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## Relationship between serum homocysteine and different menopausal stage

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### ABSTRACT

**Objective:** This study aimed to investigate the serum level of homocysteine in menopausal women and to explore the association between menopausal status, blood lipids, and homocysteine concentrations.

**Methods:** The study recruited females aged 40–60 years who were not receiving menopausal hormone therapy. The demographic characteristics and menopausal status of the women were collected in the form of questionnaires. The study analyzed the association between hyperhomocysteinemia and variables using binary logistic stepwise regression.

**Results:** Among 366 enrolled subjects, menopausal status was divided into four stages: premenopause stage ( $n = 135$ ), menopausal transition stage ( $n = 91$ ), early postmenopause stage ( $n = 87$ ), and late postmenopause stage ( $n = 53$ ). The proportion of hyperhomocysteinemia in the premenopausal stage, menopausal transition stage, and postmenopausal stage was 43%, 26.4%, and 45%, respectively ( $\chi^2 = 8.999$ ,  $p = 0.011$ ). The mean concentration of homocysteine was 9.75  $\mu\text{mol/l}$ . The level of homocysteine was higher in the postmenopause stage than in the other stages ( $p = 0.043$ ), and the difference between postmenopause and menopausal transition was statistically significant ( $p < 0.01$ ). In the binary logic analysis, menopausal transition compared with postmenopause (odds ratio = 2.027, 95% confidence interval = 1.117–3.679,  $p = 0.005$ ).

**Conclusions:** Serum homocysteine levels are associated with menopausal status. Homocysteine concentrations were progressively higher across menopausal stages. The transformation in the female body across the menopause transition stages may cause elevations in the homocysteine level in postmenopausal women.

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### KEYWORDS

Homocysteine; menopause; blood lipids; cardiovascular and cerebrovascular risk factors

### Background

Cardiovascular diseases (CVD) are the major diseases that endanger middle-aged people. Hyperhomocysteinemia has been considered a significant independent risk factor for the development of atherosclerosis and related diseases in prior studies<sup>1–3</sup>, and morbidity from CVD in elder persons is increasing significantly<sup>4</sup>. Factors affecting the serum homocysteine (Hcy) level include deficiency of certain vitamins and folic acid in the body and deficiency of the enzyme genes involved in Hcy metabolism<sup>5,6</sup>. A former study found that serum Hcy levels were significantly higher in men than women, but the differences tended to disappear with age<sup>7</sup>. The incidence of CVD in postmenopausal women increased significantly<sup>8,9</sup>. Several studies have linked increased risk of CVD to low estrogen levels<sup>10,11</sup>. However, there is no consistent conclusion about the effect of low estrogen levels on serum Hcy at home and abroad. Several studies recently showed an association between the serum concentrations of Hcy and osteoporosis in postmenopausal women<sup>12,13</sup>. In addition, studies of the effects of menopausal hormone therapy on serum Hcy are rare. Some studies have shown that supplementation of exogenous estrogen reduces serum Hcy levels<sup>14,15</sup>.

Our study aimed to investigate the levels of blood lipids, Hcy, and its influencing factors in different menopausal women who attended menopausal specialist outpatient clinics in Shanghai, and to provide evidence for the monitoring and prevention of metabolic diseases in menopausal women.

### Materials and methods

#### Sample calculation

We calculated the sample size required by the research using PASS for Windows. The prevalence of hyperhomocysteinemia in middle-aged women was 30–40%<sup>16</sup> from reading previous literature, and the sample size using PASS for Windows was calculated as 341 being sufficient for study.

#### Object of study

The study recruited females who were examined at the Physical Examination Center of Shanghai Jiao Tong University Affiliated Sixth People's Hospital from April 2014 to December 2015. The inclusion criteria are as follows: aged 40–60 years; menopausal transition naturally; and no

hormone replacement therapy for the past 6 months. The exclusion criteria were: surgical menopause; having received exogenous estrogen oral administration during the study period; women with chronic illness such as CVD or cancer; taken medicine or chemotherapy treatment such as aspirin; and chronic irregular menstruation including primary ovarian insufficiency/premature ovarian failure. The study passed the approval of the Ethical Committee of Shanghai Sixth People's Hospital, obtaining the subject's consent and their signed informed consent.

