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Association between Exposure Biomarkers of Phthalates and Early Menarche in Adolescents

청소년에서 프탈레이트의 노출 바이오마커와 이른 초경과의 관련성

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서울대학교 보건대학원

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박 완

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이 논문을 보건학 석사학위논문으로 제출함

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ABSTRACT

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Phthalates are a group of chemicals to which people have constant environmental exposure while using plastics, toys, food packaging, and personal care products. Phthalates can influence pubertal timing through disruption of multiple systems of hypothalamus-pituitary-gonadal axis. Average age at menarche has been declining through the years worldwide as well as in Korea. Although experimental studies partly held exposure to endocrine-disrupting chemicals responsible for the change in pubertal onset, human epidemiologic evidence on relationship between phthalates and menarche is still inconsistent.

In Chapter II, we conducted a systematic review to examine the association of prenatal and peripubertal exposure to phthalates with age at menarche. We searched relevant papers in databases of PubMed, EMBASE, and Web of Science, and

included 13 studies in our review. Types of phthalates that repeatedly presented association with menarche were mono-ethyl phthalate (MEP) and metabolites of di-(2-ethylhexyl) phthalate (DEHP), although the direction of association varied. Inconsistency of the results from the included studies might be related to the period of exposure and distinct levels and distributions of phthalates in study regions. We reviewed covariates for the relationship between phthalate exposure and menarche, and identified age, race/ethnicity, maternal age at menarche, body fat status, poverty index, caregiver education, and co-exposure to bisphenol A. Race/ethnicity, family poverty, caregiver education, and co-exposure to bisphenol A can have confounding effect, while body fat status might also work as a partial mediator in the biological pathway.

In Chapter III, we investigated the association between exposure to phthalates and early menarche in Korean middle-school girls, using data from Korean National Environmental Health Survey 2015–2017. Using linear regression analysis, we probed for possible linear association of eight phthalate metabolites with age at menarche. We also performed multiple logistic regression analysis to evaluate association between levels of phthalate metabolites and early onset of menarche. As a result, no significant association was found for any phthalate metabolites in linear regression. In logistic regression analysis, however, odds ratios (ORs) of early menarche were significantly increased for mono-n-butyl phthalate (MnBP) and for sum of all phthalates. When compared to group with the lowest level, high concentration group for MnBP presented significantly increased risk of early menarche (OR: 2.12; 95% confidence interval [CI]: 1.04, 4.30) after adjusting for household income, caregiver education, and body mass index. Furthermore, high

concentrations of sum of all phthalates were associated with significant increase of risk for early menarche (OR: 2.10; 95% CI: 1.03, 4.31) after adjustment, compared to the lowest concentration group. Our results suggest that higher levels of MnBP and total phthalates might have association with early menarche in Korea.

In this thesis, we conducted a systematic review and found that association between phthalate exposure and menarche existed with varying degrees and directions, especially for MEP and metabolites of DEHP. Distribution of phthalate exposure, along with covariates such as race/ethnicity, body fat status, family poverty, caregiver education, and co-exposure to bisphenol A might have affected the association. Furthermore, we discovered that exposure to certain phthalate metabolites might be associated with early menarche in Korean population.

Keywords: Phthalate; Menarche; Early menarche; Puberty; Endocrine-disrupting chemicals

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Chapter I

Introduction

I-1. Background

Phthalates are a group of chemicals used as plasticizers, solvents, and raw materials in producing plastics and consumer products (Schettler et al., 2006). Phthalates can be classified into two categories by their molecular weights (National Research Council (US) Committee on the Health Risks of Phthalates, 2008). High-molecular-weight phthalates are the ones with five or more carbons in their ester side chains, such as di-(2-ethylhexyl) phthalate (DEHP), butyl-benzyl phthalate (BBzP), di-octyl phthalate (DOP), di-isononyl phthalate (DiNP), and di-isodecyl phthalate (DiDP). High-molecular-weight phthalates are frequently used in building materials as plasticizer of poly vinyl chloride (PVC), and also in medical tubing, toys, and food packaging (ATSDR, 1997; ATSDR, 2002; Schettler et al., 2006). Low-molecular-weight phthalates, having side chains with four or less carbons, contain di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-n-butyl phthalate (DnBP), di-isobutyl phthalate (DiBP), and so on. Types of phthalates and their urinary metabolites are listed in Table I-1. Low-molecular-weight phthalates are found in personal care products such as shampoo, nail polish, and fragrance (ATSDR, 1995; ATSDR, 2001; Latini et al., 2005). In everyday life, phthalates are readily released from their original products owing to their non-covalent bond and get absorbed into human body through ingestion, inhalation, and dermal contact (Hauser and Calafat, 2005). Exposure to phthalates is so ubiquitous that they are continuously detected in biomonitoring surveys conducted in many different countries (Koch et al., 2016; Park et al., 2017)

Table I-1 Phthalate types and their metabolites

Parent phthalate	Phthalate metabolites
Dimethyl phthalate (DMP)	Monomethyl phthalate (MMP)
Diethyl phthalate (DEP)	Monoethyl phthalate (MEP)
Di-n-butyl-phthalate (DnBP)	Mono-n-butyl phthalate (MnBP)
Di-isobutyl phthalate (DiBP)	Mono-isobutyl phthalate (MiBP)
Butyl-benzyl phthalate (BBzP)	Mono-benzyl phthalate (MBzP)
Di-(2-ethylhexyl) phthalate (DEHP)	Mono-(2-ethylhexyl)-phthalate (MEHP)
	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)
	Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)
	Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)
	Mono-3-carboxypropyl phthalate (MCPP)
Di-n-octyl phthalate (DOP)	Mono-*n*-octyl phthalate (MOP)
	Mono-3-carboxypropyl phthalate (MCPP)
Di-isononyl phthalate (DiNP)	Mono-carboxyoctyl phthalate (MCOP)
Di-isodecyl phthalate (DiDP)	Mono-(carboxy-isononyl) phthalate (MCNP)

During the last century, average age at menarche has been in deceasing trend throughout the world (Euling et al., 2008; Ong et al., 2006). Concerns around this worldwide phenomenon surfaced since early onset of menarche was reported to be associated with hypertension, type 2 diabetes, coronary heart disease, and breast cancer (Bubach et al., 2018; He et al., 2010; Canoy et al., 2015; Collaborative Group on Hormonal Factors in Breast Cancer, 2012). Earlier onset of menarche was assumed to be attributable to genetic factors such as race and ethnicity, increased adiposity, and environmental factors such as exposure to endocrine-disrupting chemicals (Jacobson-Dickman et al., 2009). Phthalates are one of renowned endocrine-disrupting chemicals. In experimental studies, phthalates have both agonistic and antagonistic effects in hormonal receptors, interfering in systems of hypothalamic-pituitary-gonadal axis (Gore et al., 2015; Benjamin et al., 2017).

However, human epidemiological studies that investigated association between phthalate exposure and pubertal indices, including menarche, have presented inconsistent results. Some studies found association of exposure to certain phthalates with earlier onset of puberty, while there were also other studies that showed no association or delayed indices of puberty (Hart et al., 2014; Kasper-Sonnenberg et al., 2017; Wolff et al., 2017). The direction of association varied by the types of phthalates involved and the time of exposure in the studies

According to the report on Korean National Environmental Health Survey (KoNEHS) 2015-2017, children and adolescents are under steady exposure to phthalates (NIER, 2018). KoNEHS is a cross-sectional survey with weighted sampling design to represent Korean population and evaluate various

environmental exposure and related clinical and demographic features. Average age at menarche of South Korean girls has also been declining, to 12.7 years in 2011 from 13.4 years in 2001 and 14.2 years in 1980 (Cho et al., 2010; Lee et al., 2016). However, studies on effects of phthalate exposure on menarche in Korean population are insufficient.

I-2. Objectives

This thesis aimed to examine the overall effect of phthalate exposure on menarche, identify relevant factors in previous literature, and investigate the association between phthalates and early menarche in Korea. To accomplish these goals, following studies were conducted:

A systematic review of human epidemiologic studies for relationship between phthalate exposure and menarche

Analyses on risk of early menarche in Korean adolescent girls with exposure to phthalates, using data retrieved from KoNEHS

Chapter II

Systematic review of association between exposure to phthalates and age at menarche

II-1. Introduction

As mentioned in Chapter I, there have been inconsistent results regarding the association between exposure to phthalate and onset of menarche. A recent systematic review and meta-analysis presented no significant change in risks of menarche for any phthalate metabolites (Golestanzadeh et al., 2020). However, due to statistical reasons, only three studies were included in the pooled analysis, limiting its representability for other regions. Another systematic review was conducted for the effect of phthalate on pubertal indices, but the study also focused on other reproductive outcomes such as time to pregnancy, preterm birth, and spontaneous abortion (Radke et al., 2019). Therefore, since menarche was only small part of many interests of the study, the reason why there had been varying results and the factors that might have influenced the association were not fully discussed. We updated systematic reviews and investigated potentially relevant variables as time of exposure (prenatal or peripubertal), types of phthalates, and other covariates that appeared in previous researches.

II-2. Methods

II-2-1. Literature search

We conducted a systematic review according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2015). We searched databases including PubMed, EMBASE, and Web of Science until April 2021 (starting year for the search was not designated). Keywords for the search were as follows: (Phthalates OR "phthalic acid" OR "endocrine disrupting chemical") AND (menarche OR puberty OR pubertal OR maturation). We also searched studies which were included in the references of previous reviews regarding phthalate exposure and pubertal onset. After removing duplicates, we screened titles and abstracts according to the eligibility criteria. Then, we performed full-text review on articles that could not be determined if they met the eligibility criteria with titles and abstracts.

