Urine di-(2-Ethylhexyl) Phthalate Metabolites Are Independently Related To Body Fluid Status in Adults: Results From a U.S. Nationally Representative Survey

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Research

Keywords: Di-(2-ethylhexyl) phthalate, Bioelectrical impedance analysis, Extracellular fluid, National Health and Nutrition Examination Survey, Obesity.

DOI: https://doi.org/10.21203/rs.3.rs-827766/v1

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Abstract

Background

Di(2-ethylhexyl) phthalate (DEHP) has been utilized in many daily used products for decades. Previous studies have reported DEHP exposure could induce renin-angiotensin-aldosterone system activation and increase epithelial sodium channel (ENaC) activity, which contributes to extracellular fluid (ECF) volume expansion. However, there is also no previous study to evaluate the association between DEHP exposure and body fluid status.

Methods

We selected 1,678 subjects (aged ≥ 18 years) from a National Health and Nutrition Examination Survey (NHANES) in 2003–2004 to determine the relationship among urine DEHP metabolites and body composition (body measures, bioelectrical impedance analysis (BIA)).

Results

After weighted for sampling strategy, we reported higher levels of DEHP metabolites was correlated with increases in body measures (body weight, body mass index (BMI), waist), parameters of BIA (estimated fat mass, percent body fat, ECF, and ECF /intracellular fluid (ICF) ratio) in multiple linear regression analysis. The relationship between DEHP metabolites with ECF/ICF ratio were more evident in subjects with younger age (20–39 year-old), women, non-Hispanic white ethnic, and subjects who were not active smokers.

Conclusions

Besides positively correlated with body measures and body fat, we found urine DEHP metabolites are positively correlated with ECF, ECF/ICF ratio in the US general adult population. It is necessary to do further research to clarify this causal relationship.

Background

Phthalates, known as EDCs, are the most common chemicals widely used in many products to increase the flexibility [1, 2]. People are widely exposed to phthalates because its metabolites are commonly detected in urine samples around the world [3]. Although the half-life of phthalates is only about 12 hours [4], due to continuous exposure, their effects are similar to persistent and bioaccumulating compounds [5]. Among the compound esters of phthalates, di-(2-ethylhexyl) phthalate (DEHP) has been the main plasticizer used for decades [6]. Recently, several epidemiological studies have linked DEHP exposure to several cardiovascular disease risk factors, such as diabetes mellitus [7] and higher systolic blood pressure [8].

In healthy adults, extracellular fluid (ECF) content composed ~ 33–40% of the total body water [9] and is regulated by the activity of the renin-angiotensin-aldosterone system (RAAS), autonomic nervous system, hormones, and natriuretic peptides [10]. The increased ECF might cause stress in cardiovascular system, which eventually contributes to the hypertension [11] and have been reported as an independent predictor of cardiovascular morbidity in patients with chronic renal failure [12]. The ECF/intracellular fluid (ICF) volume ratio also shows a significant association with chronic disease [13–16]. Recently, many environmental chemicals were reported to have influence on blood pressure and ECF. The mechanism was related to activation of RAAS by inhibiting 11β hydroxysteroid dehydrogenase, type 2 (11β-HSD-2) enzyme activity [17]. The activation of RAAS contributes to sodium retention and ECF expansion [18]. Previous animal and human studies have reported DEHP exposure were associated activation of RAAS through inhibiting 11β-HSD-2 [19, 20]. Moreover, DEHP exposure might increase the risk of obesity [21], while adipocytes are an important source of extra-adrenal aldosterone [22]. In addition, DEHP have been reported to increase epithelial sodium channel (ENaC) activity in renal cortical collecting duct cells [20], which can expand ECF volume in turn [23]. However, there is no previous epidemiological study to investigate the relationship between DEHP exposure and fluid status.

Bioimpedance analysis (BIA) is a tool for measuring electrical impedance of human tissues, which can be used to assess the proportion of lean mass, fat, and body fluids [24]. Unlike fluid volume, the association between DEHP exposure and other body measures has been extensively studied. When using body mass index (BMI), weight, and waist as parameters, some results showed higher levels of urine DEHP metabolites showed positive correlation [25–27] while others showed no correlation [28], or an inverse association [29]. The relationship between DEHP exposure and body fat percentage has been most investigated in children, and the results were inconsistent [30–33]. In adults, however, there were only few studies studying the association between DEHP exposure and lean mass/body fat percentage and the results were also inconsistent. [34, 35].

