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INDOOR EXPOSURE TO PHTHALATES AND HYPERTENSION IN THE NHANES DATA 2017-2018

by
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ABSTRACT
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Introduction: Hypertension (HT) is a common chronic disease with high prevalence worldwide, in the Arab world and in Lebanon, and is a major contributor to premature death. One of the emerging environmental potential risk factors for HT is the indoor exposure to everyday plastic products containing phthalates. However, the association of phthalates with HT is still controversial. The objective of this study is to examine if this association exists and what factors may modify it. **Methods:** The study followed a cross-sectional design using secondary data from national US NHANES 2017- 2018 population-based surveys. Multi-stage random sampling was used in this dataset. The data was collected through interviews using questionnaires. The sample size in this analysis was 1,857 participants age ≥ 20 years who had provided urinary specimens in which phthalates' concentrations were measured. Eight phthalate metabolites are used in this analysis which form low-molecular and high molecular phthalates. Bivariate and multivariate logistic regression were performed to study the association, along with other covariates. A final best-fit model was later analyzed. **Results**: At the time of the survey, 37% of the participants had HT. When phthalate monoesters were analyzed separately, an association between mono-ethyl phthalate (MEP) and HT was present (OR=1.01, 95% CI: 1.003- 1.02) at the bivariate level. Other covariates that were statistically significant with HT in the multivariate logistic regression were age, race and BMI. **Discussion**: The results of this analysis are consistent with other studies showing no association between phthalates and HT at all, and studies showing no association for MEP in specific. The results of this study can be used to evaluate the trend of association between phthalate metabolites and HT across years, since prior studies have used the NHANES datasets of different years resulting in different monoesters being associated with HT. **Conclusion:** More cohort longitudinal studies should be done to better understand the association between phthalates and HT, as results are not consistent in the literature. Based on this analysis, known and preventable risk factors for HT should be screened and controlled to reduce the additive risk of phthalate on the risk of HT. These may include lowering the BMI to reduce the odds of having HT.

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ABBREVIATIONS

HT: Hypertension
NHANES: National Health and Nutrition Examination Survey
SBP: Systolic Blood Pressure
DBP: Diastolic Blood Pressure
EDCs: Endocrine-Disrupting Compounds
DiNP: Di-isononyl phthalate
DEHP: Di(2-ethylhexyl) phthalate
DnOP: Di-n-octyl phthalate
PVC: Polyvinyl Chloride
DBP: Dibutyl phthalate
DEP: Diethyl phthalate
BC: Breast Cancer
MEHP: Mono(2-ethylhexyl) phthalate
HPLC: High-performance liquid chromatography
LOD: Limit of Detection
LMW: Low-molecular weight
HMW: High-molecular weight
MEP: Mono-ethyl phthalate (MEP)
MBP: Mono-n-butyl-phthalate
MECPP: Mono-(2-ethyl-5-carboxypentyl) phthalate
MEHPP: Mono-(2-ethyl-5-hydroxyhexyl) phthalate
MEOHP: Mono-(2-ethyl-5-oxohexyl) phthalate
MEHP: Mono(2-ethylhexyl) phthalate
IV: Intravenous
LMIC: Low-to-Middle-Income Countries
PPAR-α and PPAR-γ: Peroxisome proliferator-activated receptor-alpha and gamma
BP: blood pressure
CVD: Cardio-vascular disease
NCD: Non-communicable disease
LC- MS liquid chromatography-electrospray ionization-tandem mass spectrometry
NCHS: National Center for Health Statistics
CDC: Center for disease control and prevention
FPG: Fasting Plasma Glucose
HbA1c: hemoglobin A1C
OR: Odds Ratio
CI: Confidence Interval
IRB: Institutional Review Board
SD: Standard Deviation
NICU: Neonatal Intensive Care Unit

BMI: Body Mass Index

CHAPTER 1

INTRODUCTION

1.1. Perspective on hypertension globally and in Lebanon.

Hypertension (HT), or high blood pressure, is a common chronic disease with a worldwide prevalence of an estimated 1.28 billion adults aged 30-79 years, which doubled from 1990 to 2019. HT, as well as pre-hypertension and other elevated blood pressure, leads to 8.5 million fatalities globally due to stroke, ischemic heart conditions, various vascular ailments, and kidney disease [1]. Worldwide, HT is a major cause of premature death where 1 in 4 men and 1 in 5 women have HT [2]. A systematic review of 13 studies about HT in the Arab world from 1980 to 2011 showed that the overall estimated prevalence of HT was 29.5% (n=45,379). This prevalence is shown to be higher than the prevalence in the USA (28%) and sub-Saharan African (27.6%) [3]. The prevalence of HT in Lebanon was reported to be 23.1% in 2005 and increased to reached 36.4% in 2018 [4] [5] .

1.2. Rationale of this study

One of the emerging environmental risk factors for HT is exposure to everyday plastic products containing phthalates. In this study, we explore the potential association between domestic exposure to phthalates and HT in the US- National Health and Nutrition Examination Survey (NHANES) dataset 2017-2018.

The association of phthalates with HT is still controversial. An Australian study showed an association between urinary total phthalate concentrations and cardiovascular disease, diabetes type-2 and HT [6]. On the other hand, one Chinese study showed no

association between phthalates and HT among 2330 participants aged> 18 years [7]. An analysis of the NHANES data from 1999 to 2008 did not find a significant association between urinary phthalate metabolites and cardiovascular mortality [8]. The metabolic link between phthalates and HT is explained by many mechanisms such as elevating oxidative stress levels, activating receptors which play a role in adipogenesis and modulating inflammatory pathways. In view of contradictory findings, the use of phthalates for domestic purposes continues to be largely tolerated and arguably on the increase, especially in less developed nations. In particular, to link this topic to our country, the use of phthalates in plastic devices is still not a concern in any legal restriction in Lebanon. Plastic products with phthalates are used without any regulation, and they are dumped as household solid wastes with phthalates in water sources reaching concentrations higher than the permissible limits proposed by different agencies [9]. Results from this analysis may contribute to regulating the production of plastics and banning of products including phthalates, thus ultimately positively impacting the public health. These results can then be used as evidence about the negative effects of phthalates on human health, which can be later used to advocate for regulations in the Lebanese community.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview about HT

HT, being a key risk factor for CVDs mortality and morbidity, is characterized by a systolic blood pressure (SBP) measuring 140 mmHg or above, or a diastolic blood pressure (DBP) of 90 mmHg or above [10]. Despite significant advancements in research on HT, the cause of nearly 90% of cases remains unknown. This type of hypertension is referred to as primary hypertension (idiopathic) which is characterized by elevated blood pressure not associated with any underlying medical condition. The remaining 10% of hypertension cases are categorized as secondary hypertension which is high blood pressure caused by another medical condition such as obesity, diabetes, heart disease, or vascular abnormalities [11].

One of the non-preventable risk factors for HT is age where prevalence of elevated BP and HT increases with age for both males and females. Race and ethnicity play a role in HT also where a study with a sample of 4,821 US adults aged ≥20 years analyzing National Health and Nutrition Examination Survey (NHANES) 2015–2016 data showed higher age-standardized prevalence of hypertension in non-Hispanic Black individuals (57.3%) than in non-Hispanic White individuals (43.8%) and Hispanic Americans (44.7%). There is no evidence that genetic factors account for racial and ethnic disparities in the risk of hypertension. However, the likely contributors to these differences are sociodemographic, environmental, and behavioral factors [10]. Heredity and genetic factors also play an important role in HT; heredity accounts for 25% to 60% of HT in the general population and substantial clinical data have shown that HT occurs due to the

interplay between genetics and the environment [12]. Moreover, modifiable risk factors for HT include obesity, lack of physical activity, alcohol consumption, smoking, unhealthy diet characterized by high sodium intake and low potassium intake [10].

2.2 Phthalates and cardio-metabolic health

Experimental research showed that exposure to environmental chemicals such as phthalates can potentially trigger atherosclerosis, a known risk factor for hypertension, by elevating oxidative stress levels or generating reactive oxygen species like superoxide ions, hydrogen peroxide and hydroxyl radicals [13].