II-2-2. Eligibility criteria

Inclusion criteria:

We included cohort, case-control, and cross-sectional studies on human that investigated exposure to phthalates and its association with menarche. Studies that contained risk estimates for age at menarche or occurrence of menarche were included.

Exclusion criteria:

We excluded studies that were not performed on human, such as animal or experimental studies. We excluded review articles, systemic reviews and meta-analyses, case report or series, conference abstracts, commentaries, and non-English articles. We also excluded studies that did not provide information on menarche as a separated measure or outcome. For instance, case-control studies that evaluated exposure to phthalate in precocious puberty patients were excluded since precocious puberty was usually defined with exhibition of any secondary sex characteristics (thelarche, pubarche, or menarche) before 8 years old in girls.

II-2-3. Data extraction

We extracted following data from the included studies: first author, publication year, study country, type of study, study period, period of exposure, time of sample collection, types of phthalates, covariates in analysis, study size, age, and risk estimates for menarche with 95% confidence intervals if provided. Risk estimates adjusted for covariates were collected preferably to unadjusted ones. We retrieved statistically significant estimates as well as estimates with p-value under 0.1 as "marginally significant" results.

II-2-4. Endpoints of the review

Epidemiological studies generally investigated exposure to phthalates at either of two different periods: prenatal exposure where phthalate concentration in mother during pregnancy were measured or peripubertal exposure where concentration in girls around puberty were measured. Therefore, we categorized included studies according to the time of exposure (prenatal or peripubertal) and reviewed them separately.

We reviewed the main results of included studies, including the risk estimates for menarche and their implications. We focused on which type of phthalate metabolites frequently presented association with menarche. Then, for each metabolite, we also reviewed if the direction of association was in advancing the menarche or delaying it. Finally, we identified covariates that required adjustment in evaluating relationship between phthalate exposure and menarche.

II-3. Results

II-3-1. Characteristics of included studies

1332 articles were searched in databases of PubMed, EMBASE, and Web of Science. We attempted to find additional articles in the references of recent review articles, but all of them were already identified in our query of databases. We removed duplicate articles and screened 732 articles with their titles and abstracts. After 689 articles were excluded in the screening process, 43 articles entered the full-text assessment. Finally, 13 studies were eligible for review and data extraction (Figure II-1).

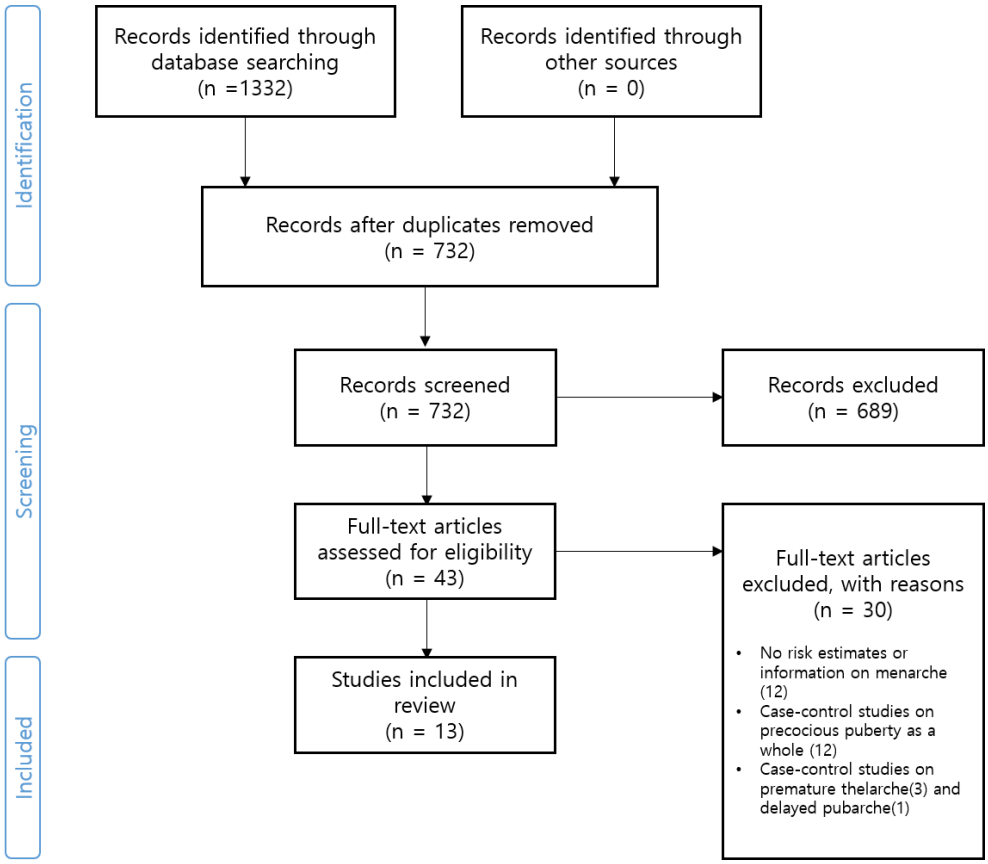

Figure II-1 PRISMA flow diagram for the systematic review

The studies we reviewed were conducted in Mexico, USA, Taiwan, China, Germany, and Chile with publication year between 2012 and 2021. To assess exposure to phthalates, all studies used urine as biological samples. Types of phthalate metabolites that were measured in the studies included mono-(2-ethylhexyl)-phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), which are metabolites of DEHP. Also, mom-methyl phthalate (MMP), mono-ethyl phthalate (MEP), Mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP), mono-carboxyoctyl phthalate (MCOP), mono-(carboxy-isononyl) phthalate (MCNP), and mono-(3-carboxypropyl) phthalate (MCPP) were measured. Out of 13 studies included, five studies measured only prenatal exposure, nine studies measured only peripubertal exposure, and one study (Watkins et al., 2014) measured both prenatal and peripubertal exposure. All included studies were either cohort or cross-sectional studies. We presented risk estimates for the phthalate metabolites that showed significant or marginally significant association with menarche (Table II-1, II-2).

Table II-1. Characteristics of studies with prenatal exposure to phthalates

Author	Country	Study type	Time of Phthalate Measurement	Phthalate type	Covariates	Main Findings	Size	Child Age	Period of Exposure
Watkins (2014)	Mexico	Cohort	3rd trimester	MEP, MnBP, MiBP, MBzP, MCPP, MEHP, MEHHP, MEOHP, MECPP	Age, BMI Z-score	MEP was marginally associated with earlier menarche (OR 2.66; 95% CI 0.91, 7.76)	116	8-13	1997-2004
Watkins (2017)	Mexico	Cohort	1st, 2nd, 3rd trimester	MEP, MnBP, MiBP, MBzP, MCPP, MEHP, MEHHP, MEOHP, MECPP	Age, BMI Z-score	MEP was associated earlier menarche (OR 3.88; 95% CI 1.24-12.1)	119	8-13	1997-2004
Berger (2018)	USA	Cohort	Twice (Mean: 14.0 and 26.9 weeks)	MBzP, MEHP, MEHHP, MEOHP, MECPP, MCOP, MCNP, MCPP	Maternal education, years in US, family poverty, Diet Quality Index, maternal prepregnancy BMI	Sum of DEHP metabolites[a] was associated with later menarche (Mean shift 2.5 months; 95% CI 1.1, 4.1) For normal-weight girls, MCNP, MCOP, and MCPP was associated with later menarche (MCNP mean shift 2.4 months; MCOP mean shift 2.1 months; MCPP mean shift 3.3 months)	165	9-13	1999-2000

Harley (2019)	USA	Cohort	Twice (Mean: 14.0 and 26.9 weeks)	MEP, MnBP, MiBP	Maternal education, years in US, family poverty, maternal prepregnancy BMI	No significant association was found	172-177	9-13	1999-2000
Cathey (2020)	Mexico	Cohort	1st, 2nd, 3rd trimester	MEP, MnBP, MiBP ,MBzP, MCPP, MEHP, MEHHP, MEOHP, MECPP	Age, BMI Z-score	MBzP was marginally associated with higher risk of menarche at 1st visit (OR 2.24; 95% CI 0.96-15.5), but also with slower progression to menarche at 2nd visit (OR 0.69; 95% CI 0.47-1.02)	103	8-13 at 1st visit 9-18 at 2nd visit	1997-2004

* OR: odds ratio, CI: confidence interval, BMI: body mass index

[a] molar sum of MEHP, MEHHP, MEOHP, and MECPP

Table II-2 Characteristics of studies with peripubertal exposure to phthalates

Author	Country	Study type	Time of Phthalate Measurement	Phthalate type	Covariates	Main Results	Size	Child Age	Period of Study
Buttke (2012)	USA	Cross-sectional	2005-2008	MEP, MBP, MiBP, MCPP, MEHP, MBzP, MEOHP, MEHHP, MECPP, MCOP, MCNP	Race/ethnicity, BMI percentile	No significant association was found for sum of all phthalate metabolites	437	12-16	2005-2008
Watkins (2014)	USA	Cohort	2010	MEP, MnBP, MiBP ,MBzP, MCPP, MEHP, MEHHP, MEOHP, MECPP	Age, BMI Z-score	MEP was marginally associated earlier menarche (OR 2.58; 95% CI 0.90-7.39)	129	8-13	1997-2010
Hou (2015)	Taiwan	Cross-sectional	2012-2013	MMP, MEP, MiBP, MnBP, MBzP, MEHP, MEOHP, MEHHP, MECPP	Age, maternal age at menarche	No significant association was found.	118	6.5-15.0	2012-2013
Shi (2015)	China	Cross-sectional	2010	MnBP, MMP, MEP, MEHP, MEHHP, MEOHP	Age, body fat composition, parental education	Compared to age- and body fat%-matched peers, MEP was associated earlier menarche in univariate analysis (OR 1.18; 95% CI not shown) No significant association was found in multivariate analysis	248 (univariate) 178 (multivariate)	7-14	2010

