 993 (3) | 0.73 (-0.17 to 1.63) |
| **Non-persistent** | | | | | | | | | | |
| BPA‖ | 8268 (3) | 1.19 (-0.28 to 2.66) | 3423 (1) | 0.00 (-8.08 to 8.08) | 8268 (3) | 0.16 (-0.16 to 0.49) | | N/A | 4934 (3) | 0.80 (0.36 to 1.25) |
| Phthalates¶ | 2350 (1) | 0.78 (-0.40 to 1.96) | | N/A | | N/A | | N/A | 4809 (3) | 0.42 (-0.16 to 0.99) |

SI conversion factors: To convert glucose values to mmol/L, multiply 0.056; to convert insulin values to pmol/L, multiply 6.945.

*HOMA-IR is calculated as fasting glucose (mg/dL) × fasting insulin (μIU/mL) / 405.

†Highest concentration categories: corresponding to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) >2.0 to >5.2 pg/g lipid; lowest concentration categories: corresponding to TCDD ≤1.0 to ≤2.8 pg/g lipid.

‡Highest concentration categories: corresponding to PCB153 >104 to >1348 ng/g lipid; lowest concentration categories: corresponding to PCB153 ≤60 to ≤455 ng/g lipid.

§Highest concentration categories: corresponding to *p,p'*-dichlorodiphenyldichloroethylene (DDE) >545 to >9258 ng/g lipid; lowest concentration categories: corresponding to DDE ≤112 to ≤3602 ng/g lipid.

‖Highest concentration categories: >1.43 to >4.20 ng/mL; lowest concentration categories: ≤0.47 to ≤1.36 ng/mL.

¶Highest concentration categories: corresponding to monoethyl phthalate (MEP) >17.5 ng/mL; lowest concentration categories: corresponding to MEP ≤7.2 ng/mL.

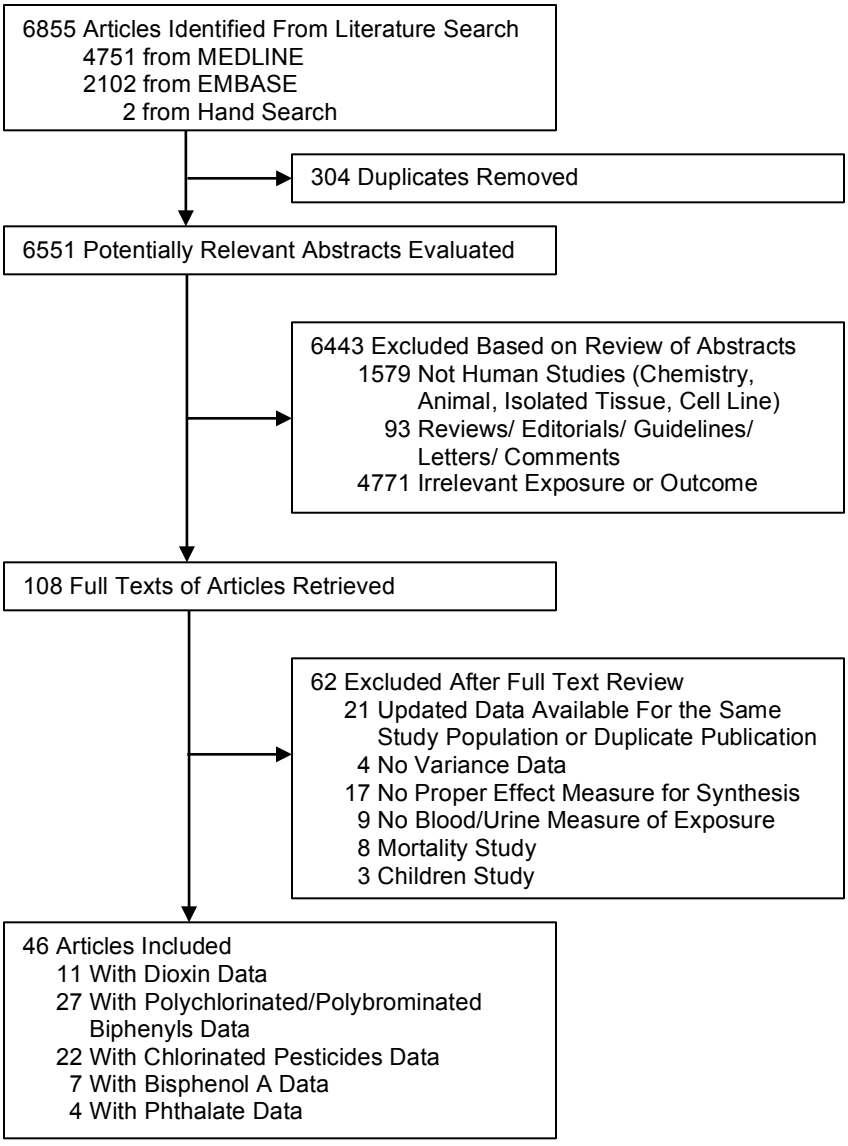


**Figure 1.1 Study Selection Process for Meta-analysis**

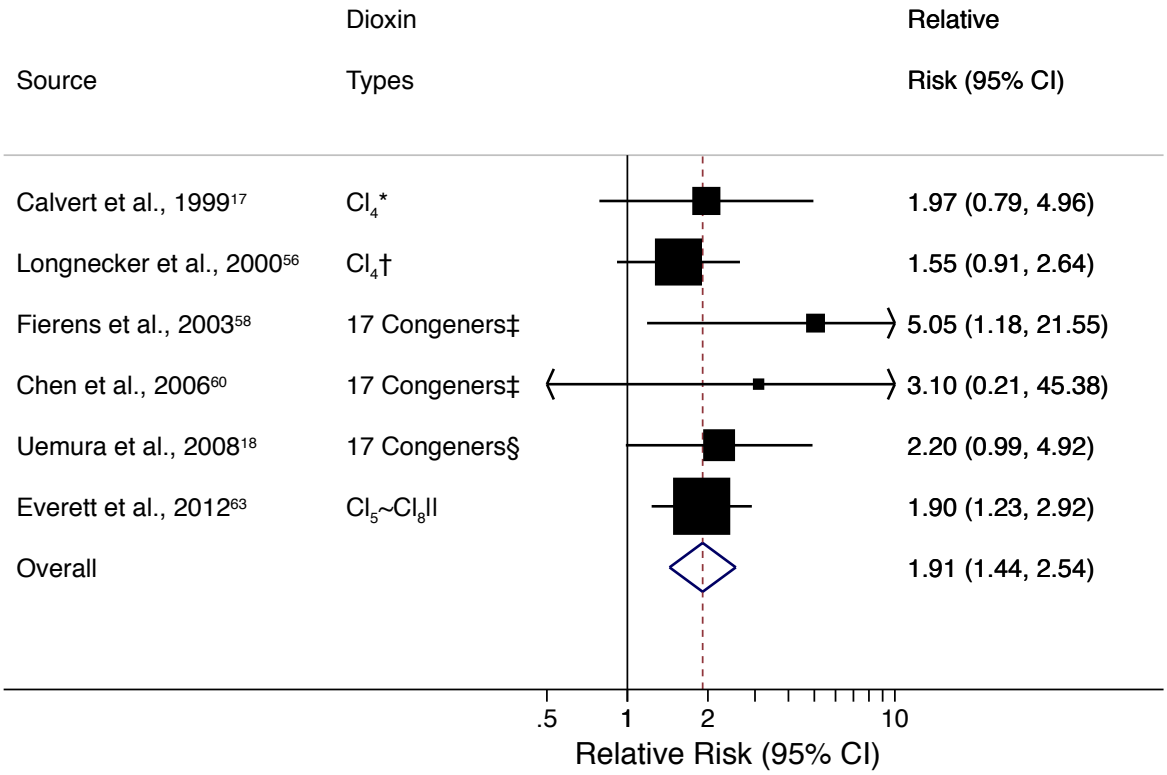


**Figure 1.2 Pooled relative risks of polychlorinated dibenzo-*p*-dioxin/dibenzofurans (dioxins) and type 2 diabetes**

Relative risks reported for highest (corresponding to TCDD concentrations of >2.0 to >5.2 pg/g lipid) vs lowest (corresponding to TCDD concentrations of ≤1.0 to ≤2.8 pg/g lipid) serum concentration categories of dioxins. Size of data markers represents the statistical weight that each study contributed to the overall random-effect estimate. 95% CI indicates 95% confidence interval.

Abbreviations: $Cl_4$: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); $Cl_5$: 2,3,4,7,8-pentachlorodibenzofuran; $Cl_6$: 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin; $Cl_7$: 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin; $Cl_8$: 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin.

*Fourth quartile of occupationally exposed workers (TCDD concentrations ≥ 238 pg/g lipid) vs referent population without occupational exposure (TCDD concentrations < 20 pg/g lipid). †Fourth quartile vs first quartile. ‡Tenth decile vs first decile. §Fourth quartile vs first and second quartiles. ||Third tertile vs first tertile.

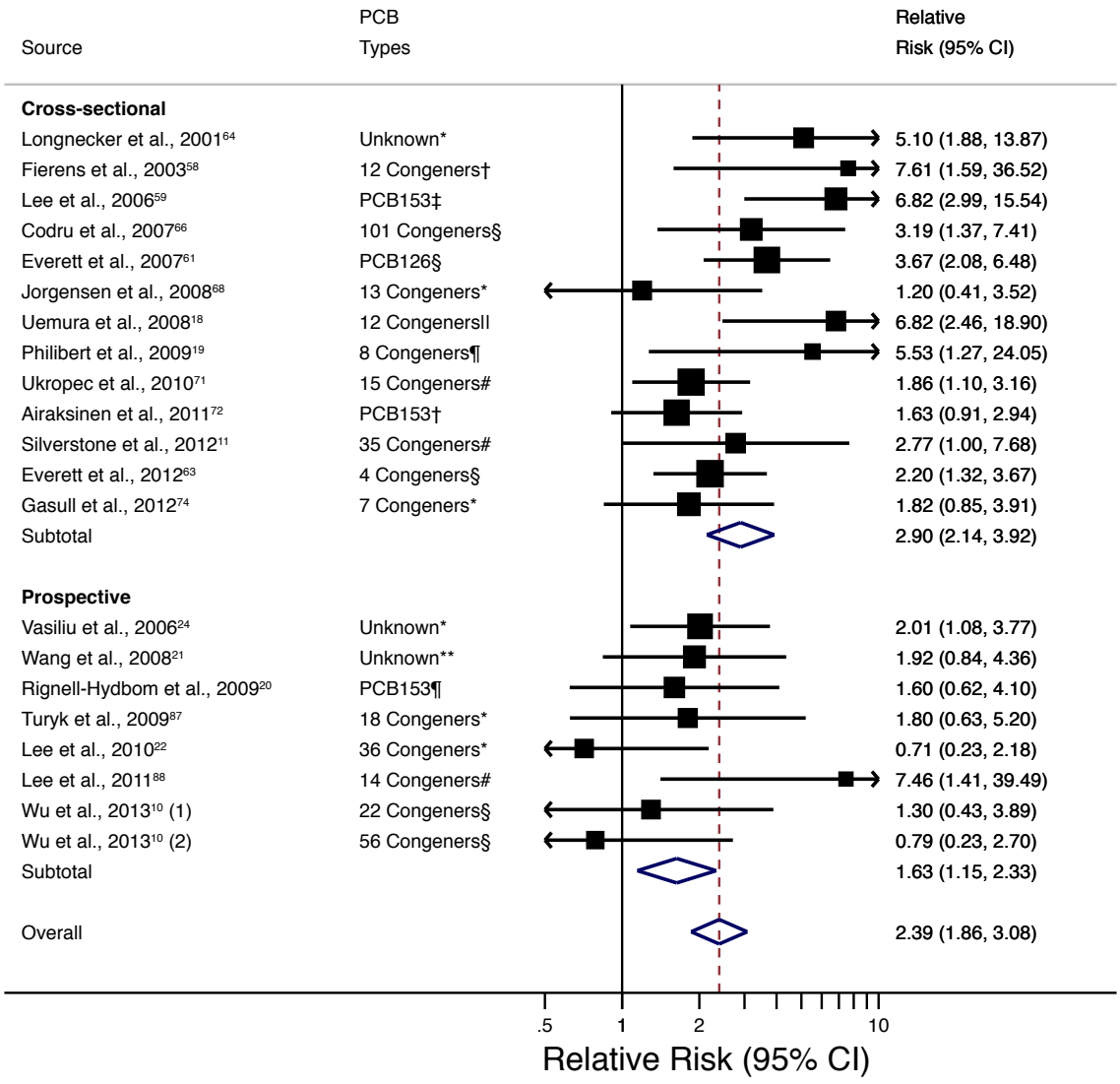


**Figure 1.3A Pooled relative risks of polychlorinated biphenyls (PCB) and type 2 diabetes**
Relative risks comparing the highest (corresponding to PCB153 concentrations of >104 to >1348 ng/g lipid) and lowest (corresponding to PCB153 concentrations of ≤60 to ≤455 ng/g lipid) serum concentration categories of PCB. Size of data markers represents the statistical weight that each study contributed to the overall random-effect estimate. 95% CI indicates 95% confidence interval.
*Fourth quartile vs first quartile. †Tenth decile vs first decile. ‡Tenth decile vs non-detectable. §Third tertile vs first tertile. ||Fourth quartile vs first and second quartiles. ¶Fourth quartile vs first to third quartiles. #Fifth quintile vs first quintile. **PCB-poisoning victims vs referent population.

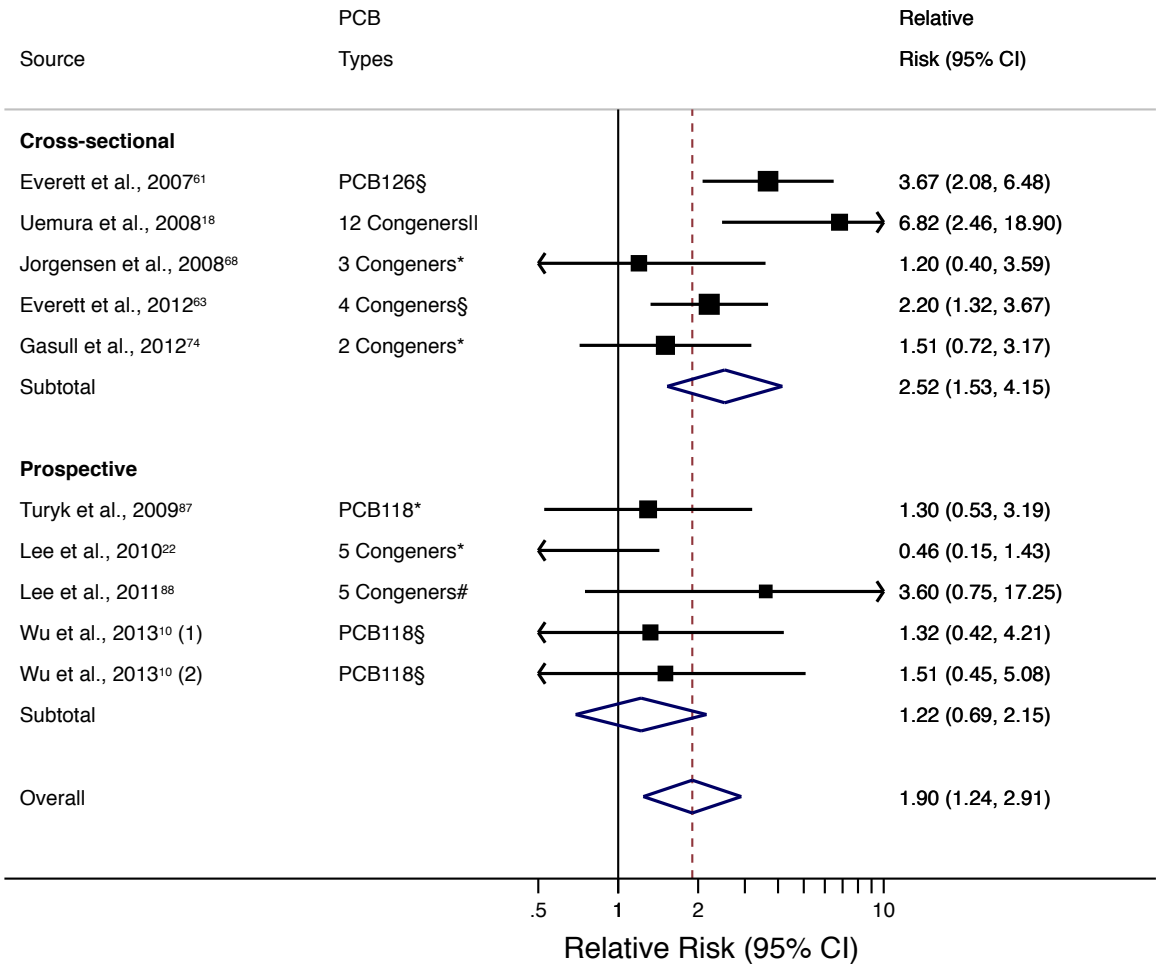


**Figure 1.3B Pooled relative risks of dioxin-like polychlorinated biphenyls (PCB) and type 2 diabetes**

Size of data markers represents the statistical weight that each study contributed to the overall random-effect estimate. 95% CI indicates 95% confidence interval.

*Fourth quartile vs first quartile. †Tenth decile vs first decile. ‡Tenth decile vs non-detectable. §Third tertile vs first tertile. ||Fourth quartile vs first and second quartiles. ¶Fourth quartile vs first to third quartiles. #Fifth quintile vs first quintile. **PCB-poisoning victims vs referent population.

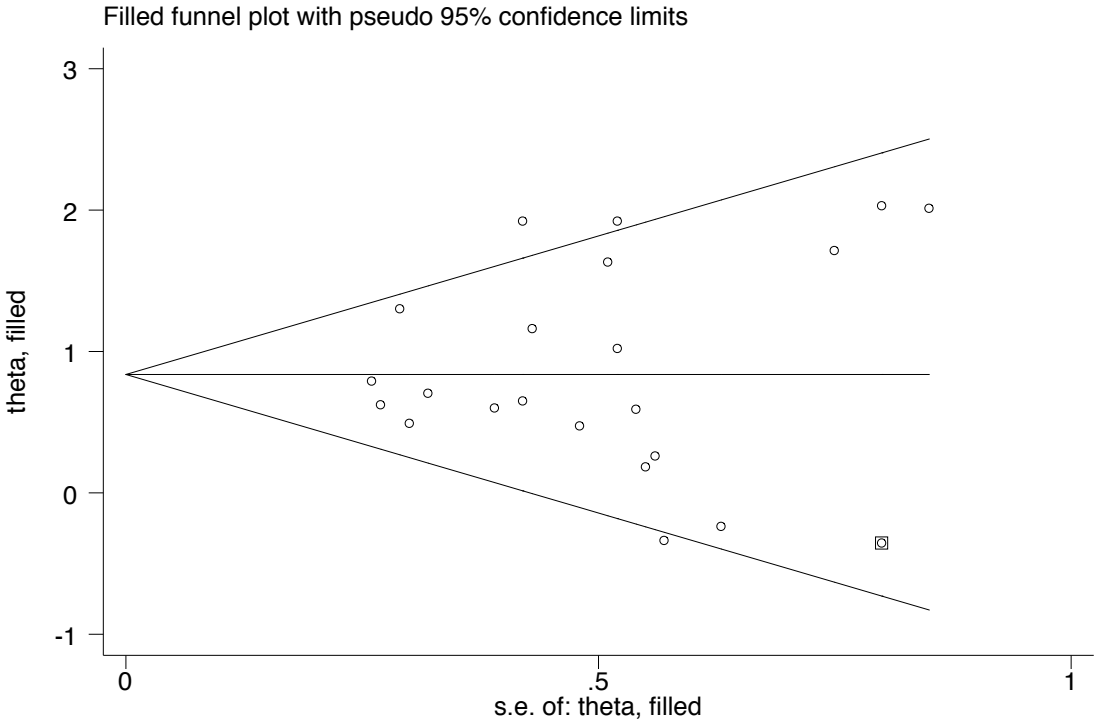


**Figure 1.4 Trim-and-fill methods for publication bias correction for the study bodies of polychlorinated biphenyls (PCBs).**
The funnel plots show the filled estimates of RR (as the horizontal line) and augmented data (as the points), along with pseudo confidence limits. A square around the data symbol indicates an imputed data point.

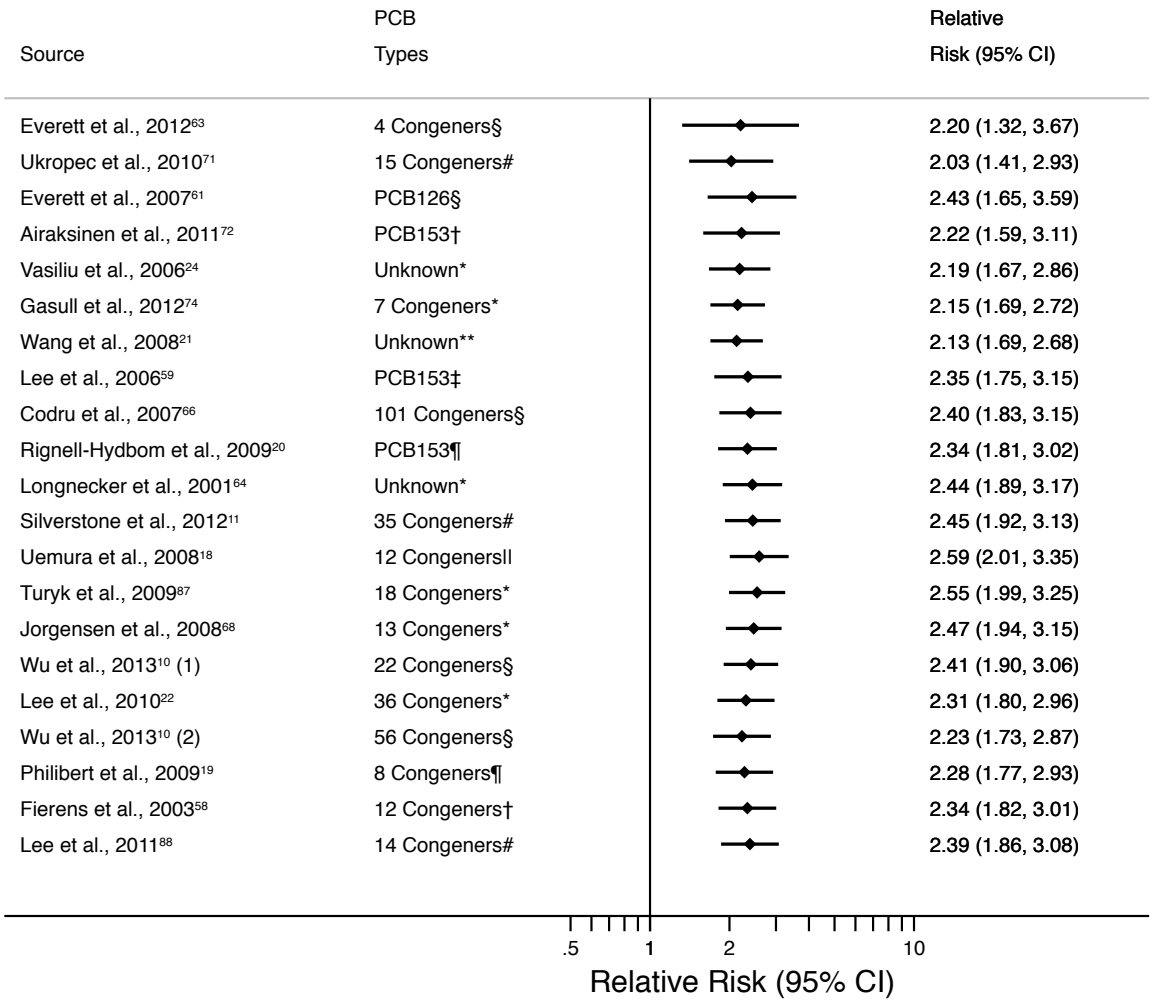


**Figure 1.5 Cumulative meta-analysis of relative risks of polychlorinated biphenyls (PCBs) and type 2 diabetes.**

Studies were sorted by the precision of their estimates (standard errors). Each estimate was pooling the corresponding study per se and all the studies with smaller precision.
*Fourth quartile vs first quartile. †Tenth decile vs first decile. ‡Tenth decile vs non-detectable. §Third tertile vs first tertile. ||Fourth quartile vs first and second quartiles. ¶Fourth quartile vs first to third quartiles. #Fifth quintile vs first quintile. **PCB-poisoning victims vs referent population.

