Mediation effects of thyroid function in the associations between phthalate exposure and lipid metabolism in adults

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Abstract
Phthalates are a group of industrial chemicals widely used in everyday products including cosmetics, food packaging and containers, plastics, and building materials. Previous studies have indicated that urinary phthalate metabolites are associated with metabolic effects including those on lipid metabolism, but the results are mixed. Furthermore, whether thyroid function mediates the association between phthalate exposure and lipid metabolism remains unclear. In the present study, we explored whether changes in thyroid function markers mediate the associations between phthalate exposure and lipid metabolism indicators in Taiwanese adults. The cross-sectional data were obtained from the Taiwan Environmental Survey for Toxicants conducted in 2013. Levels of 11 urinary phthalate metabolites, levels of 5 thyroid hormones, and 8 indicators of lipid metabolism were assessed in 222 Taiwanese adults. The relationships of urinary phthalate metabolite levels with serum thyroid hormone levels and lipid metabolism indicators were explored using multiple regression models. Mediation analysis was conducted to evaluate the role of thyroid function in the association between phthalate exposure and lipid metabolism. The metabolite of di(−2-ethylhexyl) phthalate (ΣDEHPm) exhibited a significant positive association with the lipid metabolite indicator of high-density lipoprotein cholesterol (HDL-C; β = 0.059, 95% confidence interval [CI] = 0.009, 0.109) in adults, and the thyroid function indicator thyroxine (T4) had a significant negative association with the metabolite ΣDEHPm (β = −0.059, 95% CI = −0.101, −0.016) and a significant negative association with HDL-C (β = −0.284, 95% CI = −0.440, −0.128). The T4 indirect effect was 0.015 (95% CI = −0.0087, 0.05), and the mediation effect was 32.2%. Our results support the assumption that exposure to phthalates influences the homeostasis of lipid metabolism by interfering with thyroid function.

Keywords: Phthalates, Metabolic effect, Thyroid function, Mediating effect, Lipid metabolism

Introduction
Plastics have become an essential part of everyday life. Plasticizers are indispensable substances in the manufacture of plastic goods. They are used as lubricants, stabilizers, and flavoring agents in various products, including medical devices, pharmaceuticals, personal hygiene products, food packaging, and containers. In Taiwan, a plasticizer scandal occurred on 2011 when the Taiwan Food and Drug Administration detected plasticizers in a batch of probiotic ingredients, which led to revelations that unscrupulous manufacturers were including inedible additives (instead of clouding agents) in beverages, pastries, bread, medicines, and other consumables to save costs [1]. This caused shock and panic throughout Taiwan. Although the scandal receives less attention today, plasticized products that are hazardous to health are still around us and threaten the next generation.
Plasticizers are wide ranging, with the commonly used type of compound being phthalates such as di-(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate (BBzP), diethyl phthalate (DEP), dibutyl phthalate (DBP), dimethyl phthalate (DMP), di-isonylnyl phthalate (DINP), di-n-octyl phthalate (DNOBP), di-isodecyl phthalate (DIDDP).

In Taiwan, these common types of phthalates are regulated by the Environmental Protection Administration’s Poisons and Chemicals Bureau and are classified as toxic chemical substances (categories 1 and 2) with the characteristics of being difficult to decompose and chronically toxic. Among these common types of phthalates, DEHP was the most widely used phthalate in Taiwan at 2013, and it is often added to food containers, construction materials, medical devices, and toys. DEHP is colorless and odorless at room temperature and pressure and is a water-insoluble, fat-soluble, viscous liquid with a molecular weight of 390.56. Its molecular formula is C_{20}H_{24}O_{4}. The International Agency for Research on Cancer classified DEHP as a 2B human carcinogen, which led the European Union (EU) to restrict its use in toys in 1999 [2].

Phthalates, also known as endocrine disruptors, may have a profound effect on body metabolism and exert related metabolic effects. Bajkin et al. suggested that prolonged low-dose phthalate exposure poses a health risk either through interference with endocrine system effects (anti-androgen, thyroid hormone) or gene expression, leading to systemic diseases such as abdominal obesity [3]. In an animal study, phthalates were discovered to impair reproductive function or cause liver tumors [4]. Exposure to phthalates like DEHP can affect thyroid signaling by interfering with thyroid-stimulating hormone (TSH) receptors, binding to transporter proteins, and altering thyroid follicle cells’ iodine uptake through several potential mechanisms [5, 6]. Experimental data from animal cells indicate that exposure to phthalates alters adipogenesis and lipid metabolism [7]. Exposure to phthalates also promotes weight gain by binding to the peroxisome proliferator–activated receptor (PPAR), which regulates fatty acid storage [8].

Thyroid function indicators are TSH, triiodothyronine (T3), thyroxine (T4), free triiodothyronine (free T3), and free thyroxine (free T4). These hormones regulate the body’s energy metabolism, growth, development, and reproductive system. In a study of phthalate exposure and indicators of thyroid function in adults, negative correlations were observed between urinary DEHP metabolites and T3 as well as free T3 [9]. Another study noted a negative correlation between DEHP and T4 in adults but a positive association between T4 and DEHP in adolescents [10]. Similar results have been reported in other studies. For example, Huang et al. discovered that DEHP metabolites were negatively correlated with free T4 and T3 in adults, but BBzP metabolites were positively associated with free T4 in minors [11]. A study in children revealed a positive relationship between free T3 and DEHP metabolites and negative relationships of both DBP and BBzP metabolites with T4 [12]. A Korean study demonstrated that DEHP metabolites were only negatively correlated with T4 in men; in women, DBP and BBzP metabolites were each negatively correlated with both TSH and T3 [13]. Phthalate exposure is also correlated with thyroid function indicators in pregnant women and children [14]. However, another study noted no such association with these indicators [15].