Zhang (2015)	China	Cross-sectional	Twice (2010, 2012)	MnBP, MMP, MEP, MEHP, MEHHP, MEOHP	Age, body fat composition, parental education	MEP was marginally associated with higher risk of menarche at the 1st visit (OR 1.25; 95% CI 0.98-1.59) MEHHP and MEOHP was marginally associated with faster menarche progression at the 2nd visit. (MEHHP OR 1.72; 95% CI 0.93-3.20; MEOHP OR 1.71; 95% CI 0.93-3.14)	135 (1st visit) 199 (2nd visit)	7-14 Mean age: 9.8 (2010) 11.4 (2012)	2010-2012
Kasper-Sonnenberg (2017)	Germany	Cohort	2009-2010	MEP, MMP, MBzP, MiBP, MnBP, MEHP, MEHHP, MEOHP, MECPP	Age, BMI	MEP was marginally associated with later menarche (OR 0.64; 95% CI 0.39-1.04)	198	8-10 at baseline	2009-2013
Wolff (2017)	USA	Cohort	Within a year after enrollment at 2004-2007	MEP, MNBP, MBzP, MCPP, MEHHP, MEOHP, MECPP, MEHP	Race/ethnicity, caregiver education	MEP was marginally associated with earlier menarche (OR 1.06; 95% CI 0.99-1.14) MCPP was associated with later menarche (OR 0.89; 95% CI 0.81-0.98)	1051	6-8 at enrollment	2004-2015

Binder (2018)	Chile	Cohort	At Tanner stage B1 and B4 (B1 median age 7.9, B4: median age 11.2)	MnBP, MBzP, MCNP, MCPP, MECPP, MEHHP, MEHP, MEOHP, MEP, MiBP, MMP	BMI Z-score, maternal education, Tanner stage	Middle tertile of MBzP was associated with later menarche compared to the lowest tertile (OR 0.70; 95% CI 0.54-0.93) MEHHP, MEOHP, and MEHP at B1 were associated with later menarche (MEHHP OR 0.77; 95% CI 0.62-0.96; MEOHP OR 0.78; 95% CI 0.63-0.97; MEHP OR 0.80; 95% CI 0.65-0.98) MMP at B4 was associated with higher risk of menarche (OR 1.30; 95% CI 1.10-1.53)	200	9-13	2009-2016
Oskar (2021)	USA	Cross-sectional	2005-2008	MEHP, MEOHP, MEHHP, MECPP, MCNP, MBzP, MnBP, MiBP, MEP, MCPP, MMP,	Poverty income ratio, BMI percentile, race/ethnicity, exposure to bisphenol A	Lower MEHP was associated with earlier menarche (OR 1.36; 95% CI 1.02-1.80) Lower MEHP combined with higher BPA was marginally associated with earlier menarche (OR 1.31; 95% CI 0.99, 1.73)	229	12-16	2005-2008

* HR: hazard ratio, OR: odds ratio, CI: confidence interval, BMI: body mass index

II-3-2. Association between prenatal exposure to phthalates and menarche

All five studies that measured prenatal exposure were cohort studies conducted in Mexico and USA with urine samples collected between the range of 1997 and 2004. Watkins et al. reported that increase in concentration of MEP measured in the third trimester was marginally related with earlier onset of menarche, and that higher MEP in average of all three trimesters showed significant association. Berger et al. investigated the effect of prenatal exposure to high-molecular-weight phthalates and suggested that higher concentrations of DEHP metabolites were related with delay of menarche, and that MCNP, MCOP, and MCPP also displayed similar association for normal-weight girls. Harley et al. explored the effect of low-molecular-weight phthalates in the same cohort, but could not find any significant association between phthalate exposure and menarche. The most recent study in the Mexican cohort was conducted by Cathey et al. They performed another follow-up examination at age 9 to 18 and reported that increase in prenatal MBzP was associated higher odds of menarche at first visit, but also with slower progression to menarche until second visit.

II-3-3. Association between peripubertal exposure to phthalates and menarche

Nine studies that investigated the effect of peripubertal exposure comprised four cohort studies and five cross-sectional studies. Buttke et al. and Hou et al. found no significant association between any of the phthalate metabolites measured and age at menarche. Watkins et al. presented marginally significant increase in risk of menarche for higher peripubertal concentration of MEP. Shi et al. reported association between MEP level and early menarche when matched by age and body fat percentage. Zhang et al. paid two visits to children during puberty to investigate pubertal indices at baseline and at 18 months later. As a result, MEP level was marginally related with higher risk of menarche at baseline, and MEHHP and MEOHP also showed marginal association with progression to menarche until the second visit. Kasper-Sonnenberg et al. found marginally negative association between menarche and levels of MEP. Wolff et al. reported that MEP had marginally significant association with earlier menarche, and that MCPP was associated with later menarche. Binder et al. conducted analysis stratified by Tanner stage, where exposure was measured at Tanner stage B1 and B4. MEHHP, MEOHP, and MEHP levels at stage B1 were associated with later menarche, while MMP level at stage B4 was associated with earlier menarche. Oskar et al analyzed the effect of co-exposure by phthalates, bisphenol A, and other endocrine-disrupting chemicals and found that MEHP level was inversely associated with risk of menarche, with and without the co-exposure effect of bisphenol A.

II-3-4. Covariates adjusted in the analyses

Studies included adjusted for several covariates that were related with exposure to phthalate and menarche. Adjusted covariates included age, genetic factors, factors related with body fat status, factors regarding socioeconomic status, and exposure to bisphenol A. Genetic factors included race/ethnicity and maternal age at menarche. Factors related with body fat status contained maternal pre-pregnancy body mass index (BMI), child BMI, BMI Z-score, BMI percentile, and body fat percentage. Socioeconomic status factors were composed of caregiver (or maternal) education, diet quality index, family poverty, and years residing in the United States.

II-4. Discussion

We performed a systematic review of the association between exposure to phthalates and menarche. We sorted out the included studies in accordance with time of exposure and types of phthalates involved. Metabolites of phthalates that presented associated with menarche most frequently were MEP and metabolites of DEHP such as MEHHP, MEOHP, MECPP, and MEHP.

In studies with prenatal exposure, increase in MEP level was associated with earlier menarche in the Mexican cohort (Watkins et al., 2014; Watkins et al., 2017). Association with earlier menarche was also observed for peripubertal exposure of MEP in Mexico, China, and USA (Watkins et al., 2014; Shi et al., 2015; Zhang et al., 2015; Wolff et al., 2017). However, in Germany, MEP was related with lower risk of menarche (Kasper-Sonnenberg et al, 2017). One of the factors that might have contributed to this discrepancy is difference in distribution of phthalate exposure between study period and regions. Phthalate concentrations in German population has consistently decreased from 2002 to 2008, and MEP level has also been significantly declining since 2009 (Wang et al., 2009). A study that compared national environmental survey of Germany and USA stated that MEP value was generally lower in Germany in the 2000s (Koch et al., 2016). Interestingly, this was also consistent with the studies included in our review. Subjects in the study from Germany (Kasper-Sonnenberg et al., 2017) had relatively low MEP level, with geometric mean (GM) of 25 μg/L. On the contrary, MEP levels were higher in the studies from Mexico (Watkins et al., 2014), with GM of 114 μg/L *in utero* and

97.5 μg/L prepubertal, and from USA with median of 89-124 μg/L (Watkins et al, 2014; Berger et al., 2018).

Metabolites of DEHP generally showed association with later menarche in studies from USA and Chile (Berger et al., 2018; Binder et al., 2018). However, a cross-sectional study from China linked higher concentration of MEHHP and MEOHP with faster progression of menarche (Zhang et al., 2015). Exposure of the study was measured between 2010 and 2012, which is more recent, and exposure level might have generally declined compared to exposure in 90s and 2000s. In addition, major metabolites of phthalates found in China are MnBP and MiBP (Wang et al., 2019), so exposure to different type of phthalates between regions might also be related to the differing results. The study by Zhang et al. showed similar distribution, with high MnBP concentration (GM: 21.4-23.8 μg/L) and lower concentration of DEHP metabolites (GM: MEHHP 9.4-16.1, MEOHP 3.6-5.3, MEHP 1.0-1.9 μg/L) compared to studies from USA (GM: MEHHP 18.4, MEOHP 13.4, MEHP 4.4 μg/L) and Chile (GM: MEHHP 17.3-24.7, MEOHP 11.2-15.1, MEHP 2.2-2.4 μg/L).

Race/ethnicity frequently appeared as a covariate for studies conducted in multiethnic countries. It seems natural since it affects age at menarche as a genetic factor and also is associated with regions of exposure and socioeconomic status (Huang et al., 2019). Child age is one of the most crucial covariates for a few researches that analyzed the risk of having experienced menarche until the survey or examination during puberty. However, it wasn't controlled in other studies

where age at menarche was surveyed after the menarche had already occurred.

