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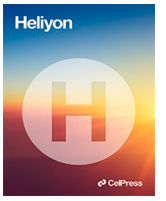

Urine levels of estrogen and its metabolites in premenopausal middle-aged women with different degrees of obesity and their correlation with glucose and lipid metabolism

Huanhuan Zhao [a], Hongfang Yang [a], Zhiwei Li [b], Zhonghuan Ge [c], Mei Zhou [c], Li Li [a], Jing He [a,*]

[a] *Department of Obstetrics and Gynecology, Fourth Hospital of Hebei Medical University, 12 Jiankang Road, Shijiazhuang, 050011, Hebei Province, China*

[b] *School of Chemistry and Pharmaceutical Engineering, Shijiazhuang University of Science and Technology, Shijiazhuang, 050018, Hebei Province, China*

[c] *Department of Laboratory, Nanpi County People's Hospital, Cangzhou Nanpi County, 061550, Hebei Province, China*

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ABSTRACT

Objectives: To determine the levels of estrogen and estrogen metabolites in the urine of premenopausal women with obesity and their correlation with glucose and lipid metabolism.

Methords: 135 premenopausal women were selected from the same area. According to the body mass index (BMI), they were divided into four different groups. High performance liquid chromatography-mass spectrometry (HPLC/MS) was adopted to detect the concentrations of estrogen and estrogen metabolites in the urine. The influencing factors of BMI were analyzed, the correlation between the urinary degrees of estrogen and estrogen metabolites and glucose and lipid metabolism levels was assessed.

Results: (1) The concentrations of 17β-estradiol (E2), estrone (E1), 16α-hydroxyestrone (16α-OHE1) and 2-hydroxyestrone (2-OHE1) gradually increased with increasing BMI ($p < 0.05$). (2) Stepwise regression analysis displayed that the concentrations of E2, 16α-OHE1 and 2-OHE1 in urine were significantly positively correlated with BMI ($p < 0.05$). (3) The concentrations of E2, E1, 16α-OHE1, 2-OHE1 and 16α-OHE1/2-OHE1 in urine were greatly positively related to fasting insulin (FIN), Triglyceride (TG), Total Cholesterol (TC) and Low-density lipoprotein (LDL) ($p < 0.05$). And they were greatly negatively related to High-density lipoprotein (HDL) ($p < 0.05$).

Conclusions: Early screening can reflect the degree of obesity and glucose and lipid metabolism disorders in premenopausal middle-aged women, thereby providing guidance for improving the prognosis of obese women.

Abbreviations: BMI, body mass index; UPLC-MS, high-performance liquid chromatography-mass spectrometry; 2-OHE1, 2-hydroxyestrone; 16α-OHE1, 16α-hydroxyestrone; MS, metabolic syndrome; SBP, systolic pressure; DBP, diastolic pressure; TC, total cholesterol; FBG, fasting blood glucose; TG, triglycerides; FIN, fasting insulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; 2-MeOE, 2-methoxyestradiol; HPLC, High-performance liquid chromatography; RE, relative error; IQR, interquartile range; HPO, hypothalamic-pituitary-ovarian.

* Corresponding author. Department of Obstetrics and Gynecology, Fourth Hospital of Hebei Medical University, No. 12 Jiankang Road, Shijiazhuang, 050011, Hebei Province, China.

E-mail address: hejingyikeda@126.com (J. He).

