

***In utero* exposure to di-(2-ethylhexyl) phthalate induces metabolic disorder and increases fat accumulation in visceral depots of C57BL/6J mice offspring**

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Abstract. Excessive visceral fat accumulation is associated with metabolic disorders. Di-(2-ethylhexyl) phthalate (DEHP), a candidate environmental obesogen, affects lipid metabolism and adipogenesis. Perinatal exposure to DEHP may be associated with metabolic disorders of dams and offspring. The aim of the present study was to explore the effects of exposure of pregnant dams to DEHP on the metabolism and fat distribution of their offspring, and to determine the mechanisms for these effects. Pregnant C57BL/6J mice were administered DEHP via gavage (0.05 or 500 mg/kg/day) from gestational days 1-19. Pups were sacrificed at nine weeks of age. Serum leptin, insulin, lipid and fasting glucose levels, and the weights of the inguinal (subcutaneous) and gonadal (visceral) fat pads were determined. mRNA expression levels of two developmental genes, T-box 15 (*Tbx15*) and glypican 4 (*Gpc4*) were detected in fat tissues. A 100% abortion rate was exhibited in 500 mg/kg DEHP-treated dams, whereas exposure to 0.05 mg/kg DEHP did not affect reproductive outcomes. Pups from the 0.05 mg/kg exposure group were used for subsequent experimentation. Serum leptin, insulin, lipid and fasting glucose concentrations

in these pups were significantly higher than those of control pups ($P < 0.05$). Although no significant change in body weight was detected, the visceral fat weights of DEHP-exposed pups were significantly higher than those of control pups ($P < 0.05$). Compared with controls, mRNA expression levels of *Tbx15* in subcutaneous fat and *Gpc4* in visceral fat were significantly increased among DEHP-exposed pups ($P < 0.01$). The present results suggest that *in utero* exposure to an environmentally safe dose of DEHP may lead to excessive visceral fat accumulation and metabolic disorders in offspring and that aberrant expression of *Tbx15* and *Gpc4* may have an important role in these effects.

Introduction

Distribution of white adipose tissues in humans is associated with metabolic disorders. Individuals who are peripherally obese (fat accumulation predominantly in the gluteofemoral region) are at little or no risk of developing metabolic disease, whereas individuals who are centrally obese (fat distribution predominantly in visceral depots) are prone to developing metabolic complications (1). However, the underlying mechanisms that regulate fat distribution and link excess visceral fat to metabolic complications are yet to be elucidated.

Environmental obesogens are chemical compounds that promote or exacerbate the development of obesity and its associated health outcomes (2) by disrupting or interfering with critical pathways associated with energy balance, adipogenesis and lipid metabolism (3). Phthalates are a class of candidate obesogens that are ingested in food. Phthalate metabolites have been detected in >80% of the population and fetal exposure levels are readily detectable (4-6). In a previous cross-sectional study, urinary phthalate metabolite concentrations were demonstrated to be associated with an increased waist circumference and insulin resistance in adult males in the USA (7,8). This correlation indicates that increased phthalate exposure may be associated with increased abdominal obesity and fat distribution. In addition, there are age and sex differ-

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ences in the association between phthalate exposure and obesity (9,10). For example, male children and adolescents are at high risk for obesity associated with urinary low molecular weight phthalate metabolites, whereas adults are at high risk for obesity associated with high molecular weight phthalate metabolites (9). Moreover, associations were positive for low molecular weight phthalate metabolites with BMI z-score in boys >10 years of age, but no association was detected in girls <10 years of age (10).

Di-(2-ethylhexyl) phthalate (DEHP), which is a phthalate ester, is predominately used as an industrial plasticizer and is found in cosmetics, industrial paints and solvents (11). The presence of DEHP metabolites in urine is associated with adiposity and insulin resistance in children (12). Previous studies have reported that perinatal exposure to DEHP may induce obesity and metabolic disorders in mice (13,14); however, the mechanisms underlying these associations are yet to be investigated.