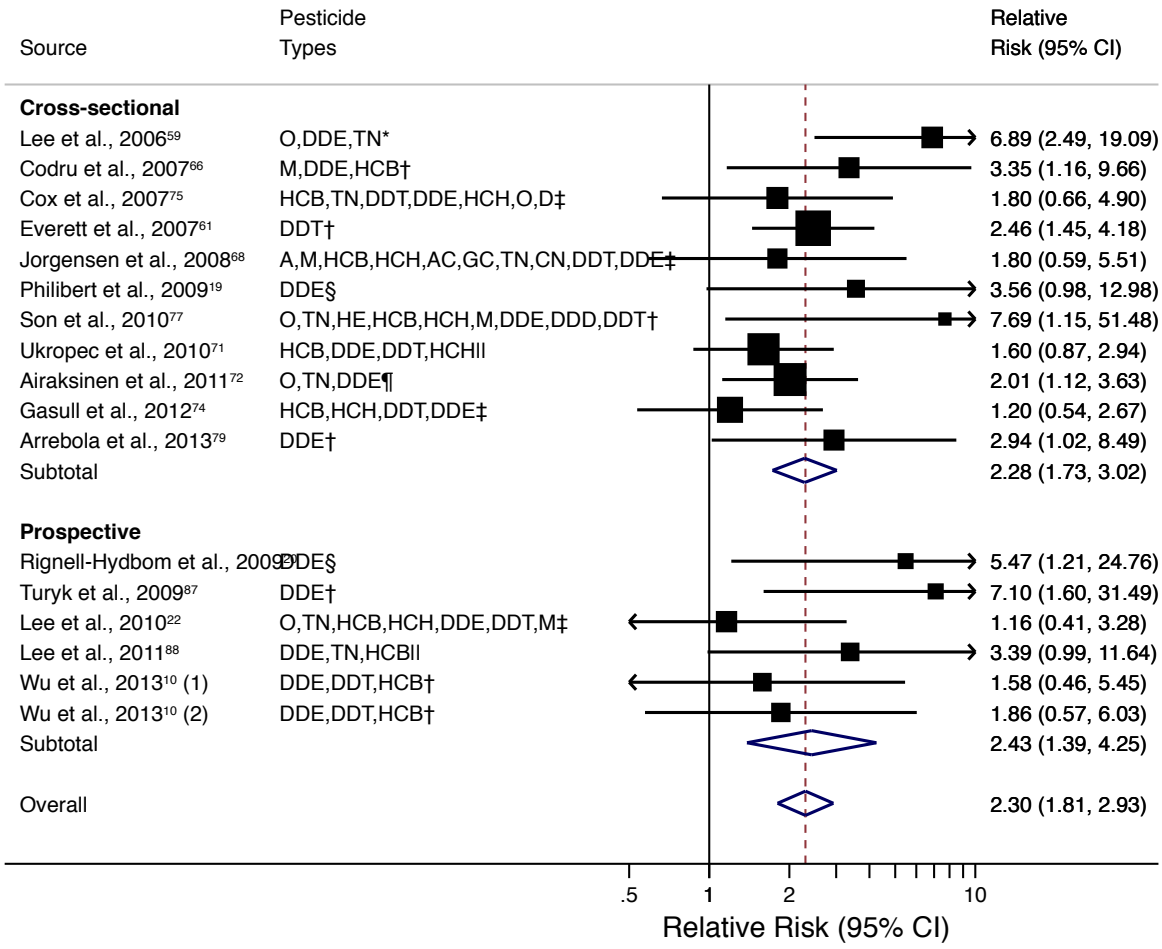


**Figure 1.6 Pooled relative risks of chlorinated pesticides and type 2 diabetes**
Relative risks comparing the highest (corresponding to DDE concentrations of >545 to >9258 ng/g lipid) and lowest (corresponding to DDE concentration of ≤112 to ≤3602 ng/g lipid) serum concentration categories of total chlorinated pesticides. Size of data markers represents the statistical weight that each study contributed to the overall random-effect estimate. 95% CI indicates 95% confidence interval.
Abbreviations: A, aldrin; AC, α-chlordane; CN, cis-nonachlor; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; GC, γ-chlordane; HCB, hexachlorobenzene; HCH, β-hexachlorocyclohexane; M, mirex; O, oxychlordane; TN, trans-nonachlor.
*Tenth decile vs first quartile. †Third tertile vs first tertile. ‡Fourth quartile vs first quartile. §Fourth quartile vs first to third quartiles. ||Fifth quintile vs first quintile. ¶Tenth decile vs first decile.

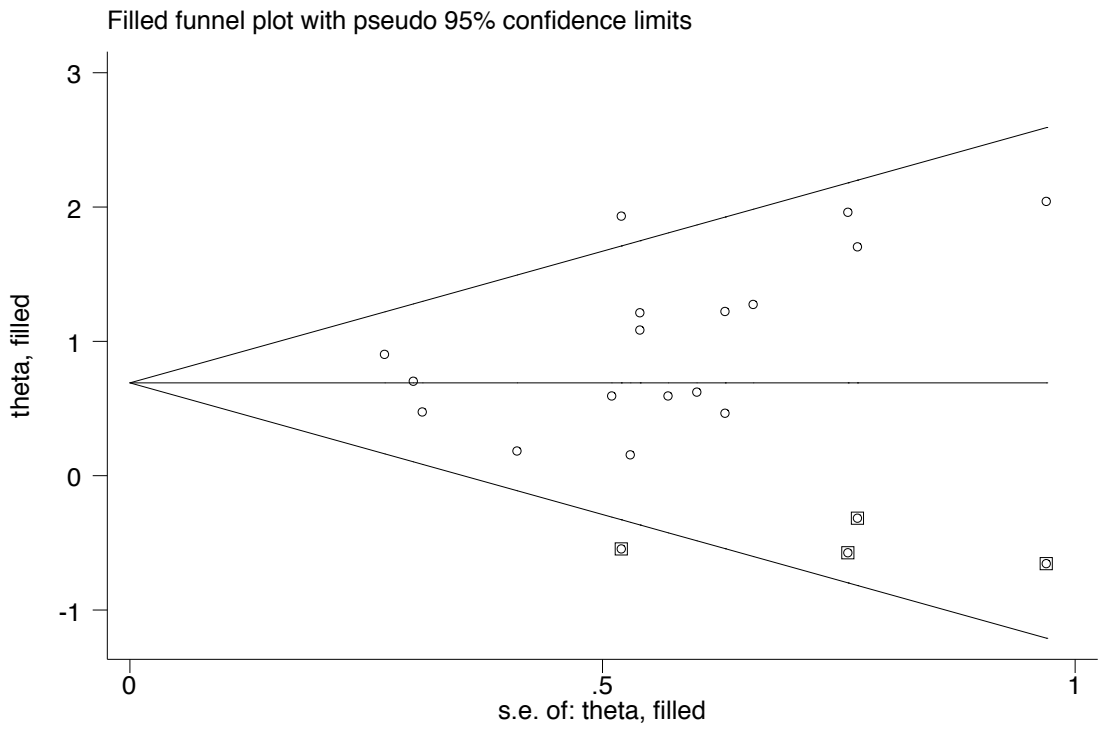


**Figure 1.7 Trim-and-fill methods for publication bias correction for the study bodies of chlorinated pesticides.**
The funnel plots show the filled estimates of RR (as the horizontal line) and augmented data (as the points), along with pseudo confidence limits. A square around the data symbol indicates an imputed data point.

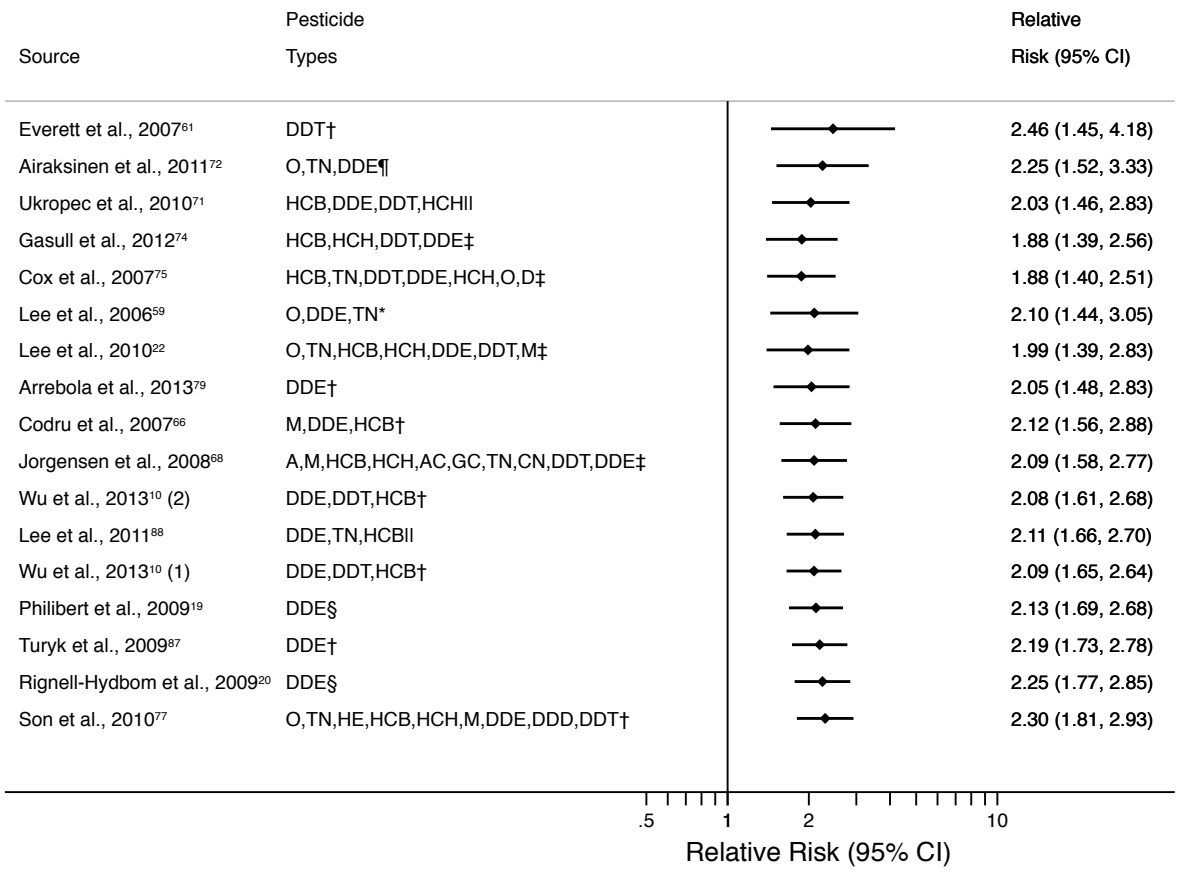


**Figure 1.8 Cumulative meta-analysis of relative risks of chlorinated pesticides and type 2 diabetes.**

Studies were sorted by the precision of their estimates (standard errors). Each estimate was pooling the corresponding study per se and all the studies with smaller precision.

*Tenth decile vs first quartile. †Third tertile vs first tertile. ‡Fourth quartile vs first quartile. §Fourth quartile vs first to third quartiles. ||Fifth quintile vs first quintile. ¶Tenth decile vs first decile.

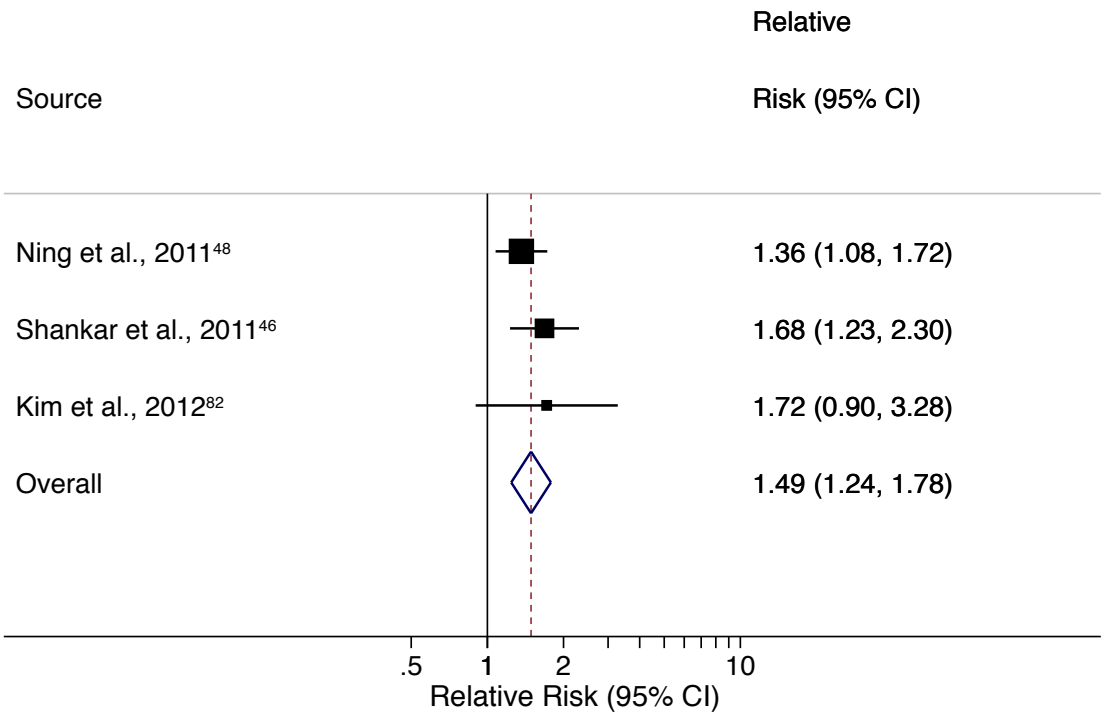


**Figure 1.9 Pooled relative risks of bisphenol A (BPA) and type 2 diabetes**
Relative risks comparing the highest (>1.43 to >4.20 ng/mL) and lowest (≤0.47 to ≤1.36 ng/mL) quartile of urinary concentrations of BPA. Size of data markers represents the statistical weight that each study contributed to the overall random-effect estimate. 95% CI indicates 95% confidence interval.

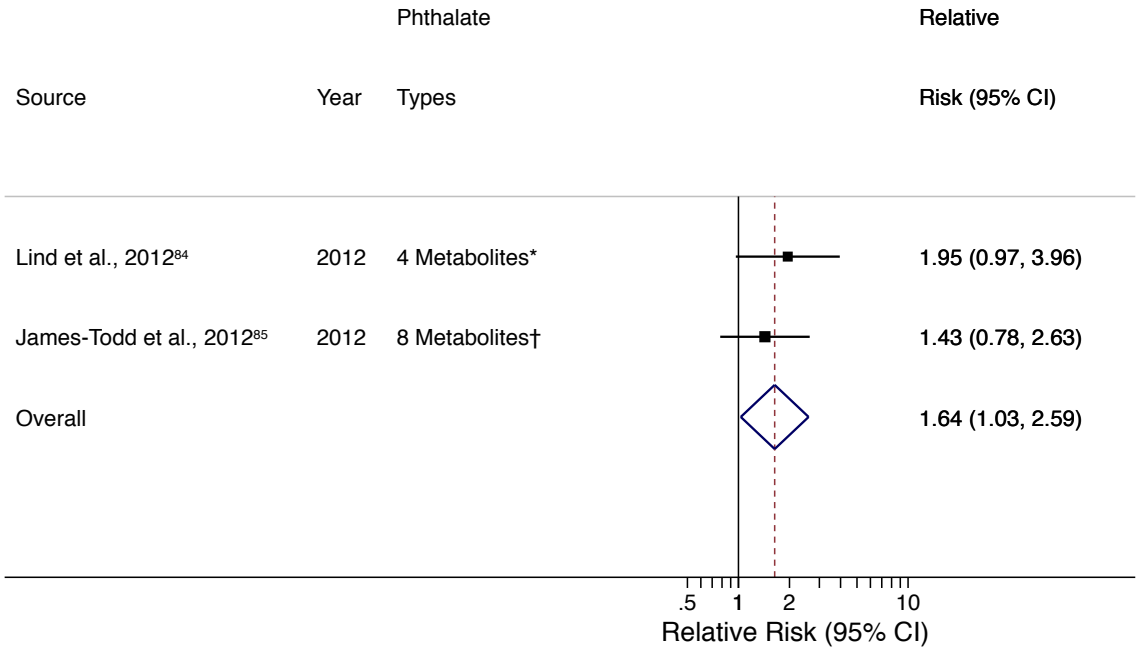


**Figure 1.10 Pooled relative risks of phthalates and type 2 diabetes**
Relative risks comparing the highest (corresponding to monoethyl phthalate concentrations of >17.5 ng/mL) and lowest (corresponding to monoethyl phthalate concentrations of ≤7.2 ng/mL) urinary concentration categories of total phthalate. Size of data markers represents the statistical weight that each study contributed to the overall random-effect estimate. 95% CI indicates 95% confidence interval.
*Fifth quintile vs first quintile, including MEHP, MEP, MiBP, and MMP.
†Fourth quartile vs first quartile, including MEP, MnBP, MiBP, MBzP, MCPP, and total DEHP metabolites (MEHP, MEHHP, and MEOHP).

## 1.7 Appendix: Search strategy for MEDLINE and EMBASE

**Pubmed:**

("Endocrine Disruptors"[MeSH Terms] OR "Endocrine Disruptors"[Title/Abstract] OR dioxins[MeSH Terms] OR benzofurans[MeSH Terms] OR dioxins[Title/Abstract] OR benzofurans [Title/Abstract] OR "Polychlorinated Biphenyls"[MeSH Terms] OR "Polybrominated Biphenyls"[MeSH Terms] OR "Polychlorinated Biphenyls"[Title/Abstract] OR "Polybrominated Biphenyls"[Title/Abstract] OR pesticides[MeSH Terms] OR Insecticides[MeSH Terms] OR Fungicides[MeSH Terms] OR "Hydrocarbons, Chlorinated"[MeSH Terms] OR pesticides[Title/Abstract] OR Insecticides[Title/Abstract] OR Fungicides[Title/Abstract] OR Phenols[MeSH Terms] OR "bisphenol A"[Title/Abstract] OR "Phthalic Acids"[MeSH Terms] OR Phthalic acids[Title/Abstract] OR phthalates[Title/Abstract]) AND ("Diabetes mellitus"[MeSH Terms] OR diabetes[Title] OR "blood glucose"[MeSH Terms] OR glucose[Title] OR insulin[MeSH Terms] OR insulin[Title] OR "insulin resistance"[MeSH Terms]) NOT (rat[Title] OR rats[Title] or rat's[Title] OR mice[Title] OR mouse[Title] OR "in vitro"[Title] OR Review[Publication Type] OR Comment[Publication Type])

**EMBASE:**

('endocrine disruptors'/exp OR 'endocrine disruptors' OR 'dioxin'/exp OR dioxin OR 'benzofuran derivative'/exp OR 'benzofuran' OR 'polychlorinated biphenyl'/exp OR 'polychlorinated biphenyl' OR 'polybrominated biphenyl'/exp OR 'polybrominated biphenyl' OR 'organochlorine pesticide'/exp OR 'organochlorine pesticide' OR '4, 4` isopropylidenediphenol'/exp OR 'bisphenol a' OR 'phthalic acid'/exp OR 'phthalate') AND ('diabetes mellitus'/exp OR 'diabetes'

OR 'blood glucose level'/exp OR 'blood glucose' OR 'insulin'/exp OR insulin OR 'insulin resistance'/exp OR 'insulin resistance') NOT(rat:ti OR rats:ti OR rat`s:ti OR mice:ti OR mouse:ti OR 'in vitro':ti) AND ('article'/it OR 'article in press'/it OR 'conference abstract'/it OR 'conference paper'/it OR 'letter'/it OR 'note'/it OR 'short survey'/it)

# CHAPTER 2. Urinary Concentrations of Bisphenol A and Phthalate Metabolites and Weight Change: A Prospective Investigation in US Women

## 2.1 Abstract

**IMPORTANCE**: Both bisphenol A (BPA) and phthalates are known endocrine-disrupting chemicals for which there is widespread general population exposure. Human exposure occurs through dietary and non-dietary routes. Although animal studies have suggested a potential role of these chemicals in obesity, evidence from human studies is sparse and inconsistent, and prospective evidence is lacking.

**OBJECTIVE**: To evaluate urinary concentrations of BPA and major phthalate metabolites in relation to prospective weight change.

**DESIGN, SETTING, AND PARTICIPANTS**: Control populations in a prospective case-control study of type 2 diabetes in the Nurses' Health Study (NHS) and NHSII.

**EXPOSURES**: A total of 977 participants provided first morning void urine samples in 1996-2002. Urinary concentrations of BPA and eight phthalate metabolites were measured using liquid chromatography – mass spectrometry.

**MAIN OUTCOMES AND MEASURES**: Body weights were self-reported at baseline and updated biennially thereafter for 10 years.

**RESULTS**: On average, the women gained 2.09 kg (95% CI, -2.27 to 6.80 kg) during the 10-year follow-up. In multivariate analysis with adjustment of lifestyle and dietary factors, in comparison to women in the lowest quartile of BPA concentration, those in the highest quartile had 0.22 kg/yr (95% CI, 0.06 to 0.37 kg/yr) greater weight gain during the 10-year follow-up (*P*-

trend=0.02). Several phthalate metabolites, including phthalic acid, monobenzyl phthalate, and monobutyl phthalate, were also associated with faster prospective weight gain in a dose-response fashion (*P*-trend<0.01), whereas other phthalates metabolites, including monoethyl phthalate and monoethylhexyl phthalate, were not monotonically associated with body weight change.

**CONCLUSION AND RELEVANCE**: These data suggest urinary concentrations of BPA and certain individual phthalate metabolites were associated with modestly greater weight gain in a dose-response fashion. These data are consistent with a potential role of BPA and phthalates in weight gain, although more prospective data are needed to corroborate these observations.

## 2.2 Introduction

Excessive body weight is associated with an increased risk of major chronic diseases including cardiovascular disease, diabetes, and certain cancers[3]. Recently, a new policy statement from the American Medical Association has officially recognized obesity as a separate disease, not simply a risk factor of other chronic diseases. Accumulating evidence has suggested that some environmental chemicals possessing endocrine-disrupting properties may lead to adiposity[10,11]. BPA and phthalates are ubiquitous endocrine-disrupting chemicals (EDCs) that have the ability to alter hormone signaling in the body[120]. Both BPA and phthalates are widely used in consumer products and exposure occurs through dietary and non-dietary routes[121,122]. Although animal studies have suggested a role of these chemicals in the etiology of obesity[58,123], evidence from human studies was inconsistent[101,103,124-135]. Furthermore, most of these studies were cross-sectional and no prospective studies in adult populations have been conducted. We aimed to evaluate urinary concentrations of BPA and major phthalate metabolites in relation to prospective weight change in the Nurses' Health Study (NHS) and NHSII.

## 2.3 Methods

### 2.3.1 Study population and sample collection

The NHS was established in 1976 when 121,700 female registered nurses aged 30-55 years were enrolled, while in 1989 the younger counterpart NHSII cohort was initiated among a total of 116,686 female registered nurses aged 25-42 years. A total of 18,717 NHS participants aged 53-79 years provided blood and urine samples in 2000-2001. In 1995-2000 blood and urine samples were collected from 29,611 NHSII participants aged 32-52 years. Urine samples were

collected without preservative in a polypropylene container and returned to a central biorepository via overnight courier with an icepack and were immediately processed upon arrival and aliquoted into polypropylene cryovials, which were stored in the vapor phase of liquid nitrogen freezers at ≤-130°C.

During the follow-up periods of 2000-2008 in the NHS and 1995-2007 in the NHS II, a total of 971 type 2 diabetes cases were identified and confirmed among participants who provided urine samples in these two cohorts[136]. A control was selected for each case and case-control pairs were matched for age at urine sample collection, race, fasting status, first morning sample, and menopausal status and use of hormone replacement therapy (NHSII only). The study population of the current analysis is from the control population of a nested case-control study of type 2 diabetes in these two cohorts[136]. All urine samples from the 977 participants were first morning void urine collected during 1996-2002. (Of note, in the NHS only, because of technical reasons, concentrations of phthalic acid were not available for 144 case-control pairs.) The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

### 2.3.2 Body weight and covariate assessments

Body weight of each individual was self-reported every two years. In these cohorts of registered nurses self-reported body weight was highly accurate: a correlation coefficient of 0.96 was observed between self-reported weight and measured weight among 184 NHS participants[137]. The major outcome of this study was the prospective weight change since urine sample collection (2000-2001 in NHS; 1995-2000 in NHSII) to the most recent follow-up cycle (2010 in NHS; 2009 in NHSII). The most recent body weights available in both NHS and NHSII were body weight at 10-years of follow-up since baseline. Body mass index (BMI) was

calculated as weight (kg) divided by squared height ($m^2$). Self-administrated questionnaires were used to collect information on demographics and lifestyle factors, including age, cigarette smoking, alcohol drinking, and physical activity. Total energy intake and alternate healthy eating index[138] (AHEI) were calculated from the data collected using a validated food frequency questionnaire (FFQ) at baseline.

