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***In utero* exposure to di(2-ethylhexyl)phthalate suppresses blood glucose and leptin levels in the offspring of wild-type mice**

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Abstract

Exposure of pregnant mice to di(2-ethylhexyl)phthalate (DEHP) induces maternal lipid malnutrition and decreases the number of live fetuses/pups. In this study, we aimed to clarify the relationship between maternal lipid malnutrition and the nutritional status of the neonatal, lactational, and adult offspring, as well as the role of peroxisome proliferator-activated receptor α (PPAR α) in these relationships. Sv/129 wild-type (mPPARA), Ppara-null, and PPAR α -humanized (hPPARA) mice were fed diets containing 0, 0.01, 0.05, or 0.1% DEHP *in utero* and/or during the lactational stage. The male offspring were killed on postnatal day 2 or 21, or after 11 weeks. Exposure to either 0.05% or 0.1% DEHP during both the *in utero* and lactational periods decreased serum glucose concentrations in 2-day-old mPPARA offspring. These dosages also decreased both serum and plasma leptin levels in both 2- and 21-day-old mPPARA offspring. In contrast, exposure to DEHP only during the lactational period did not decrease leptin levels,

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Conflict of interest

The author reports no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.tox.2019.01.008>.

suggesting the importance of *in utero* exposure to DEHP. Exposure to 0.05% DEHP during the *in utero* and lactational periods also increased food consumption after weaning in both *mPPARA* and *hPPARA* mice; this was not observed in *Ppara*-null offspring. In conclusion, *in utero* exposure to DEHP induces neonatal serum glucose malnutrition via $PPAR\alpha$. DEHP also decreases serum and plasma leptin concentrations in offspring during the neonatal and weaning periods, in association with $PPAR\alpha$, which presumably results in increased food consumption after weaning.

Keywords

DEHP; *In utero* exposure; Malnutrition; Feeding behavior; Glucose; Leptin

1. Introduction

Di(2-ethylhexyl)phthalate (DEHP) is a plasticizer used worldwide for the production of packaging, wallpaper, and agricultural vinyl film. While DEHP constituted roughly 10% of all plasticizers used in the United States in 2005, the percentage of DEHP used in other major world regions was even higher (18% in Western Europe, 52% in Japan, and 62.4% in other Asian regions) (National Toxicology Program, 2016). The toxicity of DEHP has been well studied, with hepatic carcinogenicity and adverse effects on the endocrine and reproductive systems as the major toxicities identified (Nakajima et al., 2015). Of these toxicities, recent interest in DEHP has been directed to its effects on reproduction and development, because infants are thought to be most sensitive population exposed to this chemical.

Exposure to DEHP during gestation is known to decrease the number of fetuses/pups and increase the percentage of their deformation and resorption (Lamb et al., 1987; Tyl et al., 1988). To determine the mechanism by which *in utero* DEHP exposure decreases the number of live pups, we previously investigated the role of peroxisome proliferator-activated receptor α ($PPAR\alpha$) in this process (Hayashi et al., 2011), as DEHP metabolites are ligands of $PPAR\alpha$, which regulates lipid metabolism (Grygiel-Górniak, 2014). We performed exposure experiments similar to those of Lamb et al. (1987), using wild-type (mouse $PPAR\alpha$, *mPPARA*), *Ppara*-null, and *PPAR* α -humanized (*hPPARA*) mice. *In utero* exposure to DEHP decreased the number of live fetuses and/or pups, and increased resorption of *mPPARA* and *hPPARA* offspring, but not *Ppara*-null offspring, suggesting that $PPAR\alpha$ is involved in the adverse effects of DEHP. In general, levels of plasma triglycerides (TGs) and essential fatty acids increase during pregnancy but decrease after childbirth in both dams and women (Al et al., 1995; Montes et al., 1984; Sweeney et al., 2006), suggesting that increases in the levels of these lipids may be common physiological changes during gestation. However, maternal DEHP exposure suppresses the increased levels of these lipids in only pregnant wild-type mice (Hayashi et al., 2011; Nakashima et al., 2013), suggesting that DEHP-induced lipid malnutrition in the dams is dependent on $PPAR\alpha$. In contrast, DEHP exposure influenced neither TG nor fatty acid levels in the livers of the offspring on either gestational day 18 or neonatal day 2.