Laboratory research has demonstrated that phthalates display significant variations in toxicity based on their chemical structure [14]. Accumulating scientific findings have shown in recent years links between phthalates and many health diseases, specifically cardiovascular diseases. Phthalates are PPAR-gamma agonists, binding to peroxisome proliferator-activated receptor-alpha and gamma (PPAR-α and PPAR-γ), which play roles in regulating carbohydrate metabolism and adipogenesis, another risk factor for HT [7]. When phthalates interact with these receptors, beta-cell function may be impaired, as well as influence lipid metabolism, potentially increasing the risk of DM or hyperlipidemia. Phthalates can also increase inflammatory profiles since they modulate inflammatory pathways. Both the pathways related to adipogenesis, and inflammation are recognized factors in conditions such as hypertriglyceridemia, hyperlipidemia, insulin resistance, and hypertension [7].

The mechanism of the association between phthalates and HT may be explained by that phthalates can adjust obesity and insulin resistance by activating peroxisome proliferator-activated receptors (PPAR) gamma, which in turn increases the expression

of adipogenic genes. Phthalates may also bind to PPAR alpha and influence lipid metabolism to control blood glucose levels. Second, chronic inflammation is another factor in the development of these NCDs. Certain phthalate metabolites have been shown to boost CRP production in rodent cells [15], and a recent epidemiological study found a correlation between some phthalate metabolites and increased hs-CRP levels [16]. Hs-CRP is an inflammatory biomarker which is often used as a predictor for early cardiovascular disease or systemic inflammation.

Based on experimental research, atherosclerosis may be induced by environmental chemicals since they increase oxidative stressor or produce reactive oxygen species. Previous epidemiological studies investigated this pathway by focusing on arterial and cardiovascular diseases as outcomes; the relation with hypertension and BP, however, remains unclear [13, 17].

2.3 Overview about phthalates

Despite the great benefits of plastic in daily use, invented in 1907, its wide usage is resulting in various adverse effects on the global environment and on human health. Exposure to plastic is permanent as people are exposed through various means, including contaminated food, leaching from packaging (such as water bottles and medical devices), microplastics in atmospheric fallout and urban dust, personal care products like cosmetic packaging, and synthetic clothing. Such long-term exposure inevitably results in the release of numerous harmful substances [18]. Among the most concerning ones are phthalates which were manufactured since the 1903s in big quantities and were included in up to 40% of the final plastic consumer products [19].

Phthalates are also known to be endocrine-disrupting compounds (EDCs), defined by the Endocrine Society as “chemicals, or mixtures of chemicals, that interfere with any aspect of hormone action” [19]. Phthalates. which are diesters of 1,2-benzenedicarboxylic acid (phthalic acid). are man-made chemicals used in industrial applications. Phthalates of high molecular weight, such as di-isononyl phthalate [DiNP], di(2-ethylhexyl) phthalate [DEHP], and di-n-octyl phthalate [DnOP], are used as plasticizers in the manufacture of polyvinyl chloride (PVC) plastics to enhance flexibility. These in return are used in consumer products, food applications, medical devices and blood storage bags, toys, coverings of the floor and walls. Low molecular weight phthalates, such as dibutyl phthalate [DBP] and diethyl phthalate [DEP], are utilized in personal care products (cosmetics, fragrances, and lotions), insecticides, dyes and in making coatings including pharmaceuticals [14]. Phthalates are initially used as plasticizers added to polyvinyl chloride (PVC) to increase flexibility. The endocrine system can be disrupted by their potential.

In-vivo research has shown that exposure to phthalates can lead to endocrine disruption with effects on the reproductive tract of males, being most sensitive to this influence, resulting in permanent consequences. Besides, high levels of certain phthalates have been linked to infertility in men [20]. Human studies have also shown an association between maternal exposure to phthalates and the reproductive health of male offsprings [21]. As for the effects of phthalates on females, certain phthalates increase the risk of developing breast cancer (BC) especially hormone-receptor positive cancer [22], proliferates BC cells by interfering with progesterone receptor system [23] and can even cause malignant invasion of BC cells that are low in hormone receptors [24].

In recent decades, there have been growing concerns regarding the negative impacts of phthalates on developmental processes and the reproductive system [19]. The quantity of phthalates being used is more than 18 billion pounds each year. Worldwide, the production of the main phthalate di(2-ethylhexyl) phthalate [DEHP] reaches around 3.5 million tons annually. DEHP is widely recognized as one of the most potent phthalate types in terms of its capacity to induce adverse effects on reproduction and development in various animal studies. DEHP is also acknowledged as a ubiquitous environmental contaminant, since it can easily reach the environment due to not covalently bonding to plastic matrix. It is released into the environment during the manufacturing of plastics and plastic products, during their utilization, and after their disposal [25].

In-vivo animal studies had also examined the association between phthalates exposure and blood pressure. When 48 mice were randomly divided and exposed to different dosages of DEHP for 6 weeks, a significant increase in systolic blood pressure (133.87 ± 2.2 mmHg) resulted after 10 mg/kg/day DEHP exposure compared to the saline-control group. The study suggested that the increase in BP is due to activating angiotensin converting enzyme [26].

2.4 Phthalates' metabolism

Human body metabolism processes phthalates quickly due to their short biological half-lives, which are about 12 hours. The initial phase of such metabolism involves hydrolysis once the substance is absorbed into cells. The subsequent phase consists of conjugation, wherein the hydrophilic glucuronide conjugate is formed through the catalytic action of the enzyme uridine 50-diphosphoglucuronyl transferase. Short-branched phthalates typically undergo hydrolysis to form monoester phthalates, which

are subsequently eliminated through urine. Whereas long-branched phthalates primarily undergo various biotransformation processes like hydroxylation and oxidation before being excreted in both urine and feces as phase 2 conjugated compounds. After exposure, phthalates are metabolized in their respective monoesters, converting the diester into monoester, which is the major metabolite and more bioactive [19]. Example of an important diester studied throughout this thesis is DEHP which is metabolized into mono(2-ethylhexyl) phthalate MEHP (figure 1). DEHP Phthalate monoesters are considered biologically active molecules which adds an advantage of to using them as indicators [18].

2.5 How phthalates are measured

Most phthalates and their metabolites are measurable in urine and feces, but certain phthalate compounds, such as DEHP, and their metabolites can also be detectable through sweat. Metabolites can also be measured from other body fluids such as amniotic fluid, saliva, serum, milk, semen, and ovarian follicular fluid. The most preferred medium to analyze phthalates metabolites is urine [27]. The method used is chromatography coupled with mass spectrometric techniques. The most common method is the High-performance liquid chromatography (HPLC). Another method that can be used to measure the monoesters after their conversion to volatile derivatives is gas chromatography [14].

The reportable range of results is determined by the standard calibration curves' linear range and the method limit of detection (LOD). It is essential for the reportable range to fall within the calibration curves' range. The limits of detection (LOD) for different metabolites are present in Figure 2 [28].

As indicators of exposure, urinary phthalate monoester metabolites are measured rather than the phthalates diesters and they are used to quantify the levels of exposure to phthalates [19]. LMW phthalates monoesters include mono-ethyl phthalate (MEP), mono-n-butyl-phthalate (MBP), and mono-isobutyl phthalate; HMW phthalate monoester include mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(3-carboxypropyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethylhexyl) phthalate (MEHP), and mono-benzyl phthalate [29].

2.6 Exposure to phthalates

Humans are exposed to phthalates via several means, such as oral exposure: phthalate-contaminated food (bottles, food packaging, cooking tools, casings, wrappers, mouthing of teethers and toys in children), water and other liquids (carbonated drinks, liquors, infant formulae); dermal exposure: personal care products, perfumes and cosmetics (creams, deodorants; hair dyes and nail polish, etc.) and skin contact with surfaces and plastic toys for children; and inhalation (household material and indoor air) [27].

The major route of exposure for most phthalates is food and water ingestion. However, for certain phthalates, the main route of exposure is via inhalation and the use of personal care products. Ingestion of breast milk if phthalates are present in the mother's body or ingestion of formula milk containing phthalates contribute to infant exposure. Moreover, infants and children can be exposed to phthalates through hand-to-hand activities by ingesting phthalates present in dust or through sucking toys containing phthalates [30]

Other minor routes of exposure to phthalates are inhalation, drinking and dermal exposure. Phthalates can be released in minimal quantities into the indoor air of homes or schools and thus inhalation of indoor air in rooms with large surfaces of PVC-containing products increases exposure. The most common phthalate present indoor is DEHP [31]. Individuals residing in proximity to facilities manufacturing phthalates or locations designated as hazardous waste sites could potentially encounter phthalate exposure from the release of these compounds into the surrounding air or groundwater in their residential areas. Dermal exposure occurs due to the usage of personal care products. Finally, certain medical devices containing phthalates such as implants, blood transfusion, IV bags, and dialysis material are another route of exposure for phthalates; and it is a significant route for premature infants in intensive care units [30]. As for hospitalized people, parenteral exposure to phthalates, mostly DEHP, from medical products and devices is critical [32].