### 2.3.3 Laboratory measurements

Urinary concentrations of BPA and eight phthalate metabolites [mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), monobutyl phthalate (MBP), mono-isobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), and phthalic acid (PA)] were measured using liquid chromatography – mass spectrometry[139,140]. The average intra-batch coefficients of variation (CVs) were <10% for most metabolites (including creatinine), except MEHP (NHS 11.4%, NHSII 10.0%) and BPA (NHS 11.5%, NHSII 13.0%). We grouped these metabolites according to their parent chemicals: DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and butyl phthalates metabolites (MBP and MiBP). To be consistent with previous research on phthalates, we excluded phthalic acid in calculation of total phthalate metabolite concentration. The presence of parent phthalates (diesters) is due to external contamination and they can break down to monoesters, although the process occurs very slowly and minimal given the samples were frozen within 24 hours. To test for potential environmental contamination of samples, we compared measurements of urinary concentrations of phthalate metabolites treated with and without β-glucuronidase and sulfatase in a pilot study[141]. Among 44 NHS and NHSII participants, the two measurements with and without β-glucuronidase and sulfatase were highly correlated. The intraclass coefficients (ICC) were

>0.99 for MEP, MBP, MEOHP, and MBzP, >0.96 for MEHHP and MECPP, >0.94 for MiBP and MEHP, suggesting that the impacts of the use of these enzymes on the measurements of these chemicals were minimal.

### 2.3.4 Statistical methods

Analysis was conducted among controls to facilitate the generalizability of results to the entire NHS studies and to minimize the potential confounding effect of diabetes on the association between the metabolites and weight change. Baseline characteristics were summarized according to quartiles of BPA and total phthalates concentrations. Categorical variables were shown as percentages; normal-distributed continuous variables were expressed as the mean (standard deviation); non-normal-distributed continuous variables were shown as median (interquartile range). Missing data of covariates were imputed as medians of the study population (94% participants had valid values on covariates, 6% had 1~4 missing values). General linear regression was used to model the relation of urinary concentrations of BPA and phthalates with BMI at baseline. The basic models were adjusted for urinary creatinine concentration (log-transformed). In the multivariable model, we additionally adjusted for cohort origin (NHS or NHSII), age at baseline (yr), smoking (never, past, or current smoker), alcohol consumption (g/day, log-transformed), physical activity (MET-hours/week, log-transformed), AHEI score, and total energy intake (kcal/day). *P* values for linear trend were obtained by including the median concentration of each quartile as a continuous variable in the regression models. To model prospective annual weight change rate by quartiles of urinary BPA and phthalate concentrations, we used mixed-effects models with product terms between the concentrations and year after baseline. Women with at least two measurements of body weights were included in the analysis. *P* values for linear trend were obtained by examining an

interaction term between follow-up time and median concentration of the quartile in the mixed-effects models. All *P* values were two-sided. Data were analyzed with the SAS software, version 9.3 (SAS Institute, Inc., Cary, North Carolina).

## 2.4 Results

Baseline characteristics of participants were summarized in Table 2.1. Women with higher urinary concentration of BPA were on average younger, more likely to be current smokers, had a lower level of physical exercise, higher concentrations of creatinine and phthalate metabolites, and greater weight gain during follow-up. Similarly, women with higher urinary concentration of total phthalates were on average younger, had a lower level of physical exercise, higher concentrations of creatinine and BPA, and greater weight gain during follow-up.

Associations between urinary concentrations of BPA and phthalate metabolites and baseline BMI are shown in Table 2.2. Urinary BPA concentration was not associated with baseline BMI (*P*-trend=0.79). Likewise, total phthalate metabolites concentration was not associated with baseline BMI (*P*-trend=0.29). Sum of monobutyl phthalate metabolites, however, was inversely associated with baseline BMI (*P*-trend=0.02). The nonspecific metabolite – phthalic acid – was positively associated with baseline BMI (*P*-trend=0.02). The adjusted mean of baseline BMI of women in the highest quartile of phthalic acid was 26.9 (95% CI, 26.1 to 27.7), whereas the adjusted mean for women in the lowest quartile was 25.6 (95% CI, 24.8 to 26.4). All other phthalate metabolites were not associated with baseline BMI.

Table 2.3 presents the associations between quartiles of urinary concentrations of BPA and phthalate metabolites and prospective annual weight change rate. Higher quartiles of urinary BPA concentration were associated with faster weight gain during follow-up (*P*-trend=0.02).

Compared to women in the lowest BPA quartile (median 3.61 nmol/L), those in the highest quartile (median 21.91 nmol/L) had 0.22 kg greater annual weight gain (95% CI, 0.06 to 0.37 kg) during 10 years of follow-up.

Associations between urinary phthalate concentrations and weight gain showed apparent heterogeneity among different metabolites. Phthalic acid, MBzP, and sum of monobutyl phthalates showed significant associations with faster weight gain during follow-up. Specifically, compared to women in the lowest quartile of phthalic acid concentration (median 212 nmol/L), those in the highest quartile (median 1326 nmol/L) had 0.32 kg/yr faster weight gain (95% CI, 0.15 to 0.50 kg; *P*-trend=0.002). Compared to women in the lowest quartile of MBzP concentration (median 20 nmol/L), those in the highest quartile (median 252 nmol/L) had 0.41 kg/yr faster weight gain (95% CI, 0.25 to 0.56 kg; *P*-trend<0.001). Compared to women in the lowest quartile of sum monobutyl phthalates (median 67 nmol/L), those in the highest quartile (median 481 nmol/L) had 0.33 kg/yr faster weight gain (95% CI, 0.17 to 0.49 kg; *P*-trend<0.001). In contrast, MEP and DEHP metabolites were not associated with weight change in a monotonic fashion (*P*-trend=0.27 and 0.40, respectively). For the sum of all metabolites, higher total phthalate concentration (excluding phthalic acid) was associated with faster weight gain during follow-up (*P*-trend=0.04).

In secondary analysis when the analyses were stratified by age (Table 2.4) the trends of BPA and monobutyl phthalates were attenuated to lack of statistical significance probably due to diminished statistical power, although phthalic acid and MBzP were still significantly associated with faster weight gain in young and old women, respectively.

When we further stratified the analysis by baseline BMI levels, we observed a similar pattern that overall associations were attenuated although in certain strata statistically significant relationships remained (Table 2.5).

## 2.5 Discussion

To our knowledge, this is the first report of prospective associations between BPA and phthalates and weight change. In the current study among US women, we observed that higher urinary concentrations of BPA and certain phthalate metabolites (phthalic acid, MBzP, and butyl phthalates) were significantly associated with modestly faster weight gain during follow-up. Other phthalate metabolites, including MEP and DEHP metabolites, were not monotonically associated with rate of weight gain. Analyses stratified by baseline age or BMI generated somewhat heterogeneous results probably due to lower statistical power, although positive, significant associations remained in certain strata.

Previous cross-sectional studies observed significant correlations between urinary BPA concentration and obesity in children[124-129] and adults[101,130,131], with adjustment of potential confounders. In addition, the association in children differed by age (association observed in older but not younger children[126,128]), gender (in girls but not boys[125,128]), and race/ethnicity (in whites but not other races[124,129]). Evidence for adults in this regard was generated from the NHANES[130,131] and a Chinese community-based survey.[101] These studies found significant association of higher BPA concentrations with general obesity and abdominal obesity: odds ratios comparing the highest quartile to the lowest ranged from 1.50 to 1.76. Our current study did not demonstrate the same cross-sectional correlation between urinary BPA concentration and BMI at baseline. Likewise, in the EPIC-Norfolk cohort baseline BPA concentrations were not

correlated with BMI[142]. The discrepancy regarding the cross-sectional correlations between BPA and adiposity may be due to the heterogeneous routes of exposure in different populations and presence and amounts of these chemicals in foods across countries[122,143,144], various confounding pattern unique to study populations, or chance, especially for smaller studies. Nonetheless, the cross-sectional evidence cannot help establish the temporal relationship between BPA exposure and body weight. Similarly, evidence for phthalate exposures in relation to obesity is exclusively from cross-sectional investigations among children[132,133,135] and adults[103,134,135], and the results were highly inconsistent. Two studies found association between low molecular weight metabolites (e.g., MEP) and BMI in children[15,16], but the association was not replicated in another study[135]. These studies also reported sex heterogeneity of the association between phthalate metabolites and obesity. For example, studies based on NHANES data found that MEP, MBzP, MEHHP, and MEOHP were associated with larger waist circumference in men, but not in women[103,135]. A study in a Swedish population found the opposite in that a positive association between MiBP with waist circumference was observed in women but not in men[134]. Lastly, a cross-sectional study using NHANES data found inverse association between MBP concentration and BMI in older women. The current study extends this research to elucidate the longitudinal relationships between these chemicals and weight change and provides supportive evidence that these pollutants are potential obesogens[145].

Several mechanisms have been proposed to explain potential biological pathways of BPA and phthalate exposure leading to obesity. In vitro studies have suggested that BPA exposure induces the differentiation of 3T3-L1 fibroblasts into adipocytes, and accelerates the adipocyte conversion process[146]. Moreover, BPA exposure was shown to cause triacylglycerol accumulation in adipocytes, which is associated with obesity and metabolic syndrome[55]. Animal

models also show that BPA exerts estrogenic effects through binding to estrogen receptor β to cause insulin resistance and obesity[147].

Phthalates are a group of chemicals with heterogeneous structures, and may have diverse effect on health outcomes[148]. Previous animal studies document that phthalate exposures, primarily DEHP, lead to weight gain through a PPAR-mediated pathway[149]. Several phthalate monoesters have been identified as PPAR-γ agonists and capable of promoting adipocyte maturation, which is a possible mechanism linking phthalate exposure to obesity development and adipose tissue distribution[149-151]. Some nonlinear relations were observed for certain phthalate metabolites in the analyses, which have also been previously described for other endocrine disrupting chemicals[42,152]. However, such nonlinear relations need to be interpreted with caution.

There are several limitations in the current study. First, as the biologic half-lives of BPA and phthalates are relatively short, a single measurement of urine levels may not be able to represent long-time exposure levels. Specifically, in NHS and NHSII population, Townsend et al. found that within-person variability of urinary BPA concentrations was quite high (intraclass correlation coefficient=0.14), whereas most of the phthalate metabolites showed moderate within-person stability (intraclass correlation coefficient=0.39-0.55)[153]. Conversely, an investigation demonstrated that BPA levels in a single urine sample might still be reasonably informative for categorizing participants' long-term exposure levels[154]. Ideally, use of multiple 24-hr urine samples collected through an extended period of time are required to estimate long-term exposures, although in large epidemiological investigations it is challenging to obtain such data. Second, there might be potential contamination from sample containers or during sample processing. However, as indicated above, the impacts of the use of β-glucuronidase and sulfatase

on the measurements of these chemicals were shown to be minimal. The correlation of phthalic acid measurements was somewhat weaker (ICC=0.82), although any misclassification of the true phthalic acid concentration is likely to be non-differential because contamination by environmental phthalates was unrelated with true exposures. Third, the current study only included women, most of whom are white. Future studies in other populations are warranted. Fourth, because the understanding of predictors of BPA and phthalate exposure is limited, we cannot exclude the possibility of residue confounding by factors beyond the ones we controlled in the models.

In conclusion, we observed that higher urinary concentrations of BPA, phthalic acid, MBzP, and butyl phthalates were significantly associated with faster weight gain in U.S. women. The results are consistent with an etiological role of BPA and phthalates in weight gain, although we cannot exclude the possibility of chance findings, especially when associations were attenuated in stratified analyses. Future large-scale studies with repeated assessments of the levels of these chemicals are needed to replicate these observations.

## 2.6 Tables and Figures

**Table 2.1 Baseline characteristics of participants by quartiles of urinary BPA and total phthalate metabolite concentrations.**

| Characteristics | BPA | | | | Total phthalates | | | |
|---|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| Age, mean (SD), y | 57.6 (12.0) | 54.8 (11.2) | 51.1 (9.9) | 51.7 (10.2) | 57.9 (12.3) | 53.5 (10.8) | 52.3 (10.4) | 51.4 (9.8) |
| NHS, No. (%) | 131 (53.7) | 110 (45.1) | 76 (31.3) | 81 (33.2) | 135 (55.3) | 99 (40.6) | 88 (35.9) | 78 (32.0) |
| White, No. (%) | 235 (96.3) | 237 (97.1) | 239 (98.4) | 239 (98.0) | 240 (98.4) | 236 (96.7) | 240 (98.0) | 236 (96.7) |
| Smoking status, No. (%) | | | | | | | | |
| Nonsmokers | 140 (57.4) | 145 (59.4) | 147 (60.5) | 137 (56.2) | 143 (58.6) | 132 (54.1) | 150 (61.2) | 145 (59.4) |
| Past smokers | 93 (38.1) | 81 (33.2) | 75 (30.9) | 86 (35.3) | 84 (34.4) | 90 (36.9) | 77 (31.4) | 84 (34.4) |
| Current smokers | 10 (4.1) | 18 (7.4) | 20 (8.2) | 21 (8.6) | 17 (7.0) | 22 (9.0) | 16 (6.5) | 15 (6.2) |
| Alcohol consumption, median (IQR), g/d | 1.0 (0.0-5.6) | 1.5 (0.0-4.7) | 0.9 (0.0-3.8) | 0.9 (0.0-4.9) | 1.5 (0.0-5.6) | 0.9 (0.0-5.2) | 0.9 (0.0-3.5) | 1.1 (0.0-4.7) |
| AHEI, median (IQR) | 53.0 (45.3-59.6) | 51.3 (44.9-60.0) | 52.9 (45.1-58.8) | 51.5 (43.7-58.6) | 53.1 (45.5-61.1) | 51.8 (44.6-58.7) | 51.9 (44.9-59.6) | 51.5 (45.1-58.0) |
| Physical exercise, median (IQR), MET-hr/wk | 12.9 (4.0-23.2) | 12.1 (4.6-24.9) | 12.0 (5.1-25.4) | 11.1 (5.5-21.4) | 13.8 (5.1-24.8) | 12.2 (4.6-26.2) | 11.5 (5.2-21.5) | 10.9 (3.9-22.1) |
| Creatinine, median (IQR), mg/dL | 57.1 (38.7-75.7) | 61.3 (46.5-92.7) | 92.0 (65.6-127.1) | 108.5 (71.8-157.3) | 52.3 (37.6-68.0) | 71.3 (51.2-98.3) | 92.6 (62.6-125.9) | 107.3 (65.7-157.0) |
| BPA, median (IQR), nmol/L | 3.6 (2.6-4.5) | 6.4 (5.8-7.3) | 10.5 (9.0-12.1) | 21.9 (16.8-35.7) | 6.0 (3.9-9.3) | 7.6 (4.5-12.9) | 9.0 (6.1-15.0) | 10.6 (6.5-18.6) |
| Total phthalates, median (IQR), nmol/L | 835 (503-1392) | 1261 (647-2212) | 1513 (941-2559) | 1742 (911-3531) | 500 (346-602) | 962 (841-1109) | 1688 (1495-1975) | 4094 (2952-6402) |
| Phthalic acid, median (IQR), nmol/L | 307 (210-433) | 430 (254-729) | 570 (352-926) | 742 (433-1266) | 250 (181-373) | 365 (273-570) | 602 (430-901) | 1007 (664-1600) |
| Weight at year 0, mean (SD), kg | 71.0 (14.8) | 71.5 (15.1) | 70.7 (16.0) | 70.3 (13.7) | 72.1 (15.8) | 70.0 (14.5) | 71.0 (14.3) | 70.4 (14.9) |
| 10-year weight change, mean (SD), kg | 0.7 (7.3) | 2.1 (9.0) | 2.8 (8.7) | 2.9 (8.5) | 0.9 (9.6) | 2.1 (8.0) | 3.0 (8.7) | 2.4 (7.1) |

Conversion factor: BPA (nmol/L) × 0.228 = BPA (μg/L).

**Table 2.2 Associations between urinary concentrations of bisphenol A and phthalate metabolites and baseline body mass index.**

| Chemical | Quartile | Median concentration (nmol/L) | BMI (kg/m$^2$) | |
|---|---|---|---|---|
| | | | Model 1 | Model 2 |
| BPA | Q1 | 3.61 | 26.0 (25.3 to 26.8) | 26.0 (25.3 to 26.7) |
| | Q2 | 6.39 | 26.2 (25.5 to 26.9) | 26.2 (25.5 to 26.9) |
| | Q3 | 10.48 | 26.3 (25.6 to 27.0) | 26.3 (25.7 to 27.0) |
| | Q4 | 21.91 | 26.0 (25.3 to 26.7) | 26.0 (25.3 to 26.7) |
| | *P*-trend[4] | | 0.76 | 0.79 |
| Phthalic acid | Q1 | 212 | 25.6 (24.8 to 26.4) | 25.6 (24.8 to 26.4) |
| | Q2 | 369 | 25.9 (25.2 to 26.7) | 26.0 (25.3 to 26.7) |
| | Q3 | 641 | 25.8 (25.1 to 26.6) | 25.7 (25.0 to 26.5) |
| | Q4 | 1326 | 26.8 (26.1 to 27.6) | 26.9 (26.1 to 27.7) |
| | *P*-trend | | 0.03 | 0.02 |
| MEP | Q1 | 88 | 26.3 (25.6 to 27.0) | 26.3 (25.6 to 27.0) |
| | Q2 | 321 | 25.8 (25.2 to 26.5) | 25.8 (25.1 to 26.4) |
| | Q3 | 725 | 26.5 (25.9 to 27.2) | 26.6 (25.9 to 27.2) |
| | Q4 | 2198 | 25.9 (25.2 to 26.6) | 25.9 (25.2 to 26.5) |
| | *P*-trend | | 0.52 | 0.53 |
| MBzP | Q1 | 20 | 26.4 (25.6 to 27.1) | 26.5 (25.8 to 27.2) |
| | Q2 | 47 | 26.0 (25.3 to 26.6) | 26.0 (25.3 to 26.6) |
| | Q3 | 90 | 26.6 (25.9 to 27.3) | 26.5 (25.9 to 27.2) |
| | Q4 | 252 | 25.6 (24.8 to 26.3) | 25.5 (24.7 to 26.2) |
| | *P*-trend | | 0.13 | 0.07 |
| Sum butyl phthalates[1] | Q1 | 67 | 26.4 (25.6 to 27.1) | 26.6 (25.8 to 27.3) |
| | Q2 | 140 | 26.8 (26.1 to 27.4) | 26.6 (26.0 to 27.3) |
| | Q3 | 249 | 25.8 (25.1 to 26.5) | 25.8 (25.1 to 26.5) |
| | Q4 | 481 | 25.6 (24.8 to 26.3) | 25.5 (24.7 to 26.2) |
| | *P*-trend | | 0.04 | 0.02 |
| DEHP metabolites[2] | Q1 | 115 | 26.2 (25.5 to 27.0) | 26.2 (25.5 to 26.9) |
| | Q2 | 204 | 26.5 (25.8 to 27.1) | 26.5 (25.9 to 27.2) |
| | Q3 | 353 | 25.6 (24.9 to 26.2) | 25.6 (25.0 to 26.3) |
| | Q4 | 870 | 26.3 (25.5 to 27.0) | 26.2 (25.4 to 26.9) |
| | *P*-trend | | 0.95 | 0.82 |
| Total phthalates[3] | Q1 | 500 | 26.6 (25.9 to 27.4) | 26.7 (26.0 to 27.4) |
| | Q2 | 962 | 25.9 (25.3 to 26.6) | 25.9 (25.2 to 26.5) |
| | Q3 | 1688 | 26.0 (25.4 to 26.7) | 26.1 (25.4 to 26.7) |
| | Q4 | 4094 | 25.9 (25.2 to 26.6) | 25.8 (25.1 to 26.5) |
| | *P*-trend | | 0.36 | 0.29 |

Conversion factor: BPA (nmol/L) × 0.228 = BPA (μg/L).
Numbers are least square means (95% CI) of baseline body mass index (kg/m$^2$).
Model 1: adjusted for urinary creatinine concentration.
Model 2: adjusted for urinary creatinine concentration, cohort origin, age, smoking, physical activity, alcohol consumption, AHEI, total energy intake.
[1] Include MBP and MiBP.
[2] Include MEHP, MEHHP, MCEPP, and MEOHP.
[3] Include MEP, MBzP, MBP, MiBP, MEHP, MEHHP, MCEPP, and MEOHP.
[4] *P* values for linear trend were obtained by including the median concentration of each quartile as continuous variables in the regression models.

**Table 2.3 Prospective annual weight change rate by quartiles of urinary bisphenol A and phthalates metabolite concentrations.**

| Chemical | Quartile | Median concentration (nmol/L) | Weight change rate (kg/yr) | |
|---|---|---|---|---|
| | | | Model 1 | Model 2 |
| BPA | Q1 | 3.61 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 6.39 | 0.15 (0.00 to 0.31) | 0.15 (-0.01 to 0.30) |
| | Q3 | 10.48 | 0.19 (0.03 to 0.35) | 0.19 (0.03 to 0.35) |
| | Q4 | 21.91 | 0.22 (0.06 to 0.37) | 0.22 (0.06 to 0.37) |
| | *P*-trend[4] | | 0.03 | 0.02 |
| Phthalic acid | Q1 | 212 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 369 | 0.18 (0.00 to 0.35) | 0.20 (0.03 to 0.37) |
| | Q3 | 641 | 0.19 (0.02 to 0.36) | 0.20 (0.03 to 0.37) |
| | Q4 | 1326 | 0.32 (0.15 to 0.49) | 0.32 (0.15 to 0.50) |
| | *P*-trend | | 0.001 | 0.002 |
| MEP | Q1 | 88 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 321 | -0.01 (-0.17 to 0.15) | -0.01 (-0.17 to 0.14) |
| | Q3 | 725 | 0.16 (0.00 to 0.32) | 0.16 (0.00 to 0.32) |
| | Q4 | 2198 | 0.07 (-0.09 to 0.22) | 0.08 (-0.08 to 0.24) |
| | *P*-trend | | 0.40 | 0.27 |
| MBzP | Q1 | 20 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 47 | 0.27 (0.12 to 0.43) | 0.29 (0.13 to 0.44) |
| | Q3 | 90 | 0.34 (0.19 to 0.50) | 0.33 (0.18 to 0.49) |
| | Q4 | 252 | 0.42 (0.26 o 0.57) | 0.41 (0.25 to 0.56) |
| | *P*-trend | | <0.001 | <0.001 |
| Sum butyl phthalates[1] | Q1 | 67 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 140 | 0.20 (0.04 to 0.35) | 0.16 (0.00 to 0.32) |
| | Q3 | 249 | 0.23 (0.08 to 0.39) | 0.21 (0.05 to 0.37) |
| | Q4 | 481 | 0.36 (0.20 to 0.52) | 0.33 (0.17 to 0.49) |
| | *P*-trend | | <0.001 | <0.001 |
| DEHP metabolites[2] | Q1 | 115 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 204 | 0.04 (-0.12 to 0.20) | 0.03 (-0.13 to 0.18) |
| | Q3 | 353 | 0.28 (0.13 to 0.44) | 0.27 (0.11 to 0.43) |
| | Q4 | 870 | 0.08 (-0.08 to 0.24) | 0.09 (-0.07 to 0.24) |
| | *P*-trend | | 0.51 | 0.40 |
| Total phthalates[3] | Q1 | 500 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 962 | 0.08 (-0.08 to 0.24) | 0.07 (-0.08 to 0.23) |
| | Q3 | 1688 | 0.20 (0.05 to 0.36) | 0.20 (0.04 to 0.36) |
| | Q4 | 4094 | 0.16 (0.00 to 0.32) | 0.18 (0.02 to 0.34) |
| | *P*-trend | | 0.08 | 0.04 |

Conversion factor: BPA (nmol/L) × 0.228 = BPA (μg/L).

Numbers are adjusted prospective weight change rate (kg/year) and 95% CI, relative to the rate of individuals in the 1st quartile.

Model 1: adjusted for urinary creatinine concentration.

Model 2: adjusted for urinary creatinine concentration, cohort origin, age, smoking, physical activity, alcohol consumption, AHEI, total energy intake, and baseline body weight.

[1] Include MBP and MiBP.

[2] Include MEHP, MEHHP, MCEPP, and MEOHP.

[3] Include MEP, MBzP, MBP, MiBP, MEHP, MEHHP, MCEPP, and MEOHP.