### Survey methods

The study adopted a questionnaire designed by ourselves<sup>17</sup>. A face-to-face questionnaire was conducted among women aged between 40 and 60 years by a professionally trained person. Investigation contents include age, height, body weight, body mass index (BMI), blood pressure, occupational status, occupation, marital status, menstrual status, reproductive history, history of gynecological disease, surgical history, history of hypertension, diabetes mellitus, blood lipid metabolic abnormalities, stroke history, family history of breast disease, and history of estrogen replacement therapy.

### Reproductive staging

According to the Stage of Reproductive Aging Workshop (STRAW + 10) hosted at the 2011 Annual Meeting of the North American Menopause Society<sup>18</sup>, natural reproductive staging was classified into premenopause, menopausal transition, and postmenopause, respectively. Premenopause was defined as having regular menstrual cycles. Menopausal transition was defined as the menstrual cycle length beginning to vary or amenorrhea in the last 2 months. Postmenopause is divided into early postmenopause and late postmenopause, defined as a duration of amenorrhea between 1 and 5 years after the final menstrual period and a length of amenorrhea greater than 5 years, respectively. Surgical menopause was marked as amenorrhea caused by hysterectomy and endometrial ablation or bilateral ovariectomy.

### Anthropometric measurement

The height and weight of the subjects were measured using the human body fat meter TBF-418B (TANITA berrida) in bare feet indoors. To calculate the subjects' BMI, their weight in kilograms was divided by their height in meters squared. The classification standard was as follows:  $<18.5 \text{ kg/m}^2$  for low weight,  $18.5\text{--}24.99 \text{ kg/m}^2$  for normal,  $25\text{--}29.99 \text{ kg/m}^2$  for overweight, and higher than  $30 \text{ kg/m}^2$  for obesity<sup>19</sup>. Blood pressure was measured by the OMRON BP-203RVIIIC. After sitting for 5 min, the subjects' blood pressure was measured three times on their right upper arm successively and the mean value of the three measurements was taken for analysis. Hypertension is defined as systolic blood pressure  $>140 \text{ mmHg}$  or diastolic blood pressure  $>90 \text{ mmHg}$ , or subjects taking antihypertensive drugs currently<sup>20</sup>.

### Laboratory detection

All subjects provided 5 ml of venous blood at least 12 h after fasting at the Physical Examination Center of Shanghai Jiao Tong University Affiliated Sixth People's Hospital in the morning. The venous blood samples were anticoagulated with ethylenediamine tetraacetic acid and centrifuged at a rate of 4000 rpm for 15 min in the low-speed centrifuge ch80-2 Angle (Shanghai Medical Equipment Group Co. Ltd. Surgical Equipment Factory). The samples were sent to the laboratory to test the concentration of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and Hcy on the same day. The HITACHI 7600-120 automatic biochemical analyzer (Shanghai Rihe Trading Co., Ltd.) was used to test the biochemical indexes. Hcy was measured at  $3.0\text{--}50.0 \mu\text{mol/l}$  using the cyclic enzyme method, and the coefficient of variation (CV) in the batch was no more than 4.6% and the inter-batch difference was no more than 5.9%. The measured CV value of TG was less than 5% using the method of deglycerification.

TC is determined by the cholesterol oxidase method, and the measured CV value was less than 5%. LDL-C is determined by direct measurement using the selective protection method, and the measured CV value was less than 5%. HDL-C was determined by direct measurement using the antibody blocking method, and the measured CV value was less than 5%.

### Definition of dyslipidemia and hyperhomocysteinemia

Dyslipidemia is defined as elevated cholesterol or TGs in plasma, commonly known as hyperlipidemia. In fact, it also refers to all kinds of dyslipidemia including low- and high-density lipoproteinemia. Until China develops an appropriate guideline for our local population, we can use the European Society of Cardiology/European Atherosclerosis Society 2016 guidelines to evaluate dyslipidemia in our country<sup>21,22</sup>. The classification criteria for normal TC, edge elevation, and hypercholesterolemia are  $<5.2 \text{ mmol/l}$ ,  $5.2\text{--}6.2 \text{ mmol/l}$ , and  $>6.2 \text{ mmol/l}$ , respectively. Normal LDL-C level, rising edge, and high LDL-C hematic disease classification standards are  $<3.4 \text{ mmol/l}$ ,  $3.4\text{--}4.1 \text{ mmol/l}$ , and  $>4.1 \text{ mmol/l}$ , respectively. The classification criteria for normal HDL-C level and low HDL-C lipoproteinemia were  $>1.0 \text{ mmol/l}$  and  $<1.0 \text{ mmol/l}$ , respectively. The classification criteria for normal TG level, edge elevation, and hypertriglyceridemia are  $<1.7 \text{ mmol/l}$ ,  $1.7\text{--}2.3 \text{ mmol/l}$ , and  $>2.3 \text{ mmol/l}$ , respectively. In this study, the classification criterion for serum hyperhomocysteinemia is a level  $>10 \mu\text{mol/l}$ <sup>23,24</sup>.