T-box 15 (*Tbx15*) and glypican 4 (*Gpc4*) are developmental genes that have roles in the origins of obesity and body fat distribution in mice and humans (15). In humans, *Tbx15* expression is negatively correlated with waist/hip ratio (16). Low levels of *Tbx15* mRNA and high levels of *Gpc4* mRNA in visceral adipose tissue, and low levels of *Gpc4* mRNA and high levels of *Tbx15* mRNA in subcutaneous adipose tissue appear to be associated with a high waist/hip ratio (15) and, therefore, may be associated with obesity and fat distribution. These genes may be correlated with an increased risk of developing metabolic and cardiovascular complications (15).

In utero exposure to endocrine-disrupting chemicals may alter adipose tissue development by affecting the number, size, and distribution of adipocytes formed, as well as larger regulatory systems associated with body weight homeostasis (17). Therefore, it was hypothesized in the present study that *in utero* exposure to DEHP may, in part, affect obesity and fat distribution by altering the expression of *Tbx15* and *Gpc4* in murine offspring. The effects of exposure to DEHP in pregnant dams on their 9-week-old offspring was investigated by measuring: i) Serum leptin, insulin, lipid, and glucose concentrations; ii) body weight and adipose tissue deposition; and iii) mRNA expression levels of *Tbx15* and *Gpc4* in subcutaneous and visceral adipose tissues.

Materials and methods

Reagents. DEHP, serum triglyceride determination kit (TR0100) and cholesterol quantitation kit (MAK043) were purchased from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany). Leptin ELISA kit (EK0438) was obtained from Boster Biological Technology (Wuhan, China), and insulin ELISA kit (EZRMI-13K) was purchased from Merck Millipore (Shanghai, China). RNAiso Plus, PrimeScript RT Perfect Real Time reagent kit and SYBR *Premix Ex Taq* II kit (Tli RNaseH Plus) were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

Animals. Male (n=25) and female (n=50) C57BL/6J mice (age, 8 weeks; weight: male, 21.9±1.0 g; female, 19.6±0.9 g) were purchased from the Animal Center of China Medical

University (Shenyang, China). Mice were kept at a controlled temperature of 20±2°C and a relative humidity of 50±10% with a 12-h light/dark cycle. Mice were provided water and a standard chow diet containing 10% kcal from fat *ad libitum*. Animal procedures were conducted according to an animal protocol approved by the Institutional Animal Care and Use Committee of China Medical University.

Fetal exposure to DEHP. A total of 25 male and 50 female mice (gender ratio, 1:2) were mated overnight. Females were examined for a copulation plug the following day, which was designated as gestational day (GD) 1. Pregnant females were randomly divided into three groups (F0 control, F0 DEHP0.05 and F0 DEHP500) and exposed to various concentrations of DEHP (0, 0.05 and 500 mg/kg/day). DEHP (diluted in olive oil) or vehicle (olive oil) was administered via gavage every 24 h from GD1 to GD19. Food intake of F0 dams was recorded weekly. Spontaneous abortion was determined in dams that did not deliver. Pups (F1 control, n=29 (female/male ratio, 15:14) and F1 DEHP0.05, n=30 (female/male ratio, 14:16) were maintained with their dams and weaned at three weeks of age. Pups were subsequently fed a standard diet until nine weeks of age.

Serum measurements. Blood samples of F1 offspring were harvested from the abdominal aorta at nine weeks of age. Samples were centrifuged at 1,000 x g for 15 min at room temperature and stored at -20°C prior to analysis. Levels of serum leptin and insulin were determined using ELISA kits. Serum triglyceride and total cholesterol levels were determined using commercial kits. Serum glucose concentration was measured via the glucose oxidase method (18). All experimental protocols were performed according to the manufacturers' protocols. All data were obtained from three independent experiments.

Adipose tissue collection. F1 offspring were anesthetized with ether (Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China) and sacrificed at nine weeks of age via cervical dislocation. Gonadal and inguinal fat pads were immediately removed, weighed, frozen in liquid nitrogen and stored at -80°C prior to RT-qPCR analysis. Gonadal and inguinal fat pads were representative of visceral and subcutaneous fat, respectively.