[4] *P* values for linear trend were obtained by examining an interaction term between follow-up duration and median concentration of the quartiles in the mixed-effects models.

**Table 2.4 Prospective annual weight change rate by quartiles of urinary bisphenol A and phthalates metabolite concentrations by age at baseline.**

| Chemical | Quartile | Median concentration (nmol/L) | Weight change rate (kg/yr) | |
|---|---|---|---|---|
| | | | Age ≤ 53 | Age > 53 |
| BPA | Q1 | 3.61 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 6.39 | 0.09 (-0.17 to 0.34) | 0.15 (-0.04 to 0.33) |
| | Q3 | 10.48 | 0.07 (-0.17 to 0.31) | 0.08 (-0.12 to 0.29) |
| | Q4 | 21.91 | 0.14 (-0.11 to 0.39) | 0.08 (-0.12 to 0.28) |
| | *P*-trend[4] | | 0.33 | 0.67 |
| Phthalic acid | Q1 | 212 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 369 | 0.21 (-0.04 to 0.45) | 0.04 (-0.20 to 0.28) |
| | Q3 | 641 | 0.18 (-0.06 to 0.43) | 0.10 (-0.14 to 0.34) |
| | Q4 | 1326 | 0.29 (0.06 to 0.52) | 0.13 (-0.14 to 0.40) |
| | *P*-trend | | 0.04 | 0.32 |
| MEP | Q1 | 88 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 321 | -0.19 (-0.44 to 0.05) | 0.11 (-0.08 to 0.31) |
| | Q3 | 725 | 0.02 (-0.21 to 0.26) | 0.10 (-0.11 to 0.30) |
| | Q4 | 2198 | 0.03 (-0.22 to 0.27) | 0.03 (-0.17 to 0.23) |
| | *P*-trend | | 0.33 | 0.85 |
| MBzP | Q1 | 20 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 47 | 0.22 (-0.09 to 0.52) | 0.10 (-0.08 to 0.27) |
| | Q3 | 90 | 0.18 (-0.12 to 0.48) | 0.05 (-0.15 to 0.25) |
| | Q4 | 252 | 0.20 (-0.09 to 0.50) | 0.24 (0.01 to 0.48) |
| | *P*-trend | | 0.56 | 0.06 |
| Sum butyl phthalates[1] | Q1 | 67 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 140 | -0.02 (-0.32 to 0.27) | 0.06 (-0.11 to 0.24) |
| | Q3 | 249 | -0.03 (-0.31 to 0.26) | -0.03 (-0.23 to 0.18) |
| | Q4 | 481 | 0.08 (-0.21 to 0.36) | 0.17 (-0.06 to 0.40) |
| | *P*-trend | | 0.34 | 0.23 |
| DEHP metabolites[2] | Q1 | 115 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 204 | 0.02 (-0.23 to 0.28) | -0.03 (-0.22 to 0.15) |
| | Q3 | 353 | 0.13 (-0.11 to 0.37) | 0.26 (0.05 to 0.46) |
| | Q4 | 870 | -0.08 (-0.33 to 0.17) | 0.10 (-0.10 to 0.29) |
| | *P*-trend | | 0.32 | 0.24 |
| Total phthalates[3] | Q1 | 500 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 962 | 0.01 (-0.25 to 0.26) | 0.07 (-0.11 to 0.26) |
| | Q3 | 1688 | 0.10 (-0.14 to 0.35) | 0.10 (-0.10 to 0.29) |
| | Q4 | 4094 | 0.10 (-0.15 to 0.34) | 0.07 (-0.14 to 0.27) |
| | *P*-trend | | 0.41 | 0.61 |

Conversion factor: BPA (nmol/L) × 0.228 = BPA (μg/L).

Numbers are adjusted prospective weight change rate (kg/year) and 95% CI, relative to the rate of individuals in the 1st quartile.

Model adjusted for urinary creatinine concentration, cohort origin, age, smoking, physical activity, alcohol consumption, AHEI, total energy intake, and baseline body weight.

[1] Include MBP and MiBP.

[2] Include MEHP, MEHHP, MCEPP, and MEOHP.

[3] Include MEP, MBzP, MBP, MiBP, MEHP, MEHHP, MCEPP, and MEOHP.

[4] *P* values for linear trend were obtained by examining an interaction term between follow-up duration and median concentration of the quartiles in the mixed-effects models.

**Table 2.5 Prospective annual weight change rate by quartiles of urinary bisphenol A and phthalates metabolite concentrations by baseline BMI.**

| Chemical | Quartile | Median concentration (nmol/L) | Weight change rate (kg/yr) | |
|---|---|---|---|---|
| | | | Baseline BMI ≤ 25 | Baseline BMI > 25 |
| BPA | Q1 | 3.61 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 6.39 | 0.19 (0.01 to 0.38) | 0.16 (-0.11 to 0.44) |
| | Q3 | 10.48 | 0.21 (0.03 to 0.39) | 0.20 (-0.08 to 0.49) |
| | Q4 | 21.91 | 0.24 (0.06 to 0.43) | 0.19 (-0.10 to 0.48) |
| | *P*-trend[4] | | 0.04 | 0.30 |
| Phthalic acid | Q1 | 212 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 369 | 0.10 (-0.10 to 0.30) | 0.28 (-0.04 to 0.60) |
| | Q3 | 641 | 0.10 (-0.10 to 0.30) | 0.31 (-0.02 to 0.63) |
| | Q4 | 1326 | 0.21 (0.00 to 0.41) | 0.49 (0.18 to 0.81) |
| | *P*-trend | | 0.06 | 0.006 |
| MEP | Q1 | 88 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 321 | -0.17 (-0.35 to 0.01) | 0.13 (-0.16 to 0.42) |
| | Q3 | 725 | -0.10 (-0.30 to 0.09) | 0.36 (-0.16 to 0.42) |
| | Q4 | 2198 | -0.05 (-0.23 to 0.13) | 0.25 (-0.05 to 0.54) |
| | *P*-trend | | 0.80 | 0.18 |
| MBzP | Q1 | 20 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 47 | 0.13 (-0.05 to 0.31) | 0.48 (0.21 to 0.75) |
| | Q3 | 90 | 0.26 (0.07 to 0.45) | 0.45 (0.19 to 0.72) |
| | Q4 | 252 | 0.29 (0.11 to 0.47) | 0.68 (0.39 to 0.96) |
| | *P*-trend | | 0.006 | 0.0001 |
| Sum butyl phthalates[1] | Q1 | 67 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 140 | 0.16 (-0.03 to 0.35) | 0.21 (-0.05 to 0.47) |
| | Q3 | 249 | 0.15 (-0.04 to 0.32) | 0.32 (0.05 to 0.60) |
| | Q4 | 481 | 0.22 (0.04 to 0.41) | 0.62 (0.33 to 0.90) |
| | *P*-trend | | 0.04 | <0.0001 |
| DEHP metabolites[2] | Q1 | 115 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 204 | 0.10 (-0.09 to 0.29) | -0.02 (-0.29 to 0.26) |
| | Q3 | 353 | 0.16 (-0.02 to 0.34) | 0.48 (0.20 to 0.76) |
| | Q4 | 870 | 0.09 (-0.10 to 0.28) | 0.06 (-0.21 to 0.34) |
| | *P*-trend | | 0.64 | 0.75 |
| Total phthalates[3] | Q1 | 500 | 0.00 (Reference) | 0.00 (Reference) |
| | Q2 | 962 | -0.05 (-0.24 to 0.13) | 0.27 (-0.01 to 0.54) |
| | Q3 | 1688 | -0.06 (-0.25 to 0.12) | 0.46 (0.19 to 0.73) |
| | Q4 | 4094 | 0.05 (-0.13 to 0.24) | 0.36 (0.08 to 0.64) |
| | *P*-trend | | 0.35 | 0.04 |

Conversion factor: BPA (nmol/L) × 0.228 = BPA (μg/L).

Numbers are adjusted prospective weight change rate (kg/year) and 95% CI, relative to the rate of individuals in the 1st quartile.

Model adjusted for urinary creatinine concentration, cohort origin, age, smoking, physical activity, alcohol consumption, AHEI, total energy intake, and baseline body weight.

[1] Include MBP and MiBP.

[2] Include MEHP, MEHHP, MCEPP, and MEOHP.

[3] Include MEP, MBzP, MBP, MiBP, MEHP, MEHHP, MCEPP, and MEOHP.

[4] *P* values for linear trend were obtained by examining an interaction term between follow-up duration and median concentration of the quartiles in the mixed-effects models.

# CHAPTER 3. Birth Weight, Mediating Biological Intermediates, and the Development of Type 2 Diabetes Later in Life: a Prospective Study of Multiethnic Women

## 3.1 Abstract

**IMPORTANCE**: Previous studies have associated low birth weight (LBW) with higher risk of type 2 diabetes (T2D) although whether any of the established T2D biomarkers contribute to this association remains unclear.

**OBJECTIVE**: To investigate the prospective relation between LBW and T2D and the mediation effects of validated T2D biomarkers linking LBW to T2D risk.

**DESIGN, SETTING, AND PARTICIPANTS**: A prospective case-control study nested in the Women's Health Initiative Observational Cohort. The recruitment of participants started between 1993~1998 with a median follow-up period of 6 years. We measured baseline plasma biomarkers of insulin resistance, leptin and its receptor, sex steroids and their binding protein, inflammation, endothelial function, and cellular aging in 1259 incident T2D cases and 1790 matched controls.

**EXPOSURE**: LBW (<2.72 kg).

**MAIN OUTCOMES AND MEASURES**: Odds ratios for the LBW and T2D relation. The total effect of LBW on T2D risk was partitioned into effects that were mediated by the specific biomarkers ("indirect effect") and effects that are through other mechanisms including as yet unknown pathways ("direct effect"), using a counterfactual model based mediation analysis strategy.

**RESULTS**: LBW was significantly associated with increased risk of T2D. Compared to women with birth weight between 3.63~4.54 kg, women with LBW (<2.72 kg) had a multivariable-

adjusted odds ratio of 2.15 (95% CI, 1.54 to 3.00). Insulin resistance [indicated by homeostatic model assessment – insulin resistance (HOMA-IR)] mediated 47% of total effect (95% CI, 0 to 123%). Decreased sex hormone-binding globulin (SHBG) concentration accounted for 24% (95% CI, 5 to 51%), elevated E-selectin concentration accounted for 25% (95% CI, 5 to 63%), and increased systolic blood pressure accounted for 8% (95% CI, 2 to 19%) of the total effect of LBW on T2D risk.

**CONCLUSIONS AND RELEVANCE**: LBW is directly predictive of higher risk of T2D later in life. The effect of LBW on T2D risk seems mainly mediated by insulin resistance, which is further explained by circulating levels of SHBG, E-selectin, and systolic blood pressure. These prospective data provide quantifiable mechanistic evidence linking LBW to increased risk of T2D whilst presenting risk stratification and intervention in a population at greater risk of developing T2D later in life.

## 3.2 Introduction

Previous prospective studies have shown that low birth weight (LBW), an indicator of intrauterine growth restriction, was predictive of higher risk of type 2 diabetes (T2D) in adulthood[24]. Impairments in "fetal programming", as reflected by LBW, promote adverse effects on physiology, metabolism, and hormonal function during critical phases of fetal development[25]. Insulin insensitivity has been speculated as in mechanistic pathways by which LBW leads to T2D[155]. In previous studies, birth weight was associated with alteration in biomarkers of insulin resistance[12-14], cellular aging[15], inflammation[16], endothelial dysfunction[17,18], blood pressure[19], obesity[156], and sex steroid hormones and their binding proteins[20-23], all of which increase the risk of T2D. However, how much each of these biomarkers contributes to the LBW-T2D relation remains unknown.

An improved understanding of the mechanisms through which LBW may influence T2D risk may improve clinical risk stratification and intervention of the disease. For instance, an accurate assessment of the specific biomarker mediating the LBW-T2D relation is helpful in monitoring or preventing the development of T2D for those who had suffered from LBW. Herein we used the newly developed statistical methodology to quantify potential mediators of biological significance that may link LBW to increased T2D risk later in life. Specifically, using mediation modeling, we comprehensively assessed the effect of LBW on T2D risk that is explained by potential mediators, including validated biomarkers of insulin resistance, leptin and its receptor, sex steroid hormones and their binding protein, inflammation, endothelial function, cellular aging, and blood pressure.

## 3.3 Methods

### 3.3.1 Study population

The Women's Health Initiative (WHI) is a long-term study focused on strategies for preventing chronic diseases in postmenopausal women. The original WHI study included 161,808 postmenopausal women enrolled between 1993 and 1998. The WHI has two major components: Clinical Trial (CT) and Observational Study (OS) – both were conducted at 40 Clinical Centers nationwide. We investigated participants in the OS, which examined the relationship between lifestyle, environmental, medical, and molecular risk factors and specific measures of health or disease outcomes. This WHI-OS involves tracking the medical history and health habits of 93,676 women not participating in the CT. The current study was built on a series of prospective case-control studies we have completed previously to investigate the association between different biomarkers and risk of T2D in WHI-OS[80,157-159], in which the measurements of blood biomarkers were available. The study was reviewed and approved by human subjects review committees at each participating institution, and signed informed consent was obtained from all women enrolled.

### 3.3.2 Ascertainment of incident diabetes

Participants in WHI-OS were followed by annual mailed questionnaires and an additional clinical center visit for physical measurements 3 years after enrollment. Of the 93,676 postmenopausal women enrolled into the WHI-OS cohort, 82,069 (87.6%) women had no prior history of diabetes or CVD. Incident cases of diabetes were identified based on post-baseline self-report of first-time use of hypoglycemic medication (oral hypoglycemic agents or insulin) during a median follow-up period of 6 years. Self-reported diabetes validated against medication histories yielded a positive predictive value of 72% and negative predictive values of >99.9%[160].

Following the principle of risk-set sampling, for each incident case, appropriate control subjects were selected at random from women who remained free of CVD and/or diabetes at the diagnosed time in the case patient. Control participants were matched to the incident cases by age, race/ethnicity, clinical center, time of blood draw, and length of follow-up.

### 3.3.3 Measurement of birth weight and covariates

WHI participants were asked questions regarding their own birth weight (4 categories: <2.72 kg, 2.72 kg to 3.63 kg, 3.63 kg to 4.54 kg, ≥4.54 kg). LBW was defined as a birth weight less than 2.72 kg (6 lbs.). We excluded women who reported that they were born pre-term, or a multiple (twin, triplet, or quadruple).

Self-administered questionnaires were used to collect information on demographic characteristics and lifestyle factors at study entry into the WHI. Participants were categorized into "never smokers," "former smokers," and "current smokers" according to their smoking history. Alcohol and total energy intakes were calculated from food frequency questionnaires. Body weight, height, and waist and hip circumferences were measured at baseline entry into the WHI. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared, and waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. The level of physical activity in metabolic equivalent hours per week was estimated on the basis of self-reported duration of different types of exercise, weighted by intensity levels. Blood pressure was measured using standardized procedures and instruments at the WHI enrollment visit.

### 3.3.4 Measurement of biomarkers

Fasting blood samples were collected at study entry into the WHI. Details of the biomarker assays have been described elsewhere[80,157-159] (also see Appedix). In brief, fasting

plasma glucose and high-sensitivity C-reactive protein (hsCRP) were measured on a Hitachi 911 chemistry analyzer. We used enzyme-linked immunosorbent assays to measure plasma concentrations of fasting insulin, leptin, soluble leptin receptor, tumor necrosis factor α receptor 2 (TNF-α–R2), interleukin 6 (IL-6), soluble E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1). Plasma concentrations of estradiol, testosterone, and sex-hormone binding globulin (SHBG) were measured by electrochemiluminescence immunoassays. Leukocyte telomere length was measured using a real-time PCR-based method.

### 3.3.5 Statistical analysis

Baseline characteristics were summarized according to T2D case and control status. The distributions of all the potential intermediate variables were summarized according to birth weight. Categorical variables were shown as percentage; normal-distributed continuous variables were shown as mean (standard deviation); non-normal-distributed continuous variables were shown as geometric mean (95% confidence interval).

We excluded 167 participants with missing information on family history of diabetes. In all regression models, missing values of other covariates were imputed using median values (95.8% of the participants had no missing covariates besides family history of diabetes; 3.84% had one, and 0.37% had 2~4 missing covariates). Logistic regression was used to assess the association between birth weight and T2D risk. Our primary goal was to estimate the "indirect effect" of LBW on T2D risk, which is mediated by specific biomarkers after accounting for potential confounding by measured covariates (Figure 3.1 shows the potential causal structure in the current study). Two sets of confounders were considered: the first set ($C_1$) was determined before birth, including race/ethnicity (white, black, Hispanic, or Asian/Pacific Islander) and

family history of diabetes (yes or no); the second set ($C_2$) was determined after birth, including age (6 categories), smoking (3 categories), alcohol consumption (6 categories), physical exercise (5 categories), dietary fiber intake (4 categories), dietary glycemic load (4 categories), and BMI (6 categories). Linear regression was used to model biomarker concentrations by terms of birth weight and potential confounders, and logistic regression was used to model T2D status. The linear regression for biomarkers was weighted by $\pi/p$ for cases and by $(1-\pi)/(1-p)$ for controls to account for our case-control sampling strategy[161], where $\pi$ denotes population prevalence of T2D and $p$ denotes the proportion of cases in the current study. All the intermediate variables were log-transformed and then standardized using the mean and standard deviation of the control population (systolic and diastolic blood pressure were not log-transformed because of the normality of their distributions). After these transformations, the distributions of intermediate variables were close to normal. The regression for biomarkers and the regression for T2D risk were integrated to obtain both direct and indirect effects using odds ratios (OR) for mediation analysis for a dichotomous outcome as proposed by VanderWeele et al.[161]. The "indirect effect" was the OR for T2D for those who had LBW comparing the risk if the biomarker levels were what it would have been with low versus normal birth weight, i.e. the effect that is mediated by the specific biomarker. The "direct effect" was the OR for T2D among persons who had LBW versus those who had normal birth weight if the biomarker levels were what it would have been with normal birth weight, i.e. the effect that was through all other mechanisms besides the specific biomarker including as yet unknown pathways. The proportion of mediating effects was calculated in the risk difference scale following $OR_{direct} \times (OR_{indirect} - 1) / (OR_{direct} \times OR_{indirect} - 1)$. 95% confidence intervals (CIs) of direct effects, indirect effects, and proportions mediated were obtained via bootstrapping. *P* values for multiplicative interaction were obtained using Wald test

of the interaction coefficient in the logistic regression. All the statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC).

### 3.4 Results

A total of 1259 T2D cases and 1790 controls were included in the current study, although the actual number of participants entered in the analyses differed by specific biomarkers. T2D cases had a higher prevalence of traditional diabetes risk factors at baseline than controls (Table 3.1): They tended to be older, smokers, less physically active, overweight or obese, and had a family history of diabetes. Women with incident T2D had significantly higher levels of HOMA-IR, leptin, free estradiol, total and free testosterone, TNF-α-R2, IL-6, hsCRP, E-selection, ICAM-1, VCAM-1, and systolic blood pressure at baseline, whereas the controls had significantly higher levels of HOMA-β, soluble leptin receptor, SHBG, and leukocyte telomere length. The proportion of women with LBW was significantly higher in incident T2D cases than in controls ($P = 0.01$).

LBW women had higher levels of HOMA-IR, HOMA-β, soluble leptin receptor, E-selectin, leukocyte telomere length, and systolic blood pressure, and lower levels of leptin, total and free estradiol, total testosterone, SHBG, TNF-α-R2, IL-6, and hsCRP (Table 3.2). LBW was significantly associated with an increased risk of T2D ($P$-trend<0.001, Figure 3.2). Compared to women with a birth weight between 3.63~4.54 kg, LBW women had a multivariable-adjusted OR of 2.15 (95% CI, 1.54 to 3.00). We did not observe significant interaction between any of the mediators with LBW (Table 3.3). Thus, models assuming no interaction between exposure and mediators were adopted. Four of the biomarkers (HOMA-IR, SHBG, E-selectin, and systolic blood pressure) in our study mediated relatively minor to moderate amounts of the total effect.

Insulin resistance (indicated by HOMA-IR), being a necessary cause of T2D, mediated 47% of the total effect (95% CI, 0 to 123%). As the indicator of abdominal obesity, WHR is a direct risk factor of insulin resistance, mediating 14% of the total effect (14%; 95% CI, -9 to 37%). In contrast, the effect of LBW on T2D risk mediated by β-cell function seemed to be in the opposite direction, although it did not reach statistically significance (OR = 0.93; 95% CI, 0.84 to 1.03).

SHBG levels significantly mediated the LBW-T2D relation (OR = 1.16; 95 CI: 1.03, 1.33) accounting for 24% (95% CI, 5 to 51%) of the total effect of LBW on T2D. In contrast, neither estradiol nor testosterone mediated any significant amount of the total effect. E-selectin also accounted for 25% (95% CI, 5 to 63%) of the total effect of LBW on T2D (OR of indirect effect = 1.12; 95% CI: 1.02, 1.23), but other markers of endothelial function (ICAM-1 and VCAM-1) did not quantitatively account for the effect of LBW on T2D. The indirect effect that was mediated by systolic blood pressure was significant (OR = 1.03; 95% CI, 1.01, 1.07), although it only mediated 8% (95% CI, 2 to 19%) of the total effect. Diastolic blood pressure did not explain the total effect. In total, SHBG, E-selectin, and systolic blood pressure mediated 32% of the total effect of LBW on T2D risk (95% CI, 12 to 72%). Leptin, soluble leptin receptor, inflammation markers (TNF-α-R2, IL-6, and hsCRP), and leukocyte telomere length did not mediate any significant amount of the total effect of LBW on T2D. When we used HOMA-IR and HOMA-β as the outcomes (Table 3.4), SHBG, E-selectin, and systolic blood pressure explained 33%, 28%, and 13% of the total effect on insulin resistance due to LBW, respectively. In contrast, none of these biomarkers appeared to mediate a significant amount of the effect on β-cell function. In a sensitivity analysis using traditional "difference" method comparing the regression coefficients of the models with and without the specific biomarkers as mediators, the

proportions appeared in the same directions although the magnitudes varied (20% for SHBG, 26% for E-selection, and 11% for systolic blood pressure).

### 3.5 Discussion

In this prospective study among US women, we confirmed that LBW significantly increased T2D risk later in life. Further, we found that mainly insulin resistance mediated a considerable amount of the total effect on T2D risk due to LBW. This effect was further mediated by low SHBG concentration, elevated blood E-selectin, and increased systolic pressure. β-cell function, in contrast, may counteract a portion of the effect on T2D risk due to LBW that acts through insulin resistance, although this effect was not statistically significant.

Both animal and human studies have consistently observed significant associations between LBW – a result and surrogate of early nutrition inadequacy – and higher T2D risk. A systematic review of previous population studies investigating this relationship found that, in most populations studied, birth weight was inversely associated to T2D risk, and the pooled OR for T2D was 0.75 (95% CI, 0.70 to 0.81) per kg[24]. Although an appreciable number of studies have supported this inverse association, the potential mechanisms remain unknown[162]. To examine the potential influence of excluding prevalent cases at baseline on the estimates of the association between birth weight and T2D risk, we also modeled the relationship of birth weight to prevalent T2D cases among the whole WHI-OS cohort (unpublished data by Ryckman et al.). We observed similar inverse associations as with the incident cases ($P$ for trend < 0.001).

Previous studies indicate that the increased susceptibility of T2D may be predominantly determined by epigenetic variations in early life[163]. Nonetheless, proximal mediators of the effect, i.e. changes of T2D-related biomarkers before onset of the disease, is more relevant to

targeted T2D prevention among LBW persons yet this topic remains understudied. Statistical analysis of mediation was first proposed in psychological and social sciences[164,165] and has been further developed in the framework of causal analysis[161,166]. Under appropriate causal structures justified by substantive scientific knowledge, mediation analysis measures the extent that specific intermediate factors contribute to the total effect of an exposure on an outcome of interest, thus addressing directly the questions of how and why the specific exposures and outcomes of interest are related[161,165]. Our study evaluated the potential mediation effects of validated T2D biomarkers, and observed several biomarkers that may be partially determined by birth weight and also predict T2D risk later in life.

Insulin resistance is a characteristic underpinning for T2D pathogenesis and a defining feature of metabolic syndrome. Previous studies have associated several surrogate markers of insulin resistance with LBW[12]. Animal work has shown that insufficient intrauterine nutrition may lead to growth retardation characterized by diminished skeletal muscle mass and fewer insulin-producing pancreatic islets – both observations highly correlated respectively with insulin resistance and β-cell dysfunction in adulthood[167]. Our data appear to indicate that insulin resistance, rather than β-cell function, may play a more significant mediating effect on T2D risk due to LBW, though our assessment of β-cell function using HOMA-β model may be less than ideal in assessing β-cell function.