In addition, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and total cholesterol (TC) are indicators of lipid metabolism. The Castelli risk index I (CRI-I), Castelli risk index II (CRI-II), non-HDL cholesterol (NHC), and atherogenic coefficient (AC) are indexes derived from the lipid ratio. These indicators (and thyroid function indicators) involve potentially complex physiological mechanisms. Thyroid hormones play a key role in regulating body metabolism; for example, thyroid hormones stimulate lipid synthesis and promote lipolysis [16]. The aforementioned biological indicators are also related to obesity, cerebrovascular disease, and cardiovascular disease. In recent years, the effects of abnormal lipid metabolism and physiological effects have attracted considerable research attention. Tóth et al. using the National Health and Nutrition Examination Survey 2003–2006 reported an estimated 53% of U.S. adults have lipid abnormalities: 27% have high LDL-C, 23% have low HDL-C, and 30% have high TG, in which 21% of U.S. adults have mixed dyslipidemia (high HDL-C with either low HDL-C and/or high TG), with nearly 6% having all three lipid abnormalities [17]. High LDL-C, high TG, high HDL-C, and low HDL-C levels along with hypertension and obesity are considered risk factors for stroke and cardiovascular disease [18–20]. Epidemiological studies demonstrated a correlation between indicators of thyroid function and biological indicators of lipid metabolism, and phthalates may interfere with thyroxine and affect the regulation of metabolism [3]. This suggests that changes in thyroid function may cause imbalances in body regulation of metabolism, and that these biological indicators are pivotal factors in the prevention of disease. Our previous study indicated exposure to phthalates may affect thyroid function to increase the risk of insulin resistance, and free T4 acted as a mediating factor that affects insulin resistance [21]. Thus, exploring early biomarkers involved in exposure to phthalates and lipid metabolism is warrant. Therefore, elucidating the
changes in thyroid function and lipid metabolism indicators caused by phthalates is critical.

Materials and methods
Study participants
This cross-sectional study investigated subsamples of participants to the Taiwan Environmental Survey for Toxicants (TEST) [21–23]. We cooperated with Taiwan National and Nutrition Health Survey team (NAHSIT) to employ the same sampling method and participant recruitment strategy. For NAHSIT, participants from all age groups and 20 major cities or counties in Taiwan were selected. Each Taiwanese city or township is divided into two groups according to urbanization and population density. A city or county is represented by two townships selected at random from each group. Individuals who had a severe illness (e.g., cancer), were pregnant or breastfeeding, were imprisoned or hospitalized, or were not Taiwanese nationals were excluded from the study. All of the participants were at least 7 years old and from Taiwan. The TEST results from May through December 2013 for 11 Taiwanese cities or counties were included. We interviewed a total of 500 subjects on the day of enrollment. Before enrolment, all individuals provided informed consent and had been volunteers who had joined NAHSIT. This study comprised 296 TEST participants aged larger than 18 years, with 25 participants excluded due to insufficient urine or blood samples, 48 participants excluded due to self-reported diabetes mellitus or thyroid dysfunction, and 1 participant excluded due to missing data on self-reported cigarette smoking habits. A total of 222 participants were included in the final analysis (Fig. 1). Individual characteristics (e.g., sex, age, and BMI) as well as lifestyle exposures (e.g., alcohol and cigarette usage) were collected via a questionnaire. This study was approved by the National Health Research Institutes’ Research Ethics Committee at Taiwan (no. EC1020206).

Measurement of urinary phthalate metabolite level
Participant’s morning spot urine was analyzed for seven phthalates, namely DEHP, DiBP, DnBP, DEP, DMP, DINP, and BBzP. Additionally, 11 urinary phthalate metabolites were assessed: mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), monoethylhexyl phthalate (MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECIPP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOH), monoethyl phthalate (MEP), mono-n-butyl phthalate (MBnP), mono-(2-carboxymethylhexyl) phthalate (MCMHP), mono(isobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), monomethyl phthalate (MMP), and monoisononyl phthalate (MiNP). Phthalate metabolites concentrations were measured using online liquid chromatography instrument and an Agilent 1200/API 4000 tandem mass spectrometry system (Applied Biosystems, Foster City, CA, USA) [22, 24], detailed information is included in Supplementary materials. Furthermore, the sums of the DEHP metabolite (ΣDEHPm) and DBP metabolite (ΣDBPm) molar concentrations were derived [25, 26]. For MEOHP, MECIPP, MBzP, MEP, MMP, and MEHHP, the limit of detection (LOD) was 0.3 ng/mL. Furthermore, the LOD for MiBP and MnBP was 1.0 ng/mL, the LOD for MiNP and MCMHP was 0.1 ng/mL, and the LOD for MEHP was 0.7 ng/mL. When the phthalate metabolite levels were less than the LOD, we used half of the LOD value as a substitute [27]. Each batch of analyzed samples contained a blank, repeated quality control (QC) sample. The QC sample for each sample batch in pooled urine samples was spiked with a mixture of phthalate metabolite standards (20–50 ng/mL). The QC sample’s relative percentage difference was to be less than ±30%, and QC sample’s recovery rate was to be 100%±20% [28].

Levels of thyroid hormones and lipid metabolism indicators
Thyroid function (e.g., T3, T4, free T4, thyroxine-binding globulin [TBG], and TSH levels) and lipid metabolism indicators (e.g., HDL-C, LDL-C, TG, and TC levels) were assessed in all participants by using a morning fasting blood test. A chemistry analyzer (Beckman Coulter Unicel DxC 800), chemiluminescent microparticle immunoassay system (Beckman Coulter Inc., Brea, CA, USA), and immunoenzymometric assay (Monobind Inc., Product Code 3525–300) were employed to assess serum thyroid hormone (i.e., T3, T4, free T4, and TSH), TBG, HDL-C, LDL-C, TG, and TC levels (detailed information is included in Supplementary materials). The aforementioned analyses of thyroid function were randomly performed in Taiwan Accreditation Foundation-certified laboratories (no. 1447 and 1673), which are recognized by the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement, by a technician blinded to the participants’ thyroid condition [11, 29]. The majority of thyroid hormone levels in our population were inside the reference ranges. The adults with T3 > T3 < T4, free T4, TSH, and TBG levels that were outside the reference ranges accounted for 2.7, 4.5, 6.8, 5.0, and 29.3% of the participants, respectively. In addition, the adults with T3 > T3 < T4, free T4, TSH, and TBG levels that were below the reference ranges accounted for 0.9, 3.6, 6.3, 0.9, and 10.8% of the participants, whereas the adults with T3 > T3 < T4, free T4, TSH, and TBG levels that were above the reference ranges accounted for 1.8, 0.9, 0.5, 4.1, and 18.5%
of the participants. Due to the lack of information, the reference range for TBG was for Caucasian which may result in the high percentage exceeding rate here. The adults with TC, HDL-C, LDL-C, and TG levels outside the reference range constituted 39.2, 9.0, 29.7, and 22.1% of the participants, respectively. The CRI-I, CRI-II, AC, and NHC were calculated using the following formulas. CRI-I was derived by dividing TC by HDL-C, and CRI-II was derived by dividing LDL-C by HDL-C [30]. NHC was derived by subtracting TC by HDL-C [31], and AC was derived by subtracting TC by HDL-C and then divided by HDL-C [32].