RT-qPCR. Total RNA of gonadal and inguinal fat pads was extracted using RNAiso Plus, according to the manufacturer's protocol. RT-qPCR reactions were performed using the ABI Prism 7500 system (Thermo Fisher Scientific, Inc., Waltham, MA, USA) as described previously (19). Briefly, RT was performed with a reaction mixture containing 2 µl 5X PrimeScript buffer, 0.5 µl PrimeScript RT Enzyme Mix, 0.5 µl Oligo dT Primer (50 µM) and/or 0.5 µl Random hexamers (100 µM) and 500 ng mRNA. The mixture was maintained for 5 min at 37°C followed by 5 sec at 85°C and final hold at 4°C. Subsequently, each qPCR reaction mixture contained 10 µl 2X SYBR *Premix Ex Taq* II, 0.8 µl forward and reverse primers (10 µmol/µl), 0.4 ml Rox Reference Dye II (50X) and 2 µl cDNA. Thermal cycling was performed to amplify the respective targets in a total volume of 20 µl: Initial denatur-

ation at 95°C for 30 sec for 1 cycle, followed by denaturation at 95°C for 5 sec and annealing and extension 60°C for 34 sec for 40 cycles. β -actin was used as the endogenous control gene. RT-qPCR data were analyzed using the $2^{-\Delta\Delta C_q}$ method (20). All PCR reactions were performed in triplicate. Primers were designed as follows: β -actin, forward 5'-CATCCGTAAAGA CCTCTATGCCAAC-3' and reverse 5'-ATGGAGCCACCG ATCCACA-3'; *Gpc4*, forward 5'-AGAGCAACGCCAAC CAC-3' and reverse 5'-GCCATTCCAGCAGTCATC-3'; and *Tbx15*, forward 5'-AGCTTCTGGAGACACCTGGATGA-3' and reverse 5'-CGTGGACTCGAGGCTGGTATTTA-3'.

Statistical analysis. Data are expressed as mean \pm standard deviation. Statistical analyses were performed using the two-tailed unpaired Student's t-test with unequal variance or one-way analysis of variance and Spearman's correlation analysis using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of exposure to DEHP on reproductive outcomes of F0 dams. As indicated in Table I, the food intake of pregnant mice was not significantly altered by DEHP exposure. However, reproductive outcome was impaired in dams exposed to a high dose (500 mg/kg) of DEHP. A 100% abortion rate was exhibited by the F0 DEHP500 group, whereas no adverse reproductive effect was demonstrated by the F0 DEHP0.05 group. Therefore, the offspring of the F0 DEHP0.05 group (F1 DEHP0.05) and of the control mice (F1 control) were used for subsequent stages of the present study.

Effects of in utero exposure to DEHP on serum leptin and insulin concentrations in F1 offspring. As shown in Fig. 1, serum leptin (female, 11.09 \pm 1.53 ng/ml; male, 11.11 \pm 1.23 ng/ml) and insulin (female, 0.29 \pm 0.08 ng/ml; male, 0.28 \pm 0.05 ng/ml) concentrations in the F1 DEHP0.05 group were significantly higher than those in the F1 control group (leptin: female, 9.95 \pm 1.14 ng/ml; male, 9.86 \pm 1.19 ng/ml; and insulin: female, 0.23 \pm 0.06 ng/ml; male, 0.23 \pm 0.07 ng/ml; $P < 0.05$). No significant differences were observed between female and male F1 offspring.

Effect of in utero exposure to DEHP on body weight, fat distribution, serum lipid levels, and glucose concentrations in F1 offspring. In utero exposure to DEHP had no significant effect on the body weights (Fig. 2A) or subcutaneous fat weights (Fig. 2B) of F1 offspring in the DEHP0.05 group compared with the control group. However, as shown in Fig. 2C, visceral fat weights in the F1 DEHP0.05 group (female, 0.36 \pm 0.10 g; male, 0.41 \pm 0.10 g) were significantly higher than those in the F1 control group (female, 0.29 \pm 0.05 g; male, 0.30 \pm 0.05 g; $P < 0.05$). Consistent with this increase in visceral fat mass, serum total triglycerides, total cholesterol, and fasting glucose levels were significantly increased by ~8, 13, and 16%, respectively, compared with the controls (Fig. 2D-F; $P < 0.05$). No significant difference was observed between female and male F1 offspring.

