

Different effects of oral conjugated equine estrogens and transdermal estrogen on undercarboxylated osteocalcin concentration in postmenopausal women

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ABSTRACT

Objective: Undercarboxylated osteocalcin (ucOC) is a sensitive marker of vitamin K status, and triglyceride (TG) has been shown to be the main transporter of vitamin K. In the present study, we examined the difference between ucOC concentrations in postmenopausal women receiving hormone therapy (HT) with oral conjugated equine estrogens (CEE) and transdermal estradiol (TE₂). We also examined the associations of ucOC concentration with estradiol concentration and TG.

Design: Ninety-two postmenopausal women were recruited for this study. Serum concentrations of ucOC, intact osteocalcin, estradiol, and TG were measured before and after 12 months of HT. Forty-six women received oral administration of 0.625 mg of CEE and 2.5 mg of medroxyprogesterone acetate daily, and 46 women received transdermal administration of 50 µg of 17β-estradiol twice weekly and 2.5 mg of medroxyprogesterone acetate daily.

Results: The ucOC concentration in women during HT with oral CEE was significantly ($P < 0.01$) lower than that in women during HT with TE₂. Serum estradiol concentrations during HT with CEE showed a significant inverse correlation with ucOC concentrations and the ratio of ucOC/OC during HT ($P < 0.05$ and $P < 0.01$, respectively). In addition, the serum ucOC concentration in women with an increased percentage of change in TG was significantly ($P < 0.01$) lower than that in women with a decreased percentage of change in TG during HT with oral CEE.

Conclusion: The effect of HT with TE₂ on ucOC concentration in women is weaker than the effect of HT with oral CEE. Suppression of ucOC concentration in postmenopausal women during HT with oral CEE might be associated with the effect of vitamin K through increased TG induced by oral CEE.

Key Words: Undercarboxylated osteocalcin – Estradiol – Estrone – Postmenopausal hormone therapy.

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Conjugated equine estrogens (CEE) administered orally (0.625 mg/day) and 17β-estradiol administered transdermally (0.05 mg/day) have been shown to be equally effective for reducing levels of bone turnover markers and increasing bone mineral density (BMD) regardless of the difference in the route of administration.¹⁻³ The effects of oral hormone therapy (HT) and transdermal

HT on reducing the risk of hip fracture have also been reported to be similar.⁴ Recently, HT using transdermal estradiol (TE₂) and low-dose HT has been recommended for postmenopausal women^{5,6} from the results of the Women's Health Initiative (WHI).^{7,8}

Undercarboxylated osteocalcin (ucOC) is a valuable and sensitive nutrition marker reflecting vitamin K status⁹ because vitamin K is necessary for the posttranslational gamma-carboxylation of glutamic acid residues in pro-osteocalcin. It has been shown that circulating ucOC concentration begins to increase in early postmenopausal women¹⁰ and that the concentration is higher in older women.¹¹ Serum ucOC concentration has also been shown to be negatively correlated with BMD of the hip in elderly women¹² and to be correlated with the risk of hip fracture.^{11,13} Recently, it has been reported that postmenopausal women not receiving estrogen had a higher ucOC level than those receiving estrogen.¹⁴ In addition, the association of ucOC level with lumbar BMD has been shown to be different in postmenopausal women not receiving estrogen and those receiving estrogen.¹⁴ Triglyceride (TG) plays a role in the transport of vitamin K into bone and its use in the gamma-carboxylation of osteocalcin (OC), which is converted from ucOC to carboxylated OC.¹⁵ We previously demonstrated that ucOC concentration in women receiving HT with CEE daily, whose TG concentration was higher, was lower than that in women receiving HT with CEE on alternate days.¹⁶ CEE administered orally at a conventional dose has been reported to induce an increase in plasma TG concentration,¹⁷⁻¹⁹ but TG concentration was not increased by HT with TE₂.²⁰

The purpose of this study was to clarify the differences between serum ucOC concentrations in postmenopausal women during HT with oral CEE and in postmenopausal women during HT with TE₂. We also examined the associations of serum ucOC concentration with estradiol concentration as well as TG.

