Prenatal di(2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study

Atsuko Araki[1], Takahiko Mitsui[2,3], Houman Goudarzi[1,4], Tamie Nakajima[5,6], Chihiro Miyashita[1], Sachiko Itoh[1], Seiko Sasaki[7], Kazutoshi Cho[8], Kimihiko Moriya[9], Nobuo Shinohara[9], Katsuya Nonomura[9,10], and Reiko Kishi[1]

[1]Center for Environmental and Health Sciences, Hokkaido University, Kita 12, Nishi 7, Sapporo, Hokkaido, Japan

[2]Department of Urology, Hokkaido University Hospital, Kita 15, Nishi 7, Sapporo, Hokkaido, Japan

[3]Yamanashi University, 1110, Shimogato, Chuo, Yamanashi, Japan

[4]Division of Respiratory Medicine, Graduate School of Medicine, Hokkaido University, Kita 15, Nishi 7, Sapporo, Hokkaido, Japan

[5]Graduate School of Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi, Japan

[6]College of Life and Health Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi, Japan

[7]Department of Public Health, Graduate School of Medicine, Hokkaido University, Kita 15, Nishi 7, Sapporo, Hokkaido, Japan

[8]Department of Obstetrics and Gynecology, Hokkaido University Hospital, Kita 15, Nishi 7, Sapporo, Hokkaido, Japan

[9]Department of Renal and Genitourinary Surgery, Graduate School of Medicine, Hokkaido University,

Kita 15, Nishi 7, Sapporo, Hokkaido, Japan

[10]Kushiro Rosai Hospital, 13-23, Nakazono-cho, Kushiro, Hokkaido, Japan

Corresponding Author:

Reiko Kishi, MD, PhD, MPH

Center for Environmental and Health Sciences,

Hokkaido University,

Kita 12, Nishi 7, Kita-ku, Sapporo, Hokkaido 060-0812, Japan

Phone: +81-11-706-4746

Fax: +81-11-706-4725

E-mail: rkishi@med.hokudai.ac.jp

Disclosure Statement: The authors have nothing to disclose.

Abstract

Di(2-ethylhexyl) phthalate (DEHP) is known for its endocrine disrupting properties. We previously demonstrated that prenatal DEHP exposure is associated with decreased progesterone levels and testosterone/estradiol ratio in the cord blood. However, evidence of the effects of prenatal DEHP exposure on adrenal androgen and glucocorticoids in infants is scarce. Thus, the objectives of this study were to investigate the association between prenatal DEHP exposure and adrenal androgen and glucocorticoids, and to discuss its effects on steroid hormone profiles in infants. This is part of a birth cohort study: The Hokkaido Study on Environment and Children's Health, Sapporo Cohort. Among the 514 participants, 202 mother-infant pairs with available data on maternal mono(2-ethylhexyl) phthalate (MEHP), adrenal androgen (dehydroepiandrostenedione [DHEA] and androstenedione) and glucocorticoid (cortisol and cortisone) cord blood levels were included in this study. After adjusting for potential confounders, a linear regression analysis showed that maternal MEHP levels were associated with reduced cortisol and cortisone levels and glucocorticoid/adrenal androgen ratio, whereas increased DHEA levels and DHEA/androstenedione ratio. In a quartile model, when comparing the adjusted least square means in the 4^{th} quartile of MEHP with those in the 1^{st} quartile, cortisol and cortisone levels and glucocorticoid/adrenal androgen ratio decreased, whereas DHEA/androstenedione and cortisol/cortisone ratios increased. Significant p-value trends for cortisol and cortisone levels, cortisol/cortisone ratio, and glucocorticoid/adrenal androgen ratio were observed. In combination with the previous results of reduced progesterone levels and testosterone/estradiol

ratio, prenatal exposure to DEHP altered the steroid hormone profiles of infants. Further studies investigating the long-term effects of DEHP exposure on growth, neurodevelopment, and gonad and reproductive function are required.

Key Words: Di(2-ethylhexyl) phthalate (DEHP); mono(2-ethylhexyl) phthalate (MEHP); adrenal androgen; glucocorticoid; prenatal exposure; fetal blood

Abbreviations:

CI, confidence interval

CYP11A1, cytochrome P450 family 11 subfamily A member 1

CYP11B1, cytochrome P450 family 11 subfamily B member 1

CYP17A1, cytochrome P450 family 17 subfamily A member 1

CYP19A1, cytochrome P450 family 19 subfamily A member 1

CYP21A2, cytochrome P450 family 21 subfamily A member 2

CV, coefficient of variation

DBP, dibuthyl phthalate

DEHP, di(2-ethylhexyl) phthalate

DHEA, dehydroepiandrosterone

DHEA-S, DHEA sulfate

HSD11B2, hydroxysteroid 11-beta dehydrogenase 2

HSD17B1, hydroxysteroid 17-beta dehydrogenase 1

HSD3B1, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1

IQR, interquartile range

LSM, least square means

MEHP, mono(2-ethylhexyl) phthalate

PFOS, perfluorooctane sulfonate

MBP, Monobutyl phthalate

PPAR, peroxisome proliferator-activated receptor

SULT2A1, DHEA sulfotransferase family 2A member 1

T/E2, testosterone/estradiol

Introduction

Phthalate diesters (phthalates) have been used as plasticizers for various plastic products, including toys, food containers, furniture, personal care products, medical devices, and housing materials. According to a report from the Japan Plasticizer Industry Association and Ministry of Economy, Trade and Industry in 2012, di(2-ethylhexyl) phthalate (DEHP) constitutes >50% of the phthalates used in production in Japan. Phthalates are not chemically bonded to polyvinyl chloride in plastic products; thus, they can leach into the air, dust, foodstuffs, and other materials. Consequently, humans are constantly exposed to phthalates, and biomonitoring studies have demonstrated the widespread exposure of the general population to these chemicals (Ait Bamai et al., 2015; Fromme et al., 2007; Jensen et al., 2015; Koch et al., 2004; Wittassek et al., 2007).

DEHP is a potential endocrine-disrupting chemical, and multiple adverse effects on human health due to DEHP exposure in early life were found. Phthalate exposure has been reported to shorten the anogenital distance of infants (Bornehag et al., 2015; Swan et al., 2005; Swan et al., 2015). The associations between phthalates exposure and neurodevelopment and childhood obesity were also investigated (Ejaredar et al., 2015; Kim and Park, 2014). The underlying mechanisms of their effects were not clearly understood; however, disruption of steroidogenesis could be one of the contributing factors because steroid hormones play an important role in homeostasis. Sex steroid hormones including testosterone, progesterone, and estradiol, have effects predominantly in the gonads; and dehydroepiandrosterone (DHEA) and androstenedione, which are weak adrenal steroid precursors, are

activated to form androgens and estrogens that have important roles in sex differentiation and maturation (Labrie et al., 2001). Glucocorticoids, including cortisol, and cortisone are synthesized within the adrenal cortex, and are involved in a wide range of physiological processes. Glucocorticoids are essential for regulating and/or modulating homeostasis in metabolism, growth, neurodevelopment, and the immune system (Braun et al., 2013; Reynolds, 2010). As a whole, steroid hormones in early life play important roles in reproductive growth and neurodevelopment for later life (Hollier et al., 2014; Quinn et al., 2016).