In an epidemiological study, exposure to DEHP during gestation was also shown to influence maternal lipid levels. Jia et al. (2015a) measured maternal blood levels of mono(2-ethylhexyl)phthalate (MEHP, a DEHP metabolite), TGs, and fatty acids in 318 mother/newborn pairs residing in Sapporo, Japan. These authors analyzed the association of MEHP levels with maternal blood lipid levels and infant measurements at birth. DEHP exposure during pregnancy was inversely correlated with the maternal blood levels of TGs and palmitic, oleic, linoleic, and α -linolenic acids, similar to findings reported in wild-type mice (Hayashi et al., 2011; Nakashima et al., 2013), although MEHP levels did not affect birth outcomes. Interestingly, maternal eicosapentaenoic acid (EPA) levels were positively associated with infant chest circumference (Jia et al., 2015b). Since α -linolenic acid is an EPA precursor, the decrease in α -linolenic acid caused by DEHP during pregnancy may induce lipid malnutrition in the mother, which influences infant measurements at birth. These findings suggest that exposure to DEHP induces lipid malnutrition in both rodents and humans.

Recently, the developmental origins of health and disease concept have attracted attention because associations between fetal nutritional status and adult diseases have been observed in some epidemiological studies. Roseboom et al. (2006) investigated 2414 subjects born around the time of the Dutch famine. In this study, famine experienced *in utero* was found to be associated with glucose intolerance and coronary heart disease during adulthood. Infants exposed to famine in China also showed an increased risk of hyperglycemia during adulthood (Li et al., 2010). In an animal study, a 50% food restriction during gestational days 12.5–18.5 induced glucose intolerance in the offspring later in life (Jimenez-Chillaron et al., 2006). These findings suggest that malnutrition experienced *in utero* correlates with a variety of lifestyle diseases. Therefore, maternal lipid malnutrition caused by exposure to DEHP during pregnancy may lead to health effects in the offspring during adulthood.

In this study, we evaluated the relationship between maternal lipid malnutrition caused by exposure to DEHP during pregnancy and the nutritional status of the offspring during the neonatal, weaning, and adult stages. In this study, we used three genetically distinct mice—mPPARA, Ppara-null, and hPPARA—because PPAR α is involved in DEHP-induced developmental toxicity and maternal lipid malnutrition (Hayashi et al., 2011; Nakashima et al., 2013).

2. Materials and methods

2.1. Experimental animals

This study was conducted according to the guidelines for animal experiments provided by the Nagoya University Animal Center. Three genotyped male and female mice, mPPARA, Ppara-null (Lee et al., 1995), and hPPARA^{Tet-Off} (Cheung et al., 2004), with a Sv/129 genetic background, were obtained from the Laboratory of Metabolism, National Cancer Institute. The colonies were then expanded and maintained at Nagoya University. Male and female mice at 12 weeks of age were fed a normal diets (CE-2; Clea Japan, Tokyo, Japan) containing 0, 0.01, 0.05, or 0.1% DEHP, according to the protocol described by Hayashi et al. (2011). Four weeks after the initiation of DEHP exposure, mice that had been administered the same DEHP dose were mated. DEHP exposure was then continued until

weaning. Litter size ($n = 2$ to 8) were not standardized. The day of delivery was designated postnatal day (PND) 0. Some of the male offspring were killed on PNDs 2 and 21, and their blood and livers were collected ($n = 6$ in each group). The remaining male offspring were used in a subsequent study.

Because DEHP decreases the number of live offspring, i.e., the number of live pups per litter at PND 2 was 4.0 ± 2.7 , 3.1 ± 2.0 , 1.6 ± 1.9 and 1.4 ± 2.4 in control, 0.01%, 0.05% and 0.1% DEHP *mPPARA* mice, respectively (Hayashi et al., 2011). Living *mPPARA* offspring to PND2, all survived up to PND21 at 0%, 0.01% and 0.05% dose groups, however, no offspring in *mPPARA* exposed to 0.1% DEHP were survived until weaning. Therefore, we could not obtain *mPPARA* offspring exposed to 0.1% DEHP at PND 21. Therefore, we used off-spring exposed to 0% and 0.05% DEHP *in utero* and during lactation for further experiments in the postweaning stage ($n = 6$ in each group). Male offspring were weaned at PND 21 and fed a normal diet (CE-2; Clea Japan, Tokyo, Japan) for 8 weeks. Food consumption and body weights were monitored from the age of 4 weeks until dissection at 11 weeks of age. These offspring were killed after anesthesia using pentobarbital, and their blood, livers, and hypothalami were obtained. All samples were quickly stored at -80°C until use.