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\text{CRI-I} = \frac{\text{TC}}{\text{HDL-C}}
\]

\[
\text{CRI-II} = \frac{\text{LDL-C}}{\text{HDL-C}}
\]

\[
\text{NHC} = \text{TC} - \text{HDL-C}
\]

\[
\text{AC} = \frac{(\text{TC} - \text{HDL-C})}{\text{HDL-C}}
\]

CRI-I, also known as the cardiac risk ratio, indicates the formation of coronary plaques and has a diagnostic value comparable to total cholesterol. CRI-II has been found to be an excellent predictor of cardiovascular risk.
which TBG could be a potential confounder related to thyroid hormone levels. TBG binds thyroid hormones in circulation, in estimated coefficient larger than 10% (i.e., TBG levels) used to perform mediation analysis to evaluate indirect and direct effects and estimate the proportions of mediation when exposure of phthalate metabolite was significantly associated with distinct thyroid hormones or lipid metabolism indicators that had previously been significantly associated with each other ($p=0.05$ as significance level in multiple linear regressions). Briefly, a mediation analysis is comprised of three sets of regression: $X \rightarrow Y$, $X \rightarrow M$, and $X + M \rightarrow Y$. Suppose $Y=b_0 + b_1X + e_1$, $M=b_0 + b_2X + e_2$, $Y=b_0 + b_3X + b_4M + e_3$, the indirect effect is calculated as $b_1 - b_2$ or $b_2 \times b_4$, and direct effect is $b_4$. The proportions of mediation is calculated as indirect effect divide by total effect $b_1$. Details of mediation analysis have been described by Huang et al. [21]. R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria) was used to conduct all statistical analyses.

Results

Demographic characteristics

In this study, 105 (47.3%) and 117 (52.7%) of the participants were male and female, respectively, with an average age of 52.2 years and an average BMI of 24.8. The majority of the participants were married (72.1%). Percentage of college graduates or senior high school was 60.8%. A total of 119 (55.9%) of the participants were from households with annual incomes of less than US$15,625, and 60 (28.2%) were from households with annual incomes between US$15,625 and US$31,250. In terms of daily personal habits, 52 (23.4%) of the participants smoked cigarettes, 28 (12.8%) consumed alcohol, 89 (40.1%) drank coffee, 134 (60.6%) drank tea, and 13 (5.9%) chewed betel nut. In terms of environmental exposure assessment and the daily use of plastic products, 50 of the participants (22.5%) had used pesticides at home in the past month, 68 (30.9%) lived within 1 km of farmland, and 145 (65.6%) and 167 (75.2%) had habits of eating fried and grilled foods, respectively. Among the participants, 26 (11.7%), 161 (72.5%), 124 (55.9), and 167 (75.2%) usually used plastic tableware, plastic wrap, plastic containers, and plastic bags (for refrigeration or heating), respectively (Table 1).

Concentrations of urinary phthalate metabolite, lipid metabolism indicators, and thyroid hormones

Table 2 presents the concentrations of urinary phthalate metabolite levels, lipid metabolism indicators, and thyroid hormones. Most urine phthalate metabolites demonstrated high to moderate detection rates, with the exception of MiNP and MBzP, which showed low detection rates. Consequently, MiNP and MBzP were not included in final analysis. GMs of MiBP, MEP, MMP, MnBP, MEHP, MECPP, MCMHP, and MEOHP concentrations in urine were 3.60, 10.97, 23.05, 9.72, 3.90, 17.75, 15.84, 1.54, and 8.04 ng/mL, respectively.
ΣDBPm and ΣDEHPm GMs were respectively 0.10 and 0.20 nmol/mL. The detection rates for all thyroid hormones and lipid metabolism indicators were 100%. GMs of thyroid hormones were 1.55 μIU/mL, 105.58 ng/dL, 7.26 μg/dL, 0.92 ng/dL, and 21.26 μg/mL for TSH, T3, T4, free T4, and TBG, respectively. GMs of lipid metabolism indicators were 106.84 mg/dL, 190.62 mg/dL, 57.30 mg/dL, 109.16 mg/dL, 3.33, 1.91, 129.88, and 2.27 for TG, TC, HDL-C, LDL-C, CRI-I, CRI-II, NHC, and AC, respectively. The adults with T3, T4, free T4, TSH, and TBG levels that were inside the reference ranges accounted for 97.3, 95.5, 93.2, 95.0, and 70.7% of the participants, respectively. The adults with TC, HDL-C, LDL-C, TG, CRI-I, CRI-II, NHC, and AC levels inside the reference range constituted 60.8, 91.0, 70.3, 77.9, 82.9, 80.6, 48.6, and 77.0% of the participants, respectively.

### Associations of urinary phthalate metabolites levels with thyroid hormones and lipid metabolism indicators

Regarding the associations between urinary lipid metabolism indicators and phthalate metabolites, MMP was negatively associated with LDL-C (β = −0.036, 95% confidence interval [CI] = −0.065, −0.007). MEP exhibited negative association with TC and NHC (TC: β = −0.022, 95% CI = −0.041, −0.004; NHC: β = −0.025, 95% CI = −0.050, 0.000), and ΣDEHPm had positive association with HDL-C (β = 0.058, 95% CI = 0.008, 0.109) (Table 3). Regarding associations between thyroid hormones and urinary phthalate metabolites, MnBP was positively associated with T3 and free T4 (T3: β = 0.027, 95% CI = 0.004, 0.050; free T4: β = 0.044, 95% CI = 0.019, 0.068). ΣDBPm was negatively associated with T3 and free T4 (T3: β = −0.061, 95% CI = −0.103, −0.019; free T4: β = −0.021, 95% CI = −0.042, −0.000).