Since the relationship DEHP exposure and fluid status is unknown and there were few studies investigated the association between DEHP and lean mass/fat percentage in adults, to resolve the above questions, we included subjects enrolled by National Health and Nutrition Examination Survey (NHANES) 2003 to 2004, who have detailed information on urine DEHP metabolites, body measures, BIA, and various covariates. We hypothesized that urine DEHP metabolites may have a positive correlation with body measures, lean mass, fat mass, and body fluids in this representative sample of U.S. adults.

Materials And Methods

Study design and population:
The data for this study was obtained from NHANES 2003–2004. NHANES is a study of a representative sample of the U.S. population that collects information about family health and nutrition. The survey data is released every two years. Detailed contents of the NHANES 2003–2004 are available at the NHANES website [36]. Our analysis included 1,678 participants over the age of 18 without missing data on basic demographics, and BMI, and those who had undergone urine DEHP metabolite testing.

**Urine DEHP metabolites:**

In NHANES 2003–2004, urine DEHP metabolites including mono (2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) were tested by using high performance liquid chromatography-electrospray ionization-tandem mass spectrometry. For concentrations below detection limits, a value was assigned by NHANES. We used this value in our analyses. The detailed method is available at supplemental section.

**Body Measures:**

The body measurement assessments performed on survey participants varied according to the participants’ ages. Weight, BMI, triceps and subscapular skinfold were measured in NHANES 2003–2004. The detailed method is available at supplemental section.

**Bioelectrical Impedance Analysis (BIA):**

In NHANES 2003–2004, this examination was conducted in eligible survey participants 8–49 years of age. The BIA data were collected with a HYDRA ECF/ICF Bio-Impedance Spectrum Analyzer (Model 4200, Xitron Technologies, Inc., San Diego, California, USA). Detailed information is available at the supplemental section.

**Covariates:**

We considered age, gender, race/ethnicity, education level, household income, smoking status, caffeine intake, total energy intake, total protein intake, total sugar intake, total carbohydrate intake, total saturated fatty acid intake, metabolic equivalent intensity level for activity to be potential confounders in this study. Detailed information is available at the supplemental section.

**Statistics**

DEHP metabolites concentrations were corrected for urine creatinine and expressed as the geometric mean (geometric standard error) in different subpopulations. These variables were tested by Student’s 2-tailed t-test and one-way analysis of variance. Because DEHP is metabolized primarily into MEHP, MEOHP, MEHHP, and MECPP, we divided the level of each metabolite by its molar mass, and then added the concentrations of each metabolite to calculate Σ DEHP [37]. Due to the obvious deviation from the normal distribution, the DEHP metabolites were transformed by natural logarithm. We constructed an extended model approach with body measures/bioelectrical impedance analysis as the dependent variable and individual ln-DEHP metabolites as a predictor. Model 1 adjusted for age, gender, race and ethnicity, education level, household income, smoking status, and metabolic equivalent intensity level for activity. Model 2 adjusted for model 1 plus caffeine intake, total energy intake, total protein intake, total sugar intake, total carbohydrate intake, total saturated fatty acid intake.

We used sample weights for analysis to understand the impact of weights. The calculation of sampling weight follows the analysis guidelines of the National Center for Health Statistics, and appropriately considers the complex survey design adopted in NHANES 2003–2004 [38]. All analyses were calculated by SPSS Version 20 (SPSS Inc. Chicago, Illinois, U.S.A.). P < 0.05 was considered significant.

**Results**

MEHP, MEOHP, MEHHP, and MECPP were detectable in 69.9%, 99.1%, 99.7%, and 100% of study subjects, respectively. Table 1 shows the basic demographics of study subjects. The study participants composed of 798 men and 880 women. Subjects with aged between 40–59 years old, women, and higher education level were associated with higher all DEHP metabolites concentrations and Σ DEHP. Besides MEHP, subjects with higher income were associated with higher levels of other DEHP metabolites and Σ DEHP while these metabolites levels were different between races. Participants with BMI between 25–30 had a lower concentration of DEHP metabolites and Σ DEHP while active smokers had a lower concentration of MEOHP, MECPP, and Σ DEHP. Those who had body fat percentage ≥ 35% had a higher level of MEOHP, MECPP, and Σ DEHP.
Table 1
Basic demographics of the sample subjects including geometric means (geometric S.E.) of DEHP metabolites concentrations