### Statistical method

The data were analyzed by the statistical software SPSS 21.0 (SPSS, Chicago, IL, USA). Continuous data such as age, lipids, and Hcy are presented as mean  $\pm$  standard deviation. The skew distribution is represented as the median. The classified data such as education level and occupation status are

represented by prevalence (%). Descriptive statistics are used to analyze demographic characteristics and individual variables. The comparison between blood lipid and Hcy under different menopausal conditions was analyzed by one-way analysis of variance. An independent *t*-test was used to compare continuous data in the two groups (normal and hyperhomocysteinemia groups). The incidence of hyperhomocysteinemia was compared among the four menopausal stages using the chi-square test and results are shown as  $\chi^2$ . Binary logistic stepwise regression analysis was used to test the factors associated with the serum Hcy.  $p < 0.05$  indicates that the difference was statistically significant.

## Results

### Demographic characteristics of the subjects

According to the classification standard for hyperhomocysteinemia ( $>10 \mu\text{mol/l}$ ), the study population was divided into two groups: the normal Hcy level group and the hyperhomocysteinemia group. The test results of the two independent samples show that systolic blood pressure, TC, and LDL-C were all higher in the hyperhomocysteinemia group ( $p = 0.04$ ,  $p = 0.016$ , and  $p = 0.023$ , respectively). The demographic characteristics of the 366 subjects and comparisons between the normal group and the hyperhomocysteinemia group are presented in Table 1.

### Comparison of blood lipid and homocysteine among different menopausal status

The 366 subjects in our study were in the premenopause stage ( $n = 135$ ), the menopausal transition stage ( $n = 91$ ), and

the postmenopause stage ( $n = 140$ ) divided into early postmenopause stage ( $n = 87$ ) and late postmenopause stage ( $n = 53$ ). For all subjects, the mean  $\pm$  standard deviation age was  $49.43 \pm 5.75$  years and the concentration of Hcy was  $9.75 \pm 1.92 \mu\text{mol/l}$ . Chi-square test results are presented in Table 2. The morbidity of hyperhomocysteinemia in the premenopausal stage, menopausal transition stage, and postmenopausal stage was 43%, 26.4%, and 45%, respectively ( $\chi^2 = 8.999$ ,  $p = 0.011$ ). The difference between Hcy concentrations among the four menopausal stages was statistically significant ( $p = 0.043$ ) and the Hcy concentration in the late postmenopause stage was higher than in menopause transition ( $p < 0.01$ ) (see Table 3).

### Binary regression analysis for hyperhomocysteinemia

Adjustments for possible confounding factors were performed by binary logistic regression analysis. Stepwise logistic regression analysis was used to assess the significance of the possible value of menopausal status as a determinant of the presence of hyperhomocysteinemia. Age, menopausal status, BMI, systolic blood pressure, diastolic blood pressure, TG, TC, HDL-C, and LDL-C were independent variables. The results were that menopause transition compared with postmenopause (adjusted odds ratio = 2.027, 95% confidence interval = 1.117–3.679,  $p = 0.02$ ) (see Tables 4 and 5).

## Discussion

The morbidity for CVD increases along with age<sup>25</sup>. Hyperhomocysteinemia is an important independent risk factor for atherosclerosis and its related diseases<sup>1–3</sup>. Some former studies have shown that Hcy is associated with age<sup>26,27</sup>.