Hepatokines, proteins that are exclusively or predominantly secreted by the liver, were known to directly affect glucose metabolism[168,169]. SHBG may be yet another important hepatokines[170] in the regulation of glucose homeostasis[20,171]. Recent prospective studies have identified SHBG as an independent risk factor of T2D[20,80]. In three prospective cohorts of men and women, we have consistently observed that higher concentration of plasma SHBG was

predictive of considerable lower risk of T2D[20,80]. Several germ-line mutations in the SHBG gene were also identified for T2D susceptibility[20]. Individuals born with LBW tend to have lower levels of plasma SHBG[22,23]. In the current study, we confirmed that decreased SHBG concentration explained approximately one quarter of the total effect due to LBW.

Endothelial dysfunction, measured by circulating E-selectin and ICAM-1 concentrations, has been associated with insulin resistance[42,43] and also elevated T2D risk[158]. LBW was associated with endothelial dysfunction measured by ultrasonic-based approach in different populations[17,18]. E-selectin is arguably a more sensitive and robust biomarker of early endothelial dysfunction given its exclusive expression in endothelial cells, compared to other biomarkers of endothelial function[172]. However, no previous studies have reported the association between birth weight and E-selectin concentration. In the current study, E-selectin concentration was significantly higher in LBW women than normal birth weight counterparts. In addition, we observed that increased E-selectin concentration accounted for 25% of the total effect on T2D risk due to LBW. High blood pressure was also shown to be associated with endothelial dysfunction[173]. In addition, high blood pressure is regarded as an important component of the metabolic syndrome, and is often concomitant with the presence of T2D. However, high blood pressure was also found to be a strong predictor of T2D, independent of traditional risk factors of T2D and antihypertensive medication[174,175]. In the current study, we found that systolic blood pressure was significantly elevated in LBW compared with normal birth weight women. Moreover, increased systolic blood pressure explains a moderate but significant amount of the total effect of LBW on T2D risk.

Several issues of our study are worthy of further discussion. First, self-report of LBW may have measurement errors. However, previous work has shown that self-reported birth

weight was correlated reliably with that from birth certificates ($r$=0.64 to 0.83)[176,177]. Moreover, characterizing birth weight data into a LBW vs. normal birth weight group may further reduce the probability of misclassification (as opposed to treating birth weight as a continuous variable in previous reliability studies). Second, the validity of our study is based on the assumption that we have sufficiently controlled the confounding of the exposure-mediator, exposure-outcome, and mediator-outcome relationships. To the extent possible, we have included all the possible covariates that may confound these relationships. Also, we have grouped all the covariates into multiple categories to avoid residual confounding due to potential nonlinear relationships. Finally, we used the novel counterfactual model-based mediation analysis over the traditional method that compares differences of the regression coefficients between the models with and without the mediators. The traditional "difference method" cannot address the issues due to noncollapsibility when logistic regression models are used[178], whereas the method we chose models the association between exposure and mediator separately to circumvent the problem of noncollapsibility.

In the current study, we confirmed that LBW was consistently associated with increased risk of T2D later in life in a multiethnic population of women. In addition, we found that the total effect of LBW on risk of T2D is mainly mediated by insulin resistance which is further explained by circulating levels of SHBG, E-selectin, and systolic blood pressure. These prospective data provide quantifiable mechanistic evidence linking LBW to increased risk of T2D whilst presenting risk stratification and intervention in a population at greater risk of developing T2D later in life.

## 3.6 Tables and Figures

### Table 3.1 Baseline Characteristics of Participants According to Type 2 Diabetes Case/Control Status.

| Characteristic | T2D Cases (*n* = 1259) | Controls (*n* = 1790) | *P*[a] |
|---|---|---|---|
| Low birth weight[b], n (%) | 149 (11.8) | 162 (9.1) | 0.01 |
| Age, mean (SD), y | 62.6 (6.9) | 62.0 (7.0) | 0.03 |
| **Genetic influence** | | | |
| Family history of diabetes, n (%) | 673 (56.7) | 612 (36.1) | <0.001 |
| Race/ethnicity, n (%) | | | <0.001 |
| White | 792 (62.9) | 834 (46.6) | |
| Black | 286 (22.7) | 588 (32.9) | |
| Hispanic | 110 (8.7) | 234 (13.1) | |
| Asian/Pacific Islander | 71 (5.6) | 133 (7.4) | |
| **Behavioral factors** | | | |
| Smoking, n (%) | | | 0.003 |
| Never | 605 (48.7) | 972 (55.0) | |
| Former | 532 (42.8) | 669 (37.8) | |
| Current | 106 (8.5) | 128 (7.2) | |
| Alcohol consumption, n (%) | | | 0.002 |
| Never | 199 (16.0) | 264 (14.8) | |
| Former | 324 (26.0) | 377 (21.1) | |
| Current | 723 (58.0) | 1143 (64.1) | |
| Physical exercise, geometric mean (95% CI), MET-h/wk | 7.89 (7.36-8.46) | 9.69 (9.19-10.22) | <0.001 |
| Dietary fiber intake, geometric mean (95% CI), g/d | 14.2 (13.8-14.6) | 13.9 (13.5-14.2) | 0.23 |
| Dietary glycemic load, geometric mean (95% CI) | 90.9 (88.4-93.5) | 86.6 (84.6-88.7) | 0.01 |
| BMI, geometric mean (95% CI), kg/m$^2$ | 31.7 (31.3-32.0) | 26.9 (26.7-27.2) | <0.001 |
| **Biomarkers** | | | |
| Insulin sensitivity and β-cell function, geometric mean (95% CI) | | | |
| WHR | 0.86 (0.86-0.87) | 0.80 (0.79-0.80) | <0.001 |
| HOMA-IR | 3.91 (3.73-4.10) | 1.50 (1.45-1.55) | <0.001 |
| HOMA-β | 69.9 (66.5-73.4) | 83.9 (81.4-86.4) | <0.001 |
| Leptin and leptin receptor, geometric mean (95% CI) | | | |
| Leptin, ng/mL | 29.1 (27.1-31.1) | 22.8 (21.7-24.1) | <0.001 |
| Soluble leptin receptor, ng/mL | 32.0 (31.1-33.0) | 33.6 (33.0-34.3) | 0.007 |
| Sex steroids and SHBG, geometric mean (95% CI) | | | |
| Total estradiol, pg/mL | 20.5 (18.7-22.5) | 19.8 (18.4-21.3) | 0.54 |
| Free estradiol, pg/mL | 0.29 (0.27-0.32) | 0.22 (0.20-0.23) | <0.001 |
| Total testosterone, ng/dL | 11.6 (10.6-12.7) | 8.9 (8.4-9.6) | <0.001 |
| Free testosterone, ng/dL | 0.12 (0.10-0.13) | 0.06 (0.06-0.07) | <0.001 |
| SHBG, nmol/L | 45.4 (42.6-48.3) | 74.0 (71.0-77.2) | <0.001 |
| Inflammation, geometric mean (95% CI) | | | |
| TNF-α-R2, pg/mL | 2660 (2610-2710) | 2350 (2320-2390) | <0.001 |
| IL-6, pg/mL | 2.94 (2.80-3.10) | 1.76 (1.68-1.83) | <0.001 |

| | | | |
|---|---|---|---|
| hsCRP, mg/L | 0.40 (0.37-0.43) | 0.19 (0.18-0.21) | <0.001 |
| Endothelial dysfunction, geometric mean (95% CI) | | | |
| E-selectin, ng/mL | 48.3 (46.7-49.9) | 34.3 (33.4-35.2) | <0.001 |
| ICAM-1, ng/mL | 320 (313-327) | 268 (263-274) | <0.001 |
| VCAM-1, ng/mL | 750 (732-767) | 668 (656-680) | <0.001 |
| Cellular aging, geometric mean (95% CI) | | | |
| Leukocyte telomere length, kb | 3.68 (3.60-3.76) | 3.87 (3.80-3.94) | <0.001 |
| Blood pressure, mean (SD) | | | |
| Systolic blood pressure, mmHg | 132 (17) | 127 (18) | <0.001 |
| Diastolic blood pressure, mmHg | 77 (10) | 76 (9) | 0.05 |

Abbreviations: BMI, body mass index; HOMA-β, homeostatic model assessment-β cell function; HOMA-IR, homeostatic model assessment-insulin resistance; hsCRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin 6; MET-h, metabolic equivalent hours; SHBG, sex hormone-binding globulin; TNF-α-R2, tumor necrosis factor α receptor 2; VCAM-1, vascular cell adhesion molecule-1; WHR, waist-to-hip ratio.

[a] *P* values were obtained from $\chi^2$ tests for categorical variables, from Student's *t*-tests for continuous variables.

[b] Low birth weight: self-reported birth weight<2.72 kg (6 lbs.).

**Table 3.2 Characteristics of Type 2 Diabetes-Related Biomarkers According to Birth Weight Groups in the Control Population.**

| Variable | Low birth weight[a] (*n* = 162) | Normal birth weight (*n* = 1628) | *P*[b] |
|---|---|---|---|
| Insulin sensitivity and β-cell function, geometric mean (95% CI) | | | |
| WHR | 0.80 (0.79-0.81) | 0.79 (0.79-0.80) | 0.30 |
| HOMA-IR | 1.56 (1.39-1.76) | 1.49 (1.44-1.55) | 0.44 |
| HOMA-β | 87.9 (79.1-97.6) | 83.5 (80.9-86.1) | 0.34 |
| Leptin and leptin receptor, geometric mean (95% CI) | | | |
| Leptin, ng/mL | 20.2 (17.1-24.0) | 23.2 (22.0-24.5) | 0.10 |
| Soluble leptin receptor, ng/mL | 34.0 (31.9-36.2) | 33.6 (33.9-34.3) | 0.74 |
| Sex steroids and SHBG, geometric mean (95% CI) | | | |
| Total estradiol, pg/mL | 16.7 (13.5-20.8) | 20.2 (18.7-21.8) | 0.10 |
| Free estradiol, pg/mL | 0.20 (0.16-0.24) | 0.22 (0.21-0.24) | 0.23 |
| Total testosterone, ng/dL | 8.20 (6.74-9.98) | 9.04 (8.42-9.72) | 0.36 |
| Free testosterone, ng/dL | 0.06 (0.05-0.08) | 0.06 (0.06-0.07) | 0.91 |
| SHBG, nmol/L | 67.3 (59.2-76.6) | 74.9 (71.7-78.3) | 0.11 |
| Inflammation, geometric mean (95% CI) | | | |
| TNF-α-R2, pg/mL | 2340 (2220-2470) | 2360 (2320-2400) | 0.83 |
| IL-6, pg/mL | 1.71 (1.49-1.97) | 1.76 (1.68-1.84) | 0.72 |
| hsCRP, mg/L | 0.18 (0.14-0.23) | 0.20 (0.18-0.21) | 0.49 |
| Endothelial dysfunction, geometric mean (95% CI) | | | |
| E-selectin, ng/mL | 38.1 (34.3-42.4) | 33.9 (33.0-34.9) | 0.02 |
| ICAM-1, ng/mL | 263 (248-280) | 269 (263-275) | 0.52 |
| VCAM-1, ng/mL | 658 (620-697) | 669 (657-681) | 0.60 |
| Cellular aging, geometric mean (95% CI) | | | |
| Leukocyte telomere length, kb | 4.06 (3.84-4.28) | 3.85 (3.78-3.92) | 0.09 |
| Blood pressure, mean (SD) | | | |
| Systolic blood pressure, mmHg | 131 (19) | 127 (17) | 0.01 |
| Diastolic blood pressure, mmHg | 77 (9) | 76 (9) | 0.07 |

[a] Low birth weight: self-reported birth weight<2.72 kg (6 lbs.).
[b] *P* values were obtained from Student's *t*-tests.

**Table 3.3 Direct and Indirect Effects of Low Birth Weight on Type 2 Diabetes Risk Later in Life with Mediation of Established Biomarkers.**

| Mediator | *n* | Effect decomposition[a] | | Proportion mediated[d] (%) |
|---|---|---|---|---|
| | | Direct effect[b] | Indirect effect[c] | |
| Insulin sensitivity and β-cell function | | | | |
| WHR | 3033 | 1.61 (1.22 to 2.17) | 1.06 (0.97 to 1.16) | 14 (-9 to 37) |
| HOMA-IR | 2379 | 1.32 (0.95 to 1.88) | 1.22 (1.02 to 1.49) | 47 (0 to 123) |
| HOMA-β | 2379 | 1.86 (1.34 to 2.64) | 0.93 (0.84 to 1.03) | --[e] |
| Leptin and leptin receptor | | | | |
| Leptin | 1312 | 2.17 (1.44 to 3.35) | 0.99 (0.95 to 1.01) | -- |
| Soluble leptin receptor | 1312 | 2.17 (1.41 to 3.35) | 0.99 (0.96 to 1.01) | -- |
| Sex steroids and SHBG | | | | |
| Total estradiol | 1311 | 2.16 (1.41 to 3.35) | 1.00 (0.97 to 1.02) | 0 (-7 to 4) |
| Free estradiol | 1311 | 2.16 (1.42 to 3.39) | 1.00 (0.94 to 1.05) | -- |
| Total testosterone | 1311 | 2.16 (1.40 to 3.39) | 1.00 (0.94 to 1.06) | -- |
| Free testosterone | 1311 | 2.11 (1.38 to 3.30) | 1.05 (0.95 to 1.17) | 8 (-10 to 29) |
| SHBG | 1311 | 1.97 (1.25 to 3.10) | 1.16 (1.03 to 1.33) | 24 (5 to 51) |
| Inflammation | | | | |
| TNFα-R2 | 2370 | 1.65 (1.17 to 2.29) | 1.01 (0.97 to 1.05) | 3 (-10 to 15) |
| IL6 | 2376 | 1.63 (1.15 to 2.31) | 1.01 (0.95 to 1.07) | 2 (-16 to 21) |
| hsCRP | 2384 | 1.64 (1.17 to 2.31) | 1.01 (0.95 to 1.08) | 3 (-16 to 23) |
| Endothelial function | | | | |
| E-selectin | 2372 | 1.56 (1.10 to 2.21) | 1.12 (1.02 to 1.23) | 25 (5 to 63) |
| ICAM-1 | 2354 | 1.67 (1.19 to 2.40) | 0.99 (0.91 to 1.07) | -- |
| VCAM-1 | 2377 | 1.68 (1.20 to 2.36) | 0.99 (0.93 to 1.04) | -- |
| Cellular aging | | | | |
| Leukocyte telomere length | 3028 | 1.68 (1.29 to 2.27) | 0.99 (0.96 to 1.01) | -- |
| Blood pressure | | | | |
| Systolic | 3048 | 1.61 (1.25 to 2.16) | 1.03 (1.01 to 1.07) | 8 (2 to 19) |
| Diastolic | 3046 | 1.65 (1.28 to 2.22) | 1.00 (0.98 to 1.01) | -- |

[a] Effects are shown as odds ratios (95% confidence intervals). Confidence intervals were calculated using bootstrapping. Models were adjusted for age, race/ethnicity, cigarette smoking, alcohol consumption, physical activity, dietary fiber intake, dietary glycemic load, BMI, and family history of diabetes.
[b] Odds ratio for T2D among persons who had low birth weight versus those who had normal birth weight if the biomarker levels were what it would have been with normal birth weight.
[c] Odds ratio for T2D for those who had low birth weight comparing the risk if the biomarker levels were what it would have been with low versus normal birth weight.
[d] Proportion of the total effects that are mediated by the mediators.
[e] Proportion mediated is undefined because the point estimate of the mediated effect ("indirect effect") is not in the same direction as the total effect.

**Table 3.4 Proportion of Effects of Low Birth Weight on Insulin Resistance and β-Cell Function Mediated by Established Biomarkers**

| | Proportion mediated[a] (%): Insulin resistance | Proportion mediated (%): β-cell function |
|---|---|---|
| Leptin and leptin receptor | | |
| Leptin | 15 (-45 to 124) | 58 (-305 to 482) |
| Soluble leptin receptor | 12 (-37 to 92) | 37 (-265 to 412) |
| Sex steroids and SHBG | | |
| Total estradiol | 0 (-10 to 11) | 1 (-34 to 30) |
| Free estradiol | --[b] | -- |
| Total testosterone | -- | -- |
| Free testosterone | 6 (-21 to 63) | -- |
| SHBG | 33 (-233 to 228) | 6 (-65 to 90) |
| Inflammation | | |
| TNFα-R2 | 2 (-10 to 19) | 0 (-5 to 8) |
| IL6 | 2 (-26 to 28) | 1 (-31 to 49) |
| hsCRP | 2 (-19 to 23) | 1 (-21 to 26) |
| Endothelial function | | |
| E-selectin | 28 (3 to 131) | 4 (-12 to 32) |
| ICAM-1 | -- | 0 (-5 to 8) |
| VCAM-1 | 0 (-5 to 6) | 4 (-30 to 55) |
| Cellular aging | | |
| Leukocyte telomere length | -- | -- |
| Blood pressure | | |
| Systolic | 13 (2 to 63) | 10 (-25 to 95) |
| Diastolic | 7 (-1 to 43) | 9 (-30 to 77) |

[a] Proportion of the total effects that are mediated by the mediators.
[b] Proportion mediated is undefined because the point estimate of the mediated effect ("indirect effect") is not in the same direction as the total effect.

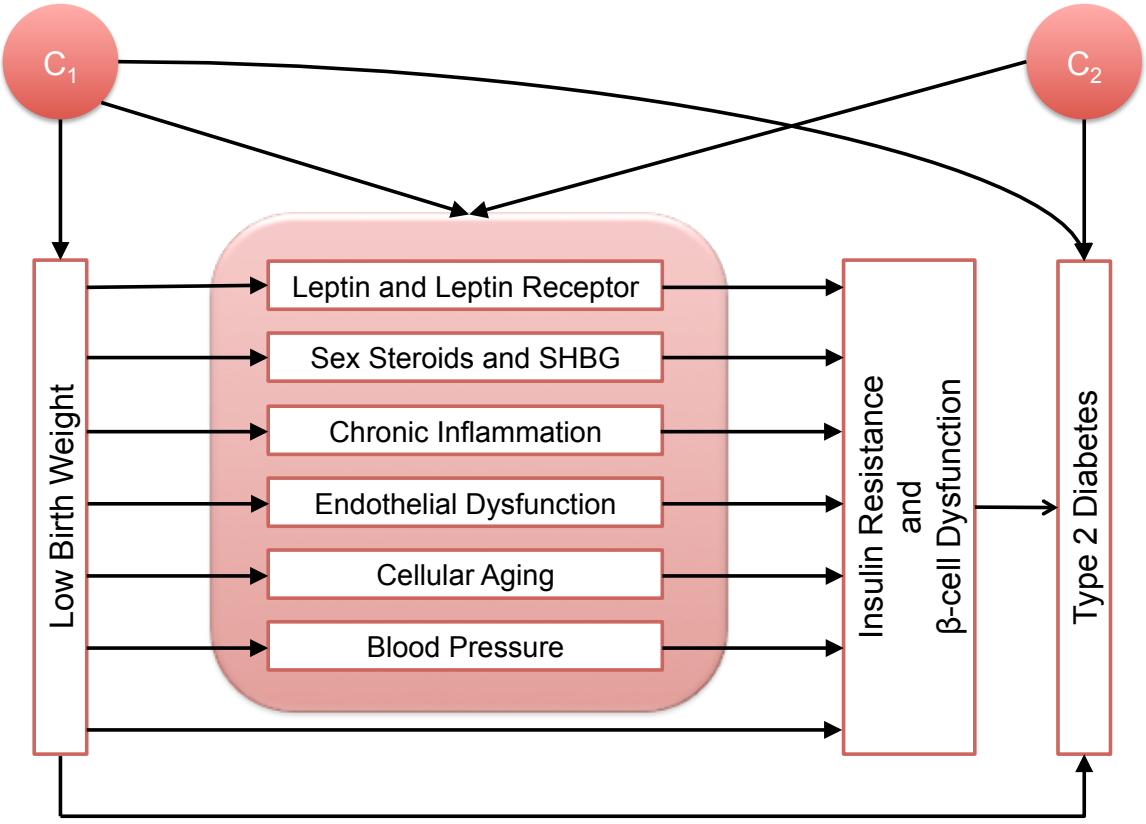


**Figure 3.1 Causal diagram hypothesized for mediation and confounding characterizing the relation between low birth weight and type 2 diabetes risk later in life.**
C1 represents the potential confounders that occurred before birth (race/ethnicity and family history of diabetes), and C2 indicates the potential confounders that occurred after birth (age, smoking, alcohol consumption, physical exercise, total fiber intake, dietary glycemic load, and BMI).

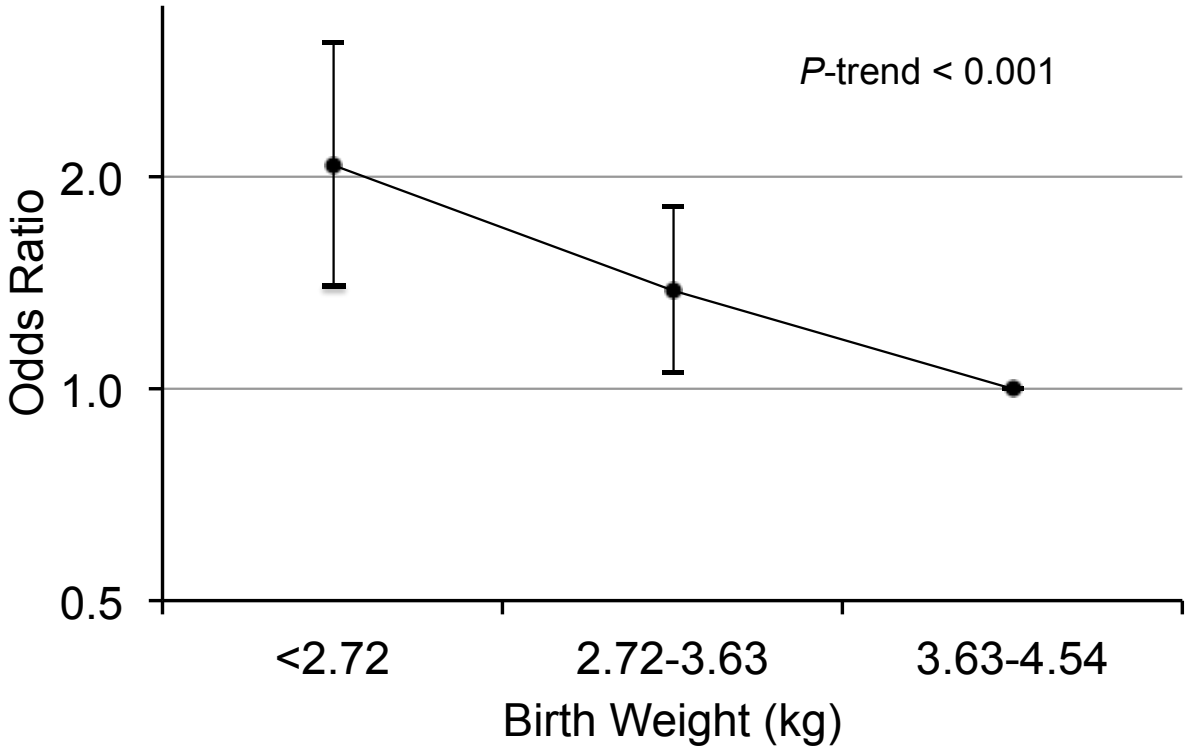


**FIGURE 3.2 Association between birth weight and type 2 diabetes risk later in life.** Women with birth weight between 3.63~4.54 kg served as the reference group. Logistic regression model was adjusted for cigarette smoking, alcohol consumption, physical activity, dietary fiber intake, dietary glycemic load, BMI, and family history of diabetes. Dots indicate point estimates of odds ratios, and bars indicate 95% confidence intervals.

## 3.7 Appendix: Measurement of blood biomarkers

Fasting serum specimens collected at baseline from each participant were processed locally, frozen, and then shipped to a central repository, where they were stored at -80 °C. All biochemical assays were processed in random order by laboratory staff blinded to case status. Samples from cases and their matched controls were handled identically, shipped in the same batch, and assayed in the same analytical run to reduce systematic bias and interassay variation. Fasting glucose was measured enzymatically on a chemistry analyzer (Hitachi 911) using reagents from Roche Diagnostics (Indianapolis, IN). Fasting insulin concentrations were determined by an ultrasensitive enzyme-linked immunosorbent assay from ALPCO Diagnostics (Windham, NH). The coefficients of variation (CVs) were 1.7% for fasting glucose and 5.7% for fasting insulin. The homeostatic model assessment of insulin resistance (HOMA-IR) and the homeostatic model assessment of β cell function (HOMA-β) were computed from the mathematical approximation equations originally described by Matthews et al.[179].