Body fat status was the single most important factor related with phthalate exposure and menarche as it was taken into account in almost every study included in our review. While it was considered a confounder in some studies (Watkins et al., 2014; Watkins et al., 2017; Cathey et al., 2020), other researches pointed it out that body fat status might serve as a partial mediator in the causal pathway of phthalates and menarche (Wolff et al., 2017; Harley et al., 2019). Phthalates influence adipogenesis and obesity through stimulation of peroxisome proliferator-activated receptor and disruption of thyroid, glucocorticoid, and sex hormones (Desvergne et al., 2009; Casals-casas and Desvergne., 2011). Epidemiologic studies conducted in countries of USA and China have shown association of phthalates and childhood obesity (Harley et al., 2019; Xia, et al., 2018). In addition, obesity is one of the factors that are associated with acceleration of pubertal timing (Li et al., 2017). The study by Binder et al. in our review presented that hazard ratio for menarche significantly increased only in obese girls with more than 85th BMI percentile (OR 1.24; 95% CI 1.05-1.47), and not in normal weight girls (OR 1.02; 95% CI 0.90-1.07), which indicate possible effect modification on relation between phthalates and menarche.

People are concurrently exposed to multiple endocrine-disrupting chemicals in real life. Levels of endocrine-disrupting chemicals often show correlation with one another (Braun et al., 2016); this might induce confounding effect as exposure to environmental chemicals other than phthalates can also affect the onset of menarche. Bisphenol A was the second most important factor following MEHP in

evaluation of conjoined effect of various endocrine-disrupting chemicals conducted in the United States (Oskar et al., 2021). Another cross-sectional study from USA also presented that higher levels of bisphenol A were related with later menarche (McGuinn et al., 2015). Results from a recent meta-analysis showed correlation of exposure to bisphenol A and obesity (Wu et al., 2021), which can eventually lead to alteration of pubertal timing in adolescents.

It was suggested that distribution of exposure to phthalates can vary according to socioeconomic factors such as caregiver education, poverty income ratio, and food security. A study conducted in USA reported that higher socioeconomic status was associated with greater exposure to DEHP, and less exposure to MBzP (Kobrosly et al., 2012). Age at menarche can also be affected by socioeconomic status, as studies conducted in China and Chile presented association between higher socioeconomic status and earlier menarche (Cheng et al., 2021; Amigo et al., 2012). Another study from the United States linked lower maternal education and household income with the risk of earlier menarche, although the results varied when stratified by race/ethnicity (Deardorff et al., 2014). In the study by Wolff et al. included in our review, MEP was associated earlier menarche (OR 1.13; 95% CI 1.07-1.20), but the risk estimate was shifted to null after adjusting for race/ethnicity and caregiver education (OR 1.06; 95% CI 0.99-1.14).

Maternal age at menarche was involved as a covariate in a few studies since it has strong concordance with age at menarche of the daughters (Sørensen et al., 2018). However, although it might reflect transgenerational socioeconomic status in relation to phthalate exposure (Binder et al., 2018), its association with exposure to

phthalates is unclear. Adjusting for maternal age at menarche as a covariate might not be necessary if other socioeconomic factors could be controlled, considering it is the effect of the possible differences in socioeconomic status.

II-5. Conclusion

Through the systematic review, we presented the types of phthalates that showed association with either earlier menarche or later menarche. MEP and metabolites of DEHP (MEHHP, MEOHP, MECPP, and MEHP) were the particular types of phthalates that often displayed varying associations, which might be attributable to different distributions and levels of phthalates between regions. Covariates such as race/ethnicity, body fat status, family poverty, caregiver education, and co-exposure to bisphenol A can also influence the relationship. In the future, meta-analyses that can incorporate and analyze the results from these heterogenous researches, taking into account distinct exposure patterns and other covariates, are needed to better understand the effect of phthalates on menarche.

Chapter III

Association of phthalates and early menarche in Korean adolescent girls from Korean National Environmental Health Survey (KoNEHS) 2015–2017

III-1. Introduction

Average age at menarche of Korean girls was 12.7 years in 2011 (Lee et al., 2016), and more girls are experiencing early menarche through the years. Although the term “early menarche” has no fixed definition, many studies regarding menarche and related factors usually define it as “menarche occurring before age 12” (McGuinn et al., 2015; Mishra et al., 2017; Oskar et al., 2021). In KoNEHS, actual ages of the subjects were unavailable owing to school-based nature of the survey. Therefore, we defined early menarche as “menarche occurring before the 6th grade”, which is a mixed group of 11- and 12-year-olds; consequently, normal menarche was defined as “menarche occurring in or after the 6th grade”.

The advancement in age at menarche can be attributable to exposure to endocrine-disrupting chemicals. Phthalate levels from the KoNEHS 2015-2017 in adults were generally lower than those from KoNEHS 2009-2011 (NIER, 2018). However, the third round of KoNEHS (2015–2017) has expanded its study population into children and adolescents in addition to adult-only design of previous rounds, and phthalate levels of infants and children turned out to be much higher than those of adults. In addition, phthalates such as MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP were detected in more than 95 percent of the subjects in the survey, which indicates the ongoing exposure to various types of phthalates in children. Considering these circumstances, we tried to investigate the effect of phthalates on age at menarche in Korean adolescents.

III-2. Methods

III-2-1. Study subjects

The study sample was retrieved from middle (junior high) and high school database of the KoNEHS 2015–2017. The database consists of 430 boys and 492 girls from middle and high schools of urban and rural districts of Korea. Since KoNEHS is a school-based survey, only grades of subjects were acquired instead of their ages. The database has subjects from 7th grade to 12th grade, which is approximately equivalent with age 12 to 17.

Due to cross-sectional design of KoNEHS and short half-life of phthalates in human body, levels of phthalate metabolites in high school students are less likely to represent levels before or upon the time of menarche that had taken place years ago. Therefore, the study population was confined to only middle school students (i.e. 7th to 9th graders). We also excluded girls with missing data of urinary phthalate metabolites. Finally, 236 girls were eligible for study subjects (Fig. III-1).

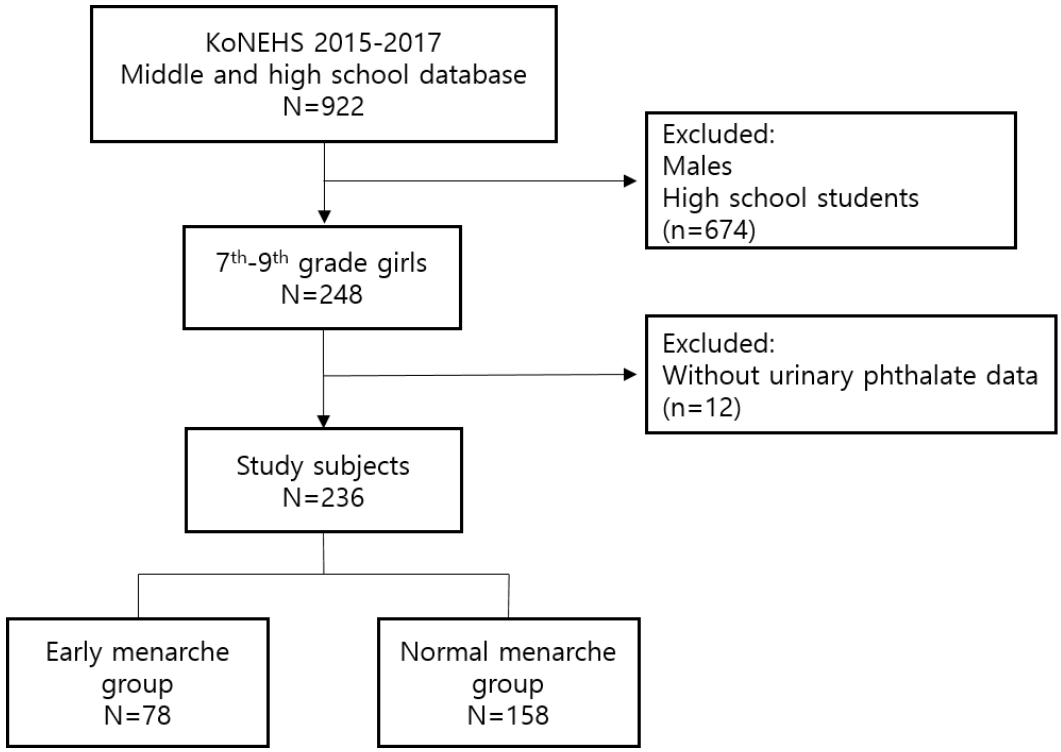

Figure III-1. Flow chart of the selection of study subjects

III-2-2. Measurement of urinary phthalate metabolites

Collection, transportation, storage, and analysis were conducted by National Institute of Environmental Research (NIER), and the summary of method described in the manual is as follows (NIER, 2018). Within 24 hours after collection, spot urine samples were transported at 2ºC–6ºC to laboratories and were stored at −20ºC before analysis. Analysis was conducted through ultra performance liquid chromatography-mass spectrometry with electrospray ionization. Urinary phthalate metabolites were measured for MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP, MCNP, and MCPP. Limit of detection (LOD) was calculated as 3.14 multiplied with standard deviation of seven samples with the lowest concentration of standard solution. Values under LOD were substituted with LOD divided by square root of 2.