SUBJECTS AND METHODS

Subjects

The subjects of this study were recruited from the outpatient clinic of the Department of Obstetrics and Gynecology, Tokushima University Hospital. Ninety-two postmenopausal women who had been suffering from vasomotor symptoms between April 2003 and April 2004 were enrolled in the study, and informed consent for participation in the study was obtained from each woman. The Ethics Committee of Tokushima

University Hospital approved the study. Postmenopausal status was defined when serum follicle-stimulating hormone (FSH) concentrations were more than 40 mIU/mL and serum estradiol concentrations were less than 20 pg/mL. Reviews of medical histories and the results of physical examinations and blood chemistry tests showed that all the women were in good health and that none of the women had previously received medication known to influence calcium and lipoprotein metabolism before the commencement of HT. Excluded from the study were women with hypertension, diabetes mellitus, clinical manifestations of arteriosclerosis (coronary heart disease, peripheral artery disease or cerebrovascular disease), venous thromboembolic disease, liver disorders, unexplained vaginal bleeding, or a personal or family history of breast cancer. No abnormalities were found in Papanicolaou smears of the endometrium in any of the women. The women were not given specific instructions regarding adequate dietary calcium intake. Serum ucOC concentration was measured to determine vitamin K status, serum concentrations of intact OC, and bone-specific alkaline phosphatase (BAP) as bone formation markers were measured, and urinary deoxypyridinoline (DPD) concentration as a bone resorption marker was measured. Measurements were made before and after 12 months of treatment. BMD at the lumbar spine was also measured before and after 12 months of HT. Serum concentrations of TG, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured before and after 12 months of HT. Serum concentrations of estradiol and estrone were measured before and after 12 months of HT, and baseline serum concentrations of luteinizing hormone (LH) and FSH were measured before treatment. Eligible women were randomly assigned in open, parallel-group fashion to HT with oral CEE or with TE₂. The women who visited our clinic on odd-numbered days were treated by HT with oral CEE, and those who visited our clinic on even-numbered days were treated by HT with TE₂. Forty-six women received daily oral administration of 0.625 mg CEE and 2.5 mg medroxyprogesterone acetate, and 46 women received transdermal administration of 17β-estradiol (absorption rate, 50 μg/d) twice weekly and 2.5 mg medroxyprogesterone acetate orally every day. Endometrial smear examination and transvaginal ultrasonography were used for endometrial assessment during treatment. Compliance was checked by pill count, and side effects were ascertained by face-to-face questionnaires at 4-week intervals. Venous blood samples were drawn into tubes between 8:00

and 10:00 AM after a 12-hour fast. Samples obtained were frozen at -70°C until use in analysis. The assay was run at the same time. Urine samples were collected over a period of about 2 hours after an overnight void on the morning of the hospital visit, and samples were stored at -70°C until use in analysis.

Measurement of ucOC

Serum ucOC concentration was assayed by an enzyme-linked immunosorbent assay (ELISA) system using two monoclonal antibodies, anti-OC (21-31) antibody and a solid-phase anti-Glu 21,24-OC (4-5) antibody, with recombinant human ucOC (Biotechnology Research Laboratory, Takara Shuzo Co., Otsu, Shiga, Japan) as a standard. The intra- and interassay coefficients of variation (CVs) were 7.3% and 9.7%, respectively, and the sensitivity of the assay was 0.5 ng/mL.¹³

Measurements of estradiol and estrone

Precise measurement of serum estradiol concentrations in women receiving oral CEE is difficult because CEE is a complex of at least 10 natural estrogens. Extraction of sex steroid hormones in serum before the assay is needed for measurement in women receiving CEE. We have developed a highly sensitive and specific assay using high-performance liquid chromatography for purification and a radioimmunoassay for measurements of serum estradiol and estrone concentrations, and we have reported the precise concentrations of estradiol and estrone in women undergoing HT.²¹ In the present study, we used this assay for precise measurements of estradiol and estrone concentrations. In brief, estradiol and estrone, obtained by solid-phase extraction using a Sep pak tC18 cartridge, were purified by HPLC. Estradiol and estrone were sufficiently separated from other steroid hormones. Antiserum, ^{125}I -labeled reagents, and a precipitating reagent were obtained from Diagnostic System Laboratories (Webster, TX). The limits of detection of estradiol and estrone were 1.04 and 0.64 pg/mL, respectively. The intra-assay CVs of estradiol and that of estrone were 8.5% and 19.5%, respectively.