|                          | Unweighted no. (%) | MEHP (µg/g) | MEOHP (µg/g) | MEHHP (µg/g) | MECPP (µg/g) | Σ DEHP (µmol/g) |
|--------------------------|--------------------|-------------|--------------|--------------|--------------|----------------|
| Overall                  | 1678 (100)         | 2.13 (1.03) | 12.18 (1.03) | 18.23 (1.03) | 29.44 (1.03) | 0.21 (1.03)    |
| Age, y                   |                    |             |              |              |              |                |
| 18–39                    | 718 (42.8)         | 2.64 (1.06) | 13.63 (1.05) | 20.15 (1.05) | 30.88 (1.04) | 0.23 (1.04)    |
| 40–59                    | 449 (26.8)         | 1.90 (1.06) | 10.67 (1.05) | 16.33 (1.05) | 25.89 (1.05) | 0.19 (1.05)    |
| ≥60                      | 511 (30.4)         | 1.72 (1.05) | 11.70 (1.04) | 17.46 (1.04) | 30.81 (1.04) | 0.21 (1.04)    |
| Gender                   |                    |             |              |              |              |                |
| Men                      | 798 (47.6)         | 1.85 (1.05) | 10.74 (1.04) | 16.52 (1.04) | 26.08 (1.04) | 0.19 (1.04)    |
| Women                    | 880 (52.4)         | 2.41 (1.04) | 13.66 (1.04) | 19.94 (1.04) | 32.86 (1.03) | 0.24 (1.03)    |
| Race                     |                    |             |              |              |              |                |
| Mexican American         | 365 (21.8)         | 1.96 (1.06) | 10.36 (1.06) | 15.18 (1.06) | 26.30 (1.05) | 0.19 (1.05)    |
| Other Hispanic           | 48 (2.9)           | 2.63 (1.17) | 12.92 (1.17) | 19.59 (1.18) | 32.47 (1.14) | 0.24 (1.15)    |
| Non-Hispanic White       | 842 (50.2)         | 2.09 (1.04) | 12.91 (1.04) | 19.19 (1.04) | 32.00 (1.04) | 0.23 (1.04)    |
| Non-Hispanic Black       | 345 (20.6)         | 2.37 (1.07) | 12.72 (1.06) | 19.64 (1.06) | 26.91 (1.06) | 0.21 (1.06)    |
| Others                   | 78 (4.5)           | 2.02 (1.14) | 11.09 (1.13) | 17.09 (1.13) | 28.41 (1.12) | 0.20 (1.12)    |
| Education levels         |                    |             |              |              |              |                |
| ≤ High school            | 927 (55.3)         | 1.94 (1.04) | 10.85 (1.03) | 16.04 (1.04) | 26.54 (1.03) | 0.19 (1.03)    |
| > High school            | 750 (44.7)         | 2.37 (1.05) | 14.06 (1.04) | 21.35 (1.04) | 33.47 (1.04) | 0.25 (1.04)    |
| Annual household income  |                    |             |              |              |              |                |
| <$25000                  | 556 (35.6)         | 2.02 (1.05) | 10.98 (1.05) | 16.50 (1.05) | 27.52 (1.04) | 0.20 (1.04)    |
| $25000–55000             | 503 (32.2)         | 2.00 (1.05) | 11.54 (1.05) | 17.25 (1.05) | 27.89 (1.05) | 0.20 (1.05)    |
| ＞$55000                 | 501 (32.2)         | 2.31 (1.06) | 14.07 (1.05) | 21.06 (1.05) | 33.01 (1.05) | 0.24 (1.05)    |
| BMI, kg/m²               |                    |             |              |              |              |                |
| <25                      | 577 (34.4)         | 2.31 (1.05) | 11.92 (1.05) | 17.77 (1.05) | 28.71 (1.05) | 0.21 (1.05)    |
| 25–30                    | 562 (33.5)         | 1.90 (1.05) | 10.93 (1.05) | 16.31 (1.05) | 27.29 (1.04) | 0.20 (1.04)    |
| ≥ 30                     | 539 (32.1)         | 2.19 (1.05) | 13.97 (1.05) | 21.06 (1.05) | 32.73 (1.04) | 0.24 (1.04)    |
| Body fat percentage (%)  |                    |             |              |              |              |                |
| <25                      | 227 (31.4)         | 2.22 (1.09) | 10.70 (1.08) | 16.81 (1.09) | 25.82 (1.08) | 0.19 (1.08)    |
| 25–35                    | 230 (31.9)         | 2.00 (1.09) | 11.33 (1.08) | 17.11 (1.08) | 25.87 (1.07) | 0.20 (1.07)    |
| ≥ 35                     | 265 (36.7)         | 2.53 (1.08) | 14.19 (1.07) | 20.83 (1.08) | 32.21 (1.07) | 0.24 (1.07)    |
| Smoking                  |                    |             |              |              |              |                |
| Nonexposed               | 353 (21.0)         | 2.16 (1.07) | 12.10 (1.06) | 18.26 (1.06) | 31.27 (1.05) | 0.22 (1.06)    |
| Expose to ETS            | 849 (50.6)         | 2.15 (1.04) | 12.99 (1.04) | 19.04 (1.04) | 31.15 (1.04) | 0.23 (1.04)    |
| Active smokers           | 476 (28.4)         | 2.07 (1.06) | 10.93 (1.05) | 18.23 (1.05) | 25.46 (1.05) | 0.19 (1.05)    |