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1. Introduction

As living standards are improved, and light physical activity becomes popular, the number of obese people in China has continued to increase. In accordance with a survey by the World Health Organization, about 13 % of the world's population is obese, approximately 57 % of obese people suffer from metabolic diseases, and approximately 2.8 million people die each year from diseases caused by obesity [1]. Obesity is one of the common manifestations of metabolic syndrome (MS). MS, also known as insulin resistance syndrome, is clinically manifested as obesity, hypertension, abnormal glucose metabolism, and hypertriglyceridemia [2]. Presently, obesity has severely affected people's quality of life. A survey showed that women showed greatly higher proportion of obesity than men, which may be related to the influence of female hormone levels [3]. Changes in the levels of insulin like growth element and endogenous estrogen in the adipose tissue of obese women disrupt the balance between cell proliferation and apoptosis, and androgen in adipose tissue is converted to estrogen by aromatase, which is the main source of endogenous estrogen [4]. Endogenous estrogen is considered a high risk element for the growth of breast cancer and endometrial cancer. A study on the epidemiology of obesity in postmenopausal females found that obesity is related to the growing risk of and mortality due to breast cancer in these women [5]. Few studies have been conducted on the correlation of estrogen metabolism levels with obesity in premenopausal, obese, healthy middle-aged women. The current research firstly examine the levels of estrogen and estrogen metabolites in the urine of middle-aged healthy premenopausal women and to evaluate the association between the urinary degrees of estrogen and estrogen toxic metabolites in overweight and obese women and MS.

2. Methods

2.1. Study subjects

According to the 2004 WHO classification standard for obesity, 60 obese women with BMI $\geq$25.0 kg/m^2 were selected from the outpatient clinic of our hospital from August 2019 to August 2020. The patients were subjected to 75 g glucose tolerance test, which helps to differentiate between patients with obesity and those with obesity with abnormal glucose metabolism. The mean age of 30 patients in the simple obesity group was 41.3 $\pm$ 3.8 years and that of 30 patients with obesity with abnormal glucose metabolism was 42.8 $\pm$ 2.9 years. Healthy women who underwent physical examination at Fourth Hospital of Hebei Medical University from August 2019 to August 2021 were chosen as the normal control group. This group included 60 women with a mean age of 43.5 $\pm$ 3.5 years and BMI between 18.5 and 24 kg/m^2; the ultrasound test of their kidney, liver abdomen, and uterus showed normal results, with negative outcomes for tumor markers in blood. These women were nonsmokers; had no breast disease, gynecological tumors, and other hormone-related tumors; and did not receive any hormonal drug therapy for at least the past 3 months. Inclusion criteria were shown below: (1) age between 35 and 55 years; (2) no previous history of metabolic disease; and (3) consent to cooperate with evaluation and follow-up. Exclusion standards were shown below (1) patients with a history of mental illness; (2) those with severe cardiovascular and cerebrovascular diseases and sequelae; (3) those with incomplete clinical data; and (4) Participants with confirmed Covid-19 cases or those who have had close contanct with individuals diagnosed with Covid-19. The Ethics Committee of the Fourth Hospital of Hebei Medical University [NO. 2020KY260] approved this research. All participants offered informed consent.

2.2. Reagents and materials

2.2.1. Waist measurement and blood pressure measurement

The subjects were asked to rest for 15 min before measurement, and the blood pressure of the left upper limb was assessed in the sitting position by using an electronic sphygmomanometer; the measurement was performed 3 times, and the mean value was taken. For waist circumference (W) measurement, the circumference of the abdomen through the umbilicus was measured. The gender and age of each subject were recorded, and their weight, height, and W were measured when fasting. The BMI was as follows: BMI = weight (kg)/height^2 (m^2). The blood pressure of the right brachial artery, including systolic pressure (SBP) and diastolic pressure (DBP), was assessed using an HEM-7124 electronic sphygmomanometer (Japan Omron Company).

2.2.2. Fasting blood glucose, blood lipid, and fasting insulin measurement

After overnight fasting for more than 10 h, blood sample collection was performed on an empty stomach on the next morning. Five milliliters of fasting cubital venous blood was gathered from every subject in the morning. Following anticoagulation with heparin, the blood sample was stored at room temperature, next to 5-min centrifugal at 3000 rpm. The storage of supernatant was made at −80 °C for subsequent testing. The oxidase method CHOD-PAP-CDC was used to detect total cholesterol (TC), and the GPO-PAP method was used to detect the levels of fasting blood glucose (FBG) and triglycerides (TG). Chemiluminescence immunoassay was used to detect fasting insulin (FIN) levels, while a high-density lipoprotein (HDL) cholesterol determination kit (direct method - selective inhibition method), catalogue number: 19–0705, and a low-density lipoprotein (LDL) cholesterol determination kit (direct method - surfactant removal method), catalogue number: 19–0514, were used to determine HDL and LDL levels, respectively.