Measurements of serum intact OC, serum BAP, and urinary deoxypyridinoline

Serum intact OC concentration was assayed by an immunoradiometric assay, which was developed utilizing two kinds of monoclonal antibodies against the midregion (12-33) and C-terminal region (34-49) of synthetic human OC (BGP immunoradiometric assay

kit, Mitsubishi Chemical Co. Ltd., Tokyo, Japan).²² The intra- and interassay CVs were 3% to 4% and 3% to 8%, respectively, and the sensitivity of the assay was 0.5 ng/mL. The serum BAP concentration was assayed by ELISA using a kit from Quidel Co. (Santa Clara, CA). The intra- and interassay CVs were 4.8% and 5.8%, respectively, and the sensitivity of the assay was 0.7 U/L.²³ The urinary concentration of DPD was measured with ELISA using a kit from Quidel Co. Urinary concentrations were corrected for creatinine excretion. The intra- and interassay CVs were less than 10% and 15%, respectively, and the sensitivity of the assay was 2 nmol/L DPD.²⁴

Measurement of bone mineral density

BMD was determined at the lumbar spine (L2-4) by the dual-energy x-ray absorptiometry (DXA) technique using a Hologic QDR 2000 densitometer (Hologic Corp., Bedford, MA) before the initiation of treatment and at 12 months after the start of treatment. The CVs of these measurements were less than 1.0% for lumbar BMD. Data for BMD are expressed as g/cm^2 .

Measurements of lipid profiles

Serum concentrations of TG, TC, and HDL-C were measured by using an enzymatic calorimetric method. The low-density lipoprotein cholesterol (LDL-C) level was calculated according to the Friedewald formula.

Measurements of serum LH and FSH

Serum concentrations of LH and FSH were measured with radioimmunoassay kits obtained from Daiichi Radioisotope Laboratories (Tokyo, Japan). The intra-assay CVs of LH and that of FSH were 6.5% and 4.1%, respectively, and the interassay CVs of LH and that of FSH were 6.1% and 6.0%, respectively.

Statistical analysis

Results are presented as means \pm SDs. One-way analysis of variance and unpaired Student's *t* test were used to compare differences between groups. Paired *t* tests were used for within-group comparisons of differences between before and during HT. To determine differences between groups, the Mann-Whitney *U* test or the Kruskal-Wallis test was used if data were not normally distributed. Serum ucOC concentrations during HT at each dose in women were tabulated using descriptive statistics and correlated with serum

estradiol and estrone concentrations during HT using linear regression analysis. The ratios of ucOC/OC during HT at each dose in women were also tabulated using descriptive statistics and correlated with serum estradiol and estrone concentrations during HT at each dose using linear regression analysis. TG concentrations during HT at each dose in women were also tabulated using descriptive statistics and correlated with serum estradiol concentrations during HT at each dose using linear regression analysis. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Baseline characteristics

Eighty-eight (95.6%) of the 92 women completed the study and four women (two women who received HT with oral CEE and two women who received HT with TE₂) dropped out of the study. The treatment groups were well matched for baseline characteristics, including age, years since menopause, body mass index, estrone, estradiol, LH, and FSH concentrations (Table 1). There were no significant differences between the baseline characteristics in the two groups. Abnormal endometrial histology, including hyperplasia and carcinoma, was not detected in any women.

Changes in serum ucOC and OC concentrations

Table 2 shows the changes in serum ucOC and intact OC concentrations after 12 months of HT. The serum ucOC concentrations during HT decreased significantly (*P* < 0.01) compared to those before treatment in women receiving HT with oral CEE and TE₂. The ucOC concentration in women during HT with oral CEE (2.73 ± 1.80 ng/mL) was significantly

TABLE 1. Characteristics of women before treatment

	HT with CEE	HT with TE ₂
No. of women	46	46
Age (y)	52.1 ± 8.4	53.6 ± 7.0
Years since menopause	4.8 ± 5.8	6.4 ± 4.8
BMI	23.2 ± 2.9	22.5 ± 3.1
Estrone (pg/mL)	19.3 ± 11.6	20.1 ± 12.0
Estradiol (pg/mL)	5.4 ± 4.8	6.4 ± 4.8
LH (mIU/mL)	28.9 ± 12.9	28.0 ± 13.3
FSH (mIU/mL)	87.4 ± 29.6	85.6 ± 27.2

Means ± SDs.