### Table 1
Demographic characteristics of the study population (N = 222)

| Variables                              | Adults (≧18 years, N = 222) |
|----------------------------------------|------------------------------|
|                                        | Mean ± SD                    |
| Age (years)                            | 222 52.2 ± 17.5              |
| BMI                                    | 222 24.8 ± 4.57              |
| Sex                                    | Female 117 52.7              |
|                                        | Male 105 47.3                |
| Region                                 | Northern Taiwan 64 28.8      |
|                                        | Central Taiwan 34 15.3       |
|                                        | Southern Taiwan 62 27.9      |
|                                        | Eastern Taiwan 39 17.6       |
|                                        | Remote islands 23 10.4       |
| Marital status                         | Single 42 18.9               |
|                                        | Married 160 72.1             |
|                                        | Divorce/widowed 20 9.0       |
| Education                              | Junior high school/elementary school (≤ 9 years) 87 39.2 |
|                                        | Senior high school (9 ~ 12 years) 48 21.6 |
|                                        | College/Graduates (≧ 12 years) 87 39.2 |
| Household annual income (USD) a        | 119 55.9                     |
|                                        | 15,625 ~ 31,250 60 28.2      |
|                                        | ≥ 31,250 34 15.9             |
| Cigarette smoking b                    | Yes 52 23.4                  |
| Alcohol consumption c                  | Yes 28 12.8                  |
| Coffee drinking d                      | Yes 89 40.1                  |
| Tea drinking e                         | Yes 134 60.6                 |
| Betel nut chewing f                    | Yes 13 5.9                   |
| Pesticide use at home                  | Yes 50 22.5                  |
| Lived near farmland (with 1 km) g      | Yes 68 30.9                  |
| Habit of eating fried food h           | Yes 145 65.6                 |
| Habit of eating grilled food i         | Yes 165 74.7                 |
| Plastic tableware use                  | Yes 26 11.7                  |
| Plastic wrap use for refrigeration or heating | Yes 161 72.5          |

### Table 1 (continued)

| Variables                              | Adults (≧18 years, N = 222) |
|----------------------------------------|------------------------------|
| Plastic containers use for refrigeration or heating | Yes 124 55.9 |
| Plastic bags use for refrigeration or heating | Yes 167 75.2 |

a 9 missing values in household annual income; currency exchange rate of USD to new Taiwan dollar is 1:32
b Participants smoking at least one cigarette per day
c 4 missing values in alcohol consumption; participants consuming at least one bottle of alcohol per week
d Participants consuming at least one cup of coffee per week
e 1 missing values in tea drinking; participants consuming at least one cup of tea per week
f Participants chewing at least one betel nut per week
g 2 missing values in living near farm land
h 1 missing value in habit of eating fried food
i 1 missing value in habit of eating grilled food

ΣDBPm and ΣDEHPm GMs were respectively 0.10 and 0.20 nmol/mL. The detection rates for all thyroid hormones and lipid metabolism indicators were 100%. GMs of thyroid hormones were 1.55 μIU/mL, 105.58 ng/dL, 7.26 μg/dL, 0.92 ng/dL, and 21.26 μg/mL for TSH, T3, T4, free T4, and TBG, respectively. GMs of lipid metabolism indicators were 106.84 mg/dL, 190.62 mg/dL, 57.30 mg/dL, 109.16 mg/dL, 3.33, 1.91, 129.88, and 2.27 for TG, TC, HDL-C, LDL-C, CRI-I, CRI-II, NHC, and AC, respectively. The adults with T3, T4, free T4, TSH, and TBG levels that were inside the reference ranges accounted for 97.3, 95.5, 93.2, 95.0, and 70.7% of the participants, respectively. The adults with TC, HDL-C, LDL-C, TG, CRI-I, CRI-II, NHC, and AC levels inside the reference range constituted 60.8, 91.0, 70.3, 77.9, 82.9, 80.6, 48.6, and 77.0% of the participants, respectively.

### Associations of urinary phthalate metabolites levels with thyroid hormones and lipid metabolism indicators

Regarding the associations between urinary lipid metabolism indicators and phthalate metabolites, MMP was negatively associated with LDL-C (β = −0.036, 95% confidence interval [CI] = −0.065, −0.007). MEP exhibited negative association with TC and NHC (TC: β = −0.022, 95% CI = −0.041, −0.004; NHC: β = −0.025, 95% CI = −0.050, 0.000), and ΣDEHPm had positive association with HDL-C (β = 0.058, 95% CI = 0.008, 0.109) (Table 3). Regarding associations between thyroid hormones and urinary phthalate metabolites, MnBP was positively associated with T3 and free T4 (T3: β = 0.027, 95% CI = 0.004, 0.050; free T4: β = 0.044, 95% CI = 0.019, 0.068). ΣDBPm was negatively associated with T3 and free T4 (T3: β = −0.061, 95% CI = −0.103, −0.019;
free T4: $\beta = -0.051$, 95% CI = −0.095, −0.006), and $\Sigma$DEHPm was negatively associated with T4 ($\beta = -0.056$, 95% CI = −0.098, −0.013) (Table 4).

**Relationship between lipid metabolism indicators and thyroid function**

The associations of lipid metabolism indicators with thyroid hormones after adjustment for sex, age, BMI, TBG

**Table 2** Distribution of urinary phthalate metabolites (ng/mL), thyroid hormone levels, and lipid metabolism indicators among adults