Circulating leptin and soluble leptin receptor were measured by enzyme-linked immunosorbent assay (R&D Systems; Minneapolis, MN). To assess the interbatch variability, high and low control samples of leptin and soluble leptin receptor were run in duplicate. Coefficients of variation were 9.5% for leptin and 7.4% for soluble leptin receptor.

Plasma concentrations of estradiol, testosterone, and sex-hormone binding globulin (SHBG) were measured by electrochemiluminescence immunoassays on the Elecsys 2010 immunoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). Competitive immunoassays were used to measure estradiol and testosterone, whereas a sandwich format was used to measure SHBG. Standardized, quality control serum samples (Liquichek Immunoassay Plus Control, Bio-Rad Laboratories, Hercules, CA, USA) were run with each batch for quality control and

evaluation of inter-batch variability. Free estradiol and free testosterone were calculated using the methods described by Vermuelen et al.[180] and Sodergard et al.[181], which have been previously validated in postmenopausal women[182,183]. CVs on quality control samples run on separate days were 5.4% for SHBG, 10.3% for total testosterone, and 12.4% for total estradiol.

Tumor necrosis factor α receptor 2 (TNF-α–R2) was measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota). Interleukin 6 (IL-6) was measured by an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems). High-sensitivity C-reactive protein (hsCRP) was measured on a chemistry analyzer (Hitachi 911; Roche Diagnostics, Indianapolis, Indiana) using reagents and calibrators from Denka Seiken Co Ltd (Niigata, Japan). Soluble E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) were measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The CVs were 3.5% for TNF-α–R2, 7.6% for IL-6, 1.61% for hsCRP, 6.5% for E-selectin, 6.7% for ICAM-1, and 8.9% for VCAM-1.

For leukocyte telomere length measurement, we applied the method proposed by O'Callaghan et al.[184] in a high-throughput 384-well format using Applied Biosystems 7900HT PCR System (Applied Biosystems by Life Technologies Corporation, Carlsbad, CA). Average telomere length per chromosome was calculated by the following formula: (telomere length/copies of diploid genome)/(23×2). The overall intraplate and interplate CVs of the telomere assays were 0.8% and 5.7%, respectively.

# CHAPTER 4. Relations of Sex Hormone Levels and Leukocyte Telomere Length in Black, Hispanic, and Asian/Pacific Islander Postmenopausal Women

## 4.1 Abstract

**Context**: Sex hormones may play important roles in sex-specific biological aging.

**Objective**: We specifically examined the associations between circulating concentrations of sex hormones and leukocyte telomere length (TL).

**Research Design and Methods**: We conducted a cross-sectional study of 1124 black, 444 Hispanic, and 289 Asian/Pacific Islander women in the Women's Health Initiative Observational Cohort. Concentrations of estradiol and testosterone were measured using electrochemiluminescence immunoassays. TL was measured using quantitative PCR.

**Results**: Levels of estradiol were not significantly associated with TL in this sample of women. The associations between total and free testosterone and TL differed by race/ethnicity (*P* for interaction=0.03 for total testosterone and 0.05 for free testosterone). Total and free testosterone concentrations were not associated with TL in black and Hispanic women, whereas in Asian/Pacific Islanders, their concentrations were inversely associated with TL (*P*-trend=0.003 for both). These associations appeared robust in multiple subgroup analysis and multivariable models adjusted for potential confounding factors. In Asian/Pacific Islanders, doubling of serum free testosterone concentration was associated with 202 bp shorter TL (95% CI, 51 to 353 bp), and doubling of total testosterone concentration was associated with 203 bp shorter TL (95% CI, 50 to 355 bp).

**Conclusions**: Serum concentration of estradiol was not associated with leukocyte TL in this large sample of postmenopausal women. Total and free testosterone levels were inversely associated with TL in Asian/Pacific Islander women but not in black and Hispanic women. Future studies to replicate our observations are warranted to address potential ethnicity-specific relations.

## 4.2 Introduction

Telomeres are DNA-protein complexes that prevent genomic loss during chromosome replications[26,185,186]. Aging has been linked to progressive shortening of the telomere length (TL), which is estimated to be at a rate of 20 to 60 base pairs (bp) per year[187,188]. Given that female life expectancy is on average 80.2 years, compared to a male life expectancy of 75.1 years in the United States[189], it is not surprising that women have longer TL than age-matched men[190,191]. Despite these observations, the mechanisms underlying the relation between sex and longevity have not been fully elucidated. Although many theories have been proposed to explain this sex divergence, including oxidative damage and chromosomal complement[27], the role of sex steroids remained untested, especially relating sex hormone concentrations to TL as a marker of biological aging.

To further elucidate the potential roles of sex hormones in biological aging, we examined the associations of circulating estradiol and testosterone with leukocyte TL in black, Hispanic, and Asian/Pacific Islander postmenopausal women participated in the Women's Health Initiative Observational Study (WHI-OS).

## 4.3 Methods

### 4.3.1 Study Subjects

The Women's Health Initiative (WHI) is a long-term national health study that has focused on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. The original WHI study included 161,808 postmenopausal women enrolled between 1993 and 1998. The WHI has two major components: a partial factorial

randomized Clinical Trial (CT) and an Observational Study (OS); both were conducted at 40 Clinical Centers nationwide. The OS examines the relationship between lifestyle, environmental, medical and molecular risk factors and specific measures of health or disease outcomes. This component involves tracking the medical history and health habits of 93,676 women not participating in the CT. In the current investigation, we included 1124 black, 444 Hispanic, and 289 Asian/Pacific Islander women whose blood samples were assayed for sex hormones (estradiol and testosterone) and leukocyte TL in a case-control study of type 2 diabetes nested in WHI-OS[157]. The study was reviewed and approved by human subjects review committees at each participating institution, and signed informed consent was obtained from all women enrolled.

### 4.3.2 Measurement of sex steroid hormones and SHBG

The measurements of circulating estradiol, testosterone, and sex hormone-binding globulin (SHBG) have been described in Chen et al.[80]. In brief, serum concentrations of estradiol, testosterone, and sex hormone-binding globulin (SHBG) were measured by electrochemiluminescence immunoassays on the Elecsys 2010 immunoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). Inter-assay imprecision (expressed as %CV) was 12.4% for estradiol, 10.3% for testosterone, and 5.4% for SHBG. Free estradiol and free testosterone were calculated using the methods described by Vermuelen et al.[180] and Sodergard et al.[181], previously validated in postmenopausal women[180-183,192].

### 4.3.3 Measurement of telomere length

The measurement of TL has been described elsewhere[157]. In brief, we adopted a qPCR method first proposed by O'Callaghan et al.[184] using a high-throughput 384-well format Applied Biosystems' 7900HT PCR System (Applied Biosystems by Life Technologies Corporation,

Carlsbad, CA). The overall intra-plate coefficient of variation (CV) was 0.8% and the inter-plate CV was 5.7%.

### 4.3.4 Measurements of covariates

Self-administered questionnaires were used to collect information on demographics and lifestyle factors. Participants were categorized according to smoking status as “never-smoker”, “former smoker”, and “current smoker”. Levels of alcohol intake and total energy intake were also calculated from the food frequency questionnaire. Information on age at menarche and menopause was collected in the questionnaire, and the difference was calculated as a surrogate of lifetime estrogen exposure. Body weight and height were measured at baseline, and BMI was calculated as body weight (kg) divided by height (m) squared. The level of physical activity in metabolic equivalent hours per week (MET-h/wk) was estimated based on the self-reported duration of exercise, weighted by intensity levels. Participants were also categorized acoording to use of hormone replacement therapy as “never-user”, “former user”, and “current user”. Tumor necrosis factor α receptor 2 (TNF-α-R2) was measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota). Interleukin 6 (IL-6) was measured by an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems). High-sensitivity C-reactive protein (hsCRP) was measured on Roche Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, Indiana) using an immunoturbidimetric assay with reagents and calibrators (Denka Seiken Co Ltd, Niigata, Japan).

### 4.3.5 Statistical Analysis

Baseline characteristics were summarized according to race/ethnicity. Categorical variables were shown as percentages; normal-distributed continuous variables were expressed as the mean (standard deviation); non-normal-distributed continuous variables were shown as median

(interquartile range). *P* values for differences among ethnic groups were obtained from $\chi^2$ tests for categorical variables, from ANOVA for normal-distributed continuous variables, and from Kruskal-Wallis tests for non-normal distributed continuous variables. General linear models were used to estimate mean TLs and their 95% confidence intervals (CIs) for different quartiles of sex hormones while adjusting for covariates. The basic models were adjusted only for age at enrollment (years, continuous). The multivariable adjusted models were additionally adjusted for race/ethnicity (Black, Hispanic, or Asian/Pacific Islander), hormone replacement therapy (HRT) use (never, former, or current user), years between menarche and menopause (years, continuous), BMI ($kg/m^2$, continuous), cigarette smoking (never, former, or current smoker), alcohol consumption (never, former, or current drinker), diabetes case in the primary case-control study (yes or no), physical activity (0, >0 to 5, >5 to 20, or >20 MET-h/wk), daily energy intake (kcal, continuous), and serum SHBG concentration (nmol/L, continuous). Additionally, the models for estradiol and testosterone were mutually adjusted for each other. Concentrations of sex hormones were categorized into quartiles among all individuals, with the fourth quartile having the highest concentration. *P* values for linear trend were obtained by including the medians of concentration levels as continuous variables in the regression models. Regression coefficients for the change in leukocyte TL for doubling of sex steroid hormones concentrations were calculated using linear regression models with log-transformed concentrations. To further assess potential effect modification by race/ethnicity, the interaction terms between race/ethnicity and log-transformed concentrations of sex steroid hormones were included in the models. In addition, subgroup analyses stratified by race/ethnicity were conducted. In the first sensitivity analysis, we fitted models with additional adjustment of serum concentrations of inflammatory biomarkers, including IL6, hsCRP, and TNF-α-R2. In the second sensitivity analysis, we stratified the

analyses by BMI with a cutoff point at 25 kg/m$^2$. To explore potential non-linear relations between sex hormone concentrations and TL, we used restricted cubic spline models. We conducted all statistical analyses using SAS (version 9.3; SAS institute, Cary, NC). All *P* values were two tailed, and false discovery rate (FDR) was adopted to control the effects of multi-testing.

## 4.4 Results

Characteristics of study participants at baseline are summarized in Table 4.1. On average, the Asian/Pacific Islanders had a lower BMI, lower proportion of current smokers and current alcohol drinkers than Black and Hispanic women. Moreover, Asian/Pacific Islander women had lower concentrations of sex hormones (estradiol and testosterone), higher concentration of SHBG, and lower concentrations of inflammation markers (hsCRP, IL6, and TNF-α) than Black and Hispanic women. The proportion of current HRT users and lifetime estrogen exposure were significantly higher in Asian/Pacific Islander women than Black and Hispanic women. In addition, Asian/Pacific Islander women had the shortest TLs among the three ethnic groups.

Total and free estradiol concentrations were positively associated with leukocyte TL in the pooled analysis, although the linear trends were not significant (*P*-trend=0.14 for free estradiol and P-trend=0.19 for total estradiol) (Table 4.2). In subgroup analysis by race/ethnicity, we did not observe significant associations between estradiol concentrations and TL in any of the three ethnic groups, although the associations using continuous measure of estradiol were in the same direction as the pooled analysis. We did not observe significant interaction between estradiol concentration and race/ethnicity (*P*=0.48 for free estradiol and 0.63 for total estradiol).

Total and free testosterone concentrations were modestly associated with TL in the pooled analysis (*P*-trend=0.04 for free testosterone and *P*-trend=0.02 for total testosterone), where the mean TL appeared shorter in higher testosterone concentrations quartiles (Table 4.3). Multivariable adjustment did not change the estimates materially, although most associations were no longer significant. The interaction between testosterone concentration and race/ethnicity was significant for both free testosterone (*P*=0.05) and total testosterone (*P*=0.03). In subgroup analyses, we observed significant inverse associations between free testosterone and TL in Asian/Pacific Islander women (*P*-trend=0.003, FDR<0.05). In multivariable-adjusted models, Asian/Pacific Islander women in the highest quartile of serum free testosterone (median, 0.241 ng/dL) had 785 bp shorter TL (95% CI, 48 to 1522 bp) than women in the lowest quartile (median 0.012 ng/dL). In Asian/Pacific Islanders, doubling of serum free testosterone concentration was associated with 202 bp shorter TL (95% CI, 51 to 353 bp). No associations between free testosterone concentration and TL were observed in black and Hispanic women. In sensitivity analyses, neither additional adjustment of inflammation markers nor stratifying on BMI changed the results materially. When we restricted our analyses to those who had never used HRT, the association between free testosterone and TL remained in the same direction but was no longer significant (*P*-trend=0.08). In cubic spline models (Figure 4.1), we observed that leukocyte TL decreased substantially with higher concentrations of free testosterone in Asian/Pacific Islander women, whereas the trends among Black and Hispanic women were not apparent.

In a similar manner, we also observed significant inverse association between total testosterone concentration and TL in Asian/Pacific Islander women (*P*-trend=0.008, FDR<0.05). In multivariable-adjusted models, Asian/Pacific Islander women with highest serum

concentration of total testosterone (median, 22.4 ng/dL) had 600 bp shorter TL (95% CI, 45 to 1274 bp) than those in the lowest concentration group (median, 1.9 ng/dL). In Asian/Pacific women, doubling of serum total testosterone concentration was associated with 203 bp shorter TL (95% CI, 50 to 355 bp). No association between total testosterone concentration and TL were observed in Black and Hispanic women. In the sensitivity analyses, neither additional adjustment of inflammation markers nor stratifying on BMI changed the results materially. When we restricted our analyses to women who had never used HRT, the association between total testosterone and TL remained in the same direction but was not longer significant (*P*-trend=0.07).

## 4.5 Discussion

Overall, we did not find significant associations between estradiol levels and leukocyte TL in this sample of multiethnic women. In Asian/Pacific Islander women, however, total and free testosterone were inversely associated with TL, independent of potential confounders. In these women, TL attrition was estimated to be approximately 22 bp per year on average. In particular, doubling of free or total testosterone concentration was associated with approximately 9.2 times of this average annual attrition. Among black and Hispanic women, however, no associations were observed between testosterone levels and TL. When we restricted our analyses to women who had never used HRT, although the magnitudes of associations did not change materially, the associations were no longer statistically significant at the conventional $\alpha$=0.05 level, probably due to lack of sufficient statistical power.

Available evidence indicates that women generally live longer than men and suffer less from certain age-related diseases, such as certain cancers and cardiovascular diseases[193,194]. There are both social lifestyle and biological factors that may account for sex-differences in these

disorders (e.g. cigarette smoking, alcohol consumption, job stress, and medical services utilization, lipids, sex steroids)[195]. Recent work has also identified altered serum lipid levels by sex steroid hormones levels as potential biological mechanisms responsible for the sex-difference in cardiovascular disease[196,197]. For women after the menopausal transition, not only does endogenous estrogen plummet, the estrogen-to-androgen ratio is also greatly altered; androgens rather than estrogens become the primary sex hormone in postmenopausal women who do not pursue hormone replacement therapy[198]. Although TL is a well-known indicator of biologic aging and senescence and has been associated with chronic diseases[199-202], few studies have investigated the relationship between sex steroid hormones and TL.

In animal studies, estrogen deficiency has been associated with telomere shortening[203,204]. Estrogen was suggested to diminish oxidative stress[205], which is fundamental to biologic aging and can accelerate telomere shortening and stimulate the transcription of the gene encoding telomerase[206]. In a human study examining the relationship between estradiol levels and TL, the duration of endogenous estrogen exposure (difference between age at menopause and age at menarche) was associated with greater TL and lower telomerase activity[207]. In the current study, we observed longer TL in women with higher concentrations of free or total estradiol, albeit these associated were not statistically significant at the conventional $\alpha$=0.05 level. One limitation of our study is that we could not accurately evaluate and control the lifetime exposure of sex hormones. However, we did assess the difference between age at menopause and age at menarche as a surrogate to adjust for potential confounding of lifetime exposure of estrogen.

Previous studies investigating the association between androgen levels and TL are scarce. One study in healthy elderly men in Belgium reported no statistically significant association between age-corrected testosterone concentrations and TL[208]. In this study, we observed racial

heterogeneity of the association between testosterone concentration and TL; the significant associations were only observed in Asian/Pacific Islander women. However, the reason for this significant interaction with race/ethnicity is still not clear. In Asian/Pacific Islander women, both higher total and free testosterone concentrations were associated with shorter TL and the associations were robust in the sensitivity analyses. High testosterone levels have been associated with insulin resistance, metabolic syndrome, and cardiovascular disease in elderly women[209] and also associated with higher levels of cardiovascular risk factors in a multiethnic women population[210]. Interestingly, our data suggest that the Asian/Pacific Islander population generally had elevated markers typically associated with good health, including lower BMI, lower levels of inflammation, higher SHBG concentrations, and resultant lower free testosterone levels compared to black and Hispanic women.

One possible explanation of the racial heterogeneity is differences in SHBG levels and sex steroid hormone clearance rates. A previous study observed that Asians undergoing ovarian stimulation for in vitro fertilization developed higher estradiol levels compared to non-Asians. As elevated estradiol levels were the only differentiating characteristic of the stimulation, the authors propose that Asians may have difficulty clearing their serum estradiol levels due to inherent enzymatic differences, thus displaying a relatively elevated level of sex steroids[211]. It is possible that Asian women have decreased clearance of testosterone as opposed to their Black and Hispanic counterparts, allowing the testosterone to have increased receptor interactions, with altered end organ effects. If a negative relationship between free testosterone and TL is subtle, it may be amplified in the Asian population, allowing us to observe the association. Another possible mechanism for the ethnic discrepancy is related to genetic diversity of the androgen receptor. It has been demonstrated that Asians have the lowest prevalence of CAG

microsatellites of exon 1 of the androgen receptor (AR) gene[212], and fewer CAG repeats in the AR gene result in higher transcriptional activity and higher levels of serum androgens[213]. Moreover, given the shape of the relationship between testosterone and TL in Asian/Pacific Islanders seems to be linear when testosterone concentrations were higher in the spline analysis, and because women in this ethnic group generally have lower testosterone levels, we cannot rule out the possibility that more prevalent or severe metabolic abnormalities (e.g. insulin resistance) among Asian/Pacific Islander women with extremely high testosterone concentrations could explain the findings.

In conclusion, higher total and free testosterone concentrations appeared significantly associated with shorter TL in Asian/Pacific Islander women but not in black or Hispanic women. These findings suggest that Asian/Pacific Islander women may be susceptible to the potential detrimental effects of high testosterone level on biologic aging. Future prospective studies will require larger numbers of ethnic minorities and multiple assessments of sex hormones and TL to further verify and confirm our observations.

## 4.6 Tables and Figures

**Table 4.1 Baseline characteristics of 1857 postmenopausal women by race/ethnicity.**

| | Race/ethnicity | | | *P*[a] |
|---|---|---|---|---|
| | Black (n=1124) | Hispanic (n=444) | Asian/Pacific Islander (n=289) | |
| Age, mean (SD), y | 60.9 (6.7) | 60.2 (6.8) | 63.6 (7.8) | <0.001 |
| Smoking, % | | | | <0.001 |
| Never | 49.2 | 68.6 | 71.2 | |
| Former | 38.9 | 26.8 | 25.0 | |
| Current | 11.9 | 4.6 | 3.8 | |
| Alcohol intake, % | | | | <0.001 |
| Never | 16.9 | 22.5 | 40.1 | |
| Former | 31.4 | 24.1 | 21.5 | |
| Current | 51.8 | 53.4 | 38.4 | |
| Physical activity, median (IQR), MET-h/wk | 6.0 (0.8-15.0) | 6.8 (1.2-15.2) | 8.6 (3.0-18.4) | 0.001 |
| BMI, mean (SD), kg/m$^2$ | 30.9 (7.0) | 28.9 (5.7) | 24.9 (4.6) | <0.001 |
| Lifetime estrogen exposure[b], mean (SD), year | 33.9 (7.4) | 35.2 (6.3) | 35.9 (6.3) | <0.001 |
| Hormone replacement therapy, % | | | | <0.001 |
| Never | 56.4 | 48.0 | 33.7 | |
| Former | 12.5 | 10.1 | 15.3 | |
| Current | 31.1 | 41.9 | 51.0 | |
| Biomarkers, median (IQR)[c] | | | | |
| Free estradiol, pg/mL | 0.29 (0.15-0.46) | 0.26 (0.14-0.43) | 0.20 (0.08-0.39) | <0.001 |
| Total estradiol, pg/mL | 21.4 (12.0-38.9) | 19.3 (10.5-45.6) | 16.6 (6.6-38.1) | 0.001 |
| Free testosterone, ng/dL | 0.095 (0.036-0.212) | 0.076 (0.026-0.162) | 0.061 (0.021-0.135) | <0.001 |
| Total testosterone, ng/dL | 12.2 (5.3-22.5) | 10.0 (4.4-19.0) | 8.4 (2.8-16.4) | <0.001 |
| SHBG, nmol/L | 59.1 (37.7-99.1) | 64.5 (37.6-121.4) | 67.5 (42.7-116.1) | 0.019 |
| Leukocyte telomere length, kb | 4.13 (3.20-5.08) | 4.20 (3.37-5.24) | 3.78 (2.96-4.72) | <0.001 |
| hsCRP, mg/L | 3.00 (1.22-6.65) | 2.99 (1.57-5.63) | 0.92 (0.39-2.18) | <0.001 |
| IL6, pg/mL | 2.19 (1.31-4.24) | 2.08 (1.31-3.59) | 1.29 (0.84-2.31) | <0.001 |
| TNF-α, pg/mL | 2290 (1880-2770) | 2430 (1950-2890) | 2190 (1820-2590) | <0.001 |

[a] *P* values were obtained from $\chi^2$ tests for categorical variables, from Student's t-tests for normal-distributed continuous variables, and from Kruskal-Wallis tests for non-normal-distributed continuous variables.
[b] Calculated as the duration between menarche and menopause.
[c] SI conversion factors: estradiol (pg/mL) × 3.67 = (pmol/L); testosterone (ng/dL) × 0.0347 = (nmol/L).

**Table 4.2 Leukocyte telomere length in base pairs according to serum levels of estradiol.**

| Model | Quartiles of estradiol | | | | *P*-trend | Continuous (per doubling) |
|---|---|---|---|---|---|---|
| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | | |
| Free estradiol | | | | | | |
| Pooled | | | | | | |
| Median, pg/mL | 0.07 | 0.20 | 0.34 | 0.61 | | |
| Age-adjusted | 0 (Reference) | 14 (-179 to 207) | -123 (-316 to 71) | 20 (-177 to 216) | 0.99 | -23 (-72 to 27) |
| Multivariable | 0 (Reference) | 149 (-80 to 378) | 109 (-130 to 347) | 223 (-34 to 480) | 0.14 | 21 (-46 to 89) |
| Blacks | | | | | | |
| Median, pg/mL | 0.09 | 0.22 | 0.36 | 0.62 | | |
| Age-adjusted | 0 (Reference) | -64 (-306 to 179) | -139 (-382 to 104) | -27 (-273 to 219) | 0.85 | -35 (-101 to 30) |
| Multivariable | 0 (Reference) | -4 (-290 to 282) | 76 (-224 to 376) | 97 (-224 to 417) | 0.48 | 9 (-79 to 96) |
| Hispanics | | | | | | |
| Median, pg/mL | 0.06 | 0.20 | 0.34 | 0.62 | | |
| Age-adjusted | 0 (Reference) | 99 (-319 to 518) | 34 (-389 to 458) | 103 (-324 to 529) | 0.72 | 31 (-73 to 135) |
| Multivariable | 0 (Reference) | 356 (-158 to 870) | 223 (-303 to 749) | 359 (-214 to 931) | 0.36 | 79 (-72 to 230) |
| Asians/Pacific Islanders | | | | | | |
| Median, pg/mL | 0.04 | 0.14 | 0.28 | 0.56 | | |
| Age-adjusted | 0 (Reference) | -232 (-719 to 254) | -307 (-791 to 178) | -385 (-874 to 103) | 0.15 | -74 (-185 to 37) |
| Multivariable | 0 (Reference) | -212 (-803 to 380) | -110 (-712 to 491) | -167 (-832 to 498) | 0.77 | 6 (-147 to 158) |
| Total estradiol | | | | | | |
| Pooled | | | | | | |
| Median, pg/mL | 6.3 | 15.3 | 27.4 | 59.1 | | |
| Age-adjusted | 0 (Reference) | -70 (-264 to 123) | -75 (-267 to 118) | -24 (-220 to 172) | 0.99 | -17 (-64 to 30) |
| Multivariable | 0 (Reference) | -26 (-255 to 203) | 134 (-112 to 381) | 161 (-112 to 435) | 0.19 | 20 (-46 to 87) |
| Blacks | | | | | | |
| Median, pg/mL | 7.5 | 16.3 | 27.5 | 58.6 | | |
| Age-adjusted | 0 (Reference) | 51 (-191 to 293) | -60 (-301 to 181) | -11 (-257 to 236) | 0.78 | -34 (-97 to 29) |
| Multivariable | 0 (Reference) | 114 (-171 to 400) | 166 (-143 to 474) | 212 (-123 to 547) | 0.28 | 7 (-80 to 94) |
| Hispanics | | | | | | |
| Median, pg/mL | 6.3 | 14.3 | 28.9 | 67.6 | | |
| Age-adjusted | 0 (Reference) | 148 (-275 to 571) | 48 (-374 to 469) | 144 (-282 to 570) | 0.66 | 28 (-69 to 125) |
| Multivariable | 0 (Reference) | 166 (-355 to 687) | -7 (-557 to 544) | 320 (-299 to 938) | 0.29 | 72 (-78 to 222) |
| Asians/Pacific Islanders | | | | | | |
| Median, pg/mL | 2.5 | 11.9 | 24.3 | 54.5 | | |
| Age-adjusted | 0 (Reference) | -178 (-663 to 306) | -291 (-777 to 194) | -89 (-574 to 396) | 0.88 | -51 (-154 to 53) |
| Multivariable | 0 (Reference) | -122 (-723 to 478) | 29 (-614 to 671) | 138 (-580 to 856) | 0.61 | 11 (-140 to 163) |

Numbers are adjusted difference of telomere length (base pairs) with the reference group (95% CI). * False discovery rate < 0.05.
Multivariable: adjusted for age, race/ethnicity, HRT, years between menarche and menopause, case/control, BMI, physical exercise, total energy intake, smoking, alcohol consumption, SHBG, and estradiol.