2.3. Determination of urine estrogen and its metabolites

2.3.1. Main materials

The AB 4500 Triple Quad 4500/LC-20AD spectrophotometer was acquired from AB SCIEX Corporation (USA). The electronic

balance Q65-1CN was from Sartorius, Germany. Methanol and acetonitrile were acquired from Merck. n-Octanol formate, sodium hydroxide, acetone, and hydrochloric acid were acquired from Tianjin Damao Company. Estradiol standard was purchased from Sigma-Aldrich, USA. Standards for estrone, 4-hydroxyestradiol (4-OHE2), 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 16α-hydroxyestrone (16α-OHE1), and 2-methoxyestradiol (2-MeOE) were provided by Toronto Research Chemicals, Canada. The creatinine determination kit (sarcosine oxidase method) was purchased from Mike Biological Co., Ltd.

2.3.1. Urine sample collection

Morning urine samples were gathered on the 2–3 days of menstrual period. Ten milliliters of urine samples was taken, and 10 mg vitamin C was put to stop oxidation. The urine specimens were stored at −80 °C until use.

2.3.2. Instruments and chromatography and mass spectrometry coefficients

An AB SCIEX 4500 Triple Quad mass spectrometer coupled to an LC-20AD system (AB SCIEX Corporation) was adopted to perform high performance liquid chromatography (HPLC)-tandem mass spectrometry (MS) analysis. Chromatographic separation was performed on a Luna Omega 3 μm polar C18 column (100 mm × 2.1 mm [ID], Agilent), with the mobile eluent containing mobile phase A (0.1 % formic acid and 99.9 % acetonitrile) and mobile phase B (0.1 % formic acid and 99.9 % water) at the flow rate of 0.4 ml/min and 40 °C. The gradient program was shown below: 1 min, 90 % A; 10 min, 40 % A; 11–12 min, 90 % A. The injection volume was 10 μl.

Standard solution preparation: HPLC-grade methanol was used to dilute the stock solutions (1000 μg/ml) of E2, E1, 2-OHE2, 2-OHE1, 4-OHE2, 16α-OHE1, 2-MeOE1, and 2-MeOE2 to 10 μg/ml. The storage of all standard solutions was made at 4 °C refrigerator. Diluting standard solutions with water was adopted to freshly prepare a series of working solutions of E2, E1 and estrogen metabolites to produce different concentrations (1000, 500, 50, 5, 2, 1, and 0.1 pg/ml).

Sample preparation: Seven milliliters of urine specimen was taken, and 0.28 g NaOH was put to the urine specimen. The 10-min boiling of urine specimen was made. Next, concentrated HCl was put to the urine specimen to modify the pH to 3, and the 3-min centrifugal of urine specimen was made at 4000 rpm. The precipitate was discarded, and the supernatant was diluted to 7 ml. The hollow fibers were cut into 11 cm pieces. After the 20-min acetone and ultrasonic washing of sections for impurities removal, they were dried directly in air. The 10-min infiltration of hollow fiber sections was made with n-octanol and ultrasonicated to fill the pores of the fiber walls with n-octanol. N-octanol was slowly pushed into the hollow fiber cavity using a syringe. The hollow fibers were then placed in a centrifuge tube including the urine specimen and vortexed for 1 h at room temperature. The fibers were then pushed into a drying centrifuge tube and dried under a nitrogen blower at 40 °C. Next, the slow rinsing of fibrous lumen was made with 0.5 ml methanol. Next, 100 μl of reconstitution solution (50 % methanol in water) was put, and the mixture was vortexed to reconstitute. Next, the injection of 10 μl of this solution into an LC-MS instrument was made for detection.