III-2-3. Covariates

We examined potential covariates for phthalate exposure and menarche surveyed in KoNEHS. Potential covariates included demographic and socioeconomic characteristics such as grade, maternal age of menarche, body fat status, household income, and caregiver education. Maternal age of menarche was presented with grade as was the daughter's, and maternal early menarche was defined as "menarche reached before 7th grade", reflecting the change of mean age at menarche over the years. Body fat status was categorized as normal (BMI < 23), overweight (23 ≤ BMI < 25), and obese (BMI ≥ 25). Monthly household income was allocated into 4 categories: I (under 3 million KRW), II (3–5 million KRW), III (5–7 million KRW), and IV (over 7 million KRW). Caregiver education was represented with maternal education, classified as "less than high school degree", "high school graduate", and "college degree or above". If the information on maternal education was unavailable, paternal education level was employed for caregiver education.

II-2-4. Statistical analysis

MEHHP, MEOHP, and MECPP are secondary metabolites of DEHP, so sum of these three were also calculated under the name of 'DEHP metabolites.' Total sum of eight metabolites measured were added as 'total phthalates.' Concentration of each metabolite was divided by its molecular mass and added together for summation. For all phthalate metabolites, level above LOD was detected in more than 75% of subjects.

Due to the fact that significant portion of elimination of phthalates is processed through active tubular secretion of kidney, altogether with variable level of urinary creatinine affected by diet, muscularity, and other health status, it has been suggested that specific gravity (SG) is a better tool to adjust for urinary dilution (Hauser et al., 2004). In addition, specific gravity was reported to have more reliability than creatinine does, with more agreement across different methods of adjustment in MnBP concentration (Kuiper et al., 2021). Therefore, we employed formula $Pc = P \times [(1.024 - 1)/(SG - 1)]$ to calculate SG corrected urinary phthalate concentration (Pc is SG corrected phthalate metabolite concentration, P is phthalate metabolite concentration). We presented geometric means (GMs) and 95% confidence intervals (CI) of phthalate metabolite levels in accordance with categories of demographic and socioeconomic traits.

We selected covariates using a directed acyclic graph shown in Appendix Figure 1, which was drawn with regard to the review of associated covariates that appeared in previous literature from Chapter II. Selected covariates included household

income, caregiver education, exposure to bisphenol A, and BMI group.

Levels of urinary phthalate metabolites were positively skewed; therefore, natural log-transformation was applied to SG-corrected concentrations. We conducted linear regression analysis to evaluate association between urinary phthalate metabolite levels and age at menarche. Nineteen girls who had not yet reached menarche at the time of survey were excluded from linear regression analysis (n = 217). Also, we divided study subjects into three groups according to concentration tertiles of each metabolite and conducted logistic regression. We compared the odds of early menarche of group with low concentrations to that of groups with moderate and high concentrations respectively. Since BMI group might act as a mediator in biological pathway, we modelled logistic regression analyses adjusted with and without BMI group, in addition to household income and caregiver education. We also conducted additional analysis adjusting for levels of bisphenol A to address the effect of co-exposure to another major endocrine-disrupting chemical that has been reported to have association with menarche. Data analysis was performed with SAS statistical software ver 9.4 (SAS Institute, Cary, NC, USA).

III-3. Results

III-3-1. Characteristics of the subjects

We described demographic and socioeconomic characteristics of 236 girls from 7th to 9th grade in KoNEHS 2015–2017. Seventy-eight girls were classified as early menarche group and 158 girls were classified as normal menarche group. Distributions of characteristics between groups were compared using Fisher's exact test for caregiver education and $\chi 2$ test for the other characteristics. Maternal early menarche was the only factor that showed difference between early menarche and normal menarche groups ($p = 0.003$) (Table III-1).

Table III-1 Demographic and socioeconomic characteristics of the subjects

Characteristics	Category	All subjects (n = 236)	Early menarche (n = 78)	Normal menarche (n = 158)	*p*-value
Grade	7	79 (33.5)	31 (39.7)	48 (30.4)	0.358[a]
	8	77 (32.6)	23 (29.4)	54 (34.2)	
	9	80 (33.9)	24 (30.8)	56 (45.4)	
Maternal early menarche	No	170 (72.0)	46 (59.0)	124 (78.5)	0.003[a]
	Yes	42 (17.8)	23 (29.5)	19 (12.0)	
	Unknown	24 (10.2)	9 (11.5)	15 (9.5)	
BMI	< 23	192 (81.4)	60 (76.9)	132 (84.5)	0.352[a]
	23–25	20 (8.5)	7 (9.0)	13 (8.2)	
	> 25	24 (10.2)	11 (14.1)	13 (8.2)	
Household income	I (lowest)	65 (27.5)	26 (33.3)	39 (24.7)	0.317[a]
	II	73 (30.9)	20 (25.6)	53 (33.5)	
	III	57 (24.2)	21 (26.9)	36 (22.8)	
	IV (highest)	28 (11.9)	6 (7.7)	22 (13.9)	
	Unknown	13 (5.5)	5 (6.4)	8 (5.1)	
Caregiver education	< High school	6 (2.6)	1 (1.3)	5 (3.2)	0.421[b]
(1 missing)	High school	96 (40.9)	36 (46.2)	60 (38.2)	
	College +	133 (56.6)	41 (52.6)	92 (58.6)	

Values are presented as number (%).

BMI: body mass index.

[a]Analyzed by χ^2 test; [b]Analyzed by Fisher's exact test.

III-3-2. Levels of urinary phthalate metabolites

GMs and 95% CIs of SG-adjusted concentrations of urinary phthalate metabolites are presented according to demographic and socioeconomic characteristics. Concentrations within each characteristic were compared using analysis of variance test or Kruskal-Wallis test. Difference of concentration was noted for BMI, household income, and caregiver education, specifically for MBzP (p = 0.017) level among BMI groups, MECPP (p = 0.042) and MCPP (p = 0.041) level among household income groups, and MECPP (p = 0.005), MCOP (p = 0.024), MCNP (p = 0.025), and MCPP (p = 0.026) level among caregiver education groups (Table III-2).

Table III-2 Concentrations of urinary phthalate metabolites according to the characteristics of the subjects

Characteristics	Categorys	Geometric mean (μg/L) (95% CI)							
		MEHHP	MEOHP	MECPP	MnBP	MBzP	MCOP	MCNP	MCPP
All subjects		16.19 (14.76, 17.76)	10.33 (9.19, 11.62)	34.62 (32.56, 36.82)	37.86 (34.09, 42.05)	2.90 (2.45, 3.43)	2.09 (1.90, 2.31)	0.56 (0.52, 0.61)	1.67 (1.58, 1.77)
Early menarche	No	16.13 (14.36, 18.12)	10.06 (8.64, 11.71)	34.15 (31.57, 36.94)	35.63 (31.21, 40.66)	2.72 (2.24, 3.32)	2.07 (1.82, 2.35)	0.55 (0.51, 0.61)	1.65 (1.54, 1.78)
	Yes	16.32 (14.06, 18.95)	10.91 (9.16, 12.99)	35.61 (32.35, 39.20)	42.83 (36.22, 50.65)	3.29 (2.41, 4.50)	2.14 (1.87, 2.46)	0.58 (0.51, 0.67)	1.71 (1.55, 1.89)
	p-value[a]	0.906	0.524	0.528	0.104	0.294	0.734	0.542	0.565
Grade	7	16.50 (14.37, 18.94)	10.38 (8.67, 12.42)	34.58 (31.55, 37.91)	40.25 (33.58, 48.24)	3.14 (2.49, 3.96)	2.19 (1.92, 2.51)	0.56 (0.49, 0.64)	1.74 (1.58, 1.93)
	8	15.18 (12.54, 18.39)	9.95 (7.79, 12.72)	33.45 (29.84, 37.50)	35.75 (29.82, 42.86)	2.47 (1.77, 3.46)	1.91 (1.55, 2.34)	0.57 (0.51, 0.65)	1.67 (1.51, 1.86)
	9	16.91 (14.62, 19.56)	10.66 (8.90, 12.77)	35.83 (32.05, 40.06)	37.67 (31.39, 45.20)	3.13 (2.36, 4.19)	2.18 (1.86, 2.56)	0.56 (0.49, 0.63)	1.60 (1.44, 1.78)
	p-value[a]	0.621	0.895	0.670	0.665	0.428	0.425	0.941	0.500
Maternal early menarche	No	15.78 (14.18, 17.56)	9.95 (8.64, 11.45)	33.34 (31.07, 35.77)	36.22 (32.04, 40.93)	2.78 (2.29, 3.36)	2.02 (1.78, 2.28)	0.55 (0.51, 0.60)	1.63 (1.53, 1.75)
	Yes	18.01 (14.66, 22.13)	11.73 (9.36, 14.71)	39.47 (34.34, 45.37)	38.14, (30.15, 48.24)	2.99 (1.96, 4.57)	2.26 (1.88, 2.72)	0.60 (0.49, 0.73)	1.71 (1.47, 1.98)
	Unknown	16.16 (11.49, 22.72)	10.83 (7.10, 16.54)	35.08 (28.83, 44.99)	51.21 (35.81, 73.25)	3.73 (2.13, 6.51)	2.37 (1.88, 2.99)	0.57 (0.46, 0.70)	1.89 (1.57, 2.28)
	p-value[b]	0.483	0.353	0.053	0.229	0.475	0.661	0.996	0.283
BMI	< 23	16.03 (14.47, 17.75)	10.30 (9.03, 11.75)	34.36 (32.10, 36.79)	38.07 (33.79, 42.89)	2.71 (2.26, 3.26)	2.12 (1.92, 2.34)	0.57 (0.52, 0.62)	1.67 (1.57, 1.77)