Similar to any other hazardous chemical, phthalates' level of exposure is expressed in its standard unit in μg/kg body weight/day.

2.6.1. Exposure to phthalates in Low-to-Middle-Income Countries (LMIC)

Populations living in LMICs have higher exposure to phthalates than those living in high-income countries [33]. In indoor environments, the most prevalent of all the semi-volatile organic compounds are phthalates. High exposure to phthalates may be explained by the consumption of more affordable processed food items, usually packaged in containers lined with plasticizers. For example, a study in Egypt showed that food storage in plastics is an important route of exposure to phthalates [34]. Moreover, some cultural norms may increase the exposure to phthalates. For example, many of the phthalates have been detected in silicone wristband worn in some tribal groups in Senegal and South

Africa, thus increasing human exposure. Studies involving human biomonitoring consistently demonstrate that higher levels of restricted phthalates can be observed in people living in poverty [33] [35]. Exposure due to phthalates is present in rivers carrying waste waters in South Africa in levels higher than the recommended levels of 7.5 to 38.5 μg/ℓ for drinking water, and higher than those reported in rivers polluted with industrial chemicals [36].

2.7 Epidemiological findings about phthalates

Many diseases have been found to be associated with phthalates exposure, such as insulin resistance, obesity, type 2 diabetes, and atherosclerosis which are all high cardiovascular risk conditions [37]. Phthalates were studied at first as associated with cancer, followed by reproductive and developmental risks [14]. Shiu aimed to study the association of several urinary concentrations of environmental chemicals and risk of high BP using a national population-based study, the United States National Health and Nutrition Examination Surveys 2011–2012 [13]. Among the 9,756 participants, it was noted that an association exists between higher urinary concentrations of several phthalates, such as mono-*n*-butyl phthalate mono-*n*-methyl phthalate, mono-2-ethyl-5-hydroxyhexyl, monobenzyl phthalate, mono-2-ethyl-5-oxohexyl and mono-2-ethyl-5-carboxypentyl phthalate, and high BP. This association was found in similar reports in children [29], which also used the NHANES data but between 2003 and 2008. The levels of DEHP were associated with higher standardized BP (sex-, age-, and height-standardized), where the systolic blood pressure z-score increases by 0.051 SD, 0.042 SD and 0.043 SD for each log unit increase in the following DEHP metabolites, commonly identified in human studies: MEHP, MEHPP and MEOHP, consecutively. Low-

molecular weight (LMW) phthalates' metabolites were not associated with high BP, but an association was found between MEP (LMW metabolites) and prehypertension. Prehypertension is defined as a systolic BP from 120–139 millimeters of mercury (mm Hg) or a diastolic pressure from 80–89 mm Hg [38]. The study concluded that, in children and adolescents, phthalates present in diet are associated with higher systolic BP, whereas phthalates present in personal care product and cosmetics are not.

The association between phthalates and HT, along with CVD and type-2-diabetes, was also shown in a study of a sample of 1504 men in South Australian urban dwelling men; the prevalence ratio for HT was 1.14 (95%CI 1.01 – 1.29) when extreme quartiles of phthalates were compared [6].

However, this association between phthalates and HT was not always conclusive. One study used also NHANES data from 1999 to 2008 to evaluate the association between eight urinary phthalate metabolites and cardiovascular disease mortality in a prospective cohort study. It was found that no cardiovascular mortality was associated with urinary phthalates individually [8]. The hazard ratios ranged from 0.73 (95%CI: 0.5–1.2) for MEP to 1.4 (95%CI:0.8–2.5) for MEHHP, after adjustments. Similarly, a Chinese study with a sample of 2330 participants aged> 18 years showed no association between phthalates and CVD-related outcomes, including HT, in both the overall sample and in the separated sex samples [7]. No cardiovascular effects were reported in another study of 18 infants who had elevated levels of DEHP in their plasma from being exposed during extracorporeal membrane oxygenation (ECMO) therapy. DEHP had leached from the plastic tubing of ECMO circuits and was projected in patients to a potential exposure that is 20 to 70 times higher than what is typically encountered during other medical procedures or from the use of medical devices, for instance transfusions, dialysis, or short-

term cardiopulmonary bypass. Nevertheless, most patients eliminated this substance from their bloodstream before the decannulation process [39]. As a result of these studies, many countries have started taking actions and measures concerning the usage of phthalates in medical devices and other consumer products.

2.8 Policies about phthalates' usage

Many countries have taken measures concerning phthalates such as banning products containing more than a specific level of phthalates in the US and banning specific type of phthalates in toys and food-handling gloves in Japan in 2001. In 2007, Europe banned many phthalate compounds, including DEHP, in all toys and childcare products containing plasticized material. China, the biggest manufacturer and consumer of phthalates, also set restrictions with a latest regulation set in 2017 about the detection limits of phthalates in products [18]. Additionally, more than 100 healthcare facilities worldwide are in the process of decreasing or gradually eliminating the use of PVC and phthalates [40].

2.9 Aim and research questions

This study aimed to study the potential association between phthalates' exposure and HT in the US- National Health and Nutrition Examination Survey (NHANES) dataset 2017-2018.

The questions to be addressed in this analysis are:

- Can an association between domestic exposure to phthalates and HT be detected in the NHANES data 2017-2018?

- What demographic, lifestyle and health factors are likely to modify this association, if it exists?

CHAPTER 3

METHODS

3.1. Study design and sources of data

The design and sampling frame of the NHANES serial surveys are available elsewhere [28]. Briefly, these national, population-based, multi-year, cross-sectional surveys use a representative sample of the civilian non-institutionalized US population (adults and children) (wwwdcov/nchs/nhanestm). It is the most comprehensive survey used to assess the health status of adults and children in the US. The program began in the 1960s and since 1999 the survey has studied around 5,000 individuals each year where detailed health information is collected through interviews using questionnaires and physical examinations [41]. In this analysis, the 2017-2018 cohort was selected as it is the most recent dataset analysis available. The NHANES 2019-2020 data collection was not completed due to the COVID-19 pandemic which suspended the program's operations in March 2020. As for the 2021-2022 dataset, it is still not yet available. In the present analysis, the NHANES questionnaire, laboratory, physical examination, and demographics components will be used. The surveys capture large and nationally representative sample of the general U.S population. This sample is diverse with respect to age, race, geography, income, and other essential covariates.

3.2. Sampling design and study population

The sampling design used is multi-stage random sampling by selecting counties then block or group of blocks of household cluster. Eligible households within segments

were identified and then eligible individuals within households were invited randomly for participation using a computer process.

In the 2017-2018 wave, 2,986 participants provided urine samples in which the phthalates metabolites of the pollutants of interest could be measured. This analysis is restricted to individuals 20 years or older. After merging the data of the NHANES questionnaire, laboratory, physical examination, and demographics components, and excluding those with missing data of blood pressure (main outcome) and those who lacked data of the eight urinary phthalates, the sample thus includes 1,857 participants.

3.3. Concepts and Measures

3.3.1. Dependent variable (Outcome)

In NHANES, blood pressure (BP) is measured using an aneroid sphygmomanometer and repeated three consecutive times by trained certified nurses during the interviews in the mobile examination center. The first measurement is done after subjects have been resting quietly for five minutes and after their maximum inflated level of tolerance is established. A fourth trial may be done if the measurement is incomplete or interrupted. Exclusion criteria for BP examination was if a participant has specific conditions on both arms, such as rashes, edema, tubes, paralysis, gauzes, and open sores. All measurements were taken using the right arm unless a certain condition was present prohibiting its use. The average value of the three measurements is used in the subsequent analysis. Among those participants, they were categorized as hypertensive (yes/no) if their measured systolic BP was ≥ 140 mmHg or diastolic BP ≥ 90 mmHg [10] (calculated as the mean of the three BP measurements and the fourth measurement if

available), or if they were recently taking anti-hypertensive medication or self-reporting presence of HT (doctor told them they have high BP) [6, 7].