HPLC-MS analysis: The verification method was analyzed by the HPLC-MS method to evaluate the precision of the method. Accuracy was decided through the calculation of the relative error (RE) applying the calculation formula: RE% = [(measured value-theoretical value)/theoretical value] × 100. The recovery rate and RSD were, respectively, as follows: E2: 94.2–96.7 % and 5.2–11.2 %; E1: 88.3–98.6 % and 6.1–13.5 %; 2-OHE2: 98.4–103.5 % and 4.7–8.5 %; 2-OHE1: 89.1–107.4 % and 3.9–10.7 %; 4-OHE2: 101–109 % and 4.3–13.1 %; 16α-OHE1: 100.4–103.7 % and 4.9–10.4 %; 2-MeOE2: 99–104 % and 7.5–12.4 %; and 2-MeOE1: 89.1–107.4 % and 4.6–7.8 %. By drawing a standard curve, the weighted linear regression method was used to obtain the calibration equation. The calibration equations were as follows: E2: y = 1392.60x-227.98 (r = 0.9972), 0.014–14 ng/ml; E1: y = 1.31683e5x-3502.89 (r = 0.99580), 0.000028–1.4 ng/ml; 2-OHE2: y = 8.76344e6x-1168.57 (r = 0.99998), 0.000028–1.4 ng/ml; 2-OHE1: y = 2.96428e7-421.78 (r = 0.99986), 0.000028–1.4 ng/ml; 4-OHE2: y = 1.03842e7x+1069.24 (r = 0.99977), 0.000028–1.4 ng/ml; 16α-OHE1: y = 2.00641e6+255.07 (r = 0.99995), 0.000028–1.4 ng/ml; 2-MeOE2: y = 5202.96x-653.70 (r = 0.99967), 0.014–14 ng/ml; and 2-MeOE1: y = 29123.0x-8335.83 (r = 0.99665), 0.014–14 ng/ml.

2.4. Laboratory testing of urinary creatinine

We used creatinine values to normalize all creatinine values to adjust for urine concentrations. Take 0.5 ml of urine sample and measure the concentration of urine creatinine by creatine oxidase method. All urine samples were repeated in one run, and if the difference between repeated tests was more than 3 times, the test was repeated. The geometric mean of the replicates for each sample was adopted for the analysis, and the estrogen and its metabolite concentrations detected by HPLC-MS were compared with the creatinine value, and the result of the ratio was the final result.

2.5. Statistical methods

SPSS 13.0 software was adopted to statistically analyze all data. The experimental data followed normal distribution were shown as mean ± standard deviation ($\bar{x} \pm s$), and one-way discussion on variance was adopted to compare the groups. The measurement data failing to follow normal distribution were expressed as median (25 %, 75 %) , and non-parametric test was adopted for group comparison. Pearson correlation parameter was adopted for association analysis, and stepwise regression analysis with BMI as the dependent variable was performed for statistically significant values. Spearman rank correlation analysis was adopted for univariate correlation analysis. $P < 0.05$ was of statistically significance.

3. Results

Based on the comparison of the clinical data of each group, waist circumference and the levels of FPG, FIN, TG, TC, SBP, DBP, LDL, E2, E1, 16α-OHE1, and 2-hydroxyestrone (2-OHE1) in the obese group were larger than those in the normal and low body weight groups ($P < 0.05$), while the obese group showed lower HDL level than the normal and lower body weight groups ($P < 0.05$) (Table 1).

In the correlation analysis of obesity influencing factors, BMI was adopted as the dependent variable, and the residual variables were used as independent variables for correlation analysis. Stepwise regression analysis displayed that the regression coefficient of E2 was 0.610 ($t = 9.355$, $P = 0.000$), 2-OHE1 was 0.631 ($t = 2.346$, $P = 0.020$), and 16α-OHE1 was 0.498 ($t = 4.09$, $P = 0.000$). $P = 0.000$). The results showed that E2, 2-OHE1 and 16α-OHE1 levels had a significant positive effect on BMI ($P < 0.05$)(Table 2).