* p < 0.05; † p < 0.01; ‡ p < 0.005 (tested by Student’s 2-tailed t-test or by one-way analysis of variance)

Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; ETS, Environmental tobacco smoke; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxoehexyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate.

Σ DEHP were the sum of (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) and corrected for urine creatinine.
| Unweighted no. (%) | MEHP (µg/g) | MEOHP (µg/g) | MEHHP (µg/g) | MECPP (µg/g) | Σ DEHP (µmol/g) |
|-------------------|------------|-------------|-------------|-------------|----------------|
| <12               | 412 (29.5) | 2.01 (1.06) | 11.86 (1.05) | 17.38 (1.05) | 2.52 (1.05)    |
| ≥ 12              | 983 (70.5) | 2.05 (1.04) | 11.89 (1.04) | 17.97 (1.04) | 2.32 (1.03)    |

*, p < 0.05; †, p < 0.01; ‡, p < 0.005 (tested by Student’s 2-tailed t-test or by one-way analysis of variance)

Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; ETS, Environmental tobacco smoke; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MECPP, Mono(2-ethyl-5-carboxypentyl) phthalate.

Σ DEHP were the sum of (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) and corrected for urine creatinine.

The means and S.E. of body fluid status between basic demographic of the sample subjects are shown in Table 2. Subjects with men, higher household income, higher BMI, lower body fat percentage, active smoking, and higher alcohol consumption were associated with higher ECF and ICF volume. Higher ECF volume was also found in subjects who were non-Hispanic white. Subjects with aged between 40–59 years old, women, higher education level, lower BMI, higher body fat percentage, and lower alcohol consumption were associated with higher ECF/ICF ration while lower ECF/ICF ratio was found in other Hispanic.
| Table 2 | Basic demographics of the sample subjects including means (S.E.) of body fluid status |
|---------|------------------------------------------------------------------|
| Overall | Unweighted no. (%) | ECF (L) | ICF (L) | ECF/ICF ratio |
|         | 726 (100)         | 16.92 (0.13) | 23.35 (0.24) | 0.74 (0.00) |
| Age, y  |                  |          |         |             |
| 18–39   | 524 (42.8)        | 16.80 (0.16) | 23.42 (0.29) | 0.74 (0.00) ‡ |
| 40–50   | 202 (26.8)        | 17.24 (0.24) | 23.16 (0.42) | 0.76 (0.01) ‡ |
| Gender  |                  |          |         |             |
| Men     | 377 (47.6)        | 19.00 (0.15) | 27.61 (0.28) | 0.70 (0.00) ‡ |
| Women   | 349 (52.4)        | 14.67 (0.15) | 18.74 (0.22) | 0.79 (0.00) ‡ |
| Race    |                  |          |         |             |
| Mexican American | 172 (21.8) | 15.95 (0.23) | 22.32 (0.43) | 0.73 (0.01) * |
| Other Hispanic | 27 (2.9)   | 15.99 (0.64) | 22.92 (1.28) | 0.72 (0.02) * |
| Non-Hispanic White | 319 (50.2) | 17.45 (0.21) | 23.78 (0.38) | 0.75 (0.01) * |
| Non-Hispanic Black | 172 (20.6) | 17.13 (0.27) | 23.78 (0.49) | 0.74 (0.01) * |
| Others  | 36 (4.5)          | 16.59 (0.65) | 22.70 (1.22) | 0.75 (0.01) * |
| Education levels |      |          |         |             |
| ≦ High school | 376 (55.3) | 16.88 (0.18) | 23.58 (0.33) | 0.73 (0.00) ‡ |
| >High school | 350 (44.7) | 16.97 (0.20) | 23.10 (0.36) | 0.75 (0.00) ‡ |
| Annual household income | |          |         |             |
| <$25000  | 203 (35.6)        | 16.40 (0.23) | 22.50 (0.41) | 0.74 (0.01) |
| $25000–55000 | 227 (32.2) | 16.79 (0.24) | 23.32 (0.44) | 0.74 (0.01) |
| >$55000  | 248 (32.2)        | 17.62 (0.24) | 24.25 (0.45) | 0.75 (0.00) |
| BMI, kg/m² |              |          |         |             |
| <25     | 301 (34.4)        | 15.04 (0.18) | 20.04 (0.31) | 0.77 (0.01) ‡ |
| 25–30   | 218 (33.5)        | 17.22 (0.20) | 24.35 (0.38) | 0.72 (0.01) ‡ |
| ≥ 30    | 207 (32.1)        | 19.35 (0.23) | 27.10 (0.46) | 0.73 (0.01) ‡ |
| Body fat percentage (%) | |          |         |             |
| <25     | 229 (31.4)        | 18.52 (0.21) | 27.79 (0.38) | 0.67 (0.00) ‡ |
| 25–35   | 230 (31.9)        | 16.94 (0.26) | 23.24 (0.42) | 0.74 (0.00) ‡ |
| ≥ 35    | 267 (36.7)        | 15.54 (0.19) | 19.62 (0.29) | 0.80 (0.01) ‡ |
| Smoking |                  |          |         |             |
| Nonexposed | 109 (21.0) | 15.91 (0.35) | 21.45 (0.60) | 0.76 (0.01) |
| Expose to ETS | 356 (50.6) | 16.74 (0.19) | 23.23 (0.35) | 0.74 (0.00) |
| Active smokers | 257 (28.4) | 17.63 (0.21) | 24.39 (0.39) | 0.74 (0.01) |
| Alcohol consumption (drink/year) | |          |         |             |
| <12     | 124 (29.5)        | 16.34 (0.36) | 21.87 (0.62) | 0.77 (0.01) ‡ |