3.3.2. Independent variables

3.3.2.1. Main independent variable: Urinary phthalate metabolites

Urine specimens were processed at the National CDC, Georgia (Chen et al., 2022). Exposure to phthalates and their metabolites was measured quantitatively (in ng/mL) in human urine using "high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry" (HPLC-ESI-MS). Details on this methodology are provided elsewhere [42]. Phthalate metabolites were well-measured providing sensitive and specific measurements, NHANES updates the laboratory methods regularly for a better measurement quality, and data is collected rigorously.

Eight phthalate metabolites are used in this analysis which form low-molecular and high molecular phthalates. They consist of monoethyl phthalate (MEP), mono-n-butyl-phthalate (MBP), mono-isobutyl phthalate, mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono(2-ethylhexyl) phthalate (MEHP). Each of these eight phthalates were adjusted for creatinine to adjust for urinary dilution and thus a new variable for phthalate concentrations is used for analysis which is concentration of phthalates in ng per gram of creatinine (ng of phthalate/ g creatinine).

When the phthalate concentrations were below the limit of detection (LOD), the LOD was substituted by the LOD divided by the square root of 2 as recommended and assigned by NHANES and NCHS (National Center for Health Statistics); this method is

used by the CDC (2013) and produced nonbiased results [43]. Detailed contents of analysis methods can be found on the CDC NHANES website (https://wwwndcov/nchs/nhanes/analyticguidelinesspx).

3.3.2.2. Independent co-factors

The sociodemographic information of the participants included age, gender, BMI, race/ethnicity, income, highest education level attained, and number of people in the household.

Age was reported at the screening interview time based on date of birth or, if missing, reported age is used: in this analysis, age was used as a continuous variable; BMI (underweight/normal, overweight, obese); race (Non-Hispanic white, non-Hispanic black, others) [8]; income was defined as ratio of poverty to family income and categorized into quartiles based on the distribution of the sample: first quartile (<1.27), second quartile (1.28-2.5), third quartile (2.51-3.62), fourth quartile (>=3.63); education level (below high school, high school, above high school); number of people in the household (1, 2, 3 or more) to capture the difference in the results between those living alone, 2 people living together and 3 or more people living in the household.

Besides socio-demographic variables, other covariates included:

1. Physical activity: based on self-reporting, categorized into low, moderate, and vigorous.
2. Urinary creatinine (concentrations in mg/dL) measured on a continuous scale, was included as a separate covariate to account for urinary dilution.
3. Alcohol consumption: measured the number of drinks per day in the past 12 months (non-drinkers, 1-2 drinks, 3-4 drinks and 5+ drinks)

4. Serum cotinine levels (biomarker of smoking status) [44], reflects recent exposure to nicotine in tobacco smoke [29], measured in ng/mL by high-performance liquid chromatography–tandem mass spectroscopy, were used to determine smoking status rather than self-reporting use of cigarettes; measured on a continuous scale.
5. Total cholesterol levels (concentrations in mg/dL): continuous variable
6. Diabetes: binary(yes/no) and was defined using a previous diagnosis according to fasting plasma glucose (FPG) level ≥7.0 mmol/L or HbA1c ≥6.5% or self-reported diagnosis.
7. Prevalent chronic conditions (binary: yes/no) included the presence of at least one self-reported of the following conditions: asthma, arthritis, congestive heart failure, coronary heart disease, angina/ angina pectoris, heart attack, stroke, or thyroid problem.
8. Dietary sodium and potassium intake (in mg): continuous variable, self-reported by 24-hour recall by participants.

3.5 Statistical methods

3.5.1. Descriptive analysis

Data analysis was conducted using STATA software version 15.1 and following the guidelines set by the National Centers for Health Statistics guidelines. A baseline descriptive analysis was done for all the sample characteristics and covariates against our main outcome, hypertension. All variables were described depending on their nature: by frequencies and percentages for categorical/ordinal ones, and by means, standard deviation (SD) and ranges for continuous ones. Missing data were replaced by means of continuous variables. For the purposes of the analysis, some continuous variables were

subsequently transformed into categorical ones as detailed above. Prevalence variables were presented with their corresponding 95% confidence intervals (95% CI).

3.5.2. Bivariate analysis

The association of all independent variables with the outcome of interest was calculated and assessed for significance. This assessment was done using the Chi-squared test for categorical variables, or the t-test for continuous variables. These tests yielded a p-value which was used to assess significance. A p-value $\leq$ (0.05/8= 0.00625) was considered as an indicator of a non-significant association. The p-value of 0.05 was divided by 8 because 8 bivariate models were used.

Bivariate regression was done for each phthalate metabolite/ g creatinine separately. Phthalates were natural log transformed due to their non-normal and skewed distribution. This regression was done to examine if an association exists between each metabolite and HT. The unadjusted ORs with 95% CI and p-values were also reported.

3.5.3. Multivariate logistic analysis

All independent variables with a statistically significant association with the outcome of interest in the bivariate analysis were subsequently entered in the multivariate analysis, to assess their joint effect on the outcome of interest. The joint association was measured using the adjusted odd-ratio (OR_{adj}) and its corresponding 95% CI. When the 95% CI included the value 1, this joint association was considered as not significant. A final "best-fit" model was created with only those independent variables significantly associated jointly with the outcome of interest. The coefficient R^2 of this final model, a measure of the amount of variability in the outcome explained by the model, was reported.

3.6 Ethics

This study is a secondary analysis of previously collected de-identified data and is therefore exempted from IRB review. There is no direct contact with subjects or any potential harm associated with this analysis. Besides, NHANES follows ethical principles such as informed consents, voluntary participation, and confidentiality of data [45, 46].

CHAPTER 4

RESULTS

4.1. Descriptive Analysis

The frequencies of the sample's characteristics are presented in table 1. The total sample size was 1,875 participants. Around 1,061 (57%) of the sample were not hypertensive. The mean age was 60 years for hypertensive participants and 42 for non-hypertensive participants.

The sample was almost equally distributed between both genders; 937 (51%) were females, around 56% and 58% of males and females were not hypertensive, respectively. The majority of non-Hispanic white (56%) and those having other races (64%) were not hypertensive. Most of the participants (54%) reached above high school as their highest education level of which 57% were not hypertensive. Most of the participants having the lowest quartile of family income to poverty ratio (61%) were not hypertensive. Those who were living alone were mostly hypertensive (60%) and those who have 3 or more people living in the household were mostly not hypertensive (67%). Around 72% of participants who were underweight or had normal BMI were not hypertensive and 52% of the obese were hypertensive. Participants who engaged in vigorous physical activity were mostly not hypertensive (60%). Most of the participants who had at least one chronic condition were hypertensive (58%) and most of the diabetic patients had HT (69%). Levels of serum cotinine and total cholesterol were higher in hypertensive participants, whereas levels of dietary sodium and potassium were higher in non-hypertensive participants.

4.2. Bivariate Analysis

Each covariate was analyzed using bivariate logistic regression along with the outcome. The results of this analysis, with the unadjusted ORs, 95% CIs and p-values are presented in table 2.

The results show that as age increases by one year, the odd of having HT increases by 1.07 (95%CI: 1.06 -1.08). Those who had HT had 1.5 higher odds of being non-Hispanic black compared to non-Hispanic white, 0.8 less odds of being living with another person and 0.3 less odds of living with 3 or more people in the household compared to living alone, had 1.97 at higher odds of being overweight, and 2.75 higher odds of being obese compared to being underweight/ normal, than those not having HT.

Moreover, compared to non-drinkers, those who drank 1-2 drinks per day at the past 12 months had 0.49 less odds of having HT (95% CI: 0.38 – 0.63). Subjects that suffered from one or more of the chronic conditions had 3 higher odds of having HT, compared to those that had none (95% CI: 2.4 – 3.6). Moreover, diabetic subjects had 3.9 higher odds of having HT compared to non-diabetic subjects (95% CI: 3.1 – 4.9). As dietary sodium levels increased by 1 mg, the odds of having HT decreased by 0.99 (95%CI: 0.7 - 0.98).

Therefore, the variables that were protective factors for HT based on this bivariate analysis are: number of people in the household and alcohol consumption. Risk factors include age, race (non-Hispanic black), income ratio, BMI, chronic conditions, and diabetes.