HT, hormone therapy; CEE, conjugated equine estrogens; TE₂, transdermal estradiol; BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

TABLE 2. Changes in ucOC, bone turnover markers, and BMD after 12 months of HT in postmenopausal women

	HT with CEE	% Change	HT with TE ₂	% Change
ucOC (ng/mL)				
Before	5.60 ± 3.69	-46.1	6.25 ± 5.08	-27.6
After	2.73 ± 1.80 ^{a,b}		4.28 ± 4.01 ^a	
OC (ng/mL)				
Before	4.62 ± 2.77	-50.8	3.95 ± 2.44	-27.5
After	1.85 ± 1.17 ^a		2.34 ± 1.79 ^a	
BAP (U/L)				
Before	25.3 ± 8.87	-20.1	23.9 ± 7.99	-11.8
After	19.3 ± 6.56 ^a		20.7 ± 7.52 ^a	
DPD (nmol/L/ mmol/LCr)				
Before	8.83 ± 3.54	-23.9	7.08 ± 2.65	-10.9
After	6.63 ± 2.99 ^a		5.95 ± 2.27 ^c	
BMD (g/cm ²)				
Before	0.854 ± 0.152	4.5	0.870 ± 0.150	2.5
After	0.891 ± 0.151 ^a		0.888 ± 0.135 ^a	

Means ± SDs.

HT, hormone therapy; Before, before treatment; After, at 12 months after the start of HT; CEE, conjugated equine estrogens; TE₂: transdermal estradiol; ucOC, undercarboxylated osteocalcin; OC, osteocalcin; BAP, bone-specific alkaline phosphatase; DPD, deoxy-pyridinoline; BMD, bone mineral density.

^a*P* < 0.01 versus before treatment.

^b*P* < 0.05 versus women who received hormone therapy with transdermal estradiol.

^c*P* < 0.05 versus before treatment.

(*P* < 0.05) lower than that in women during HT with TE₂ (4.28 ± 4.01 ng/mL), while ucOC concentrations before treatment were not significantly different in the two groups. Serum ucOC concentrations in women receiving HT with oral CEE and TE₂ decreased by 46.1% and 27.6%, respectively. Serum OC concentrations during HT with oral CEE and TE₂ also decreased significantly (*P* < 0.01). Serum OC concentrations in women receiving HT with oral CEE and TE₂ decreased by 50.8% and 27.5%, respectively. However, OC concentrations at 12 months after the start of HT with oral CEE and TE₂ (1.85 ± 1.17 vs 2.34 ± 1.79 ng/mL) were not significantly different.

Changes in serum BAP and urinary DPD concentrations

Changes in serum BAP and urinary DPD concentrations after 12 months of HT are shown in Table 2. Serum BAP concentrations in women receiving HT with oral CEE and TE₂ decreased significantly (*P* < 0.01). There was no difference between serum BAP concentrations in women receiving HT with oral CEE and TE₂ (19.3 ± 6.5 U/L vs 20.7 ± 7.5 U/L). Urinary DPD concentrations in women receiving HT with oral CEE and TE₂ also decreased significantly (*P* < 0.01 and *P* < 0.05, respectively), and

TABLE 3. Changes in serum estradiol and estrone concentrations after 12 months of HT in postmenopausal women

	HT with CEE	HT with TE ₂
Estradiol (pg/mL)		
Before	5.43 ± 4.84	6.34 ± 4.80
After	26.9 ± 9.28 ^{a,b}	45.0 ± 13.3 ^a
Estrone (pg/mL)		
Before	19.3 ± 11.6	20.1 ± 12.0
After	150.5 ± 64.8 ^{a,b}	32.2 ± 11.9 ^a

Means ± SDs.

HT, hormone therapy; Before, before treatment; After, at 12 months after the start of hormone therapy; CEE, conjugated equine estrogens; TE₂, transdermal estradiol.

^a*P* < 0.01 versus before treatment.

^b*P* < 0.01 versus women who received hormone therapy with transdermal estradiol.

urinary DPD concentrations in women receiving HT with oral CEE and TE₂ (6.63 ± 3.0 nmol/L/mmol/L Cr vs 5.95 ± 2.3 nmol/L/mmol/L Cr) were not different statistically.

Changes in BMD at the lumbar spine

As shown in Table 2, the percentages of changes in BMD were increased in women receiving HT with oral CEE (4.5 ± 0.7%) and in women receiving HT with TE₂ (2.5 ± 0.7%). The difference between these two groups was not statistically significant.

Changes in serum estradiol and estrone concentrations

Table 3 shows changes in serum estradiol and estrone concentrations after 12 months of HT. The serum estradiol concentration in women during HT with TE₂ (45.0 ± 13.3 pg/mL) was significantly (*P* < 0.01) higher than that in women during HT with oral CEE (26.9 ± 9.3 pg/mL). On the other hand, the serum estrone concentration in women during HT with oral CEE (150.5 ± 64.8 pg/mL) was significantly (*P* < 0.01) higher than that in women during HT with TE₂ (32.2 ± 11.9 pg/mL).