*, p < 0.05; †, p < 0.01; ‡, p < 0.005 (tested by Student’s 2-tailed t-test or by one-way analysis of variance)
Abbreviations: ETS, Environmental tobacco smoke;
After weighting for sampling strategy, the correlations between DEHP metabolite levels and various body component measures are shown in Table 3. Levels of MEOHP, MEHHP, MECPP, and ΣDEHP was positively associated with weight, BMI, waist. The correlations between DEHP metabolite levels and parameters of bioelectrical impedance analysis are shown in Table 4. Levels of MEOHP, MEHHP, MECPP, and ΣDEHP was positively correlated with estimated fat mass, estimated percent body fat, ECF, and ECF/ICF ratio.

Table 3
Linear regression coefficients (S.E.) of body measures with one unit increase in ln-DEHP metabolites concentrations in adults, with results weighted for sam strategy

| Body measures | MEHP (µg/g) | P | MEOHP (µg/g) | P | MEHHP (µg/g) | P | MECPP (µg/g) | P | Σ DEHP (µmol/g) |
|---------------|------------|---|--------------|---|--------------|---|-------------|---|----------------|
| Body weight (Kg) |            |    |              |    |              |    |              |    |                |
| Model 1       | 0.092(0.501) | 0.856 | 2.020(0.760) | 0.018 | 2.047(0.648) | 0.007 | 2.003(0.766) | 0.019 | 2.031(0.749) |
| Model 2       | 0.023(0.452) | 0.961 | 1.679(0.572) | 0.010 | 1.704(0.538) | 0.006 | 1.520(0.616) | 0.026 | 1.627(0.593) |
| Body mass index (kg/m²) |            |    |              |    |              |    |              |    |                |
| Model 1       | 0.060(0.157) | 0.711 | 0.679(0.211) | 0.006 | 0.696(0.190) | 0.002 | 0.709(0.227) | 0.007 | 0.705(0.217) |
| Model 2       | 0.052(0.145) | 0.726 | 0.598(0.174) | 0.004 | 0.617(0.177) | 0.003 | 0.578(0.200) | 0.011 | 0.602(0.189) |
| Waist (cm)    |            |    |              |    |              |    |              |    |                |
| Model 1       | -0.012(0.409) | 0.978 | 1.549(0.534) | 0.011 | 1.532(0.468) | 0.005 | 1.512(0.515) | 0.010 | 1.533(0.521) |
| Model 2       | 0.059(0.414) | 0.888 | 1.374(0.423) | 0.005 | 1.342(0.403) | 0.005 | 1.209(0.391) | 0.007 | 1.305(0.411) |
| Subscapular Skinfold (mm) |            |    |              |    |              |    |              |    |                |
| Model 1       | -0.362(0.210) | 0.104 | 0.082(0.274) | 0.770 | 0.151(0.272) | 0.586 | 0.133(0.278) | 0.640 | 0.073(0.284) |
| Model 2       | -0.423(0.206) | 0.058 | 0.019(0.258) | 0.943 | 0.113(0.258) | 0.669 | 0.038(0.244) | 0.877 | 0.005(0.260) |
| Triceps Skinfold (mm) |            |    |              |    |              |    |              |    |                |
| Model 1       | -0.253(0.180) | 0.181 | 0.263(0.228) | 0.267 | 0.237(0.199) | 0.254 | 0.261(0.256) | 0.323 | 0.237(0.241) |
| Model 2       | -0.198(0.164) | 0.245 | 0.272(0.216) | 0.227 | 0.234(0.200) | 0.262 | 0.205(0.234) | 0.395 | 0.216(0.223) |
| Model 1 was adjusted for age, gender, race/ethnicity, educational level, household income, smoking status, and metabolic equivalent intensity level for activit |
| Model 2 was adjusted for model 1 plus caffeine intake, total energy intake, total protein intake, total sugar intake, total carbohydrates intake, total saturated f acids intake, |
| Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl oxohexyl) phthalate; MECPP, Mono(2-ethyl-5-carboxypentyl) phthalate. |
| Σ DEHP were the sum of (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) and corrected for urine creatinine |
Table 4
Linear regression coefficients (S.E.) of bioelectrical impedance analysis parameters with one unit increase in ln-DEHP metabolites concentrations in adults, results weighted for sampling strategy