The variables that were statistically significant using p-value ≤ 0.00625 and thus would be used in the multivariate regression are: age, race, number of people in the household, BMI, alcohol consumption, chronic conditions, and diabetes.

4.3. Bivariate analysis using monoesters of phthalates

Monoesters of phthalates were used to study their association with HT. Their unadjusted ORs, 95% CI and p-values are presented in table 3. Only 1 phthalate metabolite was statistically significant with the outcome, HT, which is mono-ethyl phthalate (MEP). As the odds of MEP increased by 1 ng/ g creatinine, the odds of having HT increased 1.01 multiplicatively (OR=1.01, 95% CI: 1.003- 1.02).

4.4. Multivariable logistic regression

The results of the multivariable regression analyzing the association between MEP and HT along with other significant covariates (p-value< 0.00625) are presented in table 4; adjusted ORs, 95% CI and p-values are shown. The covariates that were statistically significant in the model were age, race, and BMI. The overall model was significant with p-value< 0.01.

The analysis showed that MEP was not associated with HT anymore at the multivariate model (OR= 1.01, 95%CI: 0.99- 1.02).

Age was still statistically significant in the model, where as age increases by 1 year the odds of having HT increases by 1.06 multiplicatively, adjusting for all other covariates in the model.

Non-Hispanic black had 2.3 higher odds of having HT compared to non-Hispanic white (95%CI: 1.6- 2.98) adjusting for all the other covariates in the model. Other races, however, were not statistically significant with the outcome (p-value= 0.731). Overweight participants had 1.7 higher odds of having HT (95%CI: 1.24- 2.3) and obese

participants had 2.6 higher odds of having HT (95% CI: 1.9- 3.45) compared to participants who were underweight/ have normal BMI, adjusting for the other variables.

Goodness of fit was later done, and it showed that the multivariable model fits the data well.

Even though MEP was significant only at the bivariate level, a new best fit model was created where only the significant variables (p-value< 0.00625) were added to the model. The best fit model showed that all the variables remained significant. Results are present in detail in table 5. The coefficient of determination R^2 showed that only 25% of the variation in the dependent variable, HT, is explained by the independent variables in the model.

CHAPTER 5

DISCUSSION

This study aimed to investigate if an association exists between phthalates and HT. Around 37% of the participants had HT at the time of survey. When each metabolite of phthalates was studied against the outcome, 1 monoester was significantly associated with HT at the bivariate level which is mono-ethyl phthalate (MEP); however, this association was not significantly present anymore at the multivariate model. This thesis, thus, showed that no association was present between phthalate monoesters and HT when adjusted for the other variables.

MEP is mainly found in personal care products and cosmetic products to enhance fragrance, in insecticide sprays and in packaging. It is a LMW phthalate [47].

The results of this study are consistent with other studies showing no association between phthalates and HT and with studies that showed no association between MEP and HT even though other metabolites were statistically significant. Studies examining the association between phthalates and HT showed inconsistent results where some studies showed an association while others did not.

In one study using NHANES dataset 2011-2012, which studied the association between different chemicals, including phthalates, and high BP, showed that even though certain phthalate metabolites were associated with BP, MEP was not significantly associated with an OR of 1.09 (95% CI of 0.99- 1.19, p-value of 0.074). This OR resulted when MEP was added to the multivariate model which adjusted for urine creatinine, age, race, sex and BMI [13].

In another wave of NHANES dataset 2003- 2008, studying the association for children aged 6-19 years, the study revealed that HMW phthalate metabolites were associated with HT, but LMW phthalates were not. Since the results of phthalate monoesters being associated with HT differ by their type of being LMW or HMW, more studies should be done studying the association between each metabolite separately and HT across years.

Another wave of NHANES 2009-2010 was used to understand this association. It was found that some phthalate metabolites were associated with higher BP such as mono-n-butyl phthalate (OR= 1.19, 95% CI 1.01- 1.41, p-value= 0.042). However, many other metabolites were not associated with HT when adjusted for urinary creatinine, gender, age, race and BMI. In specific, MEP was not statistically significant with high BP with OR of 1.01 and p-value of 0.751 [48].

In a NHANES wave of 2009- 2012, similar results were revealed where certain phthalate metabolites had an association with high BP; others had not. In this study, MEP was not significantly associated with high BP (OR=1.08, 95%CI: 0.98- 1.19, p-value=0.107) in the full model when it was adjusted for specific covariates. When this association was stratified by gender, MEP remained not significantly associated with high BP (OR= 0.99, 95%CI: 0.85- 1.14) in men but was significantly associated with high BP in women (OR= 1.17, 95%CI: 1.03- 1.34, p-value= 0.018) [49]. This shows the importance of having more studies that should take place to investigate the association between phthalates and HT stratified by gender.

Besides, a study using also NHANES 2009-2012 among children aged 6-19 years old, showed that the log-transformed MEP was not statistically significant with the outcome high BP in the adjusted model (OR= 1.16, 95%CI: 0.76- 1.79). Moreover, in this

study, specific metabolites of high-molecular weight were associated with BP; low-molecular weight monoesters, however, showed an association only in the univariate analysis and was no longer significant in the full multivariable model. This result is similar to the result of this thesis where MEP was significantly associated with HT on the bivariate model but became non-significant in the full model [50].

As each wave of studied that used NHANES has shown different phthalates being associated with HT, this thesis using NHANES 2017- 2018, has shown that MEP was associated with HT at the bivariate model but lost its significance at the multivariate level and thus can be used to study the trend of association between phthalate metabolites and HT across years. It is important to mention that the difference between the results of the studies in the literature, specifically those which used NHANES datasets of different years, can also be explained by the different covariates that were adjusted for at the multivariate level. Thus, it is essential to examine this association adjusting for many covariates that could modify this association.

Moreover, MEP has no consistent association with HT. In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study, recruiting elderly sample in Sweden, MEP had negative association with diastolic BP but not with systolic BP in Caucasians whose age is 70 years [51]. As this shows that MEP has no consistent results in the literature, this thesis can thus be used to show that MEP has no association with HT in the NHANES wave 2017- 2018.

In another study examining the association between phthalates and cardiovascular disease mortality, no association was significantly present between phthalates and CVD mortality. When MEP was adjusted for age, gender and creatinine alone on one model and for race, education, BMI, alcohol, smoking, physical activity, chronic conditions and

cholesterol, the hazard ratios of CVD with 4 quartiles of MEP showed that none of the MEP quartiles were associated with CVD mortality.

Similalrly, in another study examining the association between phthalate metabolites and many diseases including HT, showed that the ten phthalate metabolites that were measured and used in the study were all not significantly associated with HT, in both the overall population and in the separated sex populations. The p-value for MEP when studied along with HT and adjusted for age, education, gender, smoking, marital status, total calories intake and total fat, and BMI was 0.246 with ORs between 1.13 and 1.26 for increasing quartiles for MEP concentrations [7].

The inconsistency of results of this association was not only present between different studies but also within the same study. In a cross-sectional study from Wuhan, China studying this association by recruiting children of the ages 6 to 8 years old, revealed that a negative association between MEP and MEOHP and SBP was present, while positive association between MEHHP and DBO was present among boys only. As for girls, no significant associations were found [52]. This shows the importance of having more longitudinal studies to assess and understand the effect of the different phthalate metabolites on both systolic and diastolic BP and thus have a clearer conclusion of the association between phthalates and HT.

Another inconsistency of the results within the same study is present in a study in Sabadell, Spain. This was a birth-cohort study among 391 pairs of mother-child, and it showed that both HMW and LMW phthalates were associated with reduced systolic BP in girls of the age 4-7 years but not in boys. As for diastolic BP, no significant association with phthalate metabolites was revealed [53]. We can conclude that phthalate metabolites

show inconsistent results when studied with HT and thus further studies should take place to have a better understanding of this association.

The trend of association between phthalate metabolites and HT across years may be of high importance, especially that one study showed noticeable changes in urinary phthalate concentrations among the US population between 2001 and 2010, where DEHP declined for about 20-50% whereas DiBP increased by >100% [43]. Studying both the trends of changes in urinary concentrations of phthalates across years along with examining the association with HT is therefore important to be established.