**Table 1.** Differences in characteristics among the two groups.

Demographic characteristic	Total	Hyperhomocysteinemia group	Normal group	$\chi^2$	p-Value
Degree of education				1.459	0.48
Primary school and below	10 (2.7%)	5 (3.4%)	5 (2.3%)		
Secondary school	154 (42.1%)	56 (38.6%)	98 (44.3%)		
College or above	202 (55.2%)	84 (57.9%)	118 (53.4%)		
Menopausal status				10.259	0.016
Premenopause	135 (36.9%)	58 (40%)	77 (34.8%)		
Menopause transition	91 (24.9%)	24 (16.6%)	67 (30.3%)		
Early postmenopause	87 (23.8%)	36 (24.8%)	51 (23.1%)		
Late postmenopause	53 (14.5%)	27 (18.6%)	26 (11.8%)		
Diabetes mellitus				1.136	0.286
Yes	21 (5.7%)	6 (4.1%)	15 (6.8%)		
No	345 (94.3%)	139 (95.9%)	206 (93.2%)		
Hypertension				0.580	0.446
Yes	76 (20.8%)	33 (22.8%)	43 (19.5%)		
No	290 (79.2%)	112 (77.2%)	178 (80.5%)		
Age (years)	$49.43 \pm 5.75$	$49.62 \pm 5.76$	$49.31 \pm 5.75$		0.616
BMI ( $\text{kg/m}^2$ )	$22.58 \pm 2.84$	$22.67 \pm 2.83$	$22.52 \pm 2.83$		0.565
SBP (mmHg)	$121.69 \pm 16.30$	$124 \pm 16.2$	$120 \pm 16.2$		0.04 <sup>a</sup>
DBP (mmHg)	$74.80 \pm 10.45$	$75.87 \pm 10.92$	$74.08 \pm 10.09$		0.152
TG (mmol/l)	$1.11 \pm 0.66$	$1.05 \pm 0.58$	$1.16 \pm 0.71$		0.13
TC (mmol/l)	$5.18 \pm 1.05$	$5.34 \pm 1.00$	$5.07 \pm 1.08$		0.016 <sup>a</sup>
HDL-C (mmol/l)	$1.56 \pm 0.34$	$1.56 \pm 0.36$	$1.55 \pm 0.33$		0.903
LDL-C (mmol/l)	$3.12 \pm 0.77$	$3.23 \pm 0.82$	$3.04 \pm 0.73$		0.023 <sup>a</sup>
Fasting blood glucose (FBG) (mmol/l)	$5.27 \pm 0.99$	$5.17 \pm 0.61$	$5.34 \pm 1.17$		0.118

Data presented as *n* (%) or mean  $\pm$  standard deviation. BMI, body mass index; DBP, diastolic blood pressure; FBG, ; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

<sup>a</sup> $p < 0.05$ , statistically significant difference between the two groups.

**Table 2.** Comparison of the incidence of hyperhomocysteinemia.

Parameter	Total	Premenopause (n = 135)	Menopause transition (n = 91)	Postmenopause (n = 140)	$\chi^2$	p-Value
Hyperhomocysteinemia	39.6% (145)	43% <sup>a</sup> (58)	26.4% <sup>b</sup> (24)	45% (63)	8.999	0.011
Normal	60.4% (221)	57% <sup>b</sup> (77)	73.6% (67)	55% (77)		

Data presented as % (n). Compared with the menopausal transition group: <sup>a</sup> $p < 0.05$ ,  $\chi^2 = 6.471$ . Compared with the postmenopause group: <sup>b</sup> $p < 0.012$ ,  $\chi^2 = 5.774$ .

**Table 3.** Comparison among the four menopausal stages.

Parameter	Premenopause (n = 135)	Menopause transition (n = 91)	Early postmenopause (n = 87)	Late postmenopause (n = 53)	p-Value
Age (years)	44.26 ± 3.07	49.26 ± 3.51 <sup>b</sup>	52.96 ± 4.04 <sup>bd</sup>	57.11 ± 3.06 <sup>bdf</sup>	< 0.001
BMI (kg/m <sup>2</sup> )	22.65 ± 2.91	22.36 ± 2.95	22.46 ± 2.74	22.97 ± 2.62	0.628
SBP (mmHg)	123.64 ± 16.95	117.57 ± 12.40 <sup>a</sup>	119.35 ± 16.41	129.60 ± 18.02 <sup>ad</sup>	< 0.001
DBP (mmHg)	74.63 ± 11.23	73.47 ± 9.10	74.43 ± 9.88	78.22 ± 11.73 <sup>ce</sup>	0.099
TG (mmol/l)	1.01 ± 0.52	1.20 ± 0.90 <sup>a</sup>	1.17 ± 0.64	1.13 ± 0.52	0.128
TC (mmol/l)	5.17 ± 0.82	4.74 ± 1.18 <sup>b</sup>	5.24 ± 1.07 <sup>d</sup>	5.76 ± 1.00 <sup>bdf</sup>	< 0.001
HDL-C (mmol/l)	1.57 ± 0.33	2.97 ± 0.79	1.56 ± 0.40	1.58 ± 0.37	0.542
LDL-C (mmol/l)	3.05 ± 0.75	2.95 ± 0.80	3.16 ± 0.71	3.42 ± 0.76 <sup>bde</sup>	0.002
Hcy (μmol/l)	9.87 ± 2.02	9.34 ± 1.81 <sup>a</sup>	9.61 ± 1.80	10.26 ± 1.88 <sup>d</sup>	0.043