| Variables | Adults (≧18 years) (N = 222) | N (%) > LOD | Inside reference range (%) | Median (P25-P75) | GM (95% CI) |
|-----------|------------------------------|-------------|---------------------------|-------------------|-------------|
| **Phthalate metabolites**<sup>a</sup> | | | | | |
| MMP       | 212 (95.5) | 91.4 | 23.23 (10.34, 53.21) | 23.05 (18.48, 28.76) |
| MEP       | 204 (91.9) | 96.8 | 12.02 (5.33, 25.97) | 10.97 (8.66, 13.88) |
| MiBP      | 158 (71.2) | 96.4 | 7.07 (ND, 17.08) | 3.60 (2.70, 4.79) |
| MnBP      | 193 (86.9) | 91.3 | 14.76 (6.16, 28.27) | 7.97 (7.53, 12.56) |
| MBzP      | 51 (23.0)  | 99.1 | ND (ND, ND) | 0.32 (0.27, 0.39) |
| MEHP      | 175 (78.8) | 99.1 | 6.90 (3.11, 12.12) | 3.90 (3.06, 4.96) |
| MEHHP     | 217 (97.7) | 95.0 | 16.24 (9.82, 30.27) | 15.84 (13.72, 18.28) |
| MEOHP     | 205 (92.3) | 96.4 | 10.28 (5.58, 16.37) | 8.04 (6.71, 9.62) |
| MECPP     | 214 (96.4) | 95.9 | 20.17 (11.30, 32.60) | 17.75 (15.06, 20.91) |
| MCMHP     | 142 (64.0) | 98.2 | 3.21 (ND, 6.39) | 1.54 (1.21, 1.96) |
| MiNP      | 23 (10.4)  | 99.5 | ND (ND, ND) | 0.21 (0.19, 0.24) |
| **ΣDEHPm**<sup>b</sup> (nmole/mL) | 0.20 (0.12, 0.33) | 0.20 (0.18, 0.22) |
| **ΣDBPm**<sup>b</sup> (nmole/mL) | 0.11 (0.05, 0.20) | 0.10 (0.08, 0.12) |
| **Thyroid Hormones**<sup>c</sup> | | | | | |
| TSH (μIU/mL) | 222 (100) | 95.0 | 1.48 (1.09, 2.22) | 1.55 (1.43, 1.69) |
| T<sub>3</sub> (ng/dL) | 222 (100) | 97.3 | 108.00 (93.00, 121.00) | 105.58 (102.81, 108.41) |
| T<sub>4</sub> (μg/dL) | 222 (100) | 95.5 | 7.38 (6.18, 8.52) | 7.26 (7.04, 7.48) |
| Free T<sub>4</sub> (ng/dL) | 222 (100) | 93.2 | 0.92 (0.82, 1.05) | 0.92 (0.90, 0.94) |
| TBG (μg/mL) | 222 (100) | 70.7 | 21.80 (19.23, 24.68) | 21.26 (20.66, 21.87) |
| **Lipid metabolism**<sup>d</sup> | | | | | |
| TC (mg/dL) | 222 (100) | 60.8 | 190.00 (168.00, 221.00) | 190.62 (185.19, 196.21) |
| HDL-C (mg/dL) | 222 (100) | 91.0 | 57.90 (46.78, 69.85) | 57.30 (55.27, 59.41) |
| LDL-C (mg/dL) | 222 (100) | 70.3 | 112.00 (90.50, 139.00) | 109.16 (104.56, 113.97) |
| TG (mg/dL) | 222 (100) | 77.9 | 100.50 (72.00, 135.75) | 106.84 (98.60, 115.77) |
| CRI-I | 222 (100) | 82.9 | 3.32 (2.76, 3.92) | 3.33 (3.20, 3.46) |
| CRI-II | 222 (100) | 80.6 | 1.96 (1.46, 2.59) | 1.91 (1.81, 2.01) |
| NHC | 222 (100) | 48.6 | 131.30 (107.20, 162.35) | 129.88 (124.87, 135.09) |
| AC | 222 (100) | 77.0 | 2.32 (1.76, 2.92) | 2.27 (2.14, 2.40) |

ND was calculated as half of detection limit. The limit of detection (LOD) for MMP, MEP, MIBP, MBzP, MEHP, MEHHP, MEOH, MECPP, MCMHP, and MiNP were 0.3, 0.3, 1.0, 1.0, 0.3, 0.3, 0.3, 0.3, 0.1, and 0.1 ng/mL, respectively.

Abbreviations: GM Geometric mean, LOD Limit of detection, ND Not detectable, MMP Mono-methyl phthalate, MEP Mono-ethyl phthalate, MiBP Mono-n-butyl phthalate, MnBP Mono-n-butyl phthalate, MEHP Mono-ethylhexyl phthalate, MEHHP Mono-(2-ethyl-5-hydroxyhexyl) phthalate, MEOHP Mono-(2-ethyl-5-oxo-hexyl) phthalate, MECPP Mono-(2-ethyl-5-carboxypentyl) phthalate, MCMHP Mono-(2-carboxymethylhexyl) phthalate, MiNP Mono-iso-nonyl phthalate, TSH Thyroid-stimulating hormone, T<sub>3</sub> Triiodothyronine, T<sub>4</sub> Thyroxine, free T<sub>4</sub> Free thyroxine, TBG Thyroxine-binding globulin, TC Total cholesterol, LDL-C Low-density lipoprotein cholesterol, TG Triglyceride, CRI-I Castelli risk indexes I, CRI-II Castelli risk indexes II, NHC Non-HDL cholesterol, AC Atherogenic coefficient.

<sup>a</sup> The reference ranges (P<sub>95</sub>) of adults for MMP, MEP, MIBP, MnBP, MBzP, MEHP, MEHHP, MEOH, MECPP, MCMHP, and MiNP were 208.2 ng/mL, 265.8 ng/mL, 204.3 ng/mL, 11.7 ng/mL, 59.2 ng/mL, 59.2 ng/mL, 35.1 ng/mL, 93.8 ng/mL, 27.7 ng/mL, and 12.1 ng/mL, respectively.

<sup>b</sup> ΣDEHPm = sum molar concentrations of MEHP + MEHHP + MEOHP + MECPP + MCMHP; ΣDBPm = sum molar concentrations of MiBP + MnBP.

<sup>c</sup> The laboratory reference ranges of adults for TSH, T<sub>3</sub>, T<sub>4</sub>, free T<sub>4</sub>, and TBG were 0.35–4.94 μIU/mL, 58–159 ng/dL, 4.87–11.72 μg/dL, 0.70–1.48 ng/dL, and 15.8–25.4 μg/mL, respectively.