To investigate the effects of DEHP exposure during lactation alone (PNDs 0–21), dams fed a 0% DEHP diet during the gestational period were switched to a diet containing 0.05% DEHP postdelivery and were continually fed 0.05% DEHP until weaning (0.05% DEHP-LP). Male *mPPARA* offspring on PND 21 were then dissected, and their blood and livers collected and stored as described above. In addition, plasma samples were collected from postpartum dams on PND 2 and from adult male mice exposed to DEHP from the ages of 12 to 20 weeks to measure leptin levels.

2.2. Glucose concentrations

Glucose concentrations in the serum of offspring on PND 2 and in the plasma of offspring on PND 21 and at 11 weeks were spectrophotometrically measured using a LabAssay™ Glucose kit (Wako, Osaka, Japan).

2.3. Leptin concentrations

Leptin concentrations in the serum of offspring on PND 2 and in the plasma of offspring on PND 21 and at 11 weeks, as well as in post-partum dams and adult male mice, were spectrophotometrically measured using Mouse Leptin ELISA kits (Morinaga Institute of Biological Science, Inc. Kanagawa, Japan).

2.4. Real-time quantitative polymerase chain reaction (RT-qPCR)

mRNA levels were measured by ABI PRISM 7000 Sequence Detection system (Applied Biosystems, Foster City, CA, USA). Total RNA was isolated from the livers and hypothalami of mice using an RNeasy Mini kit (QIAGEN, Tokyo, Japan). The Go Taq® 2-Step RT-qPCR System, containing BRYT Green in the PCR Master Mix, was used for cDNA synthesis and RT-qPCR analysis (Promega). Gene expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) mRNA expression levels using the

CT method. The primer sequences for phosphoenolpyruvate carboxykinase (*Pck1*), glucose-6-phosphatase catalytic subunit (*G6pc*), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*Pfkfb3*), glucokinase (*Gck*), *mPpara*, *hPpara*, cytochrome P450 (*Cyp* 4a14, hypothalamus neuropeptide Y (NPY), proopiomelanocortin (POMC) and *Gapdh* mRNAs are described Table S1.

2.5. Statistical analyses

The data are expressed as the mean $\pm$ SD. Statistical analyses were performed using the Student's *t* test to compare the control and 0.05% DEHP groups from each mouse strain and Dunnett's test after using a one-way analysis of variance to compare more than three dosage groups. The analyses were carried out using the EZR software package (Kanda, 2013). *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Glucose concentrations

Since exposure to DEHP *in utero* did not influence TG or fatty acid concentrations in the fetuses or pups (Nakashima et al., 2013), glucose levels, another vital nutrient source, the metabolism of which is also regulated by PPAR α , were measured (Peeters and Baes, 2010). Exposure to either 0.05% or 0.1% DEHP decreased serum glucose levels in *mPPARA* offspring on PND 2 (Fig. 1). However, decreased glucose levels were not observed in *mPPARA* offspring on PND 21 or at 11 weeks of age. DEHP exposure decreased glucose levels slightly, but not significantly, in *hPPARA* offspring. None of the DEHP doses tested influenced glucose levels in the *Ppara*-null offspring at any age.

3.2. Glucose regulation

Because exposure to DEHP *in utero* decreased glucose concentrations in neonatal *mPPARA* offspring at PND 2, we measured the expression levels of mRNAs encoded by genes involved in both gluconeogenesis, including *Pck1* and *G6pc*, and glycolysis, including *Pfkfb3* and *Gck*, in the livers of these offspring. A 0.1% dose of DEHP increased the expression of *G6pc* in *mPPARA* offspring, but did not influence the expression of the other genes examined (Fig. 2).