Data presented as mean ± standard deviation. One-way analysis of variance and least significant difference (LSD) method used for comparison. Compared with premenopausal group: <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ . Compared with menopausal transition group: <sup>c</sup> $p < 0.05$ , <sup>d</sup> $p < 0.01$ . Compared with early postmenopausal group: <sup>e</sup> $p < 0.05$ , <sup>f</sup> $p < 0.01$ . BMI, body mass index; DBP, diastolic blood pressure; Hcy, homocysteine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride. Values in bold indicate  $p < 0.05$ .

**Table 4.** Binary regression analysis for hyperhomocysteinemia (adjusted).

Parameter	$\beta$	p-Value (unadjusted)	Odds ratio (unadjusted)	95% confidence interval	
				Lower	Upper
Age (years)	-0.009	0.615	0.991	0.955	1.027
BMI (kg/m <sup>2</sup> )	-0.022	0.564	0.979	0.909	1.053
SBP (mmHg)	-0.015	0.041 <sup>a</sup>	0.985	0.971	0.999
DBP (mmHg)	-0.016	0.152	0.984	0.962	1.006
TG (mmol/l)	0.264	0.133	1.302	0.923	1.836
TC (mmol/l)	-0.255	0.018 <sup>a</sup>	0.775	0.628	0.956
HDL-C (mmol/l)	-0.038	0.903	0.963	0.522	1.774
LDL-C (mmol/l)	-0.319	0.024 <sup>a</sup>	0.727	0.551	0.959
FBG (mmol/l)	0.217	0.126	1.242	0.941	1.640
Menopausal status		0.01 <sup>b</sup>			
Premenopause	0.083	0.734	1.086	0.675	1.749
Menopausal transition	0.826	0.005 <sup>b</sup>	2.284	1.288	4.051
Postmenopause	Reference	Reference	Reference	Reference	

BMI, body mass index; DBP, diastolic blood pressure; FBG, ; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

<sup>a</sup> $p < 0.05$ .

<sup>b</sup> $p < 0.01$ .

**Table 5.** Binary regression analysis for hyperhomocysteinemia (adjusted).

Parameter	$\beta$	p-Value (adjusted)	Odds ratio (adjusted)	95% confidence interval	
				Lower	Upper
TC (mmol/l)	-0.062	0.708	0.939	0.678	1.302
LDL-C (mmol/l)	-0.219	0.320	0.803	0.521	1.238
Menopausal status		0.040 <sup>a</sup>			
Premenopause	0.013	0.957	1.013	0.624	1.645
Menopausal transition	0.707	0.020 <sup>a</sup>	2.027	1.117	3.679
Postmenopause	Reference	Reference	Reference	Reference	

LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

<sup>a</sup> $p < 0.05$ .

In our study, we did not observe an association between the level of Hcy and age. The difference between our study and the earlier research results may be due to age differences among subjects included in the previous study. The subject was selected after the age of 60 years<sup>27</sup>. The age of the subjects in our study ranged from 40 to 60 years because this age group meets the standard for menopause and

postmenopause age ranges. In addition, in the earlier study, the study subjects were women with surgical menopause (bilateral oophorectomy or hysterectomy)<sup>26</sup>. The study did not consider that surgery may affect Hcy levels.