Associations of serum estradiol concentration with ucOC concentration and ratio of ucOC/OC at 12 months after the start of HT

Figure 1 (left) shows the relationships between serum estradiol and ucOC concentrations in women who received HT with oral CEE. Serum estradiol concentrations during HT with oral CEE showed a significant inverse correlation (*r* = -0.380, *P* < 0.05) with ucOC concentrations during HT with oral CEE. In addition, serum estradiol concentration during HT with oral CEE showed a significant inverse correlation (*r* = -0.447, *P* < 0.01) with the ratio of ucOC/OC during HT with oral CEE (Fig. 1, right). However, serum estradiol concentration during HT with TE₂ did not show any correlation with either ucOC

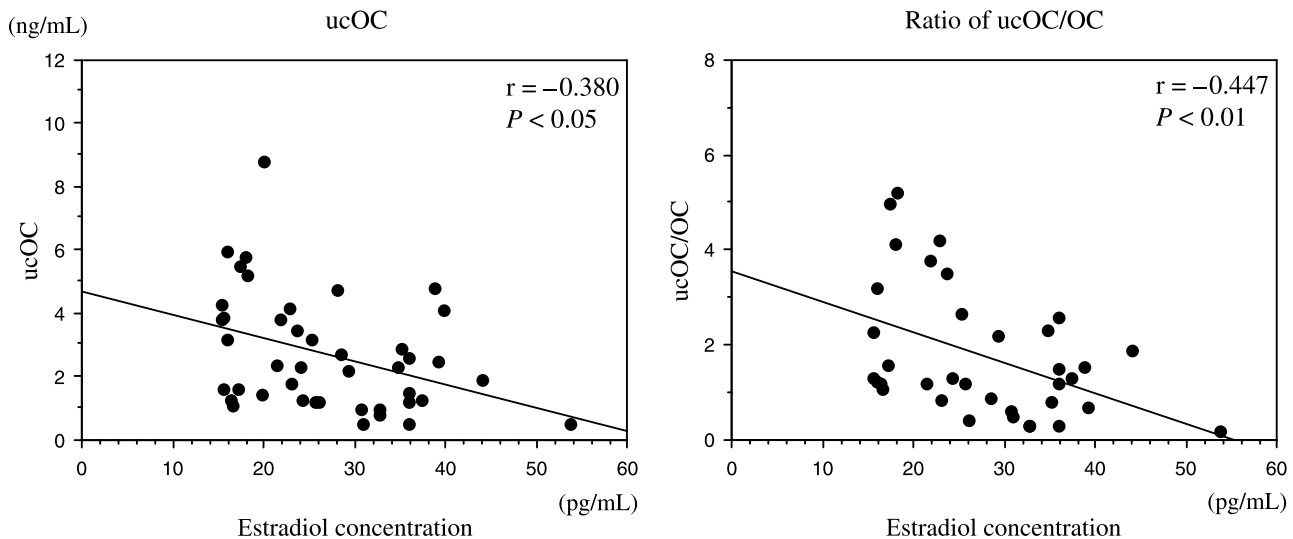


FIG. 1. Correlations of serum estradiol concentrations at 12 months after the start of hormone therapy (HT) with undercarboxylated osteocalcin (ucOC) concentration (left) and ratio of ucOC/osteocalcin (OC) (right). Serum ucOC concentration in women who received HT showed a significant (*P* < 0.05) negative correlation with estradiol concentration (the relationship being expressed by the following equation: ucOC concentration = 4.71 - 0.074 × estradiol concentration; *R* = 0.380). The ratio of ucOC/OC in women who received HT showed a significant (*P* < 0.01) negative correlation with estradiol concentration (the relationship being expressed by the following equation: ratio of ucOC/OC = 3.568 - 0.064 × estradiol concentration; *R* = 0.447).