In the correlation analysis of urine estrogen and its metabolites with blood glucose and blood lipid degrees, the levels of E1, 2-OHE1, 16α-OHE1 and E1/2- MeOE1 were positively correlated with fasting blood glucose (FBG) ($P < 0.05$). The levels of E2, E1, 2-OHE1, 16α-OHE1 and 16α-OHE1/2-OHE1 were positively correlated with fasting insulin (FIN), triglyceride (TG), total cholesterol (TC) and low-density lipoprotein (LDL) ($P < 0.05$). The levels of E2, E1, 2-OHE1, 16α-OHE1 and 16α-OHE1/2-OHE1 were negatively related to high-density lipoprotein (HDL) ($P < 0.05$). The level of 2-MeOE1 was positively related to total cholesterol (TC) and low density lipoprotein (LDL) ($P < 0.05$) (Table 3).

4. Discussion

Several anthropometric parameters are related to increased risk for different hormone-associated cancers (endometrial, ovarian, and postmenopausal breast cancers) [6]. Previous meta-analyses have estimated a 54 % increas in endometrial cancer risk and a 12 % increas in postmenopausal breast cancer risk for each 5 kg/m2 growth in BMI [7,8]. Abdominal obesity, estimated by waist-to-hip ratio (WHR), was also greatly positively related to risk for endometrial and postmenopausal breast cancer [9]. Throughout the course of life, estrogen is produced in peripheral cells through the conversion of androgens in adipose tissue. Nevertheless, during women's reproductive years, estrogen is generated intensively along the hypothalamic-pituitary-ovarian (HPO) axis, and it mainly originates from the ovaries [10]. Obesity in females of reproductive age has many adverse metabolic roles including type 2 diabetes, dyslipidemia, and cardiovascular disease. It is related to menstrual disorders, ovulation disorders, insulin resistance, and the occurrence of infertility [11].This previous study found that estradiol levels showed a downward trend with age. Related studies have shown that women's ovarian function declines with age; the E2 levels are decreased, and cholesterol and TG levels are increased, which could induce heart disease and vascular disease [12]. With a growth in the level of estrogen and a subsequent decrease in the synthesis of related apolipoprotein A1 and lecithin cholesterol, there is a decrease in fatty amidotransferase activity and a decrease in HDL in the blood, leading to increased risk of cardiovascular disease [13]. The present study showed that the BMI, waist circumference, WHR, and

Table 1
Expression and comparison of various indexes and levels of estrogen and its metabolites in different BMI groups.