Even when phthalates were studied with stroke, a key factor for HT, using NHANES 2001- 2004, the study showed an association between specific phthalate metabolites (mono-n-butyl phthalate and mono-(3-carboxypropyl) phthalate) and risk of stroke [54]. However, MEP was not associated with risk of stroke when put in 3 different models adjusting for different covariates in each model (p-values of the 3 models: 0.8, 0.914, and 0.962). This shows the importance of warranting future longitudinal studies to understand the biological mechanism of these phthalates before drawing conclusions about phthalates and HT in general.

This thesis has some limitations. The first limitation which was, however, solved is that selection bias is kept to minimal since the surveys capture large and nationally representative sample of the general U.S population which is diverse with respect to age, race, geography and income. Besides, since this is a cross-sectional study, temporal sequence cannot be established. Phthalates exposures are not known to have occurred before having HT or not. Therefore, longitudinal studies should take place to study the temporal sequence of the association between phthalates and HT. Another limitation is that many of the covariates were self-reported such as reporting the presence of chronic

conditions, so recall bias is present in this analysis. Differential misclassification bias might have occurred since those who have HT are more likely to accurately recall past events or exposures such as questions related to smoking or alcohol consumption which are being asked to them regularly than those who do not have HT. Finally, measurement bias might have occurred since phthalates have half-lives of 12 to 48 hours whereas prehypertension is a chronic process that develops from arterial wall stiffening. Thus, current urinary phthalates are weak indicators of early life exposure and thus a longitudinal study is needed to study the association of phthalates with HT across different years of exposure.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

This thesis adds to the literature where the association between phthalates and HT is already inconsistent, and the findings are limited. It helps in shaping the association in a new perspective, taking into account the variables studied in this analysis.

The results of this study showed no association between phthalates and HT, where only MEP was significantly associated with HT at the bivariate level and lost its significance when adjusted for other covariates.

As this thesis helps in studying the trends of this association, more longitudinal studies should be investigated to study this association across years. Besides, future cohort studies should take place to study the temporal sequence of phthalates and HT. Studies should also stratify the associations by gender and age where many studies, as discussed above, show difference in the significance of this association between males and females and between different age groups. Cohort studies are also essential and required to study the biological mechanism along the pathway before reaching a clearer conclusion about the relationship between phthalates and HT.

Beginning to study the above association should take place at the most immediate time to have a better understanding of the effects of each phthalate metabolite on the human body. This in return can help in advocating for policies, at the earliest time, at the national and worldwide levels to help promote health and prevent diseases among the community and the public.

Finally, based on this analysis, known and preventable risk factors for HT should be screened and controlled to reduce the additive risk of phthalate on the risk of HT. These may include lowering the BMI to reduce the odds of having HT.

APPENDIX

Figure 1: Metabolic pathway from DEHP to MEHP

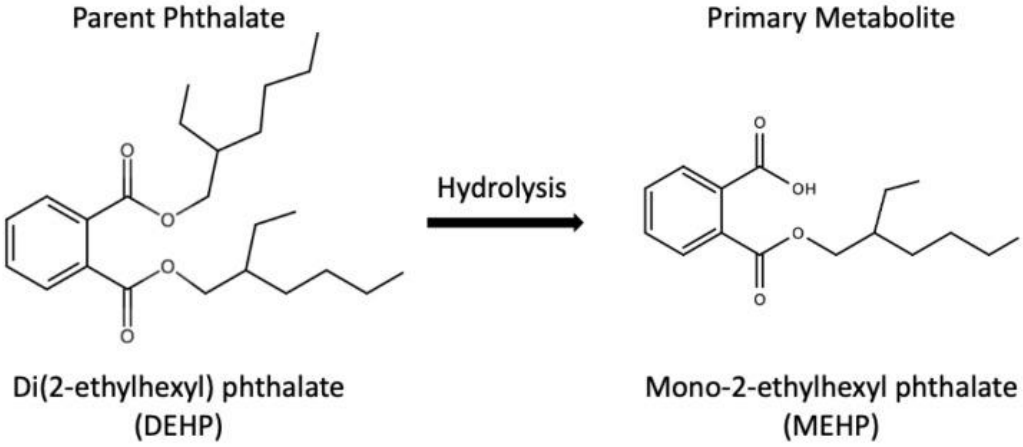

Figure 2: Different phthalates metabolites with their lower limits of detection (LLOD)

ANALYTE DESCRIPTION	LLOD
Mono(carboxyisononyl) phthalate (ng/mL)	0.2
Mono(carboxyisoctyl) phthalate (ng/mL)	0.3
Mono-2-ethyl-5-carboxypentyl phthalate (ng/mL)	0.4
Mono-2-ethyl-5-carboxypentylterephthalate (ng/mL)	0.2
Mono-2-hydroxy-iso-butyl phthalate (ng/mL)	0.4
Mono-n-butyl phthalate (ng/mL)	0.4
Mono-(3-carboxypropyl) phthalate (ng/mL)	0.4
Cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester phthalate (ng/mL)	0.5
Mono-ethyl phthalate (ng/mL)	1.2
Mono-3-hydroxy-n-butyl phthalate (ng/mL)	0.4
Mono-(2-ethyl-5-hydroxyhexyl) phthalate (ng/mL)	0.4
Mono-2-ethyl-5-hydroxyhexylterephthalate (ng/mL)	0.4
Cyclohexane 1,2-dicarboxylic acid monohydroxy isononyl ester (ng/mL)	0.4
Mono-(2-ethyl)-hexyl phthalate (ng/mL)	0.8
Mono-isobutyl phthalate (ng/mL)	0.8
Mono-isononyl phthalate (ng/mL)	0.9
Mono-(2-ethyl-5-oxohexyl) phthalate (ng/mL)	0.2
Mono-oxoisononyl phthalate (ng/mL)	0.4
Mono-benzyl phthalate (ng/mL)	0.3

Table 1: Sociodemographic, lifestyle and health factors in a sample of participants from the NHANES 2017-2018 (N= 1857)

		Main Outcome		
		Hypertensive	Not hypertensive	
		n(%) or mean ± SD*		95% CI[1]
Socio-demographics				
Age		60.4 ± 14.6	40.9 ± 17.1	48.4- 50.1
Gender	Male	404 (43.9)	516 (56.1)	
	Female	392 (41.8)	545 (58.2)	
Race	Non-Hispanic White	283 (44)	360 (56)	
	Non-Hispanic Black	233 (54.4)	195 (45.6)	
	Other	280 (35.6)	506 (64.4)	
Education level	Below high school	172 (44.3)	216 (55.7)	
	High school	184 (40.2)	274 (59.8)	
	Above high school	440 (43.5)	571 (56.5)	
Ratio of family income to poverty	1st quartile (<1.18)	182 (38.8)	287 (61.2)	
	2nd quartile (1.18- 2.04)	206 (44.8)	254 (55.2)	
	3rd quartile (2.05-4.09)	211 (45.5)	253 (54.5)	
	4th quartile (≥ 4.1)	197 (42.5)	267 (57.5)	
Number of people in the household	1 (living alone)	152 (60.1)	101 (39.9)	
	2	298 (54.6)	248 (45.5)	
	3 or more	346 (32.7)	712 (67.3)	
Lifestyle factors				
BMI	Underweight/ normal	146 (28.4)	369 (71.7)	
	Overweight	263 (43.8)	337 (56.2)	
	Obese	387 (52.2)	355 (47.8)	
Physical activity	None	446 (45)	545 (55)	
	Moderate	162 (40.2)	241 (59.8)	
	Vigorous	188 (40.6)	275 (59.4)	
Alcohol consumption	Non-drinkers	195 (59.8)	131 (40.2)	
	1-2 drinks	338 (42.1)	465 (57.9)	
	3-4 drinks	215 (36.8)	369 (63.2)	
	5+ drinks	48 (33.3)	96 (66.7)	
Health factors				
Serum Cotinine, ng/mL		56.5 ± 128.8	53.7 ± 119	49.33- 60.54
Chronic conditions	No	327 (31.4)	714 (68.6)	
	Yes	469 (57.5)	347 (42.5)	
Diabetes	No	532 (36.1)	941 (63.9)	
	Yes	264 (68.75)	120 (31.25)	
Total cholesterol level, mg/dL		186.9± 42.4	186.8± 39.7	185.01- 188.71

Sodium (mg)	3321.1 ± 1661	3510.5 ± 1776	3350.5-3508
Potassium (mg)	2523.1± 1216	2539.3± 1221.9	2476.9-2587.8

*Data are expressed as numbers (percent) for categorical variables and as means ± standard deviation (SD) for continuous variables.