In previous experimental studies in rat, Chinese rare minnow, and zebrafish, DEHP and/or its primary metabolite, mono (2-ethylhexyl) phthalate (MEHP), were reported to upregulate and/or downregulate several enzymes in the steroidogenesis pathway (Akingbemi et al., 2001, 2004; Lehmann et al., 2004; Sekaran and Jagadeesan, 2015; Thompson et al., 2005; Zhu et al., 2016a; Zhu et al., 2016b). Exposure to DEHP could modify steroidogenesis and disrupt both hypothalamic-pituitary-gonad and hypothalamic-pituitary-adrenal axes. One animal study demonstrate that dibythol phthalate initiated a rapid and dynamic change in gene expression unique to the fetal testis while in the adrenal was unaffected (Thompson et al., 2005). However, there are fundamental regulation differences of steroidogenesis in the fetal testis between rodent and human (Scott et al., 2009). In addition to species differences, exposure timing, duration, and dosage variations between studies are not relevant for the human exposure scenario. Thus, results from animal studies are limited in predicting the impact of phthalates exposure on adrenal steroid

production in human.

Despite the importance of DEHP properties on steroidogenesis, epidemiological studies on DEHP exposure, especially during early life, and its effects on adrenal androgen and glucocorticoid modulation are limited. In birth cohort studies in Mexico, the DHEA sulfate (DHEA-S) levels were increased and decreased following prenatal phthalate exposure in pubescent boys and girls, respectively (Ferguson et al., 2014; Watkins et al., 2014). In a Danish cohort, urinary phthalate metabolites in children were measured every 6 months from the baseline (aged 5.9 years) to pubertal age; girls with levels of monobutyl phthalate (MBP) and DEHP metabolites that were above the geometric group mean had lower levels of DHEA-S and androstenedione, whereas boys with higher MBP levels had lower DHEA-S levels (Mouritsen et al., 2013). Currently, only one study has investigated the effects of phthalate exposure on androstenedione and glucocorticoid levels in amniotic fluid, and the authors did not identify a significant association between phthalate and steroid hormone levels, including glucocorticoids (Jensen et al., 2015).

We recently demonstrated that prenatal DEHP exposure resulted in reduced progesterone levels and a reduced testosterone/estradiol (T/E2) ratio in cord blood (Araki et al., 2014). The aims of this study were to examine the effects of prenatal DEHP exposure on adrenal androgens (DHEA and androstenedione) and glucocorticoids (cortisol and cortisone), and to discuss its effects on the steroid hormone profile of infants, including adrenal androgens and glucocorticoids, and sex hormones (progesterone, testosterone, and estradiol) as reported by Araki et al. (2014).

Methods

Participants

This study was based on the Sapporo Cohort of the Hokkaido Study on Environment and Children's Health. Details of this study, regarding the population, data collection, sampling of the biological specimens, and contents of the questionnaire, have been previously described (Kishi et al., 2013; Kishi et al., 2011). Briefly, native Japanese women at an obstetrics and gynecology hospital in Sapporo (Hokkaido, Japan), who lived in Sapporo City or surrounding areas, were enrolled in the study at 23–35 weeks of gestation between July 2002 and October 2005. Among the 1796 pregnant women approached, 25% were excluded as they were enrolled in the Japanese Cord Blood Bank or planned to deliver the baby at another hospital. Eventually, 514 pregnant women (28.6%) were enrolled in this study. Selection of participants included in the analysis was described in our previous study (Araki et al., 2014). Briefly, of the 514 participants, 10 were excluded from the study due to miscarriage, stillbirth, relocation, or voluntary withdrawal prior to delivery. A total of 493 maternal blood samples were available for MEHP measurements. However, maternal blood samples collected during hospitalization after delivery were excluded from this analysis due to the relatively short biological half-life of DEHP. Steroid hormone measurements were available for 295 infant cord blood samples. Finally, 202 samples with available MEHP and steroid hormone levels were included in the statistical analysis.

MEHP measurement

Maternal blood samples were obtained at the time of their hospital examination following recruitment. If a blood sample could not be taken during pregnancy due to maternal anemia, a blood sample was collected during hospitalization, within a week after delivery. All samples were stored at −80 °C until analysis. MEHP, which is the primary metabolite of DEHP, was measured by gas chromatography-mass spectrometry at Nagoya University. The methods for the preparation of samples and standard solutions, and the instrumental analysis have been described previously (Araki et al., 2014; Jia et al., 2015). The detection limit was 0.278 ng/mL (1 pmol/mL). MEHP levels in a tube containing the same medium as the reaction vial were measured to determine background levels. To exclude the possibility of environmental contamination of DEHP, glassware used for MEHP measurements was heated at 200 °C for 2 h. A total of 493 maternal blood samples were analyzed for MEHP levels. The coefficient of variation (CV) of MEHP measurements taken within a single day was 2.0%–7.8% for 6 days, and the day-to-day CV for 6 days was 6.2%, at 5 pmol/mL of concentration, as described previously (Jia et al., 2015).

Measurement of steroid hormones

At the time of delivery, a blood sample was collected from the umbilical cord and stored at −80 °C

until analysis. Cord blood samples were available only from the children born vaginally. The concentrations of adrenal androgens and glucocorticoids were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS; Aska Pharma Medical Co., Ltd., Kanagawa, Japan). The methods for the preparation of samples, standard solutions, and instrumental analysis were described previously (Yamashita et al., 2007a; Yamashita et al., 2007b). The mean intra-assay CVs for steroid hormone measurements were as follows: DHEA, 2.1%–5.2%; androstenedione, 2.3%–6.8%; cortisol, 3.9%–10.9%; and cortisone, 1.3%–9.9%. The mean inter-assay CVs for steroid hormones were: DHEA, 3.3%–4.6%; androstenedione, 6.6%–7.1%; cortisol, 7.6%–11.3%; and cortisone: 7.8%–9.3% (Mitsui et al., 2016; Mitsui et al., 2015).

Questionnaire and medical record

The participants completed a self-administered questionnaire regarding information on maternal age, education level, household income, smoking and alcohol consumption during the first trimester, and medical history. Medical records were obtained at delivery for information regarding pre-pregnancy body mass index, pregnancy complications, parity, gestational age, infant sex, infant size, Apgar score, and congenital anomalies, including hypospadias and cryptorchidism, .

Statistical analyses

Distribution of adrenal androgen and glucocorticoid levels among boys and girls were

compared using the Mann-Whitney U test. Associations between maternal MEHP concentrations and infant adrenal androgen and glucocorticoid levels were initially calculated using the Spearman's rank correlation coefficient, followed by multivariate linear regression analysis. MEHP levels and adrenal androgen and glucocorticoid concentrations were converted to a log10 scale because they did not fall into a normal distribution. To evaluate whether the association between hormone and MEHP levels differed between sexes, a multivariate linear regression model for all study participants was first constructed with the interaction terms of hormone levels to sex and MEHP interaction. Since the MEHP and sex interaction was not significant for any of the hormones ($P_{interaction}>0.05$), a further analysis was performed, in which both sexes were included in the same model. The interquartile range (IQR) for MEHP concentrations and the least square means (LSM) and 95% confidence intervals (CIs) for hormone levels were calculated. To calculate a p-value for the trend, linear contrast coefficients of -3, -1, +1, and +3 were assigned to 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} quartiles, respectively (Goudarzi et al., 2016; Itoh et al., 2016). The 1^{st} quartile was compared to the 2^{nd}, 3^{rd}, and 4^{th} quartile MEHP, and the p-values were adjusted using the Bonferroni correction. When the levels were below the detection limits, half of their values were used for individual hormones. Inclusion of covariates was based on biological considerations, and included maternal age (continuous), maternal smoking during pregnancy (yes or no), maternal alcohol consumption during pregnancy (yes or no), gestational age (continuous), the week of gestation at which blood samples were taken (continuous), infant sex (boy or girl), and Apgar score (ordinal variable). A previous report from our cohort population