Analyte	BMI (kg/m^2)				F/H	*P*
	<18.5	18.5 < BMI<24.0	24.0 ≤ BMI<28.0	≥28.0		
N	30	40	35	30		
age	42.6 ± 3.8	41.8 ± 2.5	43.6 ± 2.1	42.1 ± 4.3	2.15	0.097
BMI(kg/m^2)	17.6 ± 0.7	22.91 ± 1.54	25.30 ± 1.00	28.89 ± 1.85	367.52	<0.001*
Waistline (cm)	54.56 ± 2.34	76.56 ± 3.14	98 ± 4.21	113 ± 5.06	1387.84	<0.001*
SBP(mmHg)	119.56 ± 6.03	120.75 ± 9.43	129 ± 8.01	137 ± 9.56	29.35	<0.001*
DBP(mmHg)	60.24 ± 3.12	64.31 ± 2.02	68.02 ± 3.87	95.21 ± 7.08	420.36	<0.001*
FBG (mmol/L)	4.6 ± 1.35	4.7 ± 0.85	5.13 ± 1.20	5.78 ± 1.07	7.24	<0.001*
FINS(μU/ml)	9.57 ± 2.45	10.36 ± 3.07	17.21 ± 5.02	25.35 ± 2.78	137.55	<0.001*
TG (mmol/L)	0.7 ± 0.31	0.9 ± 0.25	1.5 ± 0.28	2.0 ± 0.32	132.29	<0.001*
TC (mmol/L)	3.5 ± 0.41	4.3 ± 0.32	5.0 ± 0.52	5.92 ± 0.41	184.47	<0.001*
HDL (mmol/L)	1.7 ± 0.21	1.6 ± 0.25	1.25 ± 0.22	1.01 ± 0.24	60.91	<0.001*
LDL (mmol/L)	2.0 ± 0.45	2.6 ± 0.51	3.5 ± 0.50	4.2 ± 0.47	124.35	<0.001*
E2 (ng/mg)	1.04 (0.47,2.61)	5.44 (3.40,8.08)	5.97 (4.22,8.00)	10.51 (7.11,12.39)	72.28	<0.001*
E1 (ng/mg)	0.05 ± 0.03	0.10 ± 0.04	0.12 ± 0.07	0.17 ± 0.08	21.75	<0.001*
2-OHE2(pg/mg)	0.96 (0.41,1.60)	1.05 (0.52,1.62)	0.84 (0.26,1.60)	1.15 (0.56,2.22)	1.75	0.626
2-OHE1(pg/mg)	1.03 ± 0.52	0.88 ± 0.46	1.73 ± 1.00	2.10 ± 1.24	15.88	<0.001*
4-OHE2(pg/mg)	0.91 (0.45,1.06)	0.60 (0.25,0.85)	0.61 (0.33,1.10)	0.64 (0.33,1.07)	3.52	0.318
16α-OHE1(pg/mg)	0.78 (0.41,1.33)	0.51 (0.23,0.70)	1.72 (0.75,2.96)	2.76 (1.65,5.83)	64.92	<0.001*
2-MeOE2 (ng/mg)	2.13 (0.60,3.08)	1.35 (0.74,2.37)	2.00 (0.87,3.67)	1.51 (0.88,2.50)	1.85	0.603
2-MeOE1 (ng/mg)	1.93 (1.04,2.92)	1.08 (0.53,1.89)	1.43 (0.49,3.50)	2.09 (0.66,3.02)	5.73	0.125
E2/E1	25.11 (8.01,54.07)	53.85 (39.04,80.28)	54.60 (29.76,78.64)	58.17 (38.63,86.40)	15.44	0.001*
4- OHE2/2-MeOE2 (10^{-3})	0.37 (0.22,1.84)	0.42 (0.16,0.83)	0.29 (0.15,0.64)	0.43 (0.23,0.62)	1.78	0.619
2- OHE2/2-MeOE2 (10^{-3})	0.59 (0.21,1.21)	0.75 (0.30,1.40)	0.52 (0.19,1.19)	0.73 (0.24,1.70)	2.39	0.496
2-OHE1/2-MeOE1 (10^{-3})	0.93 (0.44,2.12)	1.30 (0.53,2.68)	0.48 (0.14,0.85)	0.66 (0.18,1.44)	9.44	0.024*
E1/2-MeOE1	0.03 (0.01,0.05)	0.08 (0.04,0.19)	0.09 (0.03,0.18)	0.08 (0.04,0.30)	23.17	<0.001*
16α-OHE1/2-OHE1	0.75 (0.36,1.59)	0.55 (0.30,0.88)	1.44 (0.44,2.09)	1.76 (0.87,4.05)	21.27	<0.001*
E2/2-MeOE2	0.79 (0.23,2.20)	3.06 (2.02,9.38)	2.84 (1.41,8.50)	5.49 (4.05,8.84)	36.34	<0.001*

*$P < 0.05$.

Table 2
Results of stepwise regression analysis of influencing factors of obesity in premenopausal middle-aged women.

	Regression Coefficient	T	95 % CI	Collinearity Diagnostics	
				VIF	Tolerance
Constant	18.323*	−36.766	17.346–19.300	–	–
E2	0.610*	−9.355	0.482–0.738	1.131	0.884
2OHE1	0.631*	−2.346	0.104–1.158	1.251	0.799
16αOHE1	0.498*	−4.09	0.259–0.736	1.299	0.77
Sample Capacity			135		
R^2			0.579		
Adjusted R^2			0.57		
F			F (3,131) = 60.134,p = 0.000		

Dependent Variable：BMI.
*P < 0.05.

Table 3
Correlation of estrogen and its metabolites with age, FBG, FINS, TG, TC, HDL and LDL.