Effects of in utero exposure to DEHP on the mRNA expression levels of *Tbx15* and *Gpc4* in subcutaneous and visceral

Table I. Reproductive outcome of F0 dams treated with DEHP from gestational day 1 to 19.

Criteria	DEHP (mg)		
	0	0.05	500
Pregnant dams (n)	6	6	7
Food intake (g/day)	4.9 \pm 0.5	5.0 \pm 0.8	5.1 \pm 0.7
Delivery (n)	6	6	0
Abortion (%)	0	0	100
F1 offspring (n)	29	30	0
F1 offspring size	4.83 \pm 1.94	4.29 \pm 1.80	-
F1 females (n)	15	14	-
F1 males (n)	14	16	-

Data are presented as mean \pm standard deviation, or as stated. DEHP, di (2-ethylhexyl) phthalate.

fat in F1 offspring. As shown in Fig. 3A, *Tbx15* mRNA expression levels in subcutaneous fat in the F1 control and F1 DEHP0.05 groups were significantly higher than those in visceral fat ($P < 0.01$). The expression of *Tbx15* mRNA expression levels in subcutaneous fat were significantly increased in the F1 DEHP0.05 group compared with the F1 control group ($P < 0.01$), whereas no significant increase was observed in visceral fat. Expression levels of *Gpc4* mRNA in subcutaneous fat in the control and DEHP-treated groups were significantly lower than those in visceral fat ($P < 0.01$; Fig. 3B). Compared with the F1 control group, the expression of *Gpc4* mRNA in visceral fat was significantly upregulated in the F1 DEHP0.05 group ($P < 0.01$), but no significant increase was detected in the subcutaneous fat.

Association between leptin, insulin, serum lipid and glucose concentration, and mRNA expression levels of *Tbx15* and *Gpc4* in subcutaneous and visceral fat of F1 offspring. As shown in Table II, Spearman's correlation analysis demonstrated that serum leptin concentration was positively correlated with *Tbx15* and *Gpc4* mRNA in visceral fat. Serum insulin and glucose concentrations were positively correlated with *Tbx15* mRNA in subcutaneous fat, as well as *Gpc4* mRNA in visceral fat. Serum total triglyceride was positively correlated with *Tbx15* mRNA in subcutaneous fat, whereas total cholesterol was positively correlated with *Gpc4* mRNA in visceral fat.

Discussion

In the present study, DEHP was administered to pregnant C57BL/6J mice by gavage. DEHP was absorbed through the intestines, a route that mimics one of the most prominent exposure routes in humans (11). DEHP was delivered at 0.05 and 500 mg/kg/day. The lower dose (0.05 mg/kg/day) is considered to be a 'safe dose' for humans and is within the tolerable daily intake (TDI) (21). In contrast, the higher dose (500 mg/kg/day) has caused adverse reproductive and developmental effects in previous animal studies (22). The principal results of the present study were as follows. Firstly,