3.3. Body and organ weight changes in offspring after weaning

Male PND 21 offspring exposed to either 0% or 0.05% DEHP *in utero* and during lactation were switched to a normal chow diet, and their body weights were monitored until 11 weeks of age. Exposure to DEHP *in utero* and during lactation did not affect the body weights of any of the offspring from the ages of 4 to 10 weeks (Fig. S1). In addition, DEHP exposure did not influence body or liver weights (Table 1), or the weights of whole brain and hypothalamus (data not shown), in any of the offspring at 11 weeks of age.

3.4. Food consumption

The average daily food consumption per mouse from 4 to 11 weeks of age is shown in Fig. 3. Exposure to DEHP *in utero* and during lactation increased food consumption in *mPPARA*

offspring between 6–8 weeks of age and in *hPPARA* offspring at 5, 6, 9, and 11 weeks of age. The average caloric intake in the 0% (control) and 0.05% DEHP groups throughout the observation period was 11.2 ± 0.94 kcal/day and 12.1 ± 0.86 kcal/day ($p < 0.05$) in *mPPARA* offspring, 10.4 ± 1.02 kcal/day and 10.7 ± 0.90 kcal/day ($p = 0.53$) in *Ppara*-null offspring, and 11.2 ± 0.58 kcal/day and 12.1 ± 0.62 kcal/day ($p < 0.05$) in *hPPARA* offspring, respectively. Thus, for *mPPARA* and *hPPARA* offspring, the average caloric intake was significantly higher in the groups exposed to DEHP *in utero* and during lactation than in the control groups. DEHP exposure did not affect food intake in *Ppara*-null offspring.

3.5. Leptin concentrations

Because exposure to DEHP *in utero* and during lactation influenced food consumption in *mPPARA* and *hPPARA* offspring, we measured serum/plasma leptin concentrations, which are reportedly related to feeding behavior (Halaas et al., 1995). On PNDs 2 and 21, leptin levels in *mPPARA* offspring decreased after exposure to 0.05 or 0.1% DEHP, and 0.05% DEHP, respectively (Table 2). As for *Ppara*-null offspring, leptin levels decreased on PND 2 after exposure to 0.01% DEHP, but this change was not dose dependent, and no differences were observed in leptin levels on PND 21. Exposure to DEHP did not alter the leptin levels in *hPPARA* offspring on PNDs 2 and 21 and at 11 weeks of age, and in *mPPARA* and *Ppara*-null offspring at 11 weeks old.

To determine the critical window of the effect of DEHP on leptin levels, we also analyzed plasma leptin levels in the postpartum dams and adult male mice exposed to DEHP for 8 weeks, as well as in off-spring exposed to 0.05% DEHP during the lactational period alone. DEHP did not influence leptin concentrations in either the postpartum dams or adult male mice (Table 2). In addition, exposure to DEHP during the lactational period alone did not decrease leptin levels compared to levels in the control group.

In addition, mRNA expression of feeding behavior-regulating factor, NPY (an orexigenic neuropeptide) and POMC (an anorexigenic neuropeptide), were measured. Exposure to DEHP *in utero* and during the lactational period decreased NPY expression in the hypothalamus of 11-week-old *hPPARA* offspring. However, expression of NPY in the other groups was unaffected, and expression of POMC was unchanged in all groups (Fig. S2)

3.6. PPAR α and the target gene *Cyp4a14*

The expression levels of *PPAR α* and the target gene *Cyp4a14* (Stauber et al., 2005) were measured in offspring on PNDs 2 and 21. Exposure to 0.1% DEHP *in utero* increased the hepatic expression of *Ppara* mRNA in *mPPARA* mice on PND 2, but did not increase the expression of *Cyp4a14* mRNA (Table 3). Exposure to DEHP *in utero* and during lactation significantly increased the expression of *Cyp4a14* on PND 21 in *mPPARA* offspring, as well as that of *Ppara* mRNA and *Cyp4a14* mRNA in *hPPARA* offspring. Exposure to DEHP during the lactational period alone also elevated the hepatic expression of *Ppara* mRNA and *Cyp4a14* mRNA in *mPPARA* offspring.