	23–25	20.12 (15.99, 25.31)	12.58 (9.54 16.60)	36.65 (29.91, 44.90)	34.65 (25.66, 46.80)	2.38 (1.31, 4.35)	1.92 (1.21, 3.05)	0.52 (0.40, 0.68)	1.82 (1.36, 2.42)
	> 25	14.66 (10.48, 20.51)	9.01 (6.02, 13.47)	35.08 (28.93, 42.54)	39.03 (28.82, 52.87)	5.83 (3.61, 9.42)	2.00 (1.36, 2.94)	0.56 (0.44, 0.71)	1.61 (1.35, 1.92)
	p-value[b]	0.544	0.749	0.766	0.882	0.017[c]	0.787	0.821	0.943
Household income	I	18.45 (15.41, 22.18)	12.21 (9.80, 15.22)	40.65 (36.02, 45.86)	47.31 (39.04, 57.34)	3.78 (2.66, 5.36)	2.20 (1.79, 2.71)	0.60 (0.52, 0.70)	1.93 (1.72, 2.17)
	II	15.01 (12.55, 17.96)	8.94 (6.99, 11.43)	33.38 (30.18, 36.92)	35.54 (30.18, 41.86)	2.63 (2.00, 3.45)	2.28 (2.01, 2.60)	0.58 (0.50, 0.66)	1.60 (1.43, 1.80)
	III	15.91 (13.78, 18.38)	10.47 (8.90, 12.32)	31.56 (28.85, 34.52)	33.01 (25.51, 42.72)	2.56 (1.81, 3.64)	1.79 (1.45, 2.20)	0.53 (0.47, 0.61)	1.60 (1.43, 1.78)
	IV	14.43 (10.68, 19.48)	9.52 (6.79, 13.37)	30.12 (25.33, 35.82)	35.05 (26.96, 45.58)	2.65 (1.70, 4.11)	2.05 (1.46, 2.88)	0.55 (0.44, 0.68	1.50 (1.30, 1.72)
	Unknown	17.67 (12.90, 24.21)	11.33 (6.56, 19.57)	38.65 (24.82, 60.20)	38.16 (25.56, 56.97)	2.81 (1.40, 5.65)	2.06 (1.44, 2.94)	0.48 (0.34, 0.68)	1.59 (1.28, 1.97)
	p-value[b]	0.315	0.275	0.042[c]	0.233	0.355	0.095	0.625	0.041[c]
Caregiver education	< High school	17.78 (11.96, 26.43)	10.38 (6.95, 15.49)	29.88 (20.36, 43.84)	24.91 (12.53, 49.52)	1.70 (0.66, 4.38)	1.31 (1.16, 1.49)	0.31 (0.22, 0.44)	1.04 (0.82, 1.31)
	High school	17.20 (14.76, 20.04)	10.78 (9.00, 12.90)	38.66 (35.28, 42.36)	28.10 (32.39, 44.81)	3.45 (2.65, 4.50)	2.12 (1.78, 2.54)	0.59 (0.53, 0.65)	1.72 (1.58, 1.88)
	College +	15.31 (13.61, 17.23)	9.95 (8.47, 11.68)	32.08 (29.55, 34.81)	37.86 (32.99, 43.46)	2.64 (2.11, 3.29)	2.11 (1.88, 2.36)	0.56 (0.50, 0.62)	1.66 (1.54, 1.80)
	p-value[b]	0.442	0.587	0.005[c]	0.403	0.181	0.024[c]	0.025[c]	0.026[c]

All concentrations were corrected with urinary specific gravity.

BMI: body mass index; CI: confidence interval.

[a]Analyzed by analysis of variance test; [b]Analyzed by Kruskal-Wallis test; [c]$p < 0.05$.

III-3-3. Linear association between phthalates and age at menarche

We calculated regression estimate for shift in age at menarche for 1-unit increase in natural log-transformed concentration of urinary phthalate metabolites. However, for both crude and adjusted regression, no significant linear association with age at menarche was found for any of the metabolites (Table III-3).

Table III-3 Regression coefficients of age at menarche for a unit increase in ln-transformed concentration of urinary phthalate metabolites

Phthalates	Crude		Adjusted[a]	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value
MEHHP	0.054 (−0.130, 0.239)	0.562	0.067 (−0.120, 0.254)	0.482
MEOHP	0.037 (−0.107, 0.182)	0.611	0.048 (−0.098, 0.193)	0.519
MECPP	−0.116 (−0.392, 0.160)	0.407	−0.055 (−0.342, 0.232)	0.706
DEHP metabolites[b]	−0.043 (−0.307, 0.220)	0.746	0.006 (−0.265, 0.278)	0.965
MnBP	−0.092 (−0.255, 0.072)	0.270	−0.067 (−0.234, 0.100)	0.431
MBzP	−0.029 (−0.130, 0.071)	0.568	0.001 (−0.104, 0.105)	0.989
MCOP	−0.111 (−0.283, 0.062)	0.207	−0.101 (−0.277, 0.074)	0.237
MCNP	−0.156 (−0.384, 0.072)	0.179	−0.112 (−0.344, 0.120)	0.342
MCPP	−0.165 (−0.453, 0.123)	0.260	−0.081 (−0.380, 0.219)	0.596
Total phthalates[c]	−0.089 (−0.348, 0.170)	0.501	−0.029 (−0.287, 0.228)	0.822

Analyzed by multiple linear regression model.

Natural log-transformed, specific gravity corrected concentrations of urinary phthalate metabolites were used.

CI: confidence interval;

[a]Model was adjusted for household income, caregiver education, and body mass index group; [b]Molar sum of MEHHP, MEOHP, and MECPP; [c]Molar sum of MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP, MCNP, and MCPP

III-3-4. Association between phthalates and early menarche

With logistic regression analysis, we compared the odds of early menarche of groups with moderate and high phthalate metabolite concentration respectively, with that of group with low concentration. High MnBP group showed adjusted OR of 2.17 (95% CI: 1.07, 4.39) in model 1, which took into account household income and caregiver education. Similar association was observed after additionally adjusting for BMI group in model 2 with OR of 2.12 (95% CI: 1.04, 4.30). None of the other individual metabolites presented notable effect size. However, for total phthalates, group with the highest tertile in model 1 had adjusted OR of 2.11 (95% CI: 1.03, 4.30), and retained significant association after adjustment of BMI with OR of 2.10 (95% CI: 1.03, 4.31) (Table III-4).

As shown in Appendix Table 1, adjustment for the levels of urinary bisphenol A by tertile had little effect on risks for early menarche,.

Table III-4 Odds ratios for early menarche based on levels of urinary phthalate metabolites

Phthalates	Moderate (OR, 95% CI)			High (OR, 95% CI)		
	Unadjusted	Model 1[a]	Model 2[b]	Unadjusted	Model 1[a]	Model 2[b]
MEHHP	0.78 (0.40, 1.52)	0.74 (0.37, 1.47)	0.74 (0.37, 1.49)	1.04 (0.54, 2.00)	0.97 (0.50, 1.90)	0.95 (0.48, 1.86)
MEOHP	0.92 (0.47, 1.83)	0.90 (0.45, 1.81)	0.89 (0.44, 1.81)	1.45 (0.75, 2.81)	1.38 (0.71, 2.71)	1.40 (0.71, 2.75)
MECPP	1.48 (0.75, 2.89)	1.43 (0.71, 2.89)	1.46 (0.73, 2.94)	1.32 (0.67, 2.60)	1.17 (0.56, 2.41)	1.17 (0.58, 2.38)
DEHP metabolites[c]	1.32 (0.67, 2.60)	1.25 (0.62, 2.50)	1.27 (0.63, 2.57)	1.48 (0.75, 2.89)	1.35 (0.67, 2.71)	1.34 (0.67, 2.69)
MnBP	1.83 (0.91, 3.69)	1.90 (0.93, 3.91)	1.86 (0.90, 3.83)	2.27 (1.14, 4.54)	2.17 (1.07, 4.39)	2.12 (1.04, 4.30)
MBzP	1.49 (0.76, 2.94)	1.35 (0.67, 2.70)	1.30 (0.65, 2.63)	1.57 (0.80, 3.10)	1.43 (0.71, 2.86)	1.29 (0.63, 2.64)
MCOP	0.69 (0.36, 1.35)	0.72 (0.36, 1.44)	0.68 (0.33, 1.38)	0.83 (0.43, 1.60)	0.77 (0.38, 1.53)	0.74 (0.37, 1.49)
MCNP	1.04 (0.53, 2.03)	1.06 (0.52, 2.13)	1.03 (0.52, 2.07)	1.10 (0.57, 2.14)	1.05 (0.53, 2.11)	1.04 (0.52, 2.08)
MCPP	1.24 (0.63, 2.43)	1.15 (0.57, 2.29)	1.16 (0.58, 2.32)	1.31 (0.67, 2.58)	1.10 (0.54, 2.23)	1.10 (0.54, 2.23)
Total phthalates[d]	1.83 (0.91, 3.69)	1.77 (0.86, 3.62)	1.81 (0.88, 3.72)	2.27 (1.14, 4.54)	2.11 (1.03, 4.30)	2.10 (1.03, 4.31)

Analyzed by multiple logistic regression model.

Groups were determined by tertile of each metabolite (low, moderate, and high); group with the lowest tertile set as reference group.