[1] 95% confidence interval

Table 2: Bivariate logistic regression of the outcome and other covariates with unadjusted ORs, 95% CI and p-values

		Unadjusted OR	CI for unadjusted OR [95%]	P -value
Socio-demograohics				
Age		1.07	1.06 ± 1.08	<0.01*
Gender	Male	Ref	---	
	Female	0.92	0.76 - 1.1	0.366
Race	Non-Hispanic White	Ref	---	
	Non-Hispanic Black	1.5	1.2 – 1.9	0.001*
	Other	0.7	0.57 - 0.87	0.001*
Education level	Below high school	Ref	---	
	High school	1.01	0.64 - 1.1	0.223
	Above high school	1.07	0.8 - 1.23	0.78
Ratio of family income to poverty	<1.18	Ref	---	
	1.18- 2.04	1.28	0.98 - 1.66	0.065
	2.05-4.09	1.32	1.01 - 1.7	0.039
	≥ 4.1	1.16	0.89 - 1.5	0.256
Number of people in the household	1 (living alone)	Ref	---	
	2	0.78	0.58 – 1.08	0.145
	3 or more	0.32	0.24 - 0.42	<0.01*
Lifestyle factors				
BMI	Underweight/ normal	Ref	---	
	Overweight	1.97	1.53 - 2.53	<0.01*
	Obese	2.75	2.17 – 3.51	<0.01*
Physical activity	None	Ref	---	
	Moderate	0.82	0.65 - 1.04	0.101
	Vigorous	0.83	0.67 - 1.05	0.115
Alcohol consumption	Non-drinkers	Ref	---	
	1-2 drinks	0.49	0.38 - 0.64	<0.01*
	3-4 drinks	0.39	0.296 - 0.52	<0.01*
	5+ drinks	0.4	0.22 - 0.51	<0.01*
Health factors				
Serum Cotinine, ng/mL		1	0.999 - 1.001	0.63
Chronic conditions		Ref	---	<0.01*

		2.95	2.4 – 3.6	
Diabetes				
		Ref	---	<0.01*
	No	3.9	3.1 - 4.9	
	Yes			
Total cholesterol		Ref	---	0.015
level, mg/dL	No	1.002	1.01- 1.005	
	Yes			
Sodium		0.999	0.99- 0.999	0.02
Potassium		0.999	0.999 – 1.0007	0.776

*Indicates significant association between covariate and outcome.
OR= Odds Ratio
95% CI: 95% Confidence Interval

Table 3: Bivariate regression of each metabolite/g creatinine with the outcome, showing unadjusted OR, 95% CI and p-values.

	Unadjusted OR	**95% CI**	**p-value**
Phthalates' metabolites, log units			
Mono(2-ethylhexyl) phthalate (MEHP)	1.005	0.995- 1.01	0.317
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	1.007	0.998 – 1.02	0.089
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	1.008	0.998 – 1.02	0.092
Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)	1.01	0.998 – 1.02	0.094
Mono-ethyl phthalate (MEP)	1.01	1.003 – 1.02	0.004*
Mono-n-butyl-phthalate (MBP)	1.007	0.998 – 1.02	0.104
Mono-isobutyl phthalate	1.01	0.998 – 1.02	0.141
Mono-(3-carboxypropyl) phthalate	1.01	1.006 – 1.03	0.065

Table 4: Multivariate logistic regression for the association between mono-ethyl phthalate (MEP) and hypertension controlling for significant co-factors.

		Adjusted OR (95%CI)	p-value
FULL MODEL			
Mono-ethyl phthalate (MEP)		1.01 (0.99 – 1.02)	0.084
Age		1.06 (1.05 – 1.07)	<0.01*
Race	Non-Hispanic White	Ref	
	Non-Hispanic Black	2.27(1.61 – 2.98)	<0.01*
	Other	1.05 (0.8 – 1.4)	0.731
Number of people in the household	1 (living alone)	Ref	
	2	0.86 (0.59 – 1.24)	0.421
	3 or more	0.81 (0.56 – 1.17)	0.259
BMI	Underweight/ normal	Ref	
	Overweight	1.67 (1.24- 2.26)	0.001*
	Obese	2.57 (1.9 – 3.45)	<0.01*
Alcohol consumption			
	Non-drinkers	Ref	
	1-2 drinks	0.832 (0.61- 1.14)	0.255
	3-4 drinks	0.84 (0.6 – 1.14)	0.314
	5+ drinks	1.1(0.67 – 1.8)	0.720
Chronic conditions	No	Ref	
	Yes	1.38 (1.09 – 1.7)	0.007
Diabetes			
	No	Ref	
	Yes	1.47 (1.1 – 1.96)	0.008
Goodness of fit: p-value =0.55 → the model fits the data well			

Table 5: Best-fit model for the association between MEP and HT, adjusting for other significant variables.

		Adjusted OR (95%CI)	p-value
BEST MODEL			
MEP		1.01 (0.99 – 1.02)	0.09
Age		1.07 (1.07– 1.08)	<0.01*
Race			
	Non-Hispanic White	Ref	
	Non-Hispanic Black	2.2 (1.61 – 2.95)	<0.01*
	Other	1.03 (0.79 – 1.33)	0.845
BMI			
	Underweight/ normal	Ref	
	Overweight	1.69 (1.26- 2.28)	<0.01*
	Obese	2.83 (2.12 – 3.77)	<0.01*
Goodness of fit: p-value =0.62 > 0.05 → good fit			

REFERENCES

1. Bernotienė G, D.V., Klumbienė J, Kriaučionienė V, Kučienė R, Lukšienė D, et al. , *Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants.* Lancet, 2021. **398**(10304): p. 957-980.

2. *Hypertension*. World Health Organization [cited 2024 Jan 6]; Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension#:~:text=Modifiable%20risk%20factors%20include%20unhealthy,and%20being%20overweight%20or%20obese

3. Tailakh, A., et al., *Hypertension prevalence, awareness, and control in Arab countries: a systematic review.* Nurs Health Sci, 2014. **16**(1): p. 126-30.

4. Tohme, R.A., A.R. Jurjus, and A. Estephan, *The prevalence of hypertension and its association with other cardiovascular disease risk factors in a representative sample of the Lebanese population.* J Hum Hypertens, 2005. **19**(11): p. 861-8.

5. Noubani, A., et al., *Prevalence, Awareness, and Control of Hypertension in Greater Beirut Area, Lebanon.* Int J Hypertens, 2018. **2018**: p. 5419861.

6. Bai PY, W.G., Taylor AW, Martin SA, Milne RW, Jenkins AJ, et al., *The association between total phthalate concentration and non-communicable diseases and chronic inflammation in South Australian urban dwelling men. .* Environ Res. , 2017. **158**: p. 366-72.

7. Dong R, Z.S., Zhang H, Chen J, Zhang M, Wang M, et al. , *Sex differences in the association of urinary concentrations of phthalates metabolites with self-reported diabetes and cardiovascular diseases in Shanghai adults. .* Int J Environ Res Public Health, 2017. **14**(6): p. 598.

8. Sturgeon SR, F.D., Kaiser AB, Reeves KW, *Urinary levels of phthalate metabolites and cardiovascular disease mortality (NHANES, 1999–2008). .* Int J Hyg Environ Health. , 2016. **219**(8): p. 876-82.

9. Borjac J, E.J.M., Youssef L, Kawach R, Blake DA, *Quantitative analysis of heavy metals and organic compounds in soil from Deir Kanoun Ras El Ain Dump, Lebanon. .* The Scientific World Journal, 2020. **2020**(8151676).

10. Mills, K.T., A. Stefanescu, and J. He, *The global epidemiology of hypertension.* Nat Rev Nephrol, 2020. **16**(4): p. 223-237.

11. Niezgoda M, K.I., *Gastrointestinal neuroendocrine cells in various types of hypertension – a review. .* Progress in Health Sciences, 2017. **7**(2): p. 117-125.

12. Manosroi, W. and G.H. Williams, *Genetics of Human Primary Hypertension: Focus on Hormonal Mechanisms.* Endocr Rev, 2019. **40**(3): p. 825-856.

13. Shiue, I., *Higher urinary heavy metal, phthalate, and arsenic but not parabens concentrations in people with high blood pressure, U.S. NHANES, 2011-2012.* Int J Environ Res Public Health, 2014. **11**(6): p. 5989-99.