This study showed that the incidence of hyperhomocysteinemia was 43%, 26.4%, and 45.0% in the premenopausal group, the menopausal transition group, and the

postmenopausal group, respectively. It showed that morbidity for hyperhomocysteine was increased across menopausal stages, which is consistent with previous studies at home and abroad<sup>26</sup>. However, there was no significant difference in hyperhomocysteine between the premenopausal group and the postmenopausal group, which is consistent with foreign studies<sup>28</sup>. But Hcy concentrations were progressively higher across menopausal stages. It is necessary for women to pay attention to the changes of Hcy after the menopause transitional period.

The design of this study was based on the STRAW + 10 standard to classify reproductive aging stages of women; namely, premenopause, menopause transition, early postmenopause, and late postmenopause. The divisions of these different periods include stages with large differences in estrogen and follicle stimulating hormone (FSH) levels, such as late menopause and premenopause, extreme fluctuations in estrogen and FSH levels in the menopausal transition stage, and a stable estrogen level that becomes variable in the premenopausal period. In this way, the association between menopausal status and Hcy concentrations can be observed objectively to the greatest extent. The results showed that serum Hcy levels gradually increased across the menopausal transition period to the postmenopause period, and there was significant difference between the menopausal transition period and the postmenopause period. At present, there is no consistent conclusion about the effect of menopause on the serum levels of Hcy. Some previous studies showed that the serum levels of Hcy were higher in the postmenopausal group than in the premenopausal group<sup>26</sup>. There are also results showing that menopausal status does not affect the serum levels of Hcy<sup>29,30</sup>. The differences in the analysis of these studies are mainly based on the division of menopausal status without following the standards of STRAW + 10 strictly, resulting in selection bias and result error for the study subjects.

Our study found that the serum levels of Hcy in the menopausal transition period were significantly lower than those in the premenopausal phase, and this result has not been reported in former studies. The aim of the study was to determine changes in the menstrual cycle in premenopausal women. Garg *et al.*<sup>31</sup> study shows that serum Hcy levels remain within a range of changes for at least 1 month. Therefore, the results of our study cannot rule out a fluctuation of Hcy caused by fluctuation of hormone levels during the menopausal transition. Tallova *et al.*<sup>32</sup> took 15 premenopausal females with luteinizing hormone/FSH peak between the 13th and 15th day of the menstrual cycle as the subject of their study, to analyze the differences of serum Hcy levels between the luteal period and the follicular period, and found that the levels of serum Hcy in the follicular period were significantly lower than in the luteal period, demonstrating that the menstrual cycle influences the concentrations of serum Hcy in premenopausal women. In our study, the changes in the menstrual cycle were not taken into account when analyzing the changes of serum Hcy level during the premenopausal stage, and extreme fluctuations in estrogen and FSH levels in the menopausal transition stage

are two factors that may jointly contribute to the result that the serum Hcy level was higher in the premenopausal period compared with the menopausal transition stage. This situation requires further expansion of sample studies to confirm.

It is not clear how hyperhomocysteinemia affects morbidity for atherosclerosis, but one mechanism may be involved in the process of Hcy induction of atherosclerosis – the effect on blood lipids, especially LDL-C<sup>29,31,32</sup>. This study showed that serum TC and LDC-C levels were higher in the hyperhomocysteine group than in the normal group. The results of the correlation analysis also showed that there was still an association between LDL-C and Hcy after controlling for confounding factors such as age and BMI. This indicates that the levels of serum Hcy and LDL-C interacted with each other, but this conclusion requires large randomized studies in the future to confirm, and the mechanism of mutual influence also requires further research.

### Significance and limitations of this study

This study investigated the serum Hcy concentration and its influencing factors in women 40–60 years old of different menopausal status through a cross-sectional study, to clarify the risk of menopause on cardiovascular disease and to improve the accuracy of monitoring the risk of cardiovascular and cerebrovascular diseases in middle-aged and elderly women and provide the basis for clinical intervention. However, this study did not conduct a large sample of epidemiological surveys in the community. Therefore, the results of the survey may be affected and bias may occur. These biases may affect the results of the analysis and affect the accuracy of the conclusions.

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### Contributions

M. Tao conceived and designed the study. Z. Zhu carried out the study and wrote the first draft. S. Jiang collected data and revised the manuscripts. J. Liu took part in the investigation. C. Li managed the data. All authors read and approved the final manuscript.

**Potential conflict of interest** All authors expressed that they did not have competing interests.

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