4. Discussion

In the present study, we found that either 0.05% or 0.1% DEHP decreased serum glucose levels in *mPPARA* offspring on PND 2. These two dosages also decreased serum/plasma leptin levels in *mPPARA* offspring on PNDs 2 and 21, which might be related to the upregulation of feeding in offspring after weaning (Fig. 4). No such findings were observed in *Ppara*-null offspring, suggesting the effects of glucose metabolism and leptin levels on the offspring were mediated by *PPARα*. It is of note that these effects disappeared before maturation (11 weeks old).

Our previous study showed that dietary exposure to either 0.05% or 0.1% DEHP during pregnancy suppressed the plasma levels of TGs and fatty acids in dams in a *PPARα*-dependent manner but did not affect these levels in their offspring (Hayashi et al., 2011; Nakashima et al., 2013). In general, blood TG and fatty acid levels increase during late pregnancy (Al et al., 1995; Liberati et al., 2004; Montes et al., 1984; Sweeney et al., 2006), suggesting that increases in blood TG and fatty acid levels are physiologically important during gestation and that DEHP-induced *PPARα*-dependent decreases in these lipid levels in the dams influence offspring development (Jia et al., 2015b). Surprisingly, exposure to DEHP *in utero* does not affect either TG or fatty acid concentrations in offspring at fetal and neonatal ages (Nakashima et al., 2013). There are at least two possible reasons for these results. First, data showing changes in lipid levels in the offspring may be missing, because only data for live offspring were measured, which might reflect lipid homeostasis. Second, maternal lipid malnutrition may lead to other nutritional deficiencies in the offspring, besides lipid deficiency. To investigate this hypothesis, we measured glucose levels, an important nutritional source, in the offspring. *In utero* exposure to DEHP only decreased serum glucose concentrations in *mPPARA* offspring. Undernutrition of dams (65% food restriction) during the last week of gestation and lactation in rats similarly lowered blood glucose levels in 10-day-old offspring (Lizarraga-Mollinedo et al., 2012). As mentioned above, exposure to DEHP during gestation induced lower blood lipid levels in the dams, similar to undernutrition. Although gluconeogenesis was upregulated via an increase in *G6pc* mRNA in the livers of offspring on PND2, along with hypoglycemia, these effects might not be sufficient to normalize glucose levels, because undernutrition during early developmental stages induces glucagon resistance and insulin hypersensitivity (Lizarraga-Mollinedo et al., 2012). In an epidemiological study, Nicolini et al. (1989) measured blood glucose levels in infants diagnosed with intrauterine growth restriction (IUGR) at both 18 and 35 weeks. Glucose levels were significantly lower in infants with IUGR than in those without IUGR, suggesting that hypoglycemia was present in infants with IUGR. Although the mechanism by which hypoglycemia is induced in *mPPARA* offspring on PND 2 as a result of *in utero* DEHP exposure is unknown, DEHP might induce IUGR without affecting body weight in offspring, which lowers serum glucose levels in the offspring. *PPARα* is thought to be an important regulator of glucose and lipid homeostasis (Kersten, 2002). Im et al. (2011) reported that *PPARα* ligands increase the expression of gluconeogenic genes in primary hepatocyte cultures. In contrast, *PPARα* ligands were reported to decrease plasma glucose levels in diabetic *db/db* mice via the downregulation of *Pck1* and *G6p* expression, key gluconeogenic genes (Jeong et al., 2011). In the current study, *in utero* exposure to

DEHP significantly increased the hepatic expression levels of *G6pc* mRNA in *mPPARA* offspring on PND 2, suggesting the importance of *PPARα* in upregulating gluconeogenesis. This upregulation was not reflected in the blood glucose levels of *mPPARA* offspring on PND 2, but serum glucose levels recovered on PND 21, and no evidence of hypoglycemia was observed.