<sup>d</sup> The laboratory reference ranges of adults for TC, LDL-C, HDL-C, and TG were <200 mg/dL, >40 mg/dL, <130 mg/dL, and <150 mg/dL, respectively. The reference ranges of adults for CRI-I, CRI-II, NHC, and AC were CRI-I <4.5 (male), CRI-I <4.0 (female), CRI-II <3.0 (male), CRI-II <2.5 (female), NHC <130 mg/dL, and AC<3.0. (Millán et al., 2009; Harari et al., 2017; Bhardwaj et al., 2013)
Table 3  Multiple linear regression coefficient (95% CI) for changes in lipid metabolism indicators associated with unit-increases in Ln-phthalate metabolites

| Variables | In-HDL-C | | In-LDL-C | | In-TC | | In-TG |  
|------------|----------| |----------| |----------| |----------| |----------|  
|            | Adjusted β (95%CI) | P value | Adjusted β (95%CI) | P value | Adjusted β (95%CI) | P value | Adjusted β (95%CI) | P value |  
| MMP(ng/mL) | -0.011 (-0.033, 0.010) | 0.303 | -0.036 (-0.065, -0.007) | 0.014* | -0.011 (-0.030, 0.008) | 0.267 | 0.022 (-0.029, 0.073) | 0.390 |  
| MEI(ng/mL) | -0.015 (-0.036, 0.006) | 0.161 | -0.013 (-0.040, 0.015) | 0.368 | -0.022 (-0.041, -0.004) | 0.016* | -0.032 (-0.081, 0.017) | 0.200 |  
| MiBP(ng/mL) | 0.007 (-0.017, 0.031) | 0.580 | -0.005 (-0.037, 0.026) | 0.744 | 0.007 (-0.014, 0.028) | 0.510 | 0.005 (-0.052, 0.061) | 0.874 |  
| MnBP(ng/mL) | 0.006 (-0.027, 0.039) | 0.714 | -0.013 (-0.056, 0.030) | 0.550 | -0.012 (-0.041, 0.017) | 0.417 | 0.017 (-0.059, 0.093) | 0.656 |  
| ΣDBPm (n mole/mL) b | -0.025 (-0.085, 0.036) | 0.421 | 0.061 (-0.017, 0.140) | 0.125 | 0.025 (-0.027, 0.078) | 0.344 | -0.034 (-0.173, 0.106) | 0.635 |  
| ΣDEHPm (n mole/mL) b | 0.058 (0.008, 0.109) | 0.024* | 0.005 (-0.061, 0.071) | 0.889 | 0.039 (-0.005, 0.084) | 0.885 | 0.063 (-0.055, 0.180) | 0.293 |  

Table 4  Multiple linear regression coefficient (95% CI) for changes in serum thyroid hormones associated with unit-increases in Ln-phthalate metabolites

| Variables | ln-T3 | | ln-T4 | | ln-free T4 | | ln-TSH |  
|------------|----------| |----------| |----------| |----------| |----------|  
|            | Adjusted β (95%CI) | P value | Adjusted β (95%CI) | P value | Adjusted β (95%CI) | P value | Adjusted β (95%CI) | P value |  
| MMP(ng/mL) | 0.006 (-0.010, 0.021) | 0.455 | 0.009 (-0.009, 0.028) | 0.329 | -0.007 (-0.024, 0.009) | 0.374 | -0.012 (-0.070, 0.045) | 0.670 |  
| MEI(ng/mL) | 0.004 (-0.011, 0.019) | 0.576 | -0.012 (-0.029, 0.006) | 0.195 | 0.008 (-0.008, 0.024) | 0.313 | 0.037 (-0.019, 0.092) | 0.195 |  
| MiBP(ng/mL) | 0.011 (-0.006, 0.028) | 0.195 | -0.017 (-0.037, 0.004) | 0.110 | 0.007 (-0.011, 0.026) | 0.423 | 0.029 (-0.035, 0.093) | 0.368 |  
| MnBP(ng/mL) | 0.027 (0.004, 0.050) | 0.022* | -0.026 (-0.054, 0.001) | 0.062 | 0.044 (0.019, 0.068) | <0.001** | 0.047 (-0.039, 0.134) | 0.280 |  
| ΣDBPm (n mole/mL) b | -0.061 (-0.103, -0.019) | 0.005** | 0.041 (-0.009, 0.092) | 0.107 | -0.051 (-0.095, -0.006) | 0.027* | -0.085 (-0.243, 0.073) | 0.290 |  
| ΣDEHPm (n mole/mL) b | -0.013 (-0.048, 0.022) | 0.466 | -0.056 (-0.098, -0.013) | 0.011* | -0.021 (-0.058, 0.017) | 0.282 | 0.010 (-0.123, 0.143) | 0.880 |  

* < 0.05; ** < 0.01

* Adjusted for age, sex, BMI, cigarette smoking, and urinary creatinine levels

* Adjusted for age, sex, BMI, cigarette smoking, TBG levels, and urinary creatinine levels

* ΣDEHPm = sum molar concentrations of MEHP + MEHHP + MEOHP + MECPP + MCMHP; ΣDBPm = sum molar concentrations of MiBP + MnBP

* ΣDEHPm = sum molar concentrations of MEHP + MEHHP + MEOHP + MECPP + MCMHP; ΣDBPm = sum molar concentrations of MiBP + MnBP
levels, and smoking are present in Table 5. T4 and HDL-C had a significant negative association (β = −0.284, 95% CI = −0.440, −0.128). Positive associations were noted between T4 and CRI-I (β = 0.245, 95% CI = 0.074, 0.415), T4 and CRI-II (β = 0.265, 95% CI = 0.025, 0.506), and T4 and AC (β = 0.370, 95% CI = 0.126, 0.613). Negative associations were observed between free T4 and AC (β = −0.278, 95% CI = −0.549, −0.006).

**Mediation analysis and BKMR results**

If exposure of phthalate metabolite was significantly associated with thyroid hormones and lipid metabolism indicators that had previously been significantly associated with each other, we then performed mediation analysis. Table 6 presents the results of the mediation analysis. T4 mediated 32.2% of the association between ∑DEHPm and HDL-C (indirect effect = 0.015, 95% CI = −0.0087, 0.05) (Fig. 2). However, the mediation effect was not significant.

We also used BKMR to explore the associations between lipid metabolism indicators and phthalate exposure, thyroid hormones and phthalate exposure, and lipid metabolism indicators and thyroid function. We observe similar associations with multiple linear regressions (Table S1, S2 and S3, Figs. S1, S2 and S3).