14. Hauser, R. and A.M. Calafat, *Phthalates and human health.* Occup Environ Med, 2005. **62**(11): p. 806-18.

15. Bility, M.T., et al., *Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters.* Toxicol Sci, 2004. **82**(1): p. 170-82.

16. Ferguson, K.K., R. Loch-Caruso, and J.D. Meeker, *Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006.* Environ Res, 2011. **111**(5): p. 718-26.

17. Lind, L. and P.M. Lind, *Can persistent organic pollutants and plastic-associated chemicals cause cardiovascular disease?* Journal of Internal Medicine, 2012. **271**(6): p. 537-553.

18. Wang Y, Q.H., *Phthalates and their impacts on human health.* Healthcare (Basel), 2021. **6**(5): p. 603.

19. Mariana, M., et al., *The effects of phthalates in the cardiovascular and reproductive systems: A review.* Environ Int, 2016. **94**: p. 758-776.

20. Gwinn, M.R., et al., *Gene expression profiling of di-n-butyl phthalate in normal human mammary epithelial cells.* J Environ Pathol Toxicol Oncol, 2007. **26**(1): p. 51-61.

21. Rudel, R.A. and L.J. Perovich, *Endocrine disrupting chemicals in indoor and outdoor air.* Atmos Environ (1994), 2009. **43**(1): p. 170-181.

22. Wu, A.H., et al., *Urinary phthalate exposures and risk of breast cancer: the Multiethnic Cohort study.* Breast Cancer Res, 2021. **23**(1): p. 44.

23. Crobeddu, B., et al., *Di(2-ethylhexyl) phthalate (DEHP) increases proliferation of epithelial breast cancer cells through progesterone receptor dysregulation.* Environ Res, 2019. **173**: p. 165-173.

24. Tranfo, G., et al., *Urinary phthalate monoesters concentration in couples with infertility problems.* Toxicol Lett, 2012. **213**(1): p. 15-20.

25. Nguyen TT, J.E., Yang H, Hyun SH, Choi KC, Jeung EB. , *Potential endocrine disrupting effects of phthalates in in vitro and in vivo models.* . Journal of Embryo Transfer, 2010. **25**(4): p. 207-13.

26. Deng, T., et al., *Di-(2-ethylhexyl) phthalate induced an increase in blood pressure via activation of ACE and inhibition of the bradykinin-NO pathway.* Environmental Pollution, 2019. **247**: p. 927-934.

27. Benjamin, S., et al., *Phthalates impact human health: Epidemiological evidences and plausible mechanism of action.* J Hazard Mater, 2017. **340**: p. 360-383.

28. *National Health and Nutrition Examination Survey*. National Center for Health Statistics 2021 [cited 2023 April 2]; Available from: https://www.cdc.gov/nchs/nhanes/about_nhanes.htm.

29. Trasande, L., et al., *Urinary phthalates are associated with higher blood pressure in childhood.* J Pediatr, 2013. **163**(3): p. 747-53 e1.

30. *America's Children and the Environment, Third Edition.* 2013, U.S. Environmental Protection Agency: Washington, D.C.

31. Lyche, J.L., *Chapter 48 - Phthalates*, in *Reproductive and Developmental Toxicology*, R.C. Gupta, Editor. 2011, Academic Press: San Diego. p. 637-655.

32. Meeker, J.D., S. Sathyanarayana, and S.H. Swan, *Phthalates and other additives in plastics: human exposure and associated health outcomes.* Philos Trans R Soc Lond B Biol Sci, 2009. **364**(1526): p. 2097-113.

33. *An Assessment Report on Issues of Concern: Chemicals and Waste Issues Posing Risks to Human Health and the Environment* UN Environment Programme. 2020 [cited 2023 April 11]; Available from: https://wedocs.unep.org/20.500.11822/33807.

34. Colacino JA, S.A., Calafat AM, Nahar MS, Van Zomeren-Dohm A, Hablas A, et al., *Exposure to phthalates among premenstrual girls from rural and urban Gharbiah, Egypt: a pilot exposure assessment study.* Environmental Health, 2011. **10**: p. 1-8.

35. Olujimi OO, F.O., Odendaal JP, Okonkwo JO, *Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring.* Water SA, 2010. **36**(5): p. 671-682.

36. Fatoki OS, B.M., Ravandhalala L, Chimuka L, Genthe B, Adeniyi AJ, *Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects.* Water SA, 2010. **36**(1): p. 117-25.

37. Mengozzi A, C.F., Pezzica S, Biancalana E, Gastaldelli A, Solini A, *High exposure to phthalates is associated with HbA1c worsening in type 2 diabetes subjects with and without edentulism: a prospective pilot study.* Diabetol Metab Syndr, 2022. **14**(1): p. 1-9.

38. Svetkey, L.P., *Management of Prehypertension.* Hypertension, 2005. **45**(6): p. 1056-1061.

39. Karle, V.A., et al., *Extracorporeal membrane oxygenation exposes infants to the plasticizer, di(2-ethylhexyl)phthalate.* Crit Care Med, 1997. **25**(4): p. 696-703.

40. *PVC Policies across the World.* Center of Health, Environment & Justice [cited 2024 Jan 4]; Available from: http://www.chej.org/pvcfactsheets/PVC_Policies_Around_The_World.html.

41. *About the National Health and Nutrition Examination Survey*. National Center for Health Statistics 2023 [cited 2023 January 10].

42. Silva, M.J., et al., *Analysis of human urine for fifteen phthalate metabolites using automated solid-phase extraction.* J Chromatogr B Analyt Technol Biomed Life Sci, 2004. **805**(1): p. 161-7.

43. Zota, A.R., A.M. Calafat, and T.J. Woodruff, *Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010.* Environ Health Perspect, 2014. **122**(3): p. 235-41.

44. Shiue, I., *Are urinary polyaromatic hydrocarbons associated with adult hypertension, heart attack, and cancer? USA NHANES, 2011-2012.* Environ Sci Pollut Res Int, 2015. **22**(21): p. 16962-8.

45. *Informed Consent*. Centers for Disease Control and Prevention 2015 [cited 2023 December, 5]; Available from: https://www.cdc.gov/nchs/nhanes/genetics/genetic_participants.htm.

46. *Your Privacy*. Centers for Disease Control and Prevention 2021 [cited 2023 December 5]; Available from: https://www.cdc.gov/nchs/nhanes/participant/participant-confidentiality.htm.

47. Dziobak, M.K., et al., *Demographic Assessment of Mono(2-ethylhexyl) Phthalate (MEHP) and Monoethyl Phthalate (MEP) Concentrations in Common Bottlenose Dolphins (Tursiops truncatus) From Sarasota Bay, FL, USA.* Geohealth, 2021. **5**(5): p. e2020GH000348.

48. Shiue, I., *Higher urinary heavy metal, arsenic, and phthalate concentrations in people with high blood pressure: US NHANES, 2009–2010.* Blood pressure, 2014. **23**: p. 1-7.

49. Shiue, I. and K. Hristova, *Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3-19% of the population attributable risk for high blood pressure: US NHANES, 2009-2012.* Hypertension research : official journal of the Japanese Society of Hypertension, 2014. **37**(12): p. 1075-1081.

50. Trasande, L. and T.M. Attina, *Association of exposure to di-2-ethylhexylphthalate replacements with increased blood pressure in children and adolescents.* Hypertension, 2015. **66**(2): p. 301-8.

51. Olsén, L., L. Lind, and P.M. Lind, *Associations between circulating levels of bisphenol A and phthalate metabolites and coronary risk in the elderly.* Ecotoxicology and Environmental Safety, 2012. **80**: p. 179-183.

52. Wu, W., et al., *Association of phthalate exposure with anthropometric indices and blood pressure in first-grade children.* Environmental Science and Pollution Research, 2018. **25**(23): p. 23125-23134.

53. Valvi, D., et al., *Prenatal Phthalate Exposure and Childhood Growth and Blood Pressure: Evidence from the Spanish INMA-Sabadell Birth Cohort Study.* Environ Health Perspect, 2015. **123**(10): p. 1022-9.

54. Shiue, I., *Urine phthalate concentrations are higher in people with stroke: United States National Health and Nutrition Examination Surveys (NHANES), 2001-2004.* Eur J Neurol, 2013. **20**(4): p. 728-31.