Similar to glucose levels, serum/plasma leptin levels also decreased only in *mPPARA* offspring on PNDs 2 and 21. During the neonatal stage, leptin is supplied via breast milk (Walker et al., 2004). Leptin levels in breast milk positively correlate with maternal blood leptin levels (Schuster et al., 2011). Since DEHP did not influence maternal plasma leptin levels, lower leptin levels in neonatal offspring might not be related to those in breast milk. Maternal malnutrition induced by a 50% food restriction between gestational day 14 to PND 21 reportedly decreased leptin concentrations in neonatal male offspring between PNDs 4 and 21 (Delahaye et al., 2008). Maternal lipid malnutrition induced by DEHP exposure during pregnancy may be related to lower leptin levels in the offspring. However, these lower leptin levels due to malnutrition caused by either food restriction or DEHP exposure normalized by PND 30 or at 11 weeks of age, respectively.

Leptin is an adipose-derived hormone that regulates food intake, body weight, and glucose levels (Pellemounter et al., 1995). In addition, leptin also influences the development of hypothalamic circuits (Bouret and Simerly, 2006). Hypothalamic NPY is an orexigenic peptide whose overexpression induces overfeeding and severe obesity in adult rats (Sousa-Ferreira et al., 2011). In contrast, hypothalamic POMC is an anorectic peptide whose expression increases in response to leptin and decreases during fasting (Mizuno et al., 1998). Hypothalamic POMC mRNA levels decreased in response to a 50% food restriction from PNDs 14 to 30, slightly after a decrease in leptin levels (Delahaye et al., 2008). *In utero* exposure to DEHP did not influence these hypothalamic circuits in *mPPARA* offspring at 11 weeks of age, although we did not measure these pathways at PND 21. Increased food intake observed in *mPPARA* offspring after weaning might be related to a decrease in leptin levels before weaning. Therefore, further investigation is needed to clarify the effects of *in utero* DEHP exposure on hypothalamic gene expression.

Our interest then focused on whether exposure *in utero* or during lactation could explain the effects of DEHP on leptin, because DEHP exposure continued until weaning. On PND 21, leptin levels were not decreased in offspring exposed to DEHP during the lactational period alone or in postpartum dams or adult male mice, suggesting that *in utero* exposure more critically causes a decrease in leptin levels in the off-spring during the neonatal and lactational periods. In a previous study, to determine DEHP exposure levels in fetal and lactational offspring, we measured the levels of MEHP, a major DEHP metabolite, in the livers of *mPPARA* offspring on gestational day 18 and PND 2 (Hayashi et al., 2012). MEHP levels were about two-times higher in the fetuses than in the offspring on PND 2 after exposure to 0.05% DEHP. Therefore, higher exposure levels *in utero* than during lactation may have a greater influence on leptin levels in the offspring. Although it is not known why DEHP did not influence glucose and leptin levels in *hPPARA* offspring, their food consumption after weaning also increased.

Finally, we examined the relationship between PPAR α and leptin levels. Exposure to DEHP *in utero* increased hepatic PPAR α levels and decreased serum leptin levels in mPPARA offspring on PND 2. Although exposure to DEHP during the lactational period alone did not decrease plasma leptin levels in offspring on PND 21, DEHP exposure increased hepatic *Ppara* and *Cyp4a14* mRNA levels, suggesting that *Ppara* and *Cyp4a14* expression during these periods may not be directly related to the decrease in leptin levels. Taken together, decreases in leptin levels during the neonatal and weaning stages may be caused by DEHP-induced maternal malnutrition. Since DEHP only induces lipid malnutrition in pregnant mPPARA mice, maternal mouse PPAR α is thought to play an important role in the decrease in leptin levels.

5. Conclusion

Exposure to DEHP during pregnancy induced neonatal malnutrition, which manifested as a decrease in glucose levels on PND 2. Within a short period of PND2 to PND21, leptin concentrations in mPPARA offspring also decreased during the neonatal and weaning stages, followed by an increase in feeding after weaning. These data suggest the importance of controlling DEHP exposure during pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

CYP	cytochrome P450
DEHP	di(2-ethylhexyl)phthalate
EPA	eicosapentaenoic acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GCK	glucokinase
G6PC	glucose-6-phosphatase catalytic subunit
hPPARA	humanized peroxisome proliferator-activated receptor α
MEHP	mono(2-ethylhexyl)phthalate
mPPARA	mouse peroxisome proliferator-activated receptor α
IUGR	Intrauterine growth restriction
LP	lactational period

PCK	1phosphoenolpyruvate carboxykinase
PCR	Polymerase chain reaction
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
PND	postnatal day
PPARα	peroxisome proliferator-activated receptor α
TGs	triglycerides

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Fig. 1.