**Discussion**

In this study, we observed that the urinary phthalate metabolite ∑DEHPm was negatively associated with HDL-C, ∑DEHPm exhibited a significant negative association with T4, and T4 showed a significant negative association with HDL-C. These findings suggest that environmental phthalate exposure alters thyroid

Table 5  Multiple linear regression coefficient (95% CI) for changes in lipid metabolism indicators associated with unit-increases in Ln-serum thyroid hormones levels a

| Variables | ln-HDL-C | ln-LDL-C | ln-TC | ln-TG |
|-----------|----------|----------|-------|-------|
| T3        | -0.031 (-0.218, 0.156) | 0.064 (-0.195, 0.323) | 0.028 (-0.147, 0.202) | 0.015 (-0.087, 0.05) |
| T4        | -0.284 (-0.440, -0.128) | < 0.001 ** | 0.866 (0.197, 0.234) | 0.005 (* | 0.265 (0.025, 0.506) | 0.031 * | 0.370 (0.126, 0.613) | 0.003 ** |
| Free T4   | 0.094 (-0.080, 0.268) | 0.288 | -0.038 (-0.279, 0.202) | 0.753 | 0.022 (-0.101, 0.145) | 0.725 |
| TSH       | -0.013 (-0.064, 0.039) | 0.634 | 0.029 (-0.042, 0.101) | 0.422 | 0.022 (-0.101, 0.145) | 0.725 |

| Variables | ln-HDL-C | ln-LDL-C | ln-TC | ln-TG |
|-----------|----------|----------|-------|-------|
| In-CRI-I  | adjusted β 95%CI | P value | adjusted β 95%CI | P value | adjusted β 95%CI | P value | adjusted β 95%CI | P value |
| T3        | 0.059 (-0.145, 0.263) | 0.570 | 0.095 (-0.194, 0.384) | 0.516 | 0.056 (-0.175, 0.287) | 0.635 | 0.087 (-0.205, 0.379) | 0.558 |
| T4        | 0.245 (0.074, 0.415) | 0.005 ** | 0.265 (0.025, 0.506) | 0.031 * | 0.086 (-0.107, 0.278) | 0.380 | 0.370 (0.126, 0.613) | 0.003 ** |
| Free T4   | -0.188 (-0.377, 0.002) | 0.053 | -0.132 (-0.401, 0.136) | 0.332 | -0.184 (-0.398, 0.031) | 0.093 | -0.278 (-0.549, -0.006) | 0.045 * |
| TSH       | 0.030 (-0.027, 0.086) | 0.299 | 0.042 (-0.038, 0.122) | 0.303 | 0.034 (-0.030, 0.097) | 0.301 | 0.046 (-0.034, 0.127) | 0.261 |

a < 0.05; ** < 0.01
* Adjusted for age, sex, BMI, cigarette smoking, and TBG

Table 6  Mediation effects of exposure to phthalates on the homeostatic model assessment of estimated lipid metabolism indicators through thyroid hormones a

| Exposure and outcome | Mediator | Estimate indirect effect (95% CI) | Estimate direct effect (95% CI) | Estimated proportion mediated |
|---------------------|----------|----------------------------------|----------------------------------|------------------------------|
| ∑DEHPm and HDL-C    | T4       | 0.015 (-0.0087, 0.05)            | 0.025 (-0.0282, 0.08)            | 32.2%                        |
| ∑DEHPm and CRI-I     | T4       | -0.019 (-0.0535, 0.001)          | 0.001 (-0.0556, 0.006)           | 46.8%                        |
| ∑DEHPm and CRI-II    | T4       | -0.031 (-0.0790, 0.000)          | -0.042 (-0.1236, 0.04)           | 38.9%                        |
| ∑DEHPm and AC        | T4       | -0.031 (-0.0813, 0.001)          | -0.008 (-0.0910, 0.007)          | 50.3%                        |
| ∑DEHPm and CRI-I     | Free T4  | -0.001 (-0.0150, 0.001)          | 0.023 (-0.0775, 0.03)            | 3.7%                         |
| ∑DEHPm and AC        | Free T4  | -0.001 (-0.0203, 0.002)          | -0.038 (-0.1156, 0.04)           | 2.2%                         |

a < 0.05; ** < 0.01
* Adjusted for adjusted for age, sex, BMI, cigarette smoking, TBG levels, and urinary creatinine levels
hormone levels, which in turn impacts lipid metabolism homeostasis.

For associations between urinary phthalate metabolites and lipid metabolism indicators, we observed positive associations between ΣDBPm and LDL-C, ΣDBPm and CRI-I, ΣDBPm and NHC, and ΣDEHPm and HDL-C; whereas negative associations were observed between MMP and LDL-C, MEP and TC, and MEP and NHC. However, Olsen et al., in a survey of 70-year-old people in Uppsala, Sweden, observed a positive association between MMP and LDL-C [41]. Perng et al. reported that ΣDBPm was correlated with lower LDL-C in boys enrolled by the Early Life Exposure in Mexico to Environmental Toxicants project from 1997 to 2004 [42]. Yaghjyan et al. found no significant associations of phthalate metabolite concentrations with TC, TG, HDL-C, or LDL-C in a study that enrolled adult women aged ≥18 years and was based on 1999–2004 US National Health and Nutrition Examination Survey data [43]. Dong et al. observed that the concentrations of MCMHP and MEHHP were positively associated with TC and TG, whereas the concentration of MECPP was negatively associated with TC, TG, and LDL-C in patients with diabetes from Shanghai, China [44]. Variations of the associations between urinary phthalate metabolites and lipid metabolism indicators could be due to differences in study design and participants (e.g., age, sex, patient).

Regarding associations between thyroid hormones and lipid metabolism indicators, we observed that T4 had a significant negative association with HDL-C, free T4 had significant negative associations with AC, and T4 had positive associations with CRI-I, CRI-II, and AC. We did not observe significant associations of free T4 with HDL-C, LDL-C, TG, or TC; TSH also was not significantly associated with HDL-C, LDL-C, TG, or TC. Studies have revealed similar results [45–52]. For example, Ren et al. discovered that free T4 was not significantly correlated with HDL-C, LDL-C, TG, or TC [49]; Roef et al. showed that free T4 was not significantly correlated with HDL-C, LDL-C, or TC, and that TSH was not significantly correlated with LDL-C or HDL-C [50]. However, some studies have revealed different findings [45, 46, 48, 50–58]. For example, Roos et al. observed that free T4 had negative associations with TG, TC, HDL-C, and LDL-C [45]; Chin et al. observed that free T4 had significant positive associations with TC, HDL-C, and LDL-C [55]; Roef et al. noted positive associations between TSH and TC, TG [50]. Few studies have reported on the associations of T3 or T4 with TG, TC, HDL-C, and LDL-C. Gutch et al. demonstrated that T4 was not significantly correlated with HDL-C, LDL-C, or TC, and TSH was not significantly correlated with LDL-C or HDL-C [56]. However, some studies have revealed different findings [45, 46, 48, 50–58]. For example, Roos et al. observed that free T4 had negative associations with TG, TC, HDL-C, and LDL-C [45]; Chin et al. observed that free T4 had significant positive associations with TC, HDL-C, and LDL-C [55]; Roef et al. reported a positive correlation between T3 and TG [50]; Kim et al. found that T3 was positively correlated with TG and HDL-C [59]; and Roef et al. discovered a positive association between T4 and body fat [60]. Variations of the associations between thyroid hormones and lipid metabolism indicators could be due to differences in study design and participants (e.g., age, location, patient).