	AGE	FBG	FINS	TG	TC	HDL	LDL
E2	−0.023	0.164	0.512**	0.528**	0.601**	−0.467**	0.569**
E1	0.03	0.237**	0.388**	0.464**	0.487**	−0.400**	0.469**
2OHE1	0.107	0.243**	0.431**	0.430**	0.413**	−0.376**	0.433**
16αOHE1	0.036	0.228**	0.525**	0.551**	0.553**	−0.449**	0.436**
2OHE2	0.023	−0.041	0.093	0.108	0.127	−0.093	0.128
4OHE2	0.008	−0.036	−0.004	−0.073	−0.045	0.109	−0.045
2MeOE2	−0.093	0.061	−0.029	−0.06	−0.014	0.033	0.045
2MeOE1	−0.002	−0.092	0.135	0.07	0.198*	−0.07	0.176*
16α-OHE1/2-OHE1	0.065	0.055	0.238**	0.266**	0.278**	−0.222**	0.193*
2-OHE1/2-MeOE1 (10–3)	0.07	−0.114	−0.073	−0.036	−0.004	0.012	−0.053
2-OHE2/2-MeOE2 (10–3)	0.102	−0.027	−0.005	0.116	0.076	−0.042	0.013
4-OHE2/2-MeOE2 (10–3)	0.152	−0.113	−0.118	−0.043	−0.086	0.065	−0.112
E1/2-MeOE1	0.003	0.257**	0.061	0.073	0.126	−0.136	−0.016
E2/2-MeOE2	0.131	−0.048	0.071	0.151	0.13	−0.138	0.1
E2/E1	0.079	−0.056	0.026	0.128	0.021	0.01	−0.059

*p < 0.05 **p < 0.01

the levels of TG, TC, LDL, FIN, and LDL were higher in the obese group than in the normal group, while the HDL degree was lower in the obese group than in the normal group. This finding suggests that the metabolism of blood lipids is disordered, the body's utilization of free fatty acids is low, and the accumulation of free fatty acids in the blood causes hypertriglyceridemia, which leads to obesity.

The loss of estrogen receptor alpha (ERα) in adipocytes has been found to exacerbate obesity in female mice during feeding. Research has revealed a close association between disruption of estrogen balance and the occurrence of obesity [14]. Research has revealed that interactions among cellular elements within the microenvironment of obese breast cancer patients can influence estrogen sensitivity, which is a critical component of hormone-related cancer progression [15]. Hormone levels in premenopausal women are affected by the menstrual cycle. Previous studies have found that the levels of estrogen and estrogen metabolites vary in the early follicular (2 ± 4 days), mid-follicular (7 ± 9 days), ovulation period (ovulation 3 to ovulation 11 days), and mid-luteal phase (5 ± 9 days after ovulation). Because the detection values significantly differ [16], in the current research, urine samples were gathered from women's early menstrual follicles to reduce the influence of ovarian factors. In the current research, HPLC-MS was addopted to detect the levels of estrogen and its metabolites in the urine of premenopausal women. It was found that the E2, 16α-OHE1 and 2-OHE1 levels were greatly grown in the obese group (P < 0.05). The study found that as the degree of obesity increases, there is an upward trend in the concentrations of E2, 16α-OHE1 and 2-OHE1. This result suggests that obesity may induce changes in estrogen metabolism, leading to an increase in genotoxic estrogen metabolites such as 16α-OHE1 and 2-OHE1. Therefore, the study proposes a potential association between obesity and the accumulation of genotoxic estrogen metabolites, including 16α-OHE1. This finding serves as a reminder that obesity not only affects body weight but also potentially exerts profound impacts on endocrine and metabolic processes, possibly increasing the risk of specific cancers. This research provides a new perspective for a deeper understanding of the relationship between obesity and health risks, offering valuable insights for the prevention and intervention of related diseases.