Glucose concentrations in sera of mice on PND 2 and the plasma of mice on PND 21 and at 11 weeks of age ($n = 6$ in each group). White columns, control; gray columns, 0.01% DEHP; black columns, 0.05% DEHP, shaded columns, 0.10% DEHP. *Significantly different from the corresponding control group ($p < 0.05$). PND, postnatal day; DEHP, di(2-ethylhexyl) phthalate; PPAR α , peroxisome proliferator-activated receptor α ; mPPARA, mouse *Ppara* gene; hPPARA, human *PPARA* gene.

Fig. 2.

The mRNA expression of hepatic factors regulating glucose levels in mice on PND 2 ($n = 6$ in each group). Each mRNA level was normalized to the level of GAPDH mRNA in the same preparation, and the mean for the control group of *mPPARA* mice was assigned a value of 1.0. Each column and bar represents the mean $\pm$ SD. White columns, control; gray columns, 0.01% DEHP; black columns, 0.05% DEHP, shaded columns, 0.10% DEHP.

*Significantly different from the corresponding control group ($p < 0.05$). PND, postnatal day; DEHP, di(2-ethylhexyl) phthalate; PPAR α , peroxisome proliferator-activated receptor α ; *mPPARA*, *mPPARA*, mouse *Ppara* gene; *hPPARA*, human *PPARA* gene; *Gapdh*, glyceraldehyde 3-phosphate dehydrogenase; *Gck*, glucokinase; *G6pc*, glucose-6-phosphatase catalytic subunit; *Pfkfb3*, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; *Pck1*, phosphoenolpyruvate carboxykinase.

Fig. 3.

Time-dependent changes in food consumption in mice from 4 to 11 weeks of age ($n = 6$ in each group). The data represent the mean. Solid line, control; dotted line, 0.05% DEHP.

*Significantly different from the corresponding control group ($p < 0.05$). DEHP, di(2-ethylhexyl) phthalate; PPAR α , peroxisome proliferator-activated receptor α ; mPPARA, mouse *Ppara* gene; hPPARA, human *PPARA* gene.

Fig. 4. Summary of the adverse effects of DEHP on *mPPARA* dams and their offspring, represented on a linear time line.

Table 1

Body and liver weights of 11-week-old offspring (n = 6 in each group).

	DEHP	mPPARA	Ppara-null	hPPARA
Body weight (g)	0%	23.7 ± 1.5	21.1 ± 1.0	21.3 ± 2.1
	0.05%	23.8 ± 1.7	21.9 ± 1.8	21.7 ± 1.9
Liver weight (g)	0%	1.13 ± 0.12	1.06 ± 0.10	1.00 ± 0.10
	0.05%	1.12 ± 0.10	1.11 ± 0.11	1.03 ± 0.12
Liver/body weight (%)	0%	4.78 ± 0.32	5.02 ± 0.40	4.68 ± 0.13
	0.05%	4.72 ± 0.27	5.09 ± 0.36	4.71 ± 0.19

The data represent the mean ± SD. DEHP, di(2-ethylhexyl) phthalate; PPARα, peroxisome proliferator-activated receptor α; mPPARA, mouse *Ppara* gene; hPPARA, human *PPARA* gene.

Table 2

Leptin concentrations in the serum/plasma of offspring on PNDs 2 and 21, and at 11 weeks of age, pregnant dams, and adult male mice (n = 6 in each group).