219 demonstrated that prenatal perfluorooctane sulfonate (PFOS) levels were associated with steroid
220 hormone levels (Goudarzi et al., 2016; Itoh et al., 2016). Therefore, PFOS was also included in the
221 adjusted model. All statistical analyses were performed using JMP Clinical 5.0 software (SAS
222 Institute Inc., NC, USA).
223
224 **Ethical approval**
225 This study was approved by the Institutional Ethical Review Board for Epidemiological Studies at
226 Hokkaido University Graduate School of Medicine, Hokkaido University Center for Environmental
227 and Health Sciences, and Nagoya University Graduate School of Medicine, in accordance with the
228 principles of the Declaration of Helsinki. All participants provided written informed consents.
229
230 **Results**
231 The characteristics of the participants included in this study are shown in Supplemental Table S1.
232 Briefly, mean ± standard deviation maternal age at delivery (years) was 29.8 ± 4.9, and 54.5% were
233 primiparous. For infants, the proportion of the boys was 46.0%, mean gestational age was 39.5 ± 1.0
234 weeks, and birth weight was 3138.6 ± 331.3 g. Apgar score at one minute after birth was nine for
235 82.7% children. Concentrations of MEHP in all samples were above the detection limit, and the
236 median (IQR) concentration was 10.4 ng/mL (5.88–15.3 ng/mL). The concentration of MEHP was not
237 significantly associated with any maternal or infant characteristics.

238 The adrenal androgen and glucocorticoid levels in infants are shown in Table 1. DHEA levels
239 were significantly higher in girls than in boys. Correlations between MEHP levels and adrenal
240 androgen and glucocorticoid concentrations are shown in Table 2, and the results of the linear
241 regression after adjusting for potential confounders are shown in Table 3. MEHP levels were inversely
242 associated with cortisol and cortisone levels and glucocorticoid/adrenal androgen ratio, but positively
243 associated with DHEA levels and DHEA/androstenedione ratios.
244 The associations between MEHP and adrenal androgen and cortisol levels were analyzed for
245 potential non-linear relationships. The LSM for each hormone in each MEHP quartile is shown in
246 Figure 1, with details of the data in Supplemental Table S2. The adjusted LSM adrenal androgen and
247 cortisol levels in relation to the MEHP quartile showed a significant p-value trend for cortisol and
248 cortisone levels, cortisol/cortisone ratio, and glucocorticoid/adrenal androgen ratio. When comparing
249 the LSM in the 4^{th} quartile of MEHP with those in the 1^{st} quartile, cortisol and cortisone
250 concentrations and glucocorticoid/adrenal androgen ratio were decreased, whereas
251 DHEA/androstenedione and cortisol/cortisone ratios were increased.
252 Since there were significant differences in DHEA levels in the cord blood with respect to sex,
253 the associations between MEHP levels and DHEA concentrations were examined separately in boys
254 and girls for both, linear and quartile models. However, there was no association found between
255 MEHP and DHEA levels in either boys or girls (data not shown).
256

257 **Discussion**

258 We have investigated the associations between maternal MEHP levels and cord blood adrenal
259 androgens (DHEA and androstenedione) and glucocorticoids (cortisol and cortisone) in mother-infant
260 pairs of a prospective birth cohort. Our results demonstrated a significant association between
261 increased maternal MEHP levels and decreased levels of cortisol and cortisone levels and
262 glucocorticoid/adrenal androgen ratio, and increased DHEA/androstenedione ratio in linear models. In
263 quartile models, p-values for trends were statistically significant for cortisol and cortisone levels, and
264 cortisol/cortisone and glucocorticoid/adrenal/androgen ratios. However, in the 4th quartile of MEHP,
265 cortisol and cortisone levels, glucocorticoid/adrenal androgen ratio were significantly lower, and
266 DHEA/androstenedione and cortisol/cortisone ratios were significantly higher compared with the 1st
267 quartile of MEHP. We have previously reported that increased maternal MEHP levels decreased
268 progesterone levels and testosterone/estradiol (T/E2) ratio in the cord blood (Araki et al., 2014). In
269 combination, the study provided novel evidence regarding the effects of prenatal DEHP exposure on
270 steroidogenesis disruption in infants, and a threshold for such effects was found.

271 In previous studies, we demonstrated that PFOS levels were inversely associated with
272 progesterone, T/E2 ratio, cortisol, and cortisone, and positively associated with DHEA (Goudarzi et
273 al., 2016; Itoh et al., 2016). Nuclear receptors, including peroxisome proliferator-activated receptor
274 (PPAR) alpha and gamma, are essential regulators of steroidogenesis, and both PFOS and DEHP are
275 known ligands of these receptors (Lovekamp-Swan et al., 2003; Vanden Heuvel et al., 2006). Thus,

276 the effects of PFOS and DEHP exposure on steroidogenesis could overlap. In addition, there was a
277 statistically significance correlation between PFOS and MEHP (Spearman's rho = 0.453, $P < 0.001$).
278 Therefore, we performed a mutual adjustment with PFOS so that the changes of steroid hormone
279 levels by MEHP in this study were independent of the effect of PFOS. For further assessment, as an
280 indicator of infant stress at birth, we have included the Apgar score (one minute after birth, ordinal
281 variable) into the adjusted model and the results remained consistent (Supplemental Table S3).
282 Therefore, stress of the birth process cannot be a confounder in our study.
283 Previous studies examining the effects of exposure to DEHP on adrenal androgen and
284 glucocorticoids in humans are limited. A Danish pregnancy-screening biobank study, which consisted
285 of cases of cryptorchidism and hypospadias and control boys, reported that increased levels of mono
286 (2-ethyl-5-carboxypentyl) phthalate, another metabolite of DEHP, was associated with higher levels of
287 androstenedione and cortisol in the second trimester amniotic fluid (Jensen et al., 2015). Further, a
288 statistically significant association between DEHP metabolites and hormones seemed more strongly in
289 cases than controls (Jensen et al., 2015). However, increased MEHP levels in the present study were
290 associated with reduced androstenedione and cortisol levels, although the p-values did not reach
291 statistically significant levels. Steroid hormone levels shift during the gestational period, such that the
292 inconsistent results could be due to the timing of the steroid measurements (Kuijper et al., 2013). In
293 addition, there were no cases of cryptorchidism or hypospadias in present study. Thus, difference of
294 the study participants could be another reason for the inconsistencies of the results.

This is the first study that examined MEHP exposure and DHEA levels at birth, and reported that MEHP levels were inversely associated with DHEA levels in a linear model. In birth cohort studies in Mexico, DHEA-S levels were increased and decreased in pubescent boys and girls, respectively, following prenatal phthalate exposure (Ferguson et al., 2014; Watkins et al., 2014). Mouristen et al.(2013) reported the results of a prospective cohort study in Denmark, in which phthalate levels in the urine of children were inversely associated with the DHEA-S levels in both sexes (Mouritsen et al., 2013). These studies in Mexico and Denmark measured hormone levels in children at puberty, whereas the present study measured hormones at birth. Another explanation for the inconsistencies between the results of this study and those of previous studies is that DHEA-S, instead of DHEA, was measured in the Mexico and Denmark studies. The levels of a DHEA sulfotransferase family 2A member 1 (SULT2A1), which converts DHEA to DHEA-S, were reported to be downregulated by phthalates in a previous study (Harris et al., 2007). Therefore, the effects of DEHP exposure on the levels of DHEA and DHEA-S could differ.