The level of estrogen and estrogen metabolites is closely associated with the occurrence of tumors. Our previous research has displayed that E2, E1, and estrogen toxic metabolites 4-OHE2 and 16α-OHE1 are highly expressed in urine of patients with endometrial cancer. 16α-OHE1 is produced by the hydroxylation reaction on the 16th carbon atom of estrogen, and the CYP3A4 enzyme exerts a significant effect on the process of C-16 hydroxylation [17]. Chagay reported that 16α-OHE1 was highly expressed in estrogen-dependent breast cancer cells [, they showed that 16α-OHE1, a metabolite of estrogen, supports the proliferation of breast cancer cells, which may be associated with the subsequent development of cancer [18]. The measurement of estrogen and estrogen metabolites in urine of premenopausal women displayed that a higher concentration of 16α-OHE1 increased the risk of breast cancer [19]. In the present study, Stepwise regression analysis showed a strong positive association between genotoxic 16α-OHE1 and 2-OHE1

concentrations and BMI($P < 0.05$). These outcomes show that the accumulation of these compounds may be caused by excessive obesity. Therefore, the present study shows that we can detect the increase in genotoxic estrogen metabolites in obese healthy women and implement treatment approaches in advance to prevent the occurrence of tumors.

Research has displayed that hyperinsulinemia is a risk element for endometrial cancer independent of E2. As a multifunctional protein hormone, insulin can inhibit the production of sex hormone-binding globulin, resulting in a growth in the level of free sex hormones and enhancement of estrogen activity [20]. Insulin can also promote the synthesis of ovarian androgens, and the increased androgens provide more substrates for the conversion of peripheral estrogen, which is particularly harmful for postmenopausal women [21]. Hyperinsulinemia can also enhance the activity of cytochrome aromatase P450, stimulate the expression and activity of aromatase in uterine glands and stroma, increase the conversion of androgens to estrogen, and promote the synthesis of local estrogen in the endometrium. This greatly increases the risk of endometrial cancer [22]. The current research found that the urine levels of E2, E1, 16α-OHE1 and 2-OHE1 in middle-aged females before menopause were positively correlated with FIN levels. As the insulin level increased, the levels of estrogen and the toxic metabolite 16a-OHE1 and 2-OHE1 also increased. Therefore, we speculate that insulin may have a synergistic effect on estrogen metabolism in some signaling pathways, which can further aggravate the progression of the disease.

5. Conclusion

Studies have demonstrated the participation of hormones in adipose tissue growth, metabolism, and inflammatory activities. Their deficiency results in overmuch fat accumulation and impairs adipocyte function, and adipose tissue in obese individuals has the characteristic such as changed expression of estrogen receptors and core enzymes taking part in their synthesis. Estrogens exert a significant effect on controlling adipogenesis and adipose tissue activity during health and obesity. This suggests a hidden herapeutic application of estrogen and its metabolites in the treatment of obesity [23]. The outcomes of the current research displayed that E2, E1, 16α-OHE1 and 2-OHE1 were negatively correlated with HDL and positively correlated with LDL. Therefore, it is necessary to monitor women with elevated estrogen levels and lipid metabolism disorders to stop and reduce the risk of cardiovascular diseases in time.

In conclusion, a great positive correlation between E2, 16α-OHE1 and 2-OHE1 levels and BMI in premenopausal women was found, and obesity was closely related to estrogen and its metabolites levels. As BMI increases, the levels of carcinogenic estrogen metabolites also increase, and 16α-OHE1 and 2-OHE1 are positively correlated with lipid metabolism levels, which explains the predisposition to malignancy in obese women. The date that support the outcomes of this research is available from the related author upon proper request.

Ethics approval and consent to participate

All studies were conducted in accordance with the protocol approved by the ethics committee of the Fourth Hospital of Hebei Medical University [No.2020K-1260]. All patients and healthy controls provided informed consent.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this. Further enquiries can be directed to the corresponding author.

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CRediT authorship contribution statement

Huanhuan Zhao: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Hongfang Yang:** Conceptualization, Data curation, Formal analysis, Methodology. **Zhiwei Li:** Conceptualization, Data curation, Formal analysis, Resources. **Zhonghuan Ge:** Conceptualization, Methodology, Writing – original draft. **Mei Zhou:** Conceptualization, Methodology, Writing – original draft. **Li Li:** Conceptualization, Formal analysis, Methodology, Writing – original draft. **Jing He:** Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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