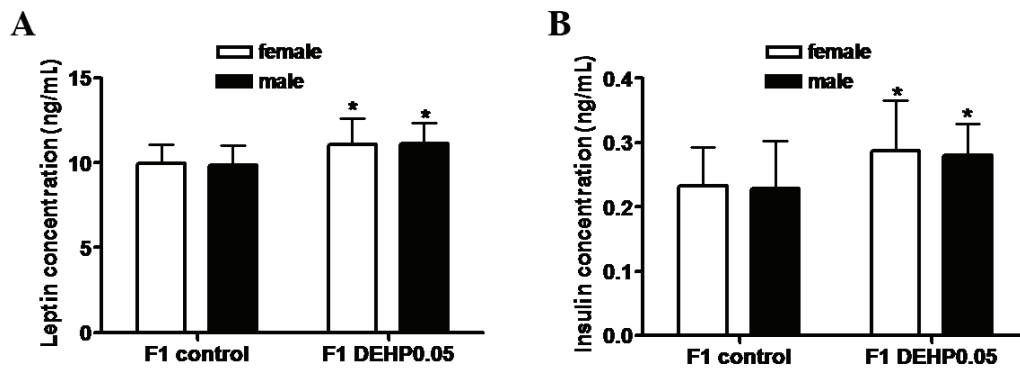


Figure 1. Effects of *in utero* exposure to DEHP on serum (A) leptin and (B) insulin concentrations of F1 offspring. DEHP or vehicle (olive oil) was administered to pregnant females by gavage (0.05 mg/kg) every 24 h from GD1 to 19. F1 offspring were weaned at three weeks of age and fed a standard diet through nine weeks of age. Results are shown as the mean \pm standard deviation (n=15 female and 14 male in the F1 control group, n=14 female and 16 male in the F1 DEHP0.05 group). DEHP, di-(2-ethylhexyl) phthalate; GD, gestational day. *P<0.05 vs. the F1 control group.