The findings of this study could be explained by previous experimental studies as shown in Figure 2. Decreased levels of progesterone following DEHP exposure likely resulted from the downregulation of hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (HSD3B1). Similarly, increases in the DHEA levels and DHEA/androstenedione ratio could also result from the downregulation of HSD3B1. Increased cortisol/cortisone ratios could be due to DEHP-mediated downregulation of hydroxysteroid 11-beta dehydrogenase 2 (HSD11B2). Finally,

decreased levels of cortisol and cortisone, together with a reduced glucocorticoid/adrenal androgen ratio, could be due to a shift of steroidogenesis from glucocorticoids to adrenal androgen by downregulation of HSD3B1 and hydroxysteroid 17-beta dehydrogenase 1 (HSD17B1). Additionally, cholesterol is a substrate of whole steroid hormones. Thus, changes in the cholesterol profile following exposure to DEHP may exist as demonstrated in a previous study of elderly patients in which reduced levels of cholesterol were detected following DEHP exposure (Olsén et al., 2012).

Previous animal and in vitro studies have provided evidence that phthalates, including DEHP and/or MEHP, modify the expression of genes encoding steroidogenesis enzymes. MEHP exposure decreased HSD11B2, cytochrome P450 family 11 subfamily A member 1 (CYP11A1), HSD3B1, and HSD17B1 (Akingbemi et al., 2001; Hong et al., 2009; Lehmann et al., 2004; Sekaran and Jagadeesan, 2015; Thompson et al., 2005; Zhao et al., 2010; Zhu et al., 2016a; Zhu et al., 2016b). Although one experimental study suggested upregulation of CYP17A1 (Wang et al., 2013), other studies found DEHP and/or MEHP exposure induced inhibition of cytochrome P450 family 17 subfamily A member 1 (CYP17A1) (Akingbemi et al., 2001; Chauvigne et al., 2011; Lehmann et al., 2004). Thus, increased levels of DHEA in this study may be better explained by inhibition of SULT2A1, HSD3B1, and HSD17B1 than activation of CYP17A1. As for cytochrome P450 family 19 subfamily A member 1 (CYP19A1), Wang et al. suggested that direction of up/downregulation differ between sex (Wang et al., 2013). Nevertheless, many of the in vitro and animal studies were conducted with concentrations and doses that are not relevant for the human exposure scenario. Moreover, the changes in gene

expression of steroidogenic enzymes may vary significantly depending on the experimental conditions, such as cell lines, species, doses, timing, and duration of exposure. (Akingbemin et al., 2001, 2004; Ge et al., 2007).

As we described in our previous study (Araki et al., 2014), the levels of MEHP in this cohort were slightly higher than those in American adults (NHANES 1999–2000), elderly Swedish subjects, and pregnant women in Australia (Hart et al., 2014; Lind et al., 2012; Silva et al., 2004). Slightly higher MEHP levels in this study can be considered acceptable as the levels of DEHP in house dust in Sapporo, Japan were higher compared with the studies from other countries, and DEHP intake in the Japanese population was higher than that of most other studies (Ait Bamai et al., 2014; Ait Bamai et al., 2015). On the other hand, as for the first limitation, it should be also noted that MEHP in blood was measured as urine samples were not available in this study. All samples were handled carefully to avoid *ex vivo* hydrolysis of DEHP; therefore, measurement errors due to contamination were minimal (Araki et al., 2014). Nevertheless, mono (2-ethyl-5-carboxypentyl) phthalate and mono (2-ethyl-5-hydroxyhexyl) phthalate have previously been detected in maternal serum (Hart et al., 2014); thus, these secondary metabolites should be measured in future studies.

Another limitation of this study was that the MEHP level was measured only once from second to third trimester. However, although there are contradicting discussions regarding this in the literature, several previous reports have found that single measurements can be useful (Hoppin et al., 2002; Townsend et al., 2013). In addition, the blood sampling week was adjusted to minimize the

effect of timing of exposure in this study. Although, we cannot rule out a possibility of the effect from acute exposure before delivery at the hospital, non-differential errors bias an effect towards the null. In this study, all participants who delivered vaginally with available cord blood samples were included in the analysis. Compared to the initial cohort population, the infants had an increased gestational age and heavier birth weight than those who were excluded from the study. As a result, healthier infants were included in the analysis; thus, the effects of MEHP may be underestimated in this study. In addition, the participation rate in our cohort study is rather low, which may limit the extrapolation of our results to the general population. Finally, the observation of only minimal reductions in the levels of cortisol in the linear model ($0.1 > P > 0.05$) may have been due to the limited sample size. Future studies with larger sample sizes are then required to confirm the results. A major strength of the present study is that this has a prospective birth cohort design, in which the effects of prenatal DEHP exposure on fetal adrenal androgen and glucocorticoids could be estimated. In addition, steroid hormones were measured by LC-MS/MS methods, which are considered more accurate than other methods, such as radioimmunoassay.

Conclusion

In summary, this study found significant associations between increased maternal MEHP levels and reduced cortisol and cortisone levels and glucocorticoid/adrenal androgen ratio and increased DHEA/androstenedione and cortisol/cortisone ratios. In combination with the previous results of

reduced progesterone levels (Araki et al., 2014), the present results suggested that prenatal DEHP exposure disrupted the steroid hormone profile of infants, despite the levels of DEHP in the general population being markedly lower than those in experimental studies. Disrupting the balance of steroid hormones may cause adverse effects on reproductive growth, development, and other health outcomes in later life. The clinical significance of these findings is unclear at present because it remains unknown whether these small hormonal alterations exert any effect on health.

Acknowledgements

We would like to thank the mothers and children that participated in the study and the staff at the Sapporo Toho Hospital. This study was supported in part by the Japan Ministry of Health, Labour and Welfare; the Environment Research and Technology Development Fund (5C-1252 and 5-2554); and Grants in Aid of Scientific Research from the Japan Society for the Promotion of Science, the Ministry of Education, Culture, Sports, Science and Technology (13307015, 16209022, 19209024, 22249021, 26740028, and 26670321).

References

Akingbemi, B.T.; Ge, R.; Klinefelter, G.R.; Zirkin, B.R.; Hardy, M.P. Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc Natl Acad Sci U S A 2004;101:775-780

Akingbemi, B.T.; Youker, R.T.; Sottas, C.M.; Ge, R.; Katz, E.; Klinefelter, G.R.; Zirkin, B.R.; Hardy, M.P. Modulation of Rat Leydig Cell Steroidogenic Function by Di(2-Ethylhexyl)Phthalate. Biol Reprod 2001;65:1252-1259

Ait Bamai, Y.; Araki, A.; Kawai, T.; Tsuboi, T.; Yoshioka, E.; Kanazawa, A.; Cong, S.; Kishi, R. Comparisons of urinary phthalate metabolites and daily phthalate intakes among Japanese families. Int J Hyg Environ Health 2015;218:461-470

Ait Bamai, Y.; Araki, A.; Kawai, T.; Tsuboi, T.; Saito, I.; Yoshioka, E.; Kanazawa, A.; Tajima, S.; Shi, C.; Tamakoshi, A.; Kishi, R. Associations of phthalate concentrations in floor dust and multi-surface dust with the interior materials in Japanese dwellings. Sci Total Environ 2014;468–469:147-157