**Table 4.3 Leukocyte telomere length in base pairs according to serum levels of testosterone.**

| Model | Quartiles of testosterone | | | | *P*-trend | Continuous (per doubling) |
|---|---|---|---|---|---|---|
| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | | |
| Free testosterone | | | | | | |
| Pooled | | | | | | |
| Median, ng/dL | 0.015 | 0.053 | 0.124 | 0.304 | | |
| Age-adjusted | 0 (Reference) | -49 (-242 to 143) | -138 (-331 to 54) | -199 (-391 to -7) | 0.04 | -39 (-78 to -1) |
| Multivariable | 0 (Reference) | -37 (-260 to 185) | -158 (-395 to 79) | -269 (-547 to 8) | 0.04 | -56 (-114 to 1) |
| Blacks | | | | | | |
| Median, ng/dL | 0.017 | 0.063 | 0.142 | 0.329 | | |
| Age-adjusted | 0 (Reference) | -187 (-428 to 54) | -240 (-481 to 1) | -88 (-329 to 153) | 0.85 | -19 (-68 to 29) |
| Multivariable | 0 (Reference) | -138 (-417 to 141) | -179 (-479 to 121) | -14 (-359 to 331) | 0.71 | -17 (-90 to 55) |
| Hispanics | | | | | | |
| Median, ng/dL | 0.015 | 0.045 | 0.115 | 0.270 | | |
| Age-adjusted | 0 (Reference) | 241 (-177 to 660) | 54 (-367 to 476) | 14 (-406 to 434) | 0.64 | -23 (-110 to 64) |
| Multivariable | 0 (Reference) | 396 (-97 to 889) | 182 (-334 to 698) | -2 (-597 to 594) | 0.52 | -19 (-148 to 110) |
| Asians/Pacific Islanders | | | | | | |
| Median, ng/dL | 0.012 | 0.034 | 0.095 | 0.241 | | |
| Age-adjusted | 0 (Reference) | 201 (-279 to 682) | -18 (-498 to 462) | -541 (-1027 to -55) | 0.003* | -150 (-245 to -55) |
| Multivariable | 0 (Reference) | 415 (-159 to 989) | -180 (-789 to 430) | -785 (-1522 to -48) | 0.003* | -202 (-353 to -51) |
| Total testosterone | | | | | | |
| Pooled | | | | | | |
| Median, ng/dL | 1.9 | 7.6 | 15.3 | 29.9 | | |
| Age-adjusted | 0 (Reference) | -41 (-233 to 151) | -220 (-411 to -28) | -198 (-391 to -6) | 0.02 | -54 (-101 to -7) |
| Multivariable | 0 (Reference) | -17 (-238 to 204) | -233 (-457 to -9) | -182 (-420 to 56) | 0.08 | -57 (-115 to 1) |
| Blacks | | | | | | |
| Median, ng/dL | 1.9 | 8.5 | 16.6 | 32.1 | | |
| Age-adjusted | 0 (Reference) | -123 (-365 to 119) | -277 (-518 to -37) | -49 (-290 to 193) | 0.78 | -30 (-89 to 29) |
| Multivariable | 0 (Reference) | -148 (-426 to 129) | -209 (-492 to 73) | -25 (-325 to 274) | 0.93 | -18 (-91 to 55) |
| Hispanics | | | | | | |
| Median, ng/dL | 1.9 | 7.0 | 13.1 | 28.2 | | |
| Age-adjusted | 0 (Reference) | 193 (-228 to 615) | 9 (-407 to 426) | -110 (-531 to 311) | 0.35 | -30 (-135 to 75) |
| Multivariable | 0 (Reference) | 324 (-170 to 817) | 68 (-444 to 581) | -22 (-540 to 497) | 0.55 | -21 (-150 to 109) |
| Asians/Pacific Islanders | | | | | | |
| Median, ng/dL | 1.9 | 5.5 | 11.6 | 22.4 | | |
| Age-adjusted | 0 (Reference) | 5 (-476 to 486) | -283 (-763 to 197) | -681 (-1161 to -202) | 0.001* | -206 (-323 to -88) |
| Multivariable | 0 (Reference) | 178 (-397 to 753) | -190 (-775 to 395) | -660 (-1274 to -45) | 0.008* | -203 (-355 to -50) |

Numbers are adjusted difference of telomere length (base pairs) with the reference group (95% CI). * False discovery rate < 0.05.
Multivariable: adjusted for age, race/ethnicity, HRT, years between menarche and menopause, case/control, BMI, physical exercise, total energy intake, smoking, alcohol consumption, SHBG, and estradiol.

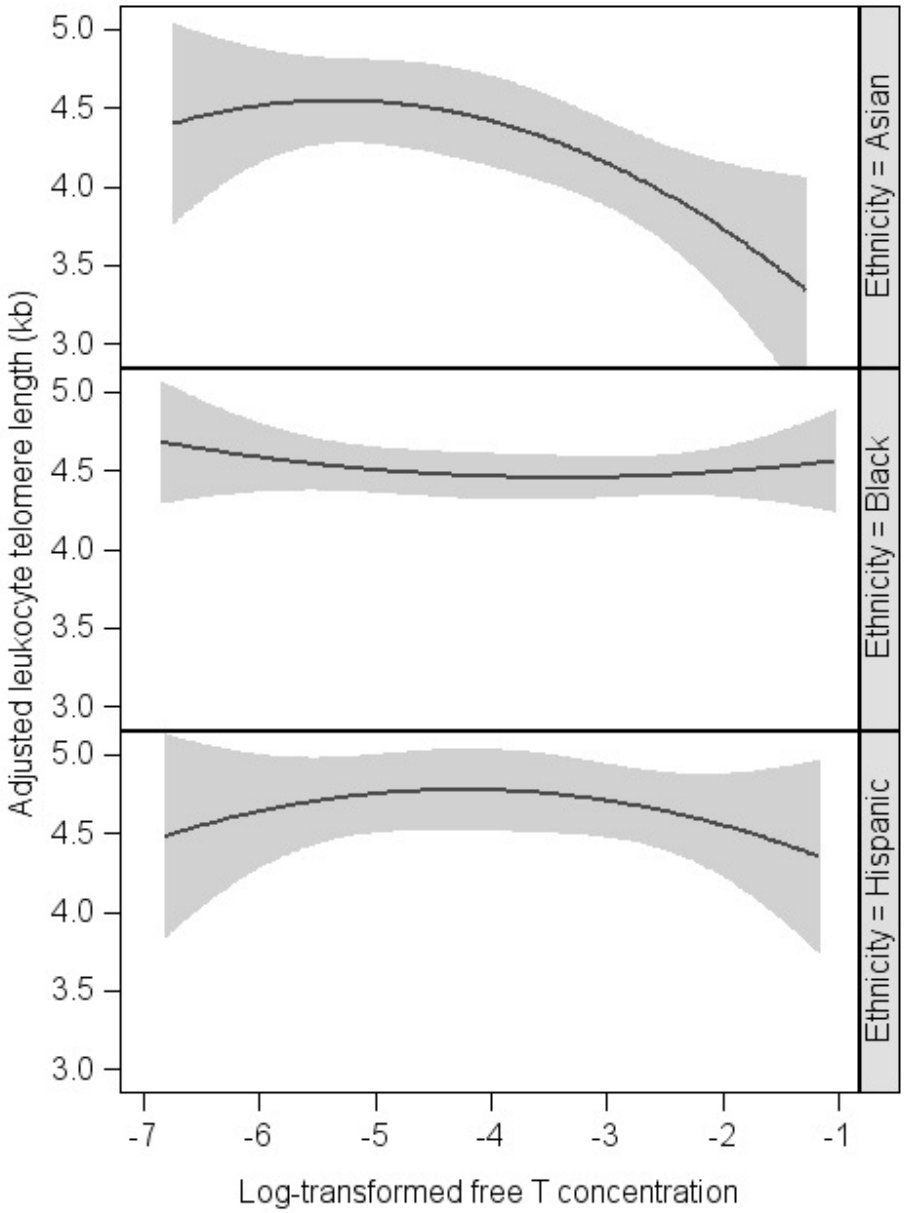


**Figure 4.1 Cubic spline models of the association between free testosterone concentration and leukocyte telomere length by race/ethnicity.**

## CONCLUSIONS AND FUTURE RESEARCH DIRESCTIONS

The current work focuses on the identification of systemic biomarkers in serum, urine, and DNA samples as indicators for the development of T2D and obesity in US women. Chapter 1 synthesized existing evidence regarding the association between different EDCs and T2D risk via a meta-analysis. Our systematic review of 46 studies comprising 51,687 individuals from different populations (including white, black, Hispanic, Asian, and Native American) indicates that higher serum concentrations of persistent EDCs and urinary concentrations of non-persistent EDCs may significantly increase T2D risk, and that the magnitudes of associations with PCBs appear sex-dependent (stronger in women than in men). Our findings emphasize the importance of environmental factors in the etiology of T2D. As the existing studies regarding the association between non-persistent EDCs and risk of T2D are all cross-sectional, large and high-quality prospective studies that comprehensively assess concentrations of these EDCs are urgently needed to clarify the role of these environmental factors in the ongoing T2D epidemics in human populations.

In Chapter 2, we examined the association between BPA and phthalate exposure and prospective weight change in NHS and NHSII. We observed that higher urinary concentrations of BPA, phthalic acid, MBzP, and butyl phthalates were significantly associated with faster weight gain in U.S. women. The results are consistent with an etiological role of BPA and phthalates in weight gain, although we cannot exclude the possibility of chance findings, especially when associations were attenuated in stratified analyses. Future large-scale studies with repeated assessments of the levels of these chemicals are needed to replicate these observations.

The results from a mediation analysis of the association between LBW and T2D risk were described in Chapter 3. In this study, we confirmed that LBW was consistently associated with increased risk of T2D later in life in a multiethnic population of women. In addition, we found that the total effect of LBW on risk of T2D is mainly mediated by insulin resistance which is further explained by circulating levels of SHBG, E-selectin, and systolic blood pressure. These prospective data provide quantifiable mechanistic evidence linking LBW to increased risk of T2D whilst presenting risk stratification and intervention in a population at greater risk of developing T2D later in life.

In Chapter 4, we investigated the association between serum sex hormone concentrations and biologic aging, as indicated by leukocyte TL. Serum concentration of estradiol was not significantly associated with leukocyte TL in the multiethnic postmenopausal women population. However, higher total and free testosterone concentrations appeared significantly associated with shorter TL in Asian/Pacific Islander women but not in black or Hispanic women. These findings suggest that Asian/Pacific Islander women may be susceptible to the potential detrimental effects of high testosterone level on biologic aging. Prospective studies incorporating larger numbers of ethnic minorities followed by serial hormonal and telomere length measurements are also essential to further justify and explain our observed associations.

Although further evidence is necessary to confirm these findings, this set of studies indicates that the systemic biomarkers in various biological samples, including serum, urine, and DNA, as indicators for environmental risk factors and the development of T2D and obesity in US women.

**REFERENCES**


1. Halter J, Ouslander J, Tinetti M, Studenski S, High K, Asthana S. *Hazzard's Geriatric Medicine and Gerontology.* 6th ed: McGraw-Hill Professional; 2009.
2. National Center for Chronic Disease Prevention and Health Promotion. *National Diabetes Fact Sheet, 2011.* Atlanta2011.
3. Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP. The continuing epidemic of obesity in the United States. *JAMA.* Oct 4 2000;284(13):1650-1651.
4. Astrup A, Finer N. Redefining type 2 diabetes: 'diabesity' or 'obesity dependent diabetes mellitus'? *Obes Rev.* Oct 2000;1(2):57-59.
5. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA : the journal of the American Medical Association.* Mar 15 2006;295(11):1288-1299.
6. Ding EL, Song Y, Manson JE, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med.* Sep 17 2009;361(12):1152-1163.
7. Ding EL, Song Y, Manson JE, Rifai N, Buring JE, Liu S. Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study. *Diabetologia.* Oct 2007;50(10):2076-2084.
8. Li C, Ford ES, Li B, Giles WH, Liu S. Association of testosterone and sex hormone-binding globulin with metabolic syndrome and insulin resistance in men. *Diabetes Care.* Jul 2010;33(7):1618-1624.

9. Calafat AM, Weuve J, Ye X, et al. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. *Environ Health Perspect.* Apr 2009;117(4):639-644.

10. Alonso-Magdalena P, Quesada I, Nadal A. Endocrine disruptors in the etiology of type 2 diabetes mellitus. *Nat Rev Endocrinol.* Jun 2011;7(6):346-353.

11. De Coster S, van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. *J Environ Public Health.* 2012;2012:713696.

12. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia.* Feb 1994;37(2):150-154.

13. Dabelea D, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC. Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. *Diabetes Care.* Jun 1999;22(6):944-950.

14. Li C, Johnson MS, Goran MI. Effects of low birth weight on insulin resistance syndrome in caucasian and African-American children. *Diabetes Care.* Dec 2001;24(12):2035-2042.

15. Kajantie E, Pietilainen KH, Wehkalampi K, et al. No association between body size at birth and leucocyte telomere length in adult life--evidence from three cohort studies. *Int J Epidemiol.* Oct 2012;41(5):1400-1408.

16. Tzoulaki I, Jarvelin MR, Hartikainen AL, et al. Size at birth, weight gain over the life course, and low-grade inflammation in young adulthood: northern Finland 1966 Birth Cohort study. *Eur Heart J.* Apr 2008;29(8):1049-1056.

17. Leeson CP, Kattenhorn M, Morley R, Lucas A, Deanfield JE. Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. *Circulation.* Mar 6 2001;103(9):1264-1268.

18. Goodfellow J, Bellamy MF, Gorman ST, et al. Endothelial function is impaired in fit young adults of low birth weight. *Cardiovasc Res.* Dec 1998;40(3):600-606.

19. Law CM, Shiell AW. Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. *J Hypertens.* Aug 1996;14(8):935-941.

20. Ding EL, Song Y, Manson JE, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med.* Sep 17 2009;361(12):1152-1163.

21. Vanbillemont G, Lapauw B, Bogaert V, et al. Birth weight in relation to sex steroid status and body composition in young healthy male siblings. *J Clin Endocrinol Metab.* Apr;95(4):1587-1594.

22. Pearce MS, Groom A, Relton CL, Peaston RT, Pollard TM, Francis RM. Birth weight and early socio-economic disadvantage as predictors of sex hormones and sex hormone binding globulin in men at age 49-51 years. *Am J Hum Biol.* Mar-Apr;23(2):185-189.

23. Ibanez L, Lopez-Bermejo A, Diaz M, Suarez L, de Zegher F. Low-birth weight children develop lower sex hormone binding globulin and higher dehydroepiandrosterone sulfate levels and aggravate their visceral adiposity and hypoadiponectinemia between six and eight years of age. *J Clin Endocrinol Metab.* Oct 2009;94(10):3696-3699.

24. Whincup PH, Kaye SJ, Owen CG, et al. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA.* Dec 24 2008;300(24):2886-2897.

25. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ.* Oct 26 1991;303(6809):1019-1022.

26. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature.* May 31 1990;345(6274):458-460.

27. Seifarth JE, McGowan CL, Milne KJ. Sex and life expectancy. *Gend Med.* Dec 2012;9(6):390-401.

28. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev.* Jun 2009;30(4):293-342.

29. Thayer KA, Heindel JJ, Bucher JR, Gallo MA. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environ Health Perspect.* Jun 2012;120(6):779-789.

30. Wu H, Bertrand KA, Choi AL, et al. Persistent Organic Pollutants and Type 2 Diabetes: A Prospective Analysis in the Nurses' Health Study and Meta-analysis. *Environ Health Perspect.* Feb 2013;121(2):153-161.

31. Silverstone AE, Rosenbaum PF, Weinstock RS, et al. Polychlorinated biphenyl (PCB) exposure and diabetes: results from the Anniston Community Health Survey. *Environ Health Perspect.* May 2012;120(5):727-732.

32. U.S. Environmental Protection Agency. Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds. 2010; http://www.epa.gov/raf/files/tefs-for-dioxin-epa-00-r-10-005-final.pdf. Accessed May 26, 2013.

33. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* Sep 1986;7(3):177-188.

34. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol.* Jun 1 1992;135(11):1301-1309.

35. Borenstein M, Hedges L, Higgins J, Rothstein H. Converting among effect sizes. *Introduction to Meta-Analysis*. Chichester: John Wiley & Sons, Ltd; 2009.

36. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics.* Jun 2000;56(2):455-463.

37. Calvert GM, Sweeney MH, Deddens J, Wall DK. Evaluation of diabetes mellitus, serum glucose, and thyroid function among United States workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Occup Environ Med.* Apr 1999;56(4):270-276.

38. Uemura H, Arisawa K, Hiyoshi M, et al. Associations of environmental exposure to dioxins with prevalent diabetes among general inhabitants in Japan. *Environ Res.* Sep 2008;108(1):63-68.

39. Philibert A, Schwartz H, Mergler D. An exploratory study of diabetes in a First Nation community with respect to serum concentrations of p,p'-DDE and PCBs and fish consumption. *Int J Environ Res Public Health.* Dec 2009;6(12):3179-3189.

40. Rignell-Hydbom A, Lidfeldt J, Kiviranta H, et al. Exposure to p,p'-DDE: a risk factor for type 2 diabetes. *PLoS One.* 2009;4(10):e7503.

41. Wang SL, Tsai PC, Yang CY, Leon Guo Y. Increased risk of diabetes and polychlorinated biphenyls and dioxins: a 24-year follow-up study of the Yucheng cohort. *Diabetes Care.* Aug 2008;31(8):1574-1579.

42. Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. *Environ Health Perspect.* Sep 2010;118(9):1235-1242.

43. Lim JS, Lee DH, Jacobs DR, Jr. Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003-2004. *Diabetes Care.* Sep 2008;31(9):1802-1807.

44. Vasiliu O, Cameron L, Gardiner J, Deguire P, Karmaus W. Polybrominated biphenyls, polychlorinated biphenyls, body weight, and incidence of adult-onset diabetes mellitus. *Epidemiology.* Jul 2006;17(4):352-359.

45. Ruzzin J, Petersen R, Meugnier E, et al. Persistent organic pollutant exposure leads to insulin resistance syndrome. *Environ Health Perspect.* Apr 2010;118(4):465-471.

46. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect.* Jan 2006;114(1):106-112.

47. Rajesh P, Sathish S, Srinivasan C, Selvaraj J, Balasubramanian K. Exposure to diethyl hexyl phthalate (DEHP) to adult male rat is associated with insulin resistance in adipose tisssue: Protective role of antioxidant vitamins (C & E). *J Cell Biochem.* Sep 18 2012;114(3):558-569.

48. Enan E, Matsumura F. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced changes in glucose transporting activity in guinea pigs, mice, and rats in vivo and in vitro. *J Biochem Toxicol.* Apr 1994;9(2):97-106.

49. Marchand A, Tomkiewicz C, Marchandeau JP, Boitier E, Barouki R, Garlatti M. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces insulin-like growth factor binding protein-1 gene

expression and counteracts the negative effect of insulin. *Mol Pharmacol.* Feb 2005;67(2):444-452.

50. Remillard RB, Bunce NJ. Linking dioxins to diabetes: epidemiology and biologic plausibility. *Environ Health Perspect.* Sep 2002;110(9):853-858.

51. Quesada I, Fuentes E, Viso-Leon MC, Soria B, Ripoll C, Nadal A. Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *Faseb J.* Oct 2002;16(12):1671-1673.

52. Wetherill YB, Akingbemi BT, Kanno J, et al. In vitro molecular mechanisms of bisphenol A action. *Reprod Toxicol.* Aug-Sep 2007;24(2):178-198.

53. Masuno H, Iwanami J, Kidani T, Sakayama K, Honda K. Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway. *Toxicol Sci.* Apr 2005;84(2):319-327.

54. Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environ Health Perspect.* 2008;116(12):1642-1647.

55. Wada K, Sakamoto H, Nishikawa K, et al. Life style-related diseases of the digestive system: endocrine disruptors stimulate lipid accumulation in target cells related to metabolic syndrome. *J Pharmacol Sci.* Oct 2007;105(2):133-137.

56. Masuyama H, Hiramatsu Y, Kunitomi M, Kudo T, MacDonald PN. Endocrine disrupting chemicals, phthalic acid and nonylphenol, activate Pregnane X receptor-mediated transcription. *Mol Endocrinol.* Mar 2000;14(3):421-428.

57. Takeshita A, Koibuchi N, Oka J, Taguchi M, Shishiba Y, Ozawa Y. Bisphenol-A, an environmental estrogen, activates the human orphan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. *Eur J Endocrinol.* Oct 2001;145(4):513-517.

58. Grun F, Blumberg B. Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. *Rev Endocr Metab Disord.* Jun 2007;8(2):161-171.

59. Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. Oestrogenic activity of parabens in MCF7 human breast cancer cells. *J Steroid Biochem Mol Biol.* Jan 2002;80(1):49-60.

60. Jung EM, An BS, Choi KC, Jeung EB. Potential estrogenic activity of triclosan in the uterus of immature rats and rat pituitary GH3 cells. *Toxicol Lett.* Jan 25 2012;208(2):142-148.

61. Janjua NR, Mogensen B, Andersson AM, et al. Systemic absorption of the sunscreens benzophenone-3, octyl-methoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans. *J Invest Dermatol.* Jul 2004;123(1):57-61.

62. LaKind JS, Goodman M, Naiman DQ. Use of NHANES data to link chemical exposures to chronic diseases: a cautionary tale. *PLoS One.* 2012;7(12):e51086.

63. Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. *Diabetologia.* Sep 2007;50(9):1841-1851.

64. Lang IA, Galloway TS, Scarlett A, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. *JAMA.* Sep 17 2008;300(11):1303-1310.

65. Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. *PLoS One.* 2010;5(1):e8673.

66. Shankar A, Teppala S. Relationship between urinary bisphenol A levels and diabetes mellitus. *J Clin Endocrinol Metab.* Dec 2011;96(12):3822-3826.

67. Silver MK, O'Neill MS, Sowers MR, Park SK. Urinary bisphenol A and type-2 diabetes in U.S. adults: data from NHANES 2003-2008. *PLoS One.* 2011;6(10):e26868.

68. Ning G, Bi Y, Wang T, et al. Relationship of urinary bisphenol A concentration to risk for prevalent type 2 diabetes in Chinese adults: a cross-sectional analysis. *Ann Intern Med.* Sep 20 2011;155(6):368-374.

69. Turyk M, Anderson HA, Knobeloch L, Imm P, Persky VW. Prevalence of diabetes and body burdens of polychlorinated biphenyls, polybrominated diphenyl ethers, and p,p'-diphenyldichloroethene in Great Lakes sport fish consumers. *Chemosphere.* May 2009;75(5):674-679.