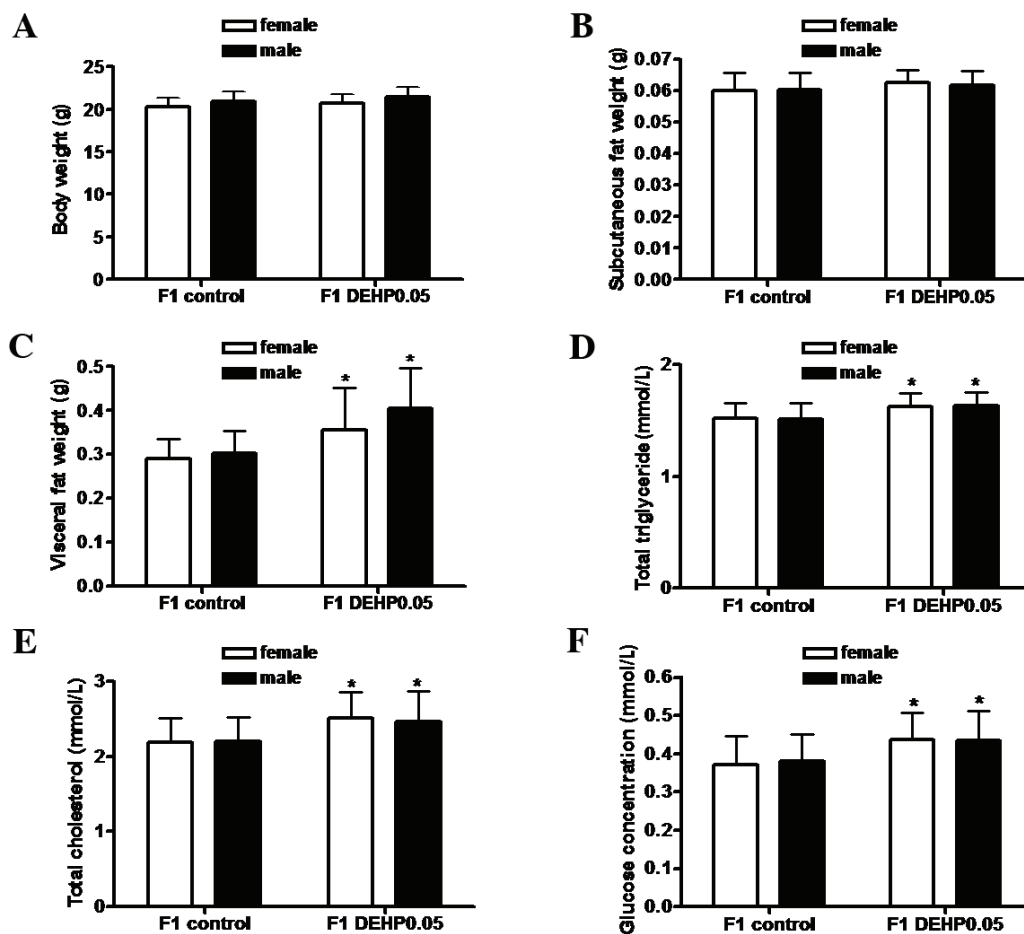


Figure 2. Effects of *in utero* exposure to DEHP on body weight, fat distribution, and serum lipid and glucose concentration in F1 offspring. (A) Body weight, (B) weight of subcutaneous fat, (C) weight of visceral fat, (D) total triglyceride levels, (E) total cholesterol levels, and (F) serum fasting glucose levels. Results are shown as the mean \pm standard deviation (F1 control group: female, n=15 and male, n=14; F1 DEHP0.05: female, n=14 and male, n=16). DEHP, di-(2-ethylhexyl) phthalate. *P<0.05 vs. the F1 control group.

exposure to 0.05 mg/kg DEHP did not significantly affect the food intake or reproductive capacity of F0 dams. Secondly, the weight of visceral fat and serum leptin, insulin, serum lipid, and glucose concentrations were significantly elevated in F1 offspring following *in utero* exposure to 0.05 mg/kg DEHP. Thirdly, expression levels of *Tbx15* mRNA in subcutaneous fat and *Gpc4* mRNA in visceral fat were significantly

upregulated in F1 offspring exposed to 0.05 mg/kg DEHP *in utero*.

Previous studies have reported that DEHP is able to induce a dose-dependent decrease in mouse fertility (23), and affect reproductive outcomes in female mice (24). In the present study, no significant decline in fertility was exhibited by F0 dams treated with 0.05 mg/kg DEHP; however, the abortion

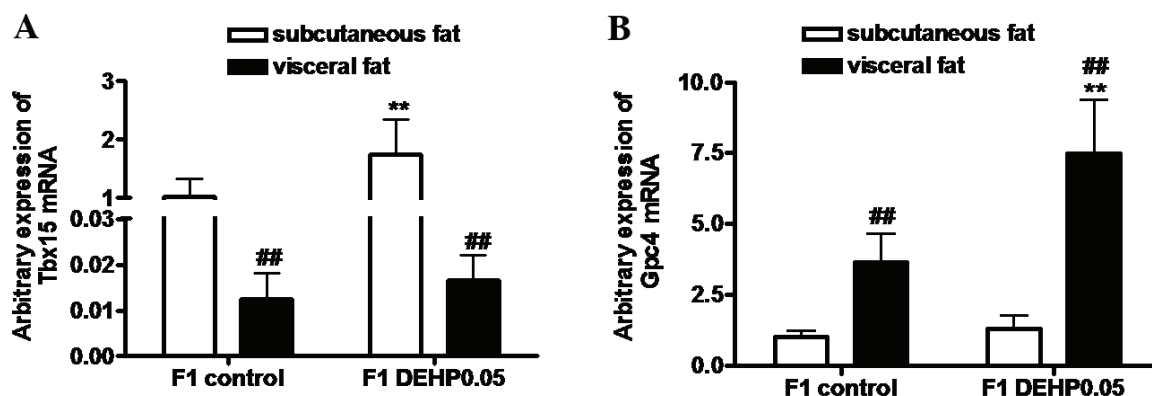


Figure 3. Effects of *in utero* exposure to DEHP on the mRNA expression levels of (A) *Tbx15* and (B) *Gpc4* in the subcutaneous and visceral fat of F1 offspring. Results are shown as the mean \pm standard deviation of two independent experiments (n=29 in the F1 control group and n=30 in the F1 DEHP0.05 group). DEHP, di-(2-ethylhexyl) phthalate. Tbx15, T-box 15; Gpc4, glypican 4. **P<0.01 vs. the F1 control group; ##P<0.01 vs. subcutaneous fat.

rate was 100% in the 500 mg/kg DEHP dose group compared with 0% in the control and 0.05 mg/kg DEHP groups. These findings indicate that exposure to DEHP at the TDI level did not affect the reproductive outcomes of mice; however, a high dose of DEHP may damage the reproductive capacity. In addition, 0.05 mg/kg DEHP exposure did not significantly affect the food intake or reproductive outcome of dams, although it did induce metabolic disorders in the offspring. Previous studies have reported a correlation between *in utero* exposure to endocrine-disrupting chemicals and the development of metabolic disorders in adulthood (2,14,25,26). In the present study, serum leptin, insulin, serum lipid, and glucose concentrations were significantly elevated in male and female offspring at postnatal week nine, indicating that *in utero* exposure to DEHP may influence metabolic function in adulthood.