Araki, A.; Mitsui, T.; Miyashita, C.; Nakajima, T.; Naito, H.; Ito, S.; Sasaki, S.; Cho, K.; Ikeno, T.; Nonomura, K.; Kishi, R. Association between maternal exposure to di(2-ethylhexyl) phthalate and reproductive hormone levels in fetal blood: the Hokkaido study on environment and children's health. PLoS One 2014;9:e109039

Bornehag, C.G.; Carlstedt, F.; Jonsson, B.A.; Lindh, C.H.; Jensen, T.K.; Bodin, A.; Jonsson, C.; Janson, S.; Swan, S.H. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ Health Perspect 2015;123:101-107

Braun, T.; Challis, J.R.; Newnham, J.P.; Sloboda, D.M. Early-life glucocorticoid exposure: the hypothalamic-pituitary-adrenal axis, placental function, and long-term disease risk. Endocr Rev 2013;34:885-916

Chauvigné, F.; Plummer, S.; Lesné, L.; Cravedi, J.-P.; Dejucq-Rainsford, N.; Fostier, A.; Jégou, B. Mono-(2-ethylhexyl) Phthalate Directly Alters the Expression of Leydig Cell Genes and CYP17 Lyase Activity in Cultured Rat Fetal Testis. PLoS One 2011;6:e27172

Ejaredar, M.; Nyanza, E.C.; Ten Eycke, K.; Dewey, D. Phthalate exposure and children's neurodevelopment: a systematic review. Environ Res 2015;142:51-60

Ferguson, K.K.; Peterson, K.E.; Lee, J.M.; Mercado-García, A.; Blank-Goldenberg, C.; Téllez-Rojo, M.M.; Meeker, J.D. Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. Reprod Toxicol 2014;47:70-76

Fromme, H.; Bolte, G.; Koch, H.M.; Angerer, J.; Boehmer, S.; Drexler, H.; Mayer, R.; Liebl, B. Occurrence and daily variation of phthalate metabolites in the urine of an adult population. Int J Hyg Environ Health 2007;210:21-33

Ge, R.-S.; Chen, G.-R.; Tanrikut, C.; Hardy, M.P. Phthalate ester toxicity in leydig cells: developmental timing and dosage considerations. Reprod Toxicol 2007;23:366-373

Goudarzi, H.; Araki, A.; Itoh, S.; Sasaki, S.; Miyashita, C.; Mitsui, T.; Nakazawa, H.; Nonomura, K.; Kishi, R. The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: the Hokkaido study. Environ Health Perspect 2016; DOI: 10.1289/EHP142

Harris, R.; Turan, N.; Kirk, C.; Ramsden, D.; Waring, R. Effects of endocrine disruptors on dehydroepiandrosterone sulfotransferase and enzymes involved in PAPS synthesis: genomic and nongenomic pathways. Environ Health Perspect 2007;115 Suppl 1:51-54

Hart, R.; Doherty, D.A.; Frederiksen, H.; Keelan, J.A.; Hickey, M.; Sloboda, D.; Pennell, C.E.; Newnham, J.P.; Skakkebaek, N.E.; Main, K.M. The influence of antenatal exposure to phthalates on subsequent female reproductive development in adolescence: a pilot study. Reproduction 2014;147:379-390

Hollier, L.P.; Keelan, J.A.; Hickey, M.; Maybery, M.T.; Whitehouse, A.J. Measurement of androgen and estrogen concentrations in cord blood: accuracy, biological interpretation, and applications to understanding human behavioral development. Front Endocrinol (Lausanne) 2014;5:64

Hong, D.; Li, X.-W.; Lian, Q.-Q.; Lamba, P.; Bernard, D.J.; Hardy, D.O.; Chen, H.-X.; Ge, R.S. Mono-(2-ethylhexyl) phthalate (MEHP) regulates glucocorticoid metabolism through 11ß-hydroxysteroid dehydrogenase 2 in murine gonadotrope cells. Biochem Biophys Res Commun 2009;389:305-309

Hoppin, J.A.; Brock, J.W.; Davis, B.J.; Baird, D.D. Reproducibility of urinary phthalate metabolites in first morning urine samples. Environ Health Perspect 2002;110:515-518

Itoh, S.; Araki, A.; Mitsui, T.; Miyashita, C.; Goudarzi, H.; Sasaki, S.; Cho, K.; Nakazawa, H.; Iwasaki, Y.; Shinohara, N.; Nonomura, K.; Kishi, R. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido study on environment and children's health. Environ Int 2016;94:51-59

Jensen, M.S.; Anand-Ivell, R.; Nørgaard-Pedersen, B.; Jönsson, B.A.G.; Bonde, J.P.; Hougaard, D.M.; Cohen, A.; Lindh, C.H.; Ivell, R.; Toft, G. Amniotic fluid phthalate levels and male fetal gonad function. Epidemiology 2015;26:91-99

Jia, X.; Harada, Y.; Tagawa, M.; Naito, H.; Hayashi, Y.; Yetti, H.; Kato, M.; Sasaki, S.; Araki, A.; Miyashita, C.; Ikeno, T.; Kishi, R.; Nakajima, T. Prenatal maternal blood triglyceride and fatty acid levels in relation to exposure to di(2-ethylhexyl) phthalate: a cross-sectional study. Environ Health Prev Med 2015;20:168-178

Kim, S.H.; Park, M.J. Phthalate exposure and childhood obesity. Ann Pediatr Endocrinol Metab 2014;19:69-75

Kishi, R.; Kobayashi, S.; Ikeno, T.; Araki, A.; Miyashita, C.; Itoh, S.; Sasaki, S.; Okada, E.; Kobayashi, S.; Kashino, I.; Itoh, K.; Nakajima, S. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile—updated 2013. Environ Health Prev Med 2013;18:429-450

460 Kishi, R.; Sasaki, S.; Yoshioka, E.; Yuasa, M.; Sata, F.; Saijo, Y.; Kurahashi, N.; Tamaki, J.; Endo,
461 T.; Sengoku, K.; Nonomura, K.; Minakami, H. Hokkaido Study Environm, C.s. Cohort
462 profile: the Hokkaido study on environment and children's health in Japan. Int J
463 Epidemiol 2011;40:611-618
464 Koch, H.M.; Drexler, H.; Angerer, J. Internal exposure of nursery-school children and their
465 parents and teachers to di(2-ethylhexyl)phthalate (DEHP). Int J Hyg Environ Health
466 2004;207:15-22
467 Kuijper, E.A.M.; Ket, J.C.F.; Caanen, M.R.; Lambalk, C.B. Reproductive hormone concentrations
468 in pregnancy and neonates: a systematic review. Reproductive BioMedicine Online
469 2013;27:33-63
470 Labrie, F.; Luu-The, V.; Labrie, C.; Simard, J. DHEA and its transformation into androgens and
471 estrogens in peripheral target tissues: intracrinology. Front Neuroendocrinol
472 2001;22:185-212
473 Lehmann, K.P.; Phillips, S.; Sar, M.; Foster, P.M.D.; Gaido, K.W. Dose-Dependent Alterations in
474 Gene Expression and Testosterone Synthesis in the Fetal Testes of Male Rats Exposed to
475 Di (n-butyl) phthalate. Toxicol Sci 2004;81:60-68
476 Lind, P.M.; Roos, V.; Ronn, M.; Johansson, L.; Ahlstrom, H.; Kullberg, J.; Lind, L. Serum
477 concentrations of phthalate metabolites are related to abdominal fat distribution two
478 years later in elderly women. Environ Health 2012;11:21
479 Lovekamp-Swan, T.; Jetten, A.M.; Davis, B.J. Dual activation of PPARα and PPARγ by
480 mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells. Mol Cell Endocrinol
481 2003;201:133-141
482 Mitsui, T.; Araki, A.; Goudarzi, H.; Miyashita, C.; Ito, S.; Sasaki, S.; Kitta, T.; Moriya, K.; Cho, K.;
483 Morioka, K.; Kishi, R.; Shinohara, N.; Takeda, M.; Nonomura, K. Effects of adrenal
484 androgens during the prenatal period on the second to fourth digit ratio in school-aged
485 children. Steroids 2016;113:46-51
486 Mitsui, T.; Araki, A.; Imai, A.; Sato, S.; Miyashita, C.; Ito, S.; Sasaki, S.; Kitta, T.; Moriya, K.; Cho,
487 K.; Morioka, K.; Kishi, R.; Nonomura, K. Effects of prenatal leydig cell function on the
488 ratio of the second to fourth digit lengths in school-aged children. PLoS One
489 2015;10:e0120636
490 Mouritsen, A.; Frederiksen, H.; Sørensen, K.; Aksglaede, L.; Hagen, C.; Skakkebaek, N.E.; Main,
491 K.M.; Andersson, A.M.; Juul, A. Urinary phthalates from 168 girls and boys measured
492 twice a year during a 5-year period: associations with adrenal androgen levels and puberty.
493 J Clin Endocrinol Metab 2013;98:3755-3764
494 Olsén, L.; Lind, L.; Lind, P.M. Associations between circulating levels of bisphenol A and
495 phthalate metabolites and coronary risk in the elderly. Ecotoxicol Environ Saf
496 2012;80:179-183
497 Quinn, T.A.; Ratnayake, U.; Dickinson, H.; Castillo-Melendez, M.; Walker, D.W. The feto-placental