OR: odds ratio; CI: confidence interval

[a]Model 1 was adjusted for household income, and caregiver education; [b]Model 2 was adjusted for body mass index group, in addition to the covariates adjusted in model 1; [c]Molar sum of MEHHP, MEOHP, and MECPP; [d]Molar sum of MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP, MCNP, and MCPP.

III-4. Discussion

We examined the association between urinary phthalate metabolites and age at menarche with data from KoNEHS 2015–2017. In linear regression analysis, no significant association with either advancing or delaying the onset of menarche was found for any of the metabolites. In logistic analysis, however, the highest concentration of MnBP was significantly associated with increased risk of early menarche, compared to the lowest concentration. The risk of early menarche was also significantly increased in high level of total phthalates. Neither BMI group nor levels of bisphenol A had notable alterations in the risks of early menarche through the adjustment. Our findings suggest that exposure to phthalates might have association with early menarche.

We found that 78 of 236 subjects (33.1%) went through early menarche, definition of which was "menarche occurring before the 6th grade." Early menarche was usually defined in previous literature by using age, as "menarche occurring before age of 12 years." A study that analyzed Korean National Health and Nutrition Examination Surveys, another nationally representative cross-sectional survey of Korea, reported the prevalence of early menarche in girls aged 10–18 years to be 21.4% in 2001 and 34.6% in 2011 (Lee et al., 2016). Another study from the United States that investigated data from National Health and Nutrition Examination Surveys 2003-2010 found early menarche in 27.8% of girls aged 12–19 years (McGuinn et al., 2015). Prevalence of early menarche in our study, even though the definition is minorly different, seems consistent with

previous reports.

In our study, only exposure to MnBP presented significant association with early menarche as an individual phthalate metabolite. Specifically, group with high MnBP level showed elevated risk of early menarche compared to low level group. In epidemiologic previous studies, MnBP exposure has presented only suggestive association with menarche and other pubertal indices (Kasper-Sonnenberg et al., 2017). In a study conducted in China, one-unit increase in natural log-transformed concentration of MnBP was related with 80% increase in the odds of menarche, but the result was not significant (Zhang et al., 2015). Another study reported that high pre-pubertal concentration of MnBP was related with both breast and pubic hair development, but not with menarche (Binder et al., 2018). *In vitro* studies using yeast cells suggested that DnBP has weak estrogenic activity (Harris et al., 1997; Zacharewski et al., 1998). Some animal studies using rats have reported that DnBP induced delaying of vaginal opening (Kay et al., 2013). However, another study that injected relatively lower dosage of DnBP, which was comparable dose to human environmental exposure, presented earlier vaginal opening in female rats (Hu et al., 2013). Comparing KoNEHS 2015-2017 and NHANES 2015-2016, Korean population has higher level of MnBP and thus greater exposure to DnBP than that of USA (Park et al., 2016). MnBP was also the type of phthalate metabolite with the highest concentration from our study sample. A recent study by Korean National Institute of Food and Drug Safety Evaluation quantitively analyzed DnBP exposure in Korea by modeling exposure scenarios through many sources like food, personal care products, indoor dust, and ambient air (KNIFDSE, 2020). The results suggested that majority of the exposure to DnBP in Korean

population derive from food and personal care products, and higher level of MnBP in Korea than in USA can be explained by different consumption of food and personal care products.

A research conducted in USA found no association between summed concentrations of 11 phthalate metabolites and age at menarche (Buttke et al., 2012). Similarly, no linear association was found from the results of our study. However, our data also suggest that group with the high total phthalates level showed significantly elevated OR of early menarche compared to low level group, and moderate concentration group, although not statistically significant, showed concordant effect size in dose-dependent trend. This indicates when compared to low concentration group, higher concentration of total phthalates might increase risks of early menarche. The mechanism that phthalates and other endocrine-disrupting chemicals intervene in the process of sexual maturation is complex. They can engage in any level of hypothalamic-pituitary-gonadal system by interacting with nuclear receptors and can interfere with multiple hormonal system (Meeker et al., 2009; Buck Louis et al., 2008). They are known to have anti-androgenic and estrogenic features, but the response is not linearly dose-dependent (Gore et al., 2015). In addition to disruption in sex and adrenal hormones, phthalates can also tamper with thyroid hormone system, insulin resistance, and adipose tissue generation, which in turn affect pubertal growth in adolescents (Benjamin et al., 2017; Combarnous et al., 2019; Roth et al., 2016). Animal studies using rodents have shown that exposure to relatively low dose of DEHP, which is comparable to environmental dose in humans, can accelerate sexual maturation and pubertal onset (Zarean et al., 2016; Ma et al., 2006). Although detailed effect of

each phthalate is not yet fully understood, our findings suggest that peripubertal exposure to certain phthalates might play a role in advancing menarche.

Since most study subjects had already reached menarche at the time of survey, urinary phthalate metabolites were measured after the onset of menarche. This temporal distance, along with short half-life of phthalates, complicates the representability for the exposure that occurred beforehand. There were three studies that investigated reliability of phthalate level in a single urine sample on average of long-term exposure, with period of one, three, and six months respectively (Peck et al., 2010; Hauser et al., 2004; Teitelbaum et al., 2008). They employed the same method where subjects were allocated into three groups by concentration tertiles (low, moderate, and high) of each sampling result. Then, average concentration of all samples in the follow-up period was compared between the groups. For each sampling, it was checked if there was monotonic increase of the average concentration of all samples from lower to higher groups, which would indicate its predictability for long-term exposure. Results from these studies presented consistent predictability for MnBP, MBzP, MEP, and MMP. DEHP metabolites did not demonstrate monotonic increase in the study with one-month follow-up, although the average level for the highest tertile group was greater than two times of that for the lowest tertile group in every sampling (Peck et al., 2010). These researches suggest that subjects with higher level of phthalates from a single sample tend to stay at higher level in the long-term as long as six months. While actual concentrations might have variability, levels from single urine samples have certain predictive value for long-term exposure of phthalates.

There are several limitations in our research. First, cross-sectional design of the study limits causal association of the results. Second, all questionnaires were conducted by parents, which makes the study susceptible to recall bias, although restriction of study subjects to middle school girls would make it less troubling for parents to remember the age at menarche. Third, co-exposure to other endocrine disrupting chemicals was not taken into consideration. Although many chemicals act together in disrupting sexual maturation, it is a challenging statistical task to analyze multiple exposure because of the issues with multicollinearity and synergistic and antagonistic effects of the chemicals. Lastly, as previously discussed, urinary metabolite concentrations might not represent exposure that occurred before the onset of menarche. Even though we confined our study population to middle school girls, temporal distance persists in hindering the inference of direct association. However, provided concentrations from single urine samples have predictive value for long-term exposure, our study can still propose possibility of association between higher level of phthalate exposure and early menarche.

III-5. Conclusion

Using data from KoNEHS 2015-2017, our study can generalize the results by adopting representative samples and directly measuring exposure metabolites. As a result, odds of early menarche were more than two times higher at higher concentrations than at lower concentrations in total phthalates and MnBP, one of the most commonly exposed phthalates in Korea. The increased risk did not change even after the effect of BMI and exposure to bisphenol A was accounted for. This implies that phthalate exposure in daily life is related to early menarche in adolescent women.

Chapter IV

Summary and conclusions

Phthalates are renowned for their endocrine-disrupting properties. Previous studies revealed that phthalates can influence thyroid hormone levels, lipogenesis, and male and female reproductive system. Although experimental and animal studies presented phthalates have impact on alteration in sexual maturation, human epidemiological studies presented varying results in accordance with types of phthalates involved and the time of exposure. Controversies developed over whether phthalates are associated with acceleration of menarche or delaying of menarche.

We first performed a systematic review for association between risk of menarche and exposure to phthalates during pregnancy and around puberty. MEP and metabolites of DEHP were the types of phthalates that showed association most frequently. The results from the studies were inconsistent, which may have been affected by period of exposure and different distribution of exposed phthalates between regions. We identified covariates related with phthalate exposure and menarche, which included age, race/ethnicity, maternal age at menarche, body fat status, poverty index, caregiver education, and co-exposure with bisphenol A.

We obtained data of middle school girls from KoNEHS 2015-2017, a nationwide survey of South Korea. We investigated association between phthalate exposure of adolescents and early onset of menarche. There were heterogeneous results, similar to previous studies. No linear association was notable between phthalate exposure and age at menarche. On the other hand, our results presented that high concentrations of MnBP and total phthalates might be associated with early menarche. This incongruity may derive from inherent complex mechanism of

phthalates in endocrine system, and also from cross-sectional design of our study. Further investigations with larger scale samples are needed, and longitudinal studies are also warranted to assess the effect of exposure to phthalates and other endocrine-disrupting chemicals in each critical point of development.