Leptin has an important role in the developmental programming of obesity and insulin resistance (27). In the present study, *in utero* DEHP exposure correlated with a significant elevation in leptin concentration in F1 offspring, which was consistent with the increased weight of visceral fat. This suggested that *in utero* DEHP exposure may cause dysregulation of the central effect of leptin in F1 offspring. Similarly, a significant increase in insulin concentration was also demonstrated, consistent with the increase in glucose levels. This implied the potential development of insulin resistance. Elevated leptin and insulin levels in adult offspring may result from hypoleptinemia and hypoinsulinemia during fetal development due to *in utero* exposure to DEHP (28). Fetal hypoinsulinemia may lead to reduced fetal glucose uptake and subsequent insulin resistance (28). In the present study, leptin and insulin levels were not detected during the fetal period. However, a previous study found that fetal rats exposed to diisobutyl phthalate, another phthalate, exhibited altered insulin and leptin levels, which implies that perinatal phthalate exposure may increase the risk of insulin resistance (27). Rajesh and Balasubramanian (29) also demonstrated that gestational exposure to DEHP may lead to β -cell dysfunction and whole body glucometabolic abnormalities in F1 offspring.

Fat distribution in F1 offspring in the present study was also affected by *in utero* exposure to DEHP. The body and subcutaneous fat weights of DEHP-exposed F1 offspring were not significantly increased in male and female mice,

whereas the visceral fat weights were significantly elevated. These results indicate that excessive visceral fat storage due to *in utero* exposure to DEHP may be associated with a high risk of developing metabolic disorders. The present results agreed in part with two previous studies, which demonstrated that direct exposure to DEHP, through placenta and milk, increased body weight and adipose storage in offspring (13,14). However, in contrast to the effects of DEHP on body weight and visceral fat tissue in the F1 offspring observed in the present study, previous studies have detected no effect (3) or reductions (30) in body weight and white adipose tissue, suggesting that developmental exposure to DEHP is unlikely to cause metabolic disorders in adulthood (3). These discrepancies may be due to the use of different exposure dosages, exposure periods (*in utero*/lactation), or strain differences due to gene mutations or enzyme inactivation accounting for differences in DEHP metabolism (11). Therefore, more extensive studies are required to determine whether there are any adverse effects of DEHP on F1 offspring.

Exposure to certain environmental chemicals, such as diethylstilbestrol may alter the expression of genes involved in fat distribution (31). Overexpression of *Tbx15* in 3T3-L1 preadipocytes has been demonstrated to impair adipocyte differentiation and decrease triglyceride content (32). Therefore, differential expression of *Tbx15* between fat depots has a principal role in the interdepot differences in adipocyte differentiation, triglyceride accumulation and mitochondrial function, which may contribute to the risk of developing diabetes and metabolic disease (32). *Gpc4* is thought to have a critical role in the control of cell growth, differentiation, and morphogenesis (33). This gene is developmentally regulated in 3T3-F442A adipocytes, and its expression level may contribute to the regulation of preadipocyte differentiation (34). Therefore, the expression of *Tbx15* and *Gpc4* mRNA in subcutaneous and visceral fat tissues was detected in the present study to investigate whether *in utero* exposure to DEHP may modify their expression levels in F1 offspring. It was demonstrated that *Tbx15* and *Gpc4* mRNA expression levels were significantly increased in subcutaneous fat and visceral fat tissues, respectively, of filial mice exposed to DEHP *in utero* compared with the control group. DEHP-mediated alteration of developmental gene levels in subcutaneous and visceral fat

Table II. Spearman correlation coefficient analysis between serum measurements and mRNA expression levels in the fat tissues of F1 offspring.