498 unit, and potential roles of dehydroepiandrosterone (DHEA) in prenatal and postnatal
499 brain development: a re-examination using the spiny mouse. J Steroid Biochem Mol Biol
500 2016;160:204-213
501 Reynolds, R.M. Corticosteroid-mediated programming and the pathogenesis of obesity and
502 diabetes. J Steroid Biochem Mol Biol 2010;122:3-9
503 Scott, H.M.; Mason, J.I.; Sharpe, R.M. Steroidogenesis in the Fetal Testis and Its Susceptibility to
504 Disruption by Exogenous Compounds. Endocr Rev 2009;30:883-925
505 Sekaran, S.; Jagadeesan, A. In utero exposure to phthalate downregulates critical genes in leydig
506 cells of F1 male progeny. J Cell Biochem 2015;116:1466-1477
507 Silva, M.J.; Barr, D.B.; Reidy, J.A.; Malek, N.A.; Hodge, C.C.; Caudill, S.P.; Brock, J.W.; Needham,
508 L.L.; Calafat, A.M. Urinary levels of seven phthalate metabolites in the U.S. population
509 from the National Health and Nutrition Examination Survey (NHANES) 1999-2000.
510 Environ Health Perspect 2004;112:331-338
511 Swan, S.H.; Main, K.M.; Liu, F.; Stewart, S.L.; Kruse, R.L.; Calafat, A.M.; Mao, C.S.; Redmon,
512 J.B.; Ternand, C.L.; Sullivan, S.; Teague, J.L. Decrease in anogenital distance among male
513 infants with prenatal phthalate exposure. Environ Health Perspect 2005;113:1056-1061
514 Swan, S.H.; Sathyanarayana, S.; Barrett, E.S.; Janssen, S.; Liu, F.; Nguyen, R.H.N.; Redmon, J.B.
515 First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod
516 2015;
517 Thompson, C.J.; Ross, S.M.; Hensley, J.; Liu, K.; Heinze, S.C.; Young, S.S.; Gaido, K.W.
518 Differential Steroidogenic Gene Expression in the Fetal Adrenal Gland Versus the Testis
519 and Rapid and Dynamic Response of the Fetal Testis to Di(n-butyl) Phthalate. Biol Reprod
520 2005;73:908-917
521 Townsend, M.K.; Franke, A.A.; Li, X.N.; Hu, F.B.; Eliassen, A.H. Within-person reproducibility of
522 urinary bisphenol A and phthalate metabolites over a 1 to 3 year period among women in
523 the Nurses' Health Studies: a prospective cohort study. Environ Health 2013;12
524 Vanden Heuvel, J.P.; Thompson, J.T.; Frame, S.R.; Gillies, P.J. Differential activation of nuclear
525 receptors by perfluorinated fatty acid analogs and natural atty Aacids: a comparison of
526 human, mouse, and rat peroxisome roliferator-Acativated receptor-α, -ß, and -γ, liver X
527 receptor-ß, and retinoid X receptor-α. Toxicol Sci 2006;92:476-489
528 Wang, X.; Yang, Y.; Zhang, L.; Ma, Y.; Han, J.; Yang, L.; Zhou, B. Endocrine disruption by
529 di-(2-ethylhexyl)-phthalate in Chinese rare minnow (Gobiocypris rarus). Environ Toxicol
530 Chem 2013;32:1846-1854
531 Watkins, D.J.; Téllez-Rojo, M.M.; Ferguson, K.K.; Lee, J.M.; Solano-Gonzalez, M.;
532 Blank-Goldenberg, C.; Peterson, K.E.; Meeker, J.D. In utero and peripubertal exposure to
533 phthalates and BPA in relation to female sexual maturation. Environ Res
534 2014;134:233-241
535 Wittassek, M.; Heger, W.; Koch, H.M.; Becker, K.; Angerer, J.; Kolossa-Gehring, M. Daily intake of

536 di(2-ethylhexyl)phthalate (DEHP) by German children - a comparison of two estimation
537 models based on urinary DEHP metabolite levels. Int J Hyg Environ Health
538 2007;210:35-42
539 Yamashita, K.; Okuyama, M.; Watanabe, Y.; Honma, S.; Kobayashi, S.; Numazawa, M. Highly
540 sensitive determination of estrone and estradiol in human serum by liquid
541 chromatography–electrospray ionization tandem mass spectrometry. Steroids
542 2007a;72:819-827
543 Yamashita, K.; Takahashi, M.; Tsukamoto, S.; Numazawa, M.; Okuyama, M.; Honma, S. Use of
544 novel picolinoyl derivatization for simultaneous quantification of six corticosteroids by
545 liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr
546 A 2007b;1173:120-128
547 Zhao, B.; Chu, Y.; Huang, Y.; Hardy, D.O.; Lin, S.; Ge, R.S. Structure-dependent inhibition of
548 human and rat 11β-hydroxysteroid dehydrogenase 2 activities by phthalates. Chem-Biol
549 Interact 2010;183:79-84
550 Zhu, Y.P.; Li, E.H.; Sun, W.L.; Xu, D.L.; Liu, Z.H.; Zhao, W.; Wood, K.; Xia, S.J.; Jiang, J.T.
551 Maternal exposure to di-n-butyl phthalate (DBP) induces combined anorectal and
552 urogenital malformations in male rat offspring. Reprod Toxicol 2016a;61:169-176
553 Zhu, Y.; Hua, R.; Zhou, Y.; Li, H.; Quan, S.; Yu, Y. Chronic exposure to
554 mono-(2-ethylhexyl)-phthalate causes endocrine disruption and reproductive dysfunction
555 in zebrafish. Environ Toxicol Chem 2016b: 35:2117-2124