Animal studies have shown that phthalates such as DEHP and DEP may affect lipid metabolism by binding to the PPAR (e.g., Pradhan et al. [61]). PPAR can be divided into three types: α, δ, and γ. Receptor γ regulates glucose metabolism and fatty acid storage [8], whereas receptor α plays a pivotal role in liver peroxidase proliferation [7, 62]. DEHP causes lipid metabolism disorders by activating the PPARα or PPARγ signal transducer and farnesoid X receptor or liver X receptor signaling pathway, respectively [63]. DBP can activate the PPARα signaling pathway and affect the expression of fatty acid

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**Fig. 2** Mediation effects of phthalate exposure on HDL-C through thyroxine (T4). The signs in the arrows represent the direction of association.
suggested that phthalates reduce LDL-C and TG pro-
phthalate exposure and lipid metabolism indicators are
logical mechanisms underlying the associations between
mal study or other work has described whether the bio-
lipid metabolism indicators. However, to date, no ani-
results could support our findings that T4 levels were neg-
T4 could lay the role of mediator in the associations between
phthalate exposure and lipid metabolism. Future studies
are required to investigate whether a complex pathway is
involved in this association.

White adipose tissue (WAT) and brown adipose tissue
are the two types of adipose tissue found in mam-
BAT inhibits obesity by metabolizing lipids via uncoupling protein 1-mediated uncoupled respiration,
whereas WAT accumulates lipids. BAT is involved in the
thermogenic response and energy balance regulation in
small mammals. Furthermore, BAT activation increases
energy expenditure, lowers adiposity, and protects against
diet-induced obesity [82]. A recent study found that
MEHP and DEHP caused browning-like effects on adipo-
cytes and mice, respectively [83]. Their findings support
the browning activity of PAEs both in vivo and in vitro.
Browning effects suggest that excess energy in WAT is
being dissipated as heat instead of being stored. The
browning of WAT is generally thought to help improve
metabolic disorders by increasing energy expenditure
and decreasing adiposity. MEHP/DEHP could be both
endocrine disrupting chemicals and browning chemicals,
which seemed contradictory when considered combined
[84]. In the present study, ΣDEHPm is positively corre-
related with HDLC, which is consistent with the findings
in Hsu et al. that DEHP cause browning-like effects on
lean mice [83, 84]. Further study on browning-like effects
with DEHP or DEHP alternatives (e.g. DBP, etc.) would
be warranted.

Our study’s key strength is its novel approach to
mediation analysis, with the associations between
phthalate exposure, lipid metabolism, and thyroid
function, along with possible mediating effects, being
investigated. To the best of our knowledge, no other study has investigated the association between phthalate exposure and lipid metabolism indicators and whether this association is mediated by thyroid function indicators. Therefore, in addition to examining the correlation between phthalate exposure and lipid metabolism indicators in Taiwanese adults, this study investigated thyroid function indicators as potential mediators of the relationship between phthalate exposure and lipid metabolism indicators in Taiwanese adults. We collaborated with the NAHSIT team and used the same sampling frame and procedure in subject recruitment in order to obtain sufficient participation from the general Taiwanese population. We followed NAHSIT’s Standard Operating Procedure [85, 86], transported and stored samples at –80°C until analysis. We analyzed the sample within weeks or 2–3 months just after the samples were collected, and did quality control in phthalate metabolite analysis. By these systematic methods in subject selection, sample collection, and quality, we were able to reduce variation and increase reliability of final analysis. This study has the following limitations. First, this is the cross-sectional study, which could not explain causality. Second, phthalate exposure measurements were derived from a single urine test and were corrected for the creatinine concentration, but they may not be representative of the participants’ complete exposure to phthalates over time. However, previous studies have indicated that a single urine test is representative of phthalate exposure over a period of 16 weeks to 6 months [87, 88]. Third, the blood samples analyzed in this study were collected only once, and thyroid hormone concentrations may have varied among individuals over time. Nevertheless, Andersen et al. suggested that the measured values of thyroid hormones in individuals do not fluctuate greatly over time [89]. Third, data on iodine or selenium exposure concentrations were not available for the study population, and deficiencies in these trace elements may have affected the presence of abnormal thyroid function indicators [90]. But, individuals with self-reported endocrine system abnormalities (e.g., abnormal thyroid function or diabetes mellitus) were excluded from this study, and approximately 90% of the study population exhibited thyroid function values that were within the reference range. Finally, though our subjects were chosen from general population, our results were limited for Taiwanese and could not represent the whole general population. Further study with larger sample size would be needed to elucidate the associations.

Conclusion
Our findings supported that thyroid hormones mediate the association between phthalate exposure and lipid metabolism. In particular, T₄ levels strongly mediate the effect of phthalate exposure on HDL-C in humans. Large-scale epidemiological and mechanistic research is required to validate these associations and determine the underlying biological mechanisms.

Supplementary Information
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Authors’ contributions
Han-Bin Huang: Conceptualization, Methodology, Software, Formal data analysis, Writing - original draft, Resources, Supervision. Po-Keng Cheng: Formal data analysis, Writing - review & editing. Chi-Ying Siao: Formal data analysis, Validation, Writing - review & editing. Yuan-Ting C. Lo: Investigation, Methodology, Resources, Writing - review & editing. Wei-Chun Chou: Writing - Validation, Writing - review & editing. Po-Chun Huang: Conceptualization, Methodology, Visualization, Validation, Data curation, Resources, Writing - review & editing, Supervision. The author(s) read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
This study was approved by the National Health Research Institutes’ Research Ethics Committee at Taiwan (no. EC1020206). All participants gave written informed consent.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

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