Measurement	Subcutaneous fat		Visceral fat	
	Tbx15	Gpc4	Tbx15	Gpc4
Leptin (ng/ml)	0.164	0.120	0.317 ^a	0.290 ^a
Insulin (ng/ml)	0.387 ^b	0.027	0.027	0.423 ^b
Total triglyceride (mmol/l)	0.462 ^b	0.260	0.178	0.160
Total cholesterol (mmol/l)	0.245	0.140	0.074	0.386 ^b
Glucose (mmol/l)	0.303 ^a	0.208	0.113	0.305 ^a

Tbx15, T-box 15; *Gpc4*, glypican 4. ^aP<0.05; ^bP<0.01.

tissues may be one explanation for the excessive visceral fat weight in the DEHP group.

The transcriptional activity and mRNA expression of *Tbx15* may be regulated by methylation status in the distal promoter region. Hypomethylation of *Tbx15* has previously been correlated with low birth weight, which is a risk factor for the development of obesity in adults (35). In the present study, DNA methylation of *Tbx15* was not detected; however, a recent study has reported that gestational exposure to DEHP at 40 mg/kg may affect the DNA methylation of imprinting genes in fetal germ cells and growing oocytes, and in the offspring's oocytes (36). Therefore, it was inferred in the present study that the differential expression levels of *Tbx15* mRNA in subcutaneous and visceral fat tissues between the control and DEHP groups may be due to an alteration in *Tbx15* methylation status following *in utero* DEHP exposure.

Another developmental gene, *Gpc4*, may be regulated by the ratio of specificity protein 3 to specificity protein 1 (Sp3/Sp1) (34,37). Moreover, there is a physical interaction between Sp1 and peroxisome proliferator activated receptor- γ (PPAR- γ) (38,39). The authors of the present study have previously demonstrated that the Sp3/Sp1 ratio is correlated with *Gpc4* expression levels in the subcutaneous and visceral fat tissues of mice treated with the PPAR- γ agonist, rosiglitazone, indicating that the Sp3/Sp1 ratio may regulate *Gpc4* expression during the PPAR- γ activation process (37). In the present study, DEHP exposure was associated with a significant increase in *Gpc4* mRNA expression levels in visceral fat in F1 offspring, which was consistent with the increase of visceral fat. This suggested that DEHP exposure may affect fat distribution by regulating *Gpc4* mRNA expression. In animals and humans, intestinal lipases convert DEHP to its monoester equivalent, monoethyl-hexyl-phthalate (MEHP), which is a selective PPAR- γ modulator (40). MEHP has previously been demonstrated to induce adipogenesis by modulating PPAR- γ activity (41). *In utero* exposure to a low dose of MEHP significantly increased the body and fat pad weights, and the serum cholesterol, triacylglycerol and glucose levels of male offspring at postnatal day 60 (42). These observations suggest that *in utero* exposure to DEHP may directly or indirectly regulate the expression levels of Sp1 and Sp3 by activating

PPAR- γ , thereby altering the expression of *Gpc4* in visceral and subcutaneous fat tissues.

In conclusion, prenatal exposure to safe, environmentally relevant concentrations of DEHP caused an increase in serum leptin and insulin levels in male and female offspring. This finding indicates that *in utero* exposure to DEHP may influence metabolic function later in life. The effects of DEHP were analyzed in terms of the increased visceral fat weight and increased expression levels of two developmental genes: *Tbx15* in subcutaneous fat and *Gpc4* in visceral fat. These data suggest that prenatal DEHP exposure may alter fat distribution by upregulating *Tbx15* and *Gpc4* in subcutaneous and visceral adipose tissues, although the exact mechanisms are yet to be elucidated. The present study stresses the importance of further investigation into the mechanisms by which prenatal and postnatal DEHP exposure may affect fat distribution and lead to metabolic disorders.

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