Table 1. Distribution of adrenal androgen and glucocorticoid concentrations

	Detection limit	>Detection limit (%)	Total (n = 202) Med (IQR)	Boys (n = 93) Med (IQR)	Girls (n = 109) Med (IQR)	[a]P-value
Adrenal androgen						
DHEA (ng/mL)	0.010	100	2.31 (1.79, 3.07)	2.08 (1.59, 2.71)	2.62 (1.99, 3.42)	<0.001
Androstenedione (ng/mL)	0.010	100	0.46 (0.36, 0.58)	0.47 (0.38, 0.59)	0.45 (0.35, 0.58)	0.572
Glucocorticoid						
Cortisol (ng/mL)	0.250	96.5	40.5 (22.7, 66.7)	41.1 (22.3, 66.8)	39.3 (24.1, 66.8)	0.902
Cortisone (ng/mL)	0.100	93.6	96.9 (70.3, 124)	97.2 (72.5, 126)	96.0 (69.2, 125)	0.734

DHEA, dehydroepiandrosterone; IQR, the interquartile range

[a]p-values were calculated by Mann-Whitney U test comparing boys and girls

Table 2. Associations between MEHP and hormone levels

	[a]ρ	p-value
DHEA	0.136	0.054
Androstenedione	-0.190	0.007
DHEA/androstenedione	0.178	0.011
Androstenedione/testosterone	0.027	0.699
Cortisol	-0.273	<0.0001
Cortisone	-0.367	<0.0001
Cortisol/cortisone	0.038	0.592
Glucocorticoid/adrenal androgen	-0.297	<0.0001

[a]Spearman's ρ

MEHP, mono(2-ethylhexyl)phthalate; DHEA, dehydroepiandrosterone

Table 3. Adjusted linear regression coefficients of adrenal androgen and glucocorticoid in the cord blood in relation to MEHP

	Crude model				Adjusted model			
	β	(95% CI)		p-value	β	(95% CI)		p-value
DHEA	0.246	0.095	0.397	0.002	0.205	0.029	0.381	0.023
Androstenedione	-0.079	-0.199	0.042	0.198	-0.082	-0.224	0.059	0.252
DHEA/androstenedione	0.325	0.147	0.503	<0.001	0.287	0.073	0.501	0.009
Androstenedione/testosterone	0.002	0.000	0.003	0.082	0.001	-0.001	0.004	0.163
Cortisol	-0.527	-0.809	-0.245	<0.001	-0.412	-0.751	-0.072	0.018
Cortisone	-0.734	-1.111	-0.358	<0.001	-0.570	-1.026	-0.114	0.015
Cortisol/cortisone	0.207	0.040	0.375	0.015	0.158	-0.041	0.358	0.119
Glucocorticoid/adrenal	-0.828	-1.258	-0.398	<0.001	-0.649	-1.167	-0.132	0.014

Steroid hormone levels and MEHP concentrations were log10-transformed and included in the model separately.

β for linear regression coefficients

Adjusted for maternal age, smoking and alcohol consumption during pregnancy, gestational age, blood sampling week, infant sex, and PFOS

95%CI, 95% confidence interval; DHEA, dehydroepiandrosterone; MEHP, mono(2-ethylhexyl)phthalate; PFOS, perfluorooctane sulfonate levels,

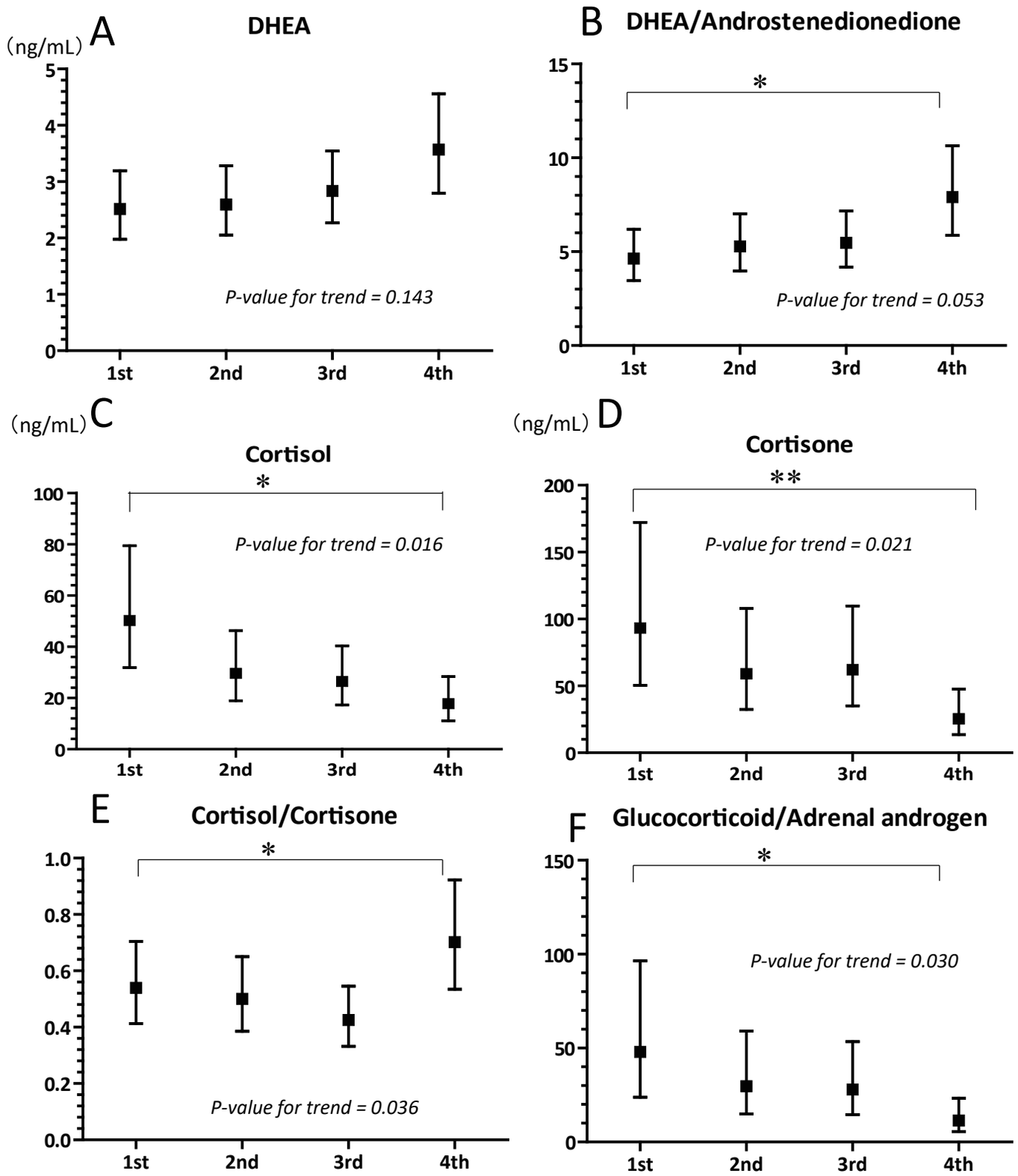
A
DHEA
(ng/mL)
P-value for trend = 0.143
1st
2nd
3rd
4th
B
DHEA/Androstenedionedione
*
P-value for trend = 0.053
C
Cortisol
(ng/mL)
*
P-value for trend = 0.016
D
Cortisone
(ng/mL)
**
P-value for trend = 0.021
E
Cortisol/Cortisone
*
P-value for trend = 0.036
F
Glucocorticoid/Adrenal androgen
*
P-value for trend = 0.030