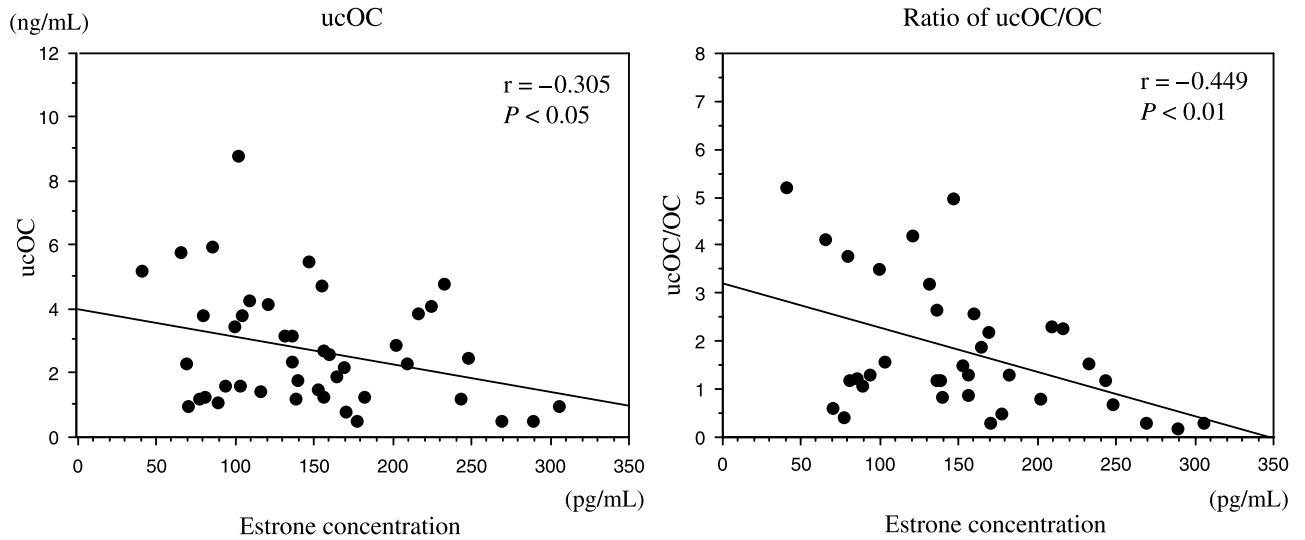


FIG. 2. Correlations of serum estrone concentrations at 12 months after the start of hormone therapy (HT) with undercarboxylated osteocalcin (ucOC) concentration (left) and ratio of ucOC/osteocalcin (OC) (right). Serum ucOC concentration in women who received HT showed a significant ($P < 0.05$) negative correlation with estrone concentration (the relationship being expressed by the following equation: ucOC concentration = $4.012 - 0.009 \times$ estrone concentration; $R = 0.305$). The ratio of ucOC/OC in women who received HT showed a significant ($P < 0.01$) negative correlation with estrone concentration (the relationship being expressed by the following equation: ratio of ucOC/OC = $3.209 - 0.009 \times$ estrone concentration; $R = 0.449$).

concentration ($r = -0.058$, $P = 0.77$) or the ratio of ucOC/OC ($r = -0.101$, $P = 0.62$) during HT with TE₂.

Associations of serum estrone concentration with ucOC concentration and ratio of ucOC/OC at 12 months after the start of HT

As shown in Figure 2, serum estrone concentrations during HT with oral CEE showed a negative correlation ($r = -0.305$, $P < 0.05$) with ucOC concentrations during HT with oral CEE. In addition, estrone concentrations during HT with oral CEE showed a significant inverse correlation ($r = -0.449$, $P < 0.01$) with the ratio of ucOC/OC during HT with oral CEE. However, serum estrone concentration during HT with TE₂ did not show any correlation with either ucOC concentration or the ratio of ucOC/OC during HT with TE₂.

Changes in TG and other lipid profiles

Table 4 shows changes in TG and other lipid profiles (TC, HDL-C, LDL-C) after 12 months of HT. TG concentration did not change significantly in women receiving HT with oral CEE but decreased significantly ($P < 0.05$) in women receiving HT with TE₂. The percentage of change in TG in women receiving HT with oral CEE was significantly ($P < 0.05$) higher than that in women receiving HT with TE₂ (+14.6% vs -7.1%). LDL-C concentration in women receiving HT with oral CEE decreased significantly ($P <$

0.01) and the concentration of LDL-C after 12 months of HT with oral CEE was significantly ($P < 0.05$) lower than that after 12 months of HT with TE₂. HDL-C

TABLE 4. Changes in lipid profiles after 12 months of HT in postmenopausal women

	HT with CEE	% Change from baseline	HT with TE ₂	% Change from baseline
TG (mg/dL)				
Before	107.6 ± 47.2	14.6	117.4 ± 47.8	-7.1
After	112.5 ± 51.6		102.6 ± 42.7 ^b	
TC (mg/dL)				
Before	210.8 ± 29.1	-2.7	219.0 ± 31.1	-4.4
After	203.9 ± 26.5 ^b		206.9 ± 31.7 ^a	
HDL-C (mg/dL)				
Before	65.1 ± 15.0	11.5	63.9 ± 14.1	-3.4
After	71.6 ± 15.5 ^{a,c}		62.2 ± 14.4	
LDL-C (mg/dL)				
Before	122.7 ± 26.5	-8.9	130.5 ± 27.8	-2.8
After	109.8 ± 28.5 ^{a,d}		125.0 ± 29.4	

Means ± SDs.
 HT, hormone therapy; Before, before treatment; After, at 12 months after the start of hormone therapy; CEE: conjugated equine estrogens; TE₂, transdermal estradiol; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
^a $P < 0.01$ versus before treatment.
^b $P < 0.05$ versus before treatment.
^c $P < 0.01$ versus women who received hormone therapy with transdermal estradiol.
^d $P < 0.05$ versus women who received hormone therapy with transdermal estradiol.