70. Grandjean P, Henriksen JE, Choi AL, et al. Marine food pollutants as a risk factor for hypoinsulinemia and type 2 diabetes. *Epidemiology.* May 2011;22(3):410-417.

71. Patel CJ, Bhattacharya J, Butte AJ. An Environment-Wide Association Study (EWAS) on type 2 diabetes mellitus. *PLoS One.* 2010;5(5):e10746.

72. Persky V, Piorkowski J, Turyk M, et al. Associations of polychlorinated biphenyl exposure and endogenous hormones with diabetes in post-menopausal women previously employed at a capacitor manufacturing plant. *Environ Res.* Aug 2011;111(6):817-824.

73. Persky V, Piorkowski J, Turyk M, et al. Polychlorinated biphenyl exposure, diabetes and endogenous hormones: a cross-sectional study in men previously employed at a capacitor manufacturing plant. *Environ Health.* 2012;11:57.

74. Svensson K, Hernandez-Ramirez RU, Burguete-Garcia A, et al. Phthalate exposure associated with self-reported diabetes among Mexican women. *Environ Res.* Aug 2011;111(6):792-796.

75. Montgomery MP, Kamel F, Saldana TM, Alavanja MC, Sandler DP. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993-2003. *Am J Epidemiol.* May 15 2008;167(10):1235-1246.

76. Longnecker MP, Michalek JE. Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure. *Epidemiology.* Jan 2000;11(1):44-48.

77. Cranmer M, Louie S, Kennedy RH, Kern PA, Fonseca VA. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is associated with hyperinsulinemia and insulin resistance. *Toxicol Sci.* Aug 2000;56(2):431-436.

78. Fierens S, Mairesse H, Heilier JF, et al. Dioxin/polychlorinated biphenyl body burden, diabetes and endometriosis: findings in a population-based study in Belgium. *Biomarkers.* Nov-Dec 2003;8(6):529-534.

79. Lee DH, Lee IK, Song K, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002. *Diabetes Care.* Jul 2006;29(7):1638-1644.

80. Chen BH, Brennan K, Goto A, et al. Sex hormone-binding globulin and risk of clinical diabetes in american black, Hispanic, and asian/pacific islander postmenopausal women. *Clin Chem.* Oct 2012;58(10):1457-1466.

81. Everett CJ, Frithsen IL, Diaz VA, Koopman RJ, Simpson WM, Jr., Mainous AG, 3rd. Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and DDT with diabetes in the 1999-2002 National Health and Nutrition Examination Survey. *Environ Res.* Mar 2007;103(3):413-418.

82. Chang JW, Chen HL, Su HJ, Liao PC, Guo HR, Lee CC. Dioxin exposure and insulin resistance in Taiwanese living near a highly contaminated area. *Epidemiology.* Jan 2010;21(1):56-61.

83. Everett CJ, Thompson OM. Associations of dioxins, furans and dioxin-like PCBs with diabetes and pre-diabetes: Is the toxic equivalency approach useful? *Environ Res.* Oct 2012;118:107-111.

84. Longnecker MP, Klebanoff MA, Brock JW, Zhou H, Collaborative Perinatal P. Polychlorinated biphenyl serum levels in pregnant subjects with diabetes. *Diabetes Care.* Jun 2001;24(6):1099-1101.

85. Rylander L, Rignell-Hydbom A, Hagmar L. A cross-sectional study of the association between persistent organochlorine pollutants and diabetes. *Environ Health.* 2005;4:28.

86. Codru N, Schymura MJ, Negoita S, Akwesasne Task Force on E, Rej R, Carpenter DO. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated

pesticides in adult Native Americans. *Environ Health Perspect.* Oct 2007;115(10):1442-1447.

87. Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. *Hum Exp Toxicol.* May 2007;26(5):447-452.

88. Jorgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland Inuit. *Diabetologia.* Aug 2008;51(8):1416-1422.

89. Chen JW, Wang SL, Liao PC, Chen HY, Ko YC, Lee CC. Relationship between insulin sensitivity and exposure to dioxins and polychlorinated biphenyls in pregnant women. *Environ Res.* Jun 2008;107(2):245-253.

90. Langer P, Kocan A, Tajtakova M, et al. Multiple adverse thyroid and metabolic health signs in the population from the area heavily polluted by organochlorine cocktail (PCB, DDE, HCB, dioxin). *Thyroid Res.* 2009;2(1):3.

91. Ukropec J, Radikova Z, Huckova M, et al. High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. *Diabetologia.* May 2010;53(5):899-906.

92. Airaksinen R, Rantakokko P, Eriksson JG, Blomstedt P, Kajantie E, Kiviranta H. Association between type 2 diabetes and exposure to persistent organic pollutants. *Diabetes Care.* Sep 2011;34(9):1972-1979.

93. Dirinck E, Jorens PG, Covaci A, et al. Obesity and persistent organic pollutants: possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. *Obesity (Silver Spring).* Apr 2011;19(4):709-714.

94. Gasull M, Pumarega J, Tellez-Plaza M, et al. Blood concentrations of persistent organic pollutants and prediabetes and diabetes in the general population of Catalonia. *Environ Sci Technol.* Jul 17 2012;46(14):7799-7810.

95. Cox S, Niskar AS, Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic health and nutrition examination survey, 1982-1984. *Environ Health Perspect.* Dec 2007;115(12):1747-1752.

96. Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. *Diabetes Care.* Mar 2007;30(3):622-628.

97. Son HK, Kim SA, Kang JH, et al. Strong associations between low-dose organochlorine pesticides and type 2 diabetes in Korea. *Environ Int.* Jul 2010;36(5):410-414.

98. Park SK, Son HK, Lee SK, et al. Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among non-diabetic adults. *J Prev Med Public Health.* Jan 2010;43(1):1-8.

99. Arrebola JP, Pumarega J, Gasull M, et al. Adipose tissue concentrations of persistent organic pollutants and prevalence of type 2 diabetes in adults from Southern Spain. *Environ Res.* Jan 2 2013.

100. Li TT, Xu LZ, Chen YH, et al. [Effects of eight environmental endocrine disruptors on insulin resistance in patients with polycystic ovary syndrome: a preliminary investigation]. *Nan Fang Yi Ke Da Xue Xue Bao.* Oct 2011;31(10):1753-1756.

101. Wang T, Li M, Chen B, et al. Urinary bisphenol A (BPA) concentration associates with obesity and insulin resistance. *J Clin Endocrinol Metab.* Feb 2012;97(2):E223-227.

102. Kim K, Park H. Association between urinary concentrations of bisphenol A and type 2 diabetes in Korean adults: A population-based cross-sectional study. *Int J Hyg Environ Health.* Aug 23 2012.

103. Stahlhut RW, van Wijngaarden E, Dye TD, Cook S, Swan SH. Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males. *Environ Health Perspect.* Jun 2007;115(6):876-882.

104. Lind PM, Zethelius B, Lind L. Circulating levels of phthalate metabolites are associated with prevalent diabetes in the elderly. *Diabetes Care.* Jul 2012;35(7):1519-1524.

105. James-Todd T, Stahlhut R, Meeker JD, et al. Urinary Phthalate Metabolite Concentrations and Diabetes among Women in the National Health and Nutrition Examination Survey (NHANES) 2001-2008. *Environ Health Perspect.* Sep 2012;120(9):1307-1313.

106. Olsen L, Lind L, Lind PM. Associations between circulating levels of bisphenol A and phthalate metabolites and coronary risk in the elderly. *Ecotoxicol Environ Saf.* Jun 2012;80:179-183.

107. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect.* Jul 2009;117(7):1076-1082.

108. Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes

in the elderly: the prospective investigation of the vasculature in Uppsala Seniors (PIVUS) study. *Diabetes Care.* Aug 2011;34(8):1778-1784.

109. Pirkle JL, Wolfe WH, Patterson DG, et al. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Vietnam Veterans of Operation Ranch Hand. *J Toxicol Environ Health.* 1989;27(2):165-171.

110. Phillips DL, Smith AB, Burse VW, Steele GK, Needham LL, Hannon WH. Half-life of polychlorinated biphenyls in occupationally exposed workers. *Arch Environ Health.* Nov-Dec 1989;44(6):351-354.

111. Rosen DH, Flanders WD, Friede A, Humphrey HE, Sinks TH. Half-life of polybrominated biphenyl in human sera. *Environ Health Perspect.* Mar 1995;103(3):272-274.

112. Ferreira CP, De-Oliveira AC, Paumgartten FJ. Serum concentrations of DDT and DDE among malaria control workers in the Amazon region. *J Occup Health.* 2011;53(2):115-122.

113. Wolff MS. Half-lives of organochlorines (OCs) in humans. *Arch Environ Contam Toxicol.* May 1999;36(4):504.

114. World Health Orgnization. Environmental health criteria 44: Mirex. 1984; http://www.inchem.org/documents/ehc/ehc/ehc44.htm - SectionNumber:3.4. Accessed May 28, 2013.

115. World Federation of Public Health Association. Persistent organic pollutants and human health. 2000; http://www.wfpha.org/tl_files/doc/about/POPs WFPHA 2000.pdf. Accessed May 28, 2013.

116. Olsen GW, Burris JM, Ehresman DJ, et al. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect.* Sep 2007;115(9):1298-1305.

117. Stahlhut RW, Welshons WV, Swan SH. Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environ Health Perspect.* May 2009;117(5):784-789.

118. Koch HM, Bolt HM, Angerer J. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch Toxicol.* Mar 2004;78(3):123-130.

119. Gilbert RJ, Williams PE. The oral retention and antiplaque efficacy of triclosan in human volunteers. *Br J Clin Pharmacol.* May 1987;23(5):579-583.

120. Crews D, McLachlan JA. Epigenetics, evolution, endocrine disruption, health, and disease. *Endocrinology.* Jun 2006;147(6 Suppl):S4-10.

121. Hauser R, Calafat AM. Phthalates and human health. *Occup Environ Med.* Nov 2005;62(11):806-818.

122. Geens T, Aerts D, Berthot C, et al. A review of dietary and non-dietary exposure to bisphenol-A. *Food Chem Toxicol.* Oct 2012;50(10):3725-3740.

123. Newbold RR, Padilla-Banks E, Jefferson WN. Environmental estrogens and obesity. *Mol Cell Endocrinol.* May 25 2009;304(1-2):84-89.

124. Bhandari R, Xiao J, Shankar A. Urinary bisphenol A and obesity in U.S. children. *Am J Epidemiol.* Jun 1 2013;177(11):1263-1270.

125. Li DK, Miao M, Zhou Z, et al. Urine bisphenol-a level in relation to obesity and overweight in school-age children. *PLoS One.* 2013;8(6):e65399.

126. Harley KG, Aguilar Schall R, Chevrier J, et al. Prenatal and Postnatal Bisphenol A Exposure and Body Mass Index in Childhood in the CHAMACOS Cohort. *Environ Health Perspect.* Feb 15 2013.

127. Wells EM, Jackson LW, Koontz MB. Association between bisphenol A and waist-to-height ratio among children: National Health and Nutrition Examination Survey, 2003-2010. *Ann Epidemiol.* Jul 3 2013.

128. Wang HX, Zhou Y, Tang CX, Wu JG, Chen Y, Jiang QW. Association between bisphenol A exposure and body mass index in Chinese school children: a cross-sectional study. *Environ Health.* 2012;11:79.

129. Trasande L, Attina TM, Blustein J. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. *JAMA.* Sep 19 2012;308(11):1113-1121.

130. Shankar A, Teppala S, Sabanayagam C. Urinary bisphenol a levels and measures of obesity: results from the national health and nutrition examination survey 2003-2008. *ISRN Endocrinol.* 2012;2012:965243.

131. Carwile JL, Michels KB. Urinary bisphenol A and obesity: NHANES 2003-2006. *Environ Res.* Aug 2011;111(6):825-830.

132. Wang H, Zhou Y, Tang C, et al. Urinary phthalate metabolites are associated with body mass index and waist circumference in chinese school children. *PLoS One.* 2013;8(2):e56800.

133. Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. Race/Ethnicity-Specific Associations of Urinary Phthalates with Childhood Body Mass in a Nationally Representative Sample. *Environ Health Perspect.* Feb 4 2013.

134. Lind PM, Roos V, Ronn M, et al. Serum concentrations of phthalate metabolites are related to abdominal fat distribution two years later in elderly women. *Environ Health.* 2012;11:21.

135. Hatch EE, Nelson JW, Qureshi MM, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. *Environ Health.* 2008;7:27.

136. Sun Q, Cornelis M, Townsend M, et al. Urinary Concentrations of Bisphenol A and Phthalate Metabolites and Risk of Type 2 Diabetes: A Prospective Investigation among U.S. Women. *Environ Health Perspect.* 2013.

137. Willett W, Stampfer MJ, Bain C, et al. Cigarette smoking, relative weight, and menopause. *Am J Epidemiol.* Jun 1983;117(6):651-658.

138. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr.* Jun 2012;142(6):1009-1018.

139. Fox SD, Falk RT, Veenstra TD, Issaq HJ. Quantitation of free and total bisphenol A in human urine using liquid chromatography-tandem mass spectrometry. *J Sep Sci.* Jun 2011;34(11):1268-1274.

140. Kato K, Silva MJ, Needham LL, Calafat AM. Determination of 16 phthalate metabolites in urine using automated sample preparation and on-line preconcentration/high-performance liquid chromatography/tandem mass spectrometry. *Anal Chem.* May 1 2005;77(9):2985-2991.

141. Blount BC, Milgram KE, Silva MJ, et al. Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. *Anal Chem.* Sep 1 2000;72(17):4127-4134.

142. Melzer D, Osborne NJ, Henley WE, et al. Urinary bisphenol A concentration and risk of future coronary artery disease in apparently healthy men and women. *Circulation.* Mar 27 2012;125(12):1482-1490.

143. Braun JM, Kalkbrenner AE, Calafat AM, et al. Variability and predictors of urinary bisphenol A concentrations during pregnancy. *Environ Health Perspect.* Jan 2011;119(1):131-137.

144. Buckley JP, Palmieri RT, Matuszewski JM, et al. Consumer product exposures associated with urinary phthalate levels in pregnant women. *J Expo Sci Environ Epidemiol.* Sep 2012;22(5):468-475.

145. Grun F, Blumberg B. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology.* Jun 2006;147(6 Suppl):S50-55.

146. Masuno H, Kidani T, Sekiya K, et al. Bisphenol A in combination with insulin can accelerate the conversion of 3T3-L1 fibroblasts to adipocytes. *J Lipid Res.* May 2002;43(5):676-684.

147. Soriano S, Alonso-Magdalena P, Garcia-Arevalo M, et al. Rapid insulinotropic action of low doses of bisphenol-A on mouse and human islets of Langerhans: role of estrogen receptor beta. *PLoS One.* 2012;7(2):e31109.

148. Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. *Int J Hyg Environ Health.* Oct 2007;210(5):623-634.

149. Desvergne B, Feige JN, Casals-Casas C. PPAR-mediated activity of phthalates: A link to the obesity epidemic? *Mol Cell Endocrinol.* May 25 2009;304(1-2):43-48.

150. Feige JN, Gelman L, Rossi D, et al. The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor gamma modulator that promotes adipogenesis. *J Biol Chem.* Jun 29 2007;282(26):19152-19166.

151. Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicol Sci.* Aug 2003;74(2):297-308.

152. Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PLoS One.* 2011;6(1):e15977.

153. Townsend MK, Franke AA, Li X, Hu FB, Eliassen AH. Within-person reproducibility of urinary bisphenol A and phthalate metabolites over a 1 to 3 year period among women in the Nurses' Health Studies: a prospective cohort study. *Environ Health.* Sep 13 2013;12(1):80.

154. Mahalingaiah S, Meeker JD, Pearson KR, et al. Temporal variability and predictors of urinary bisphenol A concentrations in men and women. *Environ Health Perspect.* Feb 2008;116(2):173-178.

155. Mericq V. Low birth weight and endocrine dysfunction in postnatal life. *Pediatr Endocrinol Rev.* Sep 2006;4(1):3-14.

156. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation.* Dec 15 1996;94(12):3246-3250.

157. You NC, Chen BH, Song Y, et al. A prospective study of leukocyte telomere length and risk of type 2 diabetes in postmenopausal women. *Diabetes.* Nov 2012;61(11):2998-3004.

158. Song Y, Manson JE, Tinker L, et al. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. *Diabetes.* Jul 2007;56(7):1898-1904.

159. Liu S, Tinker L, Song Y, et al. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Arch Intern Med.* Aug 13-27 2007;167(15):1676-1685.

160. Margolis KL, Lihong Q, Brzyski R, et al. Validity of diabetes self-reports in the Women's Health Initiative: comparison with medication inventories and fasting glucose measurements. *Clin Trials.* 2008;5(3):240-247.

161. Vanderweele TJ, Vansteelandt S. Odds ratios for mediation analysis for a dichotomous outcome. *Am J Epidemiol.* Dec 15 2010;172(12):1339-1348.

162. Couzin-Frankel J. Mysteries of development. How does fetal environment influence later health? *Science.* Jun 7 2013;340(6137):1160-1161.

163. Stoger R. The thrifty epigenotype: an acquired and heritable predisposition for obesity and diabetes? *Bioessays.* Feb 2008;30(2):156-166.

164. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol.* Dec 1986;51(6):1173-1182.

165. MacKinnon DP. *Introduction to Statistical Mediation Analysis.* New York: Taylor & Francis Group; 2008.

166. Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. *Psychol Methods.* Dec 2010;15(4):309-334.

167. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med.* Jul 3 2008;359(1):61-73.

168. Stefan N, Haring HU. The role of hepatokines in metabolism. *Nat Rev Endocrinol.* Mar 2013;9(3):144-152.

169. Hotamisligil GS. Inflammation and metabolic disorders. *Nature.* Dec 14 2006;444(7121):860-867.

170. Khan MS, Knowles BB, Aden DP, Rosner W. Secretion of testosterone-estradiol-binding globulin by a human hepatoma-derived cell line. *J Clin Endocrinol Metab.* Aug 1981;53(2):448-449.

171. Perry JR, Weedon MN, Langenberg C, et al. Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum Mol Genet.* Feb 1 2010;19(3):535-544.

172. Price DT, Loscalzo J. Cellular adhesion molecules and atherogenesis. *Am J Med.* Jul 1999;107(1):85-97.

173. Bautista LE. Inflammation, endothelial dysfunction, and the risk of high blood pressure: epidemiologic and biological evidence. *J Hum Hypertens.* Apr 2003;17(4):223-230.

174. Stolk RP, van Splunder IP, Schouten JS, Witteman JC, Hofman A, Grobbee DE. High blood pressure and the incidence of non-insulin dependent diabetes mellitus: findings in a 11.5 year follow-up study in The Netherlands. *Eur J Epidemiol.* Mar 1993;9(2):134-139.

175. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. *N Engl J Med.* Mar 30 2000;342(13):905-912.

176. Sanderson M, Williams MA, White E, et al. Validity and reliability of subject and mother reporting of perinatal factors. *Am J Epidemiol.* Jan 15 1998;147(2):136-140.

177. Kemp M, Gunnell D, Maynard M, Smith GD, Frankel S. How accurate is self reported birth weight among the elderly? *J Epidemiol Community Health.* Aug 2000;54(8):639.

178. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods.* Jun 2013;18(2):137-150.

179. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* Jul 1985;28(7):412-419.

180. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab.* Oct 1999;84(10):3666-3672.

181. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem.* Jun 1982;16(6):801-810.

182. Miller KK, Rosner W, Lee H, et al. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. *J Clin Endocrinol Metab.* Feb 2004;89(2):525-533.

183. Rinaldi S, Geay A, Dechaud H, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. *Cancer Epidemiol Biomarkers Prev.* Oct 2002;11(10 Pt 1):1065-1071.

184. O'Callaghan N, Dhillon V, Thomas P, Fenech M. A quantitative real-time PCR method for absolute telomere length. *Biotechniques.* May 2008;44(6):807-809.

185. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci.* Jun 2000;908:99-110.

186. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet.* Feb 1 2003;361(9355):393-395.

187. Song Y, You NC, Song Y, et al. Intake-of small-to-medium-chain saturated fatty acids is associated with peripheral leukocyte telomere length in postmenopausal women. *J Nutr.* 2013;143.

188. Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet.* Aug 20-26 2005;366(9486):662-664.

189. Arias E. United States life tables, 2006. *Natl Vital Stat Rep.* Jun 28 2010;58(21):1-40.

190. Benetos A, Okuda K, Lajemi M, et al. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension.* Feb 2001;37(2 Part 2):381-385.

191. Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. *Aging Cell.* Dec 2011;10(6):913-921.

192. Endogenous Hormones and Breast Cancer Collaborative Group. Free estradiol and breast cancer risk in postmenopausal women: comparison of measured and calculated values. *Cancer Epidemiol Biomarkers Prev.* Dec 2003;12(12):1457-1461.

193. Wingard DL, Suarez L, Barrett-Connor E. The sex differential in mortality from all causes and ischemic heart disease. *Am J Epidemiol.* Feb 1983;117(2):165-172.

194. Cook MB, McGlynn KA, Devesa SS, Freedman ND, Anderson WF. Sex disparities in cancer mortality and survival. *Cancer Epidemiol Biomarkers Prev.* Aug 2011;20(8):1629-1637.

195. Wingard DL. The sex differential in morbidity, mortality, and lifestyle. *Annu Rev Public Health.* 1984;5:433-458.

196. Hazzard WR, Applebaum-Bowden D. Why women live longer than men: the biologic mechanism of the sex differential in longevity. *Trans Am Clin Climatol Assoc.* 1990;101:168-188; discussion 188-169.

197. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA.* Mar 15 2006;295(11):1288-1299.

198. Fritz MA, Speroff L. *Clinical Gynecologic Endocrinology and Infertility, Eighth Edition.* Lippincott Williams & Wilkins; 2010.

199. Harte AL, da Silva NF, Miller MA, et al. Telomere length attrition, a marker of biological senescence, is inversely correlated with triglycerides and cholesterol in South Asian males with type 2 diabetes mellitus. *Exp Diabetes Res.* 2012;2012:895185.

200. Monickaraj F, Aravind S, Gokulakrishnan K, et al. Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes. *Mol Cell Biochem.* Jun 2012;365(1-2):343-350.

201. Zee RY, Castonguay AJ, Barton NS, Germer S, Martin M. Mean leukocyte telomere length shortening and type 2 diabetes mellitus: a case-control study. *Transl Res.* Apr 2010;155(4):166-169.

202. Salpea KD, Humphries SE. Telomere length in atherosclerosis and diabetes. *Atherosclerosis.* Mar 2010;209(1):35-38.

203. Bayne S, Jones ME, Li H, Pinto AR, Simpson ER, Liu JP. Estrogen deficiency leads to telomerase inhibition, telomere shortening and reduced cell proliferation in the adrenal gland of mice. *Cell Res.* Nov 2008;18(11):1141-1150.

204. Bayne S, Li H, Jones ME, et al. Estrogen deficiency reversibly induces telomere shortening in mouse granulosa cells and ovarian aging in vivo. *Protein Cell.* Apr 2011;2(4):333-346.

205. Aviv A. Telomeres, sex, reactive oxygen species, and human cardiovascular aging. *J Mol Med (Berl).* Nov 2002;80(11):689-695.

206. Wong JM, Collins K. Telomere maintenance and disease. *Lancet.* Sep 20 2003;362(9388):983-988.

207. Lin J, Kroenke CH, Epel E, et al. Greater endogenous estrogen exposure is associated with longer telomeres in postmenopausal women at risk for cognitive decline. *Brain Res.* Mar 16 2011;1379:224-231.

208. Bekaert S, Van Pottelbergh I, De Meyer T, et al. Telomere length versus hormonal and bone mineral status in healthy elderly men. *Mech Ageing Dev.* Oct 2005;126(10):1115-1122.

209. Patel SM, Ratcliffe SJ, Reilly MP, et al. Higher serum testosterone concentration in older women is associated with insulin resistance, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab.* Dec 2009;94(12):4776-4784.

210. Sutton-Tyrrell K, Wildman RP, Matthews KA, et al. Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). *Circulation.* Mar 15 2005;111(10):1242-1249.

211. Huddleston HG, Rosen MP, Lamb JD, Modan A, Cedars MI, Fujimoto VY. Asian ethnicity in anonymous oocyte donors is associated with increased estradiol levels but comparable recipient pregnancy rates compared with Caucasians. *Fertil Steril.* Nov 2010;94(6):2059-2063.

212. Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. *Cancer Res.* May 1 1995;55(9):1937-1940.

213. Westberg L, Baghaei F, Rosmond R, et al. Polymorphisms of the androgen receptor gene and the estrogen receptor beta gene are associated with androgen levels in women. *J Clin Endocrinol Metab.* Jun 2001;86(6):2562-2568.