Figure 1

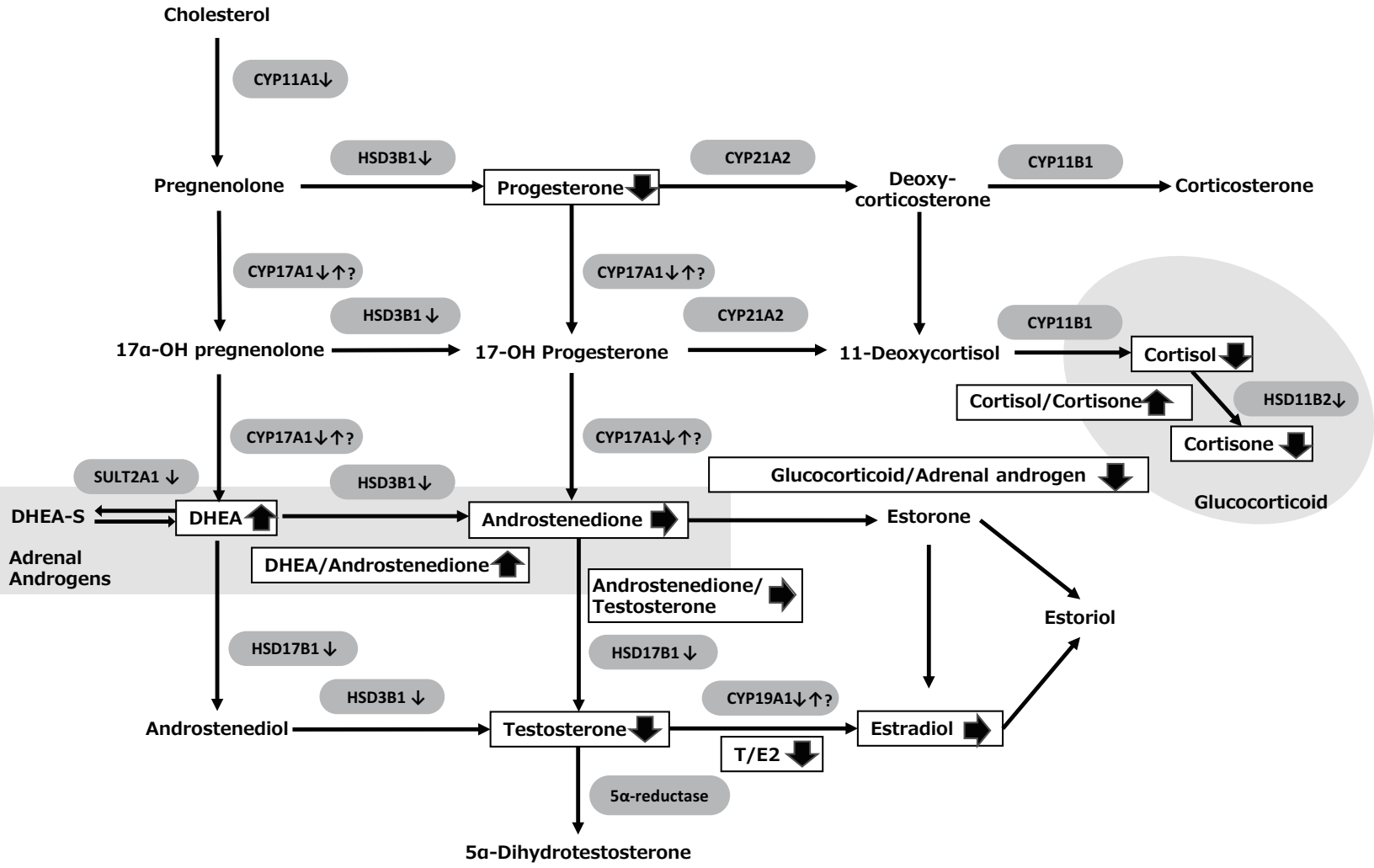
Cholesterol
CYP11A1↓
Pregnenolone
HSD3B1↓
Progesterone
CYP21A2
Deoxy-corticosterone
CYP11B1
Corticosterone
CYP17A1↓↑?
17α-OH pregnenolone
HSD3B1↓
17-OH Progesterone
CYP21A2
11-Deoxycortisol
CYP11B1
Cortisol
Cortisol/Cortisone
HSD11B2↓
Cortisone
SULT2A1 ↓
DHEA-S
DHEA
HSD3B1↓
Androstenedione
Glucocorticoid/Adrenal androgen
Glucocorticoid
Estorone
Adrenal Androgens
DHEA/Androstenedione
Androstenedione/ Testosterone
Estoriol
HSD17B1↓
HSD3B1↓
Androstenediol
Testosterone
CYP19A1↓↑?
Estradiol
T/E2
5α-reductase
5α-Dihydrotestosterone

Figure 2

Figure legend

Figure 1

X-axis shows the mono(2-ethylhexyl) phthalate (MEHP) quartiles, and Y-axis shows each hormone level. The adjusted least square means (LSMs) with 95% confidence intervals for each hormone in the cord blood in relation to the MEHP concentration quartile fit to all study participants are shown with a *p*-value trend for: (A**)** DHEA, (B) DHEA/androstenedione ratio, (C**)** cortisol, (D**)** cortisone, (E**)** cortisol/cortisone ratio, and (F**)** glucocorticoid (cortisol and cortisone)/adrenal androgen (DHEA and androstenedione) ratio. There was a significant and inverse association between MEHP levels and cortisol and cortisone levels, glucocorticoid/adrenal androgen ratio, but a positive association between MEHP levels and the cortisol/cortisone ratio. The 1st quartile (≤5.88 ng/mL) was compared with the 2nd (5.88–10.4 ng/mL), 3rd (10.4–15.3 ng/mL), and 4th (>15.3 ng/mL) quartile MEHP levels. Statistical significance of the P values were *p < 0.017 and **P < 0.0033 based on Bonferroni correction. When compared with the LSM of the 1st MEHP quartile, the 4th MEHP quartile of cortisol, cortisone, and glucocorticoid/adrenal androgen ratios were significantly decreased, whereas the DHEA/androstenedione ratio and cortisol/cortisone ratio were significantly increased. LSMs were adjusted for maternal age, smoking and alcohol consumption during pregnancy, gestational age, blood sampling week, infant sex, and perfluorooctane sulfonate level

Abbreviations: DHEA, dehydroepiandrosterone; LSM, least square means, MEHP, mono(2-ethylhexyl) phthalate

Figure 2

Steroid metabolic pathways and their disruption following mono(2-ethylhexyl) phthalate (MEHP) exposure. Squares indicate the hormones measured in this study and our previous report. Black bold arrows indicate the direction of shift following MEHP exposure. Black characters and arrows in gray background and rounded rectangles indicate enzymes reported as upregulated or downregulated by di (2-ethylhexyl) phthalate (DEHP) and/or MEHP in experimental studies.

Abbreviations: CYP11A1, cytochrome P450 family 11 subfamily A member 1; CYP11B1, cytochrome P450 family 11 subfamily B member 1; CYP17A1, cytochrome P450 family 17 subfamily A member 1; CYP19A1, cytochrome P450 family 19 subfamily A member 1; CYP21A2, cytochrome P450 family 21 subfamily A member 2; HSD11B2, hydroxysteroid 11-beta dehydrogenase 2; HSD17B1, hydroxysteroid 17-beta dehydrogenase 1; HSD3B1, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1; SULT2A1, DHEA sulfotransferase family 2A member 1