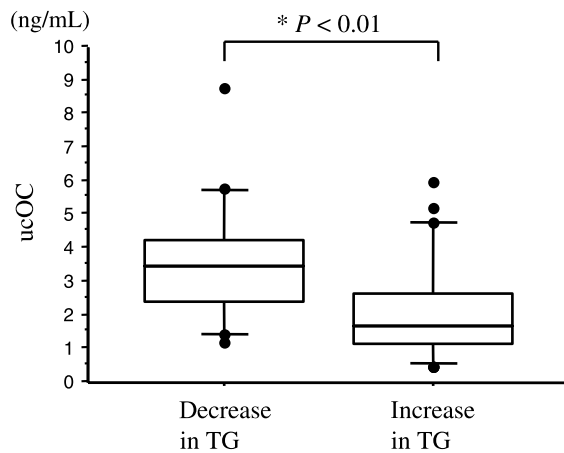


FIG. 3. Box and whisker plot representation of serum undercarboxylated osteocalcin (ucOC) concentrations in women who decreased and increased in percentage change in triglyceride (TG) during hormone therapy (HT) with oral conjugated equine estrogens. Box plots show median, 25th, and 75th percentiles as boxes, and 5th and 95th percentiles as error bars; extreme values are shown as closed circles. Decrease in TG, decrease in percentage of change in TG; increase in TG, increase in percentage of change in TG.

concentration in women receiving HT with oral CEE increased significantly ($P < 0.01$) and the concentration of HDL-C after 12 months of HT with oral CEE was significantly ($P < 0.01$) higher than that after 12 months of HT with TE₂.

Associations of serum estradiol concentration with TG concentration at 12 months after the start of HT

Serum estradiol concentrations during HT with oral CEE showed a significant positive correlation ($r = 0.344$, $P < 0.05$) with TG concentrations in women receiving HT with oral CEE (data not shown). However, serum estradiol concentrations during HT with TE₂ did not show any correlation with TG concentrations during HT with TE₂.

Serum ucOC concentration classified by percentage of change in TG during HT with CEE

Serum ucOC concentrations were classified by percentage of changes in TG in postmenopausal women during HT with oral CEE. As shown in Figure 3, serum ucOC concentration in women who increased in percentage of change in TG (2.19 ± 1.52 ng/mL) was significantly ($P < 0.01$) lower than that in women who decreased in percentage change in TG (3.67 ± 1.91 ng/mL).

DISCUSSION

In the present study, we found that there were significant differences between serum ucOC concentrations

in women during HT with oral CEE and TE₂. Serum ucOC concentration in women during HT with oral CEE was significantly lower than that in women during HT with TE₂. The decrease in percentage of ucOC in women receiving HT with oral CEE was also significantly greater than that in women receiving HT with TE₂. However, there was no significant difference in bone turnover markers such as OC, BAP, and DPD or in lumbar BMD between women during HT with oral CEE and TE₂. The results obtained in the present study for these bone turnover markers and BMD are in line with results of previous studies.¹⁻³ There are several possible reasons for the difference in the effects on ucOC in women during HT with oral CEE and TE₂.

One reason might be related to the difference between TG concentrations in women during HT with oral CEE and in women during HT with TE₂. In the present study, TG concentration in women during HT with oral CEE did not change significantly, whereas that in women during HT with TE₂ decreased. In addition, we showed that serum ucOC concentration in women with an increased percentage of change in TG was significantly lower than that in women with a decreased percentage of change in TG during HT with oral CEE. CEE administered orally at a conventional dose has been reported to induce an increase in TG concentration,¹⁷⁻¹⁹ but TE₂ did not increase TG concentrations.²⁰ TG-rich lipoproteins are thought to be the main transporters of vitamin K.^{25,26} It has been reported that plasma concentration of phylloquinone, which is one of the forms of vitamin K, was positively correlated with TG.^{14,15,27} We previously reported that ucOC concentration showed an inverse correlation with TG concentration in women receiving HT daily and in women receiving HT on alternate days.¹⁶ In the present study, we confirmed that serum estradiol concentrations during HT showed a significant positive correlation with TG concentrations in women during HT with oral CEE. Therefore, the difference between serum ucOC concentrations in women during HT with oral CEE and in women during HT with TE₂ might be due to the conversion of ucOC to carboxylated OC by the different effect of vitamin K through the change in circulating TG induced by oral CEE or TE₂.