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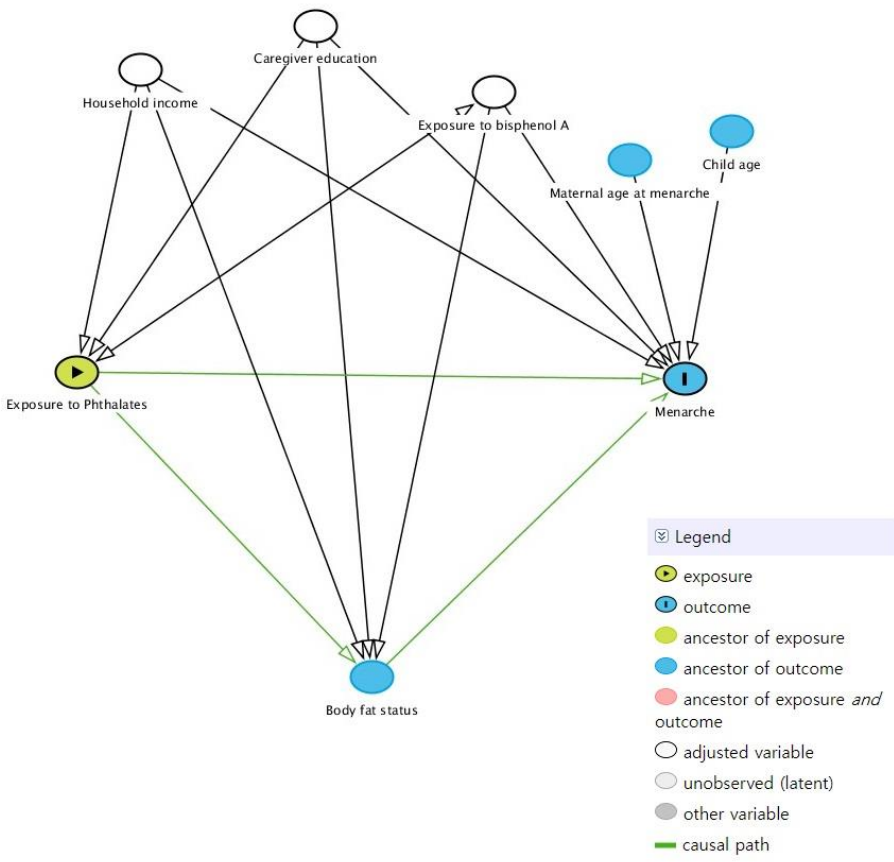
APPENDICES

Appendix Table 1 Odds ratios for early menarche based on levels of urinary phthalate metabolites, adjusting for levels of bisphenol A

Phthalates	Moderate (OR, 95% CI)		High (OR, 95% CI)	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
MEHHP	0.74 (0.37, 1.49)	0.72 (0.35, 1.46)	0.95 (0.48, 1.86)	0.92 (0.46, 1.83)
MEOHP	0.89 (0.44, 1.81)	0.89 (0.43, 1.81)	1.40 (0.71, 2.75)	1.39 (0.70, 2.75)
MECPP	1.46 (0.73, 2.94)	1.47 (0.73, 2.98)	1.17 (0.58, 2.38)	1.17 (0.56, 2.42)
DEHP metabolites[c]	1.27 (0.63, 2.57)	1.27 (0.62, 2.57)	1.34 (0.67, 2.69)	1.32 (0.65, 2.68)
MnBP	1.86 (0.90, 3.83)	1.86 (0.89, 3.87)	2.12 (1.04, 4.30)	2.12 (1.04, 4.35)
MBzP	1.30 (0.65, 2.63)	1.31 (0.64, 2.64)	1.29 (0.63, 2.64)	1.26 (0.60, 2.64)
MCOP	0.68 (0.33, 1.38)	0.67 (0.33, 1.37)	0.74 (0.37, 1.49)	0.73 (0.36, 1.48)
MCNP	1.03 (0.52, 2.07)	1.06 (0.52, 2.13)	1.04 (0.52, 2.08)	1.05 (0.53, 2.11)
MCPP	1.16 (0.58, 2.32)	1.16 (0.58, 2.35)	1.10 (0.54, 2.23)	1.10 (0.54, 2.24)
Total phthalates[d]	1.81 (0.88, 3.72)	1.81 (0.88, 3.75)	2.10 (1.03, 4.31)	2.11 (1.02, 4.38)

Analyzed by multiple logistic regression model.
Groups were determined by tertile of each metabolite (low, moderate, and high); group with the lowest tertile set as reference group.
OR: odds ratio; CI: confidence interval
[a]Model 1 was adjusted for household income, caregiver education, and body mass index group; [b]Model 2 was adjusted for levels of bisphenol A by tertile, in addition to the covariates adjusted in model 1; [c]Molar sum of MEHHP, MEOHP, and MECPP; [d]Molar sum of MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP, MCNP, and MCPP.



Appendix Figure 1. Directed acyclic graph (DAG) of covariates in association between exposure to phthalates and menarche

ABBREVIATIONS

BBzP	butyl-benzyl phthalate
BMI	body mass index
DEHP	di-(2-ethylhexy) phthalate
DEP	di-ethyl phthalate
DiBP	di-isobutyl phthalate
DiDP	di-isodecyl phthalate
DiNP	di-isononyl phthalate
DMP	di-methyl phthalate
DnBP	di-n-butyl phthalate
DOP	di-octyl phthalate
GM	geometric mean
IRB	Institutional Review Board
KoNEHS	Korean National Environmental Health Survey
LOD	limit of detection
MBzP	mono-benzyl phthalate
MCNP	mono-(carboxy-isononyl) phthalate
MCOP	mono-carboxyoctyl phthalate
MCPP	mono-(3-carboxypropyl) 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) phthalate
MEP	mono-ethyl phthalate
MiBP	mono-isobutyl phthalate
MMP	mono-methyl phthalate
MnBP	mono-n-butyl phthalate
OR	odds ratio
SG	specific gravity
PRISMA	Preferred Reporting Items for Systemic Reviews and Meta-Analysis

국문 초록

프탈레이트는 플라스틱 제품, 장난감, 식품 포장 용기 등 여러 용도에 사용되는 화학 물질로서, 일상 생활에서 꾸준한 환경적 노출이 일어난다. 프탈레이트는 시상하부-뇌하수체-생식선 축의 여러 호르몬계에 교란을 일으켜 사춘기 및 2차 성징 시기에 영향을 줄 수 있다. 한편 전세계적으로 초경 연령은 지속적으로 감소해왔으며, 한국에서도 같은 추세를 보인다. 여러 실험 연구에서 프탈레이트와 같은 내분비계 교란 물질이 사춘기 시기에 영향을 줄 수 있음이 밝혀졌지만, 사람 대상 역학 연구에서 프탈레이트 노출과 초경 연령 사이 관계는 일정하지 못한 결과를 보여주고 있다.

이에 제 2장에서는 체계적 문헌 고찰(systematic review)을 통해 산전 및 사춘기 무렵 프탈레이트 노출과 초경 연령 사이의 관련성을 알아보았다. PubMed, EMBASE, Web of Science 데이터베이스에서 프탈레이트와 초경에 대한 논문들을 검색하였고, 총 13개 연구가 문헌 고찰에 포함되었다. 가장 자주 관련성을 보였던 프탈레이트 대사산물은 MEP, 그리고 DEHP의 대사산물이었으나, 연구에 따라 초경 연령이 빨라지는 결과와 늦어지는 결과가 공존하고 있었다. 이는 연구 시기, 그리고 연구가 이루어진 국가들 사이에 프탈레이트 사용 및 노출의 분포가 달랐기 때문인 것으로 추정할 수 있다. 또한 프탈레이트와 초경 사이 관련성에

영향을 줄 수 있는 공변수들로는 연령, 인종, 어머니의 초경 연령, 체지방, 빈곤 또는 가계 수입, 보호자의 교육 수준이 조사되었다. 이들 중 인종, 가계 소득, 보호자 교육 수준, 비스페놀 A의 노출은 교란 변수로서 작용할 수 있는 반면 BMI와 같은 체지방 관련 변수들은 프탈레이트 노출에서 초경 사이 경로에서 부분적인 매개자로서 작용할 가능성이 있다.

제 3장에서는 제 3기 국민환경보건기초조사 (2015-2017) 자료를 이용하여 한국 중학생 여아에서 프탈레이트와 이른 초경 사이의 관계를 연구하였다. 우선 선형 회귀 분석을 통해 체내 프탈레이트 대사산물 농도와 초경 연령 사이 관계성이 있는지를 분석하였다. 또한 로지스틱 회귀 분석을 시행하여 프탈레이트의 삼분위 농도 수준에 따라 이른 초경의 비율에 차이가 있는지를 분석하였다. 선형 회귀 분석에서는 특별히 유의한 관련성이 나타난 대사산물은 없었다. 그러나 로지스틱 회귀 분석에서는 MnBP에서 가장 낮은 삼분위에 비해 가장 높은 삼분위에서, 가계 수입, 보호자 교육 수준 및 BMI를 보정하였을 때 이른 초경의 위험이 유의하게 증가하였다 (Adjusted OR: 2.12; 95% CI: 1.04, 4.30). 또한 프탈레이트 대사산물의 총합 (total phthalates)에서도 마찬가지로 가장 높은 삼분위에서 이른 초경의 위험이 증가함을 보였다 (Adjusted OR: 2.10; 95% CI: 1.03, 4.31). 본 연구의 결과는 한국에서 MnBP 및 전체 프탈레이트 수준이 이른 초경과 관계가 있을 수 있음을 제시하고 있다.

본 연구에서는 체계적 문헌 고찰을 통해 MEP, DEHP 대사산물 등에서 프탈레이트 노출과 초경 연령 사이 다양한 크기와 방향의 관련성이 있음을 확인할 수 있었다. 지역별 프탈레이트 노출의 분포와 함께 인종, 체지방, 가계 소득, 보호자 교육 수준, 비스페놀 A에 대한 노출과 같은 변수들이 초경과의 관련성에 영향을 줄 수 있었다. 또한 본 연구를 통해서 한국 청소년 여아에서 프탈레이트 노출과 이른 초경 연령 사이 관련성의 존재를 확인할 수 있었다.

주요어: 프탈레이트, 초경, 이른 초경, 사춘기, 이차 성징, 내분비계 교란 물질

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