Mice	DEHP	mPPARA	Ppara-null	hPPARA
Offspring ^a (PND 2)	0%	3.08 ± 3.30	1.15 ± 0.57	0.62 ± 0.44
	0.01%	0.93 ± 0.65	0.32 ± 0.16 *	0.37 ± 0.35
	0.05%	0.33 ± 0.27 *	0.62 ± 0.50	0.95 ± 0.65
	0.10%	0.48 ± 0.26 *	0.77 ± 0.52	1.04 ± 2.06
Offspring (PND 21)	0%	3.75 ± 1.06	4.16 ± 2.28	2.54 ± 1.12
	0.01%	3.14 ± 1.16	3.49 ± 1.41	2.27 ± 1.11
	0.05%	1.50 ± 0.75 *	3.82 ± 1.52	3.39 ± 2.52
	0.10%	-	3.80 ± 1.38	2.99 ± 0.58
Offspring (11 weeks old)	0.05%-LP ^b	3.13 ± 1.44	-	-
	0%	3.95 ± 1.74	5.69 ± 2.33	2.34 ± 1.75
	0.05%	3.83 ± 1.89	4.31 ± 1.80	2.80 ± 1.46
	0.10%	3.41 ± 1.14	2.11 ± 1.10	2.57 ± 1.03
Postpartum dams ^c	0%	3.11 ± 1.66	4.10 ± 2.08	5.25 ± 4.03
	0.01%	3.46 ± 1.35	1.85 ± 0.78 *	3.86 ± 2.66
	0.05%	4.97 ± 2.04	2.53 ± 1.38	2.99 ± 1.95
	0.10%	3.41 ± 1.14	2.11 ± 1.10	2.57 ± 1.03
Adult male mice ^d	0%	3.24 ± 1.57	4.11 ± 2.50	1.82 ± 1.45
	0.05%	5.07 ± 3.16	3.59 ± 1.80	2.19 ± 1.00

The data represent the mean ± SD.

* Significantly different from the corresponding 0% DEHP (control) group ($p < 0.05$). PND, postnatal day; DEHP, di (2-ethylhexyl) phthalate; PPAR α , peroxisome proliferator-activated receptor α ; mPPARA, mouse *Ppara* gene; hPPARA, human *PPARA* gene.

^a Offspring exposed to DEHP *in utero* and during the lactational period.

^b PND 21 offspring exposed to 0.05% DEHP during the lactational period (LP) alone.

^c Dams of PND 2 offspring exposed to DEHP during pregnancy.

^d Male mice exposed to DEHP from 12 to 20 weeks of age.

Table 3

Hepatic mRNA expression of *PPARα* and *CYP4A14* in offspring on PNDs 2 and 21 (n = 6 in each group).

Gene	Mice	DEHP	mPPARA	Ppara-null	hPPARA
<i>PPARα</i>	PND 2	0%	1.00 ± 0.81	-	177.2 ± 53.1
		0.01%	1.59 ± 1.09	-	149.6 ± 32.7
		0.05%	0.84 ± 0.40	-	158.8 ± 80.2
		0.10%	3.13 ± 1.19 [*]	-	248.8 ± 115.4
	PND 21	0%	1.00 ± 0.13	-	70.2 ± 9.8
		0.05%	1.31 ± 0.41	-	108.4 ± 34.3 [*]
		0.05%-LP ^a	1.59 ± 0.39 [*]	-	-
<i>CYP4A14</i>	PND 2	0%	1.00 ± 0.45	0.10 ± 0.05	1.04 ± 0.54
		0.01%	0.79 ± 0.15	0.09 ± 0.04	1.32 ± 0.26
		0.05%	0.72 ± 0.17	0.05 ± 0.04	1.16 ± 0.70
		0.10%	1.26 ± 0.32	0.05 ± 0.02	1.77 ± 0.52
	PND 21	0%	1.00 ± 0.74	0.03 ± 0.03	0.17 ± 0.08
		0.05%	2.64 ± 0.50 [*]	0.03 ± 0.03	0.32 ± 0.13 [*]
		0.05%-LP ^a	3.79 ± 1.59 [*]	-	-

Each mRNA level was normalized to the level of GAPDH mRNA in the same preparation, and the mean for the control group of the mPPARA mice was assigned a value of 1.0. The data represent the mean ± SD.

^{*} Significantly different from the corresponding control group ($p < 0.05$). PND, postnatal day; DEHP, di(2-ethylhexyl) phthalate; PPARα, peroxisome proliferator-activated receptor α; mPPARA, mouse *Ppara* gene; hPPARA, human *PPARA* gene; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

^a PND 21 offspring exposed to 0.05% DEHP during the lactational period (LP) alone.