Second, the difference in the oral and transdermal routes of administration is related to liver metabolism. The difference between the effects of oral administration and transdermal administration on ucOC may be caused by the first-pass effect on the liver. In the present study, serum ucOC concentration during HT with oral CEE showed a significant inverse correlation with estradiol concentration after

metabolism of CEE in the liver, whereas there was no correlation between serum ucOC concentration and estradiol concentration in women during HT with TE₂. Thus, the difference between ucOC concentrations in women during HT with oral CEE and in women during HT with TE₂ may be due to the difference in metabolism of CEE and TE₂ in the liver.

It has been reported that the ratio of ucOC/intact OC was related to the risk of hip fracture,²⁸ and the predictive importance of serum ucOC for the occurrence of fractures in older subjects has been suggested.²⁹ Recently, bone quality and BMD have been reported to be related to bone fractures. It has also been reported that ucOC concentration was strongly correlated with ultrasonic transmitted velocity in bone³⁰ and that the ratio of ucOC/OC was associated with the factor of elasticity.³¹ These studies suggest that the ratio of ucOC/OC might have an effect on not only BMD but also bone quality. In the present study, the ratio of ucOC/OC during HT with oral CEE was inversely associated with both serum estradiol and estrone concentrations, but no association was found between the ratio of ucOC/OC and estradiol concentration or between the ratio and estrone concentration in women during HT with TE₂. Decrease in the ratio of ucOC/OC due to increase in both estradiol and estrone concentrations may be important for bone health.

Several design limitations should be considered in assessing the results of this study. One limitation is that we did not measure BMD at the hip. We measured lumbar BMD because the subjects in this study were younger than subjects in previous studies. Further study may be needed to measure BMD at the hip. It is possible that vitamin K status plays a role in our present results. We examined serum ucOC concentration instead of vitamin K concentration because elevated ucOC has been shown to be corrected or ameliorated by vitamin K supplementation.³² Further study is needed to determine the associations of vitamin K and TG in women during HT with oral CEE and TE₂. Recently, it has been reported that vitamin K binds 17 β -hydroxysteroid dehydrogenase and modulates the metabolism of estrogen.³³ There may be a linkage between vitamin K and estrogen function. The method used for measurement of serum ucOC concentration should be taken into account. In the present study, we measured serum ucOC concentration using ELISA, whereas serum ucOC concentration was determined by hydroxyapatite binding assays in most previous studies. Measurement using ELISA might include measurements of not only ucOC but also probable products of catabolism of OC.

However, our results seem to be significant because the use of ELISA for measurement of ucOC has been reported to discriminate ucOC and total OC.³⁴

CEE is a complex of at least 10 natural estrogens, and a large proportion of CEE is estrone sulfate, whereas TE₂ contains only 17 β -estradiol. In the present study, estrone concentration during HT with CEE was significant higher than that during HT with TE₂, whereas estradiol concentration was significantly higher in women during HT with TE₂ than in women during HT with CEE. In addition, serum estrone concentrations during HT with CEE showed a significant inverse correlation with serum ucOC concentrations, but there was no correlation between serum ucOC and estrone concentrations during HT with TE₂. It has been reported that estrone had a significant bone-protecting effect, similar to that of 17 β -estradiol, in rats in a hypoestrogenic state,³⁵ although the effects of estrone on several tissues were weaker than those of 17 β -estradiol. It has also been demonstrated that estrone sulfate is a major source of local bioactive estrogen formation in human bone, and local estrogen formation from estrone sulfate may play an important role in bone maturation.³⁶ Our results did not demonstrate that suppression of ucOC might be due to the direct action of estrone because we compared the effects of oral CEE and transdermal 17 β -estradiol. Further study using oral CEE and oral 17 β -estradiol is needed.

CONCLUSION

The effect of HT with TE₂ on serum ucOC concentration in women is weaker than the effect of HT with oral CEE. Suppression of serum ucOC concentration in postmenopausal women during HT with oral CEE might be associated with the effects of vitamin K through increased TG concentration.

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