| Bioelectrical Impedance Analysis | MEHP (µg/g) | P | MEOHP (µg/g) | P | MEHHP (µg/g) | P | MECPP (µg/g) | P | Σ DEHP (µmol/g) | F |
|---------------------------------|-------------|---|-------------|---|-------------|---|-------------|---|----------------|---|
| Lean mass (kg)                  |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 674/96499431 | -0.012(0.320) | 0.970 | 0.654(0.444) | 0.162 | 0.692(0.382) | 0.090 | 0.562(0.481) | 0.262 | 0.635(0.444) | 0 |
| Model 2                         | 415/61088209 | -0.020(0.296) | 0.948 | 0.608(0.384) | 0.134 | 0.640(0.364) | 0.099 | 0.497(0.416) | 0.251 | 0.579(0.393) | 0 |
| Fat mass (kg)                   |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 674/96499431 | 0.806(0.610) | 0.206 | 1.646(0.675) | 0.028 | 1.579(0.617) | 0.022 | 1.639(0.697) | 0.033 | 1.666(0.695) | 0 |
| Model 2                         | 415/61088209 | 1.000(0.546) | 0.087 | 1.716(0.602) | 0.012 | 1.560(0.580) | 0.017 | 1.547(0.639) | 0.029 | 1.651(0.636) | 0 |
| Percent body fat (%)            |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 674/96499431 | 0.419(0.377) | 0.285 | 0.800(0.383) | 0.034 | 0.718(0.368) | 0.040 | 0.808(0.374) | 0.047 | 0.794(0.391) | 0 |
| Model 2                         | 415/61088209 | 0.632(0.352) | 0.093 | 0.951(0.337) | 0.013 | 0.789(0.336) | 0.033 | 0.865(0.348) | 0.025 | 0.894(0.354) | 0 |
| Cell membrane capacitance (nF)  |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 674/96499431 | -0.024(0.028) | 0.416 | -0.008(0.022) | 0.716 | -0.001(0.018) | 0.952 | -0.014(0.021) | 0.525 | -0.009(0.020) | 0 |
| Model 2                         | 415/61088209 | -0.022(0.029) | 0.469 | -0.002(0.018) | 0.925 | 0.004(0.018) | 0.824 | -0.009(0.020) | 0.653 | -0.004(0.020) | 0 |
| ECF (L)                         |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 674/96499431 | 0.134(0.101) | 0.203 | 0.321(0.149) | 0.049 | 0.308(0.133) | 0.035 | 0.316(0.167) | 0.078 | 0.327(0.156) | 0 |
| Model 2                         | 415/61088209 | 0.147(0.085) | 0.105 | 0.289(0.126) | 0.037 | 0.271(0.119) | 0.038 | 0.279(0.143) | 0.069 | 0.292(0.134) | 0 |
| ICF (L)                         |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 674/96499431 | -0.106(0.151) | 0.493 | 0.196(0.191) | 0.321 | 0.230(0.162) | 0.175 | 0.139(0.203) | 0.505 | 0.179(0.186) | 0 |
| Model 2                         | 415/61088209 | -0.120(0.147) | 0.430 | 0.189(0.168) | 0.278 | 0.224(0.160) | 0.184 | 0.123(0.178) | 0.498 | 0.168(0.169) | 0 |
| ECF/ICF ratio                   |             |   |             |   |             |   |             |   |                |   |
| Model 1                         | 471/68616209 | 0.008(0.003) | 0.010 | 0.008(0.002) | 0.004 | 0.006(0.002) | 0.021 | 0.009(0.003) | 0.005 | 0.008(0.003) | 0 |
| Model 2                         | 415/61088209 | 0.009(0.003) | 0.003 | 0.007(0.002) | 0.004 | 0.005(0.002) | 0.041 | 0.009(0.002) | 0.002 | 0.008(0.002) | 0 |

Model 1 was adjusted for age, gender, race/ethnicity, educational level, household income, smoking status, and metabolic equivalent intensity level for activity level.

Model 2 was adjusted for model 1 plus caffeine intake, total energy intake, total protein intake, total sugar intake, total carbohydrates intake, total saturated fatty acids intake.

Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-oxohexyl) phthalate; MECPP, Mono(2-ethyl-5-carboxypentyl) phthalate; ECF, extracellular fluid; ICF, intracellular fluid.

Σ DEHP were the sum of (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) and corrected for urine creatinine.
Identifying adiposity, because they could not distinguish half of people with high body fat. BMI and waist are generally used as screening tools for overweight or obesity with high specificity after 6 years. Analyses, MEOHP levels have positive trends with weight gain between baseline and year 3. However, no statistically significant association was observed reported the relationship between BMI and DEHP may be explained by higher energy intake and the consequent higher DEHP exposure. 1999–2002 NHANES data. Σ BMI/waist/weight gain as markers of obesity. In cross-sectional studies, one study observed higher MEHP levels were associated with increased obesity. Several recent epidemiological studies have surveyed the association between DEHP exposure and obesity in adults. All the previous studies used obesogens “. In the 3T3-L1 cell model, DEHP metabolites induce adipogenesis through this mechanism. Obesity increases the incidence of cardiovascular disease, diabetes mellitus, and certain cancers. In the United States, the prevalence of obesity was 42.4% in adults. Its related health issues have a significant economic impact on the U.S. medical system, estimated to be approximately US$147 billion in 2008. Traditionally, the causes of obesity is resulting from a combination of behavior, genetics, diseases and medications. Recently, it has been discovered that the effects of environmental toxins, especially those defined as endocrine-disrupting chemicals (EDCs), can promote obesity and are called “hypothyroidism and hypothalamic leptin resistance is a possible mechanism. In animal studies, female C3H/N mice exposed to DEHP doses similar to environmental exposures increased food intake, body weight, and visceral fat deposits. In male C3H/He mice, chronic exposure to DEHP may induce obesity through changes in energy homeostasis. The synergistic effect of hypothryoidism and hypothalmic leptin resistance is a possible mechanism.

Several recent epidemiological studies have surveyed the association between DEHP exposure and obesity in adults. All the previous studies used BMI/waist/weight gain as markers of obesity. In cross-sectional studies, one study observed higher MEHP levels were associated with increased obesity prevalence in NHANES 1999–2004. Another study using data from the 2007–2010 NHANES, the authors reported higher levels of MECPP, MEHHP, and ΣDEHP were associated with increased prevalence of obesity. However, other studies found MEHP levels have inverse associations with obesity in both 1999–2002 NHANES data and the Nurses’ Health Study (NHS) and NHS2 cohorts. By using physiologically-based pharmacokinetic model, a study reported the relationship between BMI and DEHP may be explained by higher energy intake and the consequent higher DEHP exposure. In prospective analyses, MEOHP levels have positive trends with weight gain between baseline and year 3. However, no statistically significant association was observed after 6 years. In the current study, we found higher levels of DEHP metabolites were correlated with higher body measures parameters (weight, BMI, and waist). Differences between studies may be due to a variety of factors, such as race, measurement method, lifestyle, and food.

BMI and waist are generally used as screening tools for overweight or obesity with high specificity. However, these two parameters are less sensitive in identifying adiposity, because they could not distinguish half of people with high body fat. Some experts suggest the value of assessing body-fat
The fluid volume in human depends on age, gender, and body size and is also associated with human health. Extracellular fluid (ECF) is controlled by sodium balance and total body sodium content. Osmolality of the ECF is regulated by water intake and vasopressin secretion while the ECF volume is maintained by RAAS and some natriuretic factors, including atrial/brain natriuretic peptide [10]. In addition, growth hormone and sex hormones have been found to have a role in ECF volume [23, 51]. Environmental chemicals which have capacity to inhibit 11β-HSD-2. would be predicted to elevate blood pressure and expand ECF [17]. In mice, DEHP exposure have been reported to elevate blood pressure through activation of RAAS [19]. In premature infants, higher levels of postnatal DEHP exposure are correlated with increased blood pressure and hypertension. Statistical analysis showed this relationship was mediated by the cortisol-to-cortisone ratio, suggesting the mechanism may be because DEHP inhibits the activity of 11β-HSD-2 and then activates the mineralocorticoid receptor. In this study, increased expression of ENaC and phosphorylated Na+–Cl− cotransporter were also found in hypertensive infants [20]. Moreover, adipocytes produced 30% of the total blood angiotensinogen [52] and are also an important source of extra-adrenal aldosterone [22]. It is possible that DEHP induced activation of RAAS, and then contributes to sodium retention and ECF expansion [18]. Another possible mechanism is that DEHP, as an endocrine disruptive agent, have been reported to have estrogenic activity both in vitro and in vivo [53]. The high estrogen status will then increase ENaC activity via protein kinase C δ signaling in renal cortical collecting duct cells and expands ECF volume [23]. In the current study, we provide the first evidence that levels of DEHP metabolites were positively correlated with ECF, and ECF/ICF ratio. Since increased ECF may cause stress on the cardiovascular system [12], which may be one of the possible mechanisms of DEHP causing cardiovascular disease.

In the subgroup analysis, the correlation between ΣDEHP and ECF/ICF ratio were more evident in subjects with women. Women are exposed to a higher concentration of phthalates than men because of the personal care products they used [54]. Furthermore, report has shown there were sex differences in the regulation of RAAS. The adrenal response to exogenous angiotensin II was significantly higher in women. It is possible that the effect of DEHP induced RAAS may be more evident in women than in men [55]. We also found that the association between ΣDEHP and the ECF/ICF ratio was more pronounced among subjects who were not current smokers. Studies show that body composition may be altered by smoking habits [56, 57]. A possible explanation is that DEHP has a much weaker effect on body fluids than tobacco smoke. When considering the impact of DEHP on active smokers, the trend is too small to be statistically significant.

Our research has several limitations. First, causal inference is not suitable for cross-sectional research. Secondly, when DEHP is exposed, other undetected chemicals may be exposed at the same time, and the correlation found in the research is not caused by DEHP itself. Third, our study population is mainly composed of adults, so we cannot infer that this association also exists in children. Lastly, other restriction includes over-adjustments because of the relatively small number of cases.

**Conclusions**

Although previous reports have explored the association between DEHP exposure and obesity parameters, including BMI, waist circumference, and fat percentage in adults, we present the first report identifying a positive association of urine DEHP metabolites with body fluid status in a nationally representative survey of U.S. adults. Since DEHP exposure has become a worldwide concern, further research is necessary to determine the long-term mechanism and effects of low-dose DEHP exposure on human health.

**Abbreviations**

BIA bioimpedance analysis
BMI body mass index
DEHP di(2-ethylhexyl) phthalate
ECF extracellular fluid
EDCs endocrine-disrupting chemicals
ICF intracellular fluid
MECPP mono(2-ethyl-5-carboxypentyl) phthalate
MEHP mono(2-ethyl-5-carboxypentyl) phthalate
mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHP
mono (2-ethylhexyl) phthalate
MEOHP
mono(2-ethyl-5-oxohexyl) phthalate
NHANES
National Health and Nutrition Examination Survey
NHS
Nurses’ Health Study
RAAS
renin-angiotensin-aldosterone system

Declarations

Ethics approval and consent to participate: National Center for Health Statistics Research Ethics Review Board Approval

Consent for publication: Consent documents are part of the protocol for the NHANES, conducted by the National Center for Health Statistics.

Availability of data and material: All the data are available at NHANES website.

Competing interests: The authors declared that the methods were carried out in accordance with the approved guidelines. The authors have declared that no other conflict of interest exists. No competing financial interest was declared.

Funding: There was no specific funding for this study

Author's contributions: Chien-Yu Lin developed the theoretical concept and performed the analytic calculations. Wei-Jie Wang and Chia-Sung Wang contributed to manuscript drafting. Chi-Kang Wang verified the analytical method. An-Ming Yang and Chien-Yu Lin contributed to critical discussion and the final version of the manuscript.

Acknowledgments: We would like to thank the many individuals who have contributed to the National Health and Nutrition Examination Survey data, including all of the anonymous participants in the present study. We are particularly grateful to those who carried out the laboratory assays for DEHP at the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention.

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