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Impact of Menopause on Lipid and Bone Metabolism and Effect of Hormone Replacement Therapy

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Background: **Hyperlipidemia and osteoporosis are the medical targets to improve the quality of life of increasing elderly women.** ***Objective:*** **To elucidate the effect of menopause and hormone replacement therapy (HRT) on lipid and bone metabolism.** ***Subjects:*** **With their written informed consent, studied were 89 postmenopausal with 30 premenopausal women, and postmenopausal 35 were assigned into HRT (n = 18) or control group (n = 17); the former received conjugated equine estrogen (0.625 mg/day) and medroxyprogesterone acetate (2.5 mg/day), the latter calcium aspartate (800 mg/day).** ***Outcome measured:*** **Parameters were measured for lipids; total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), triglycerides (TG), lipoproteins, and apolipoproteins as well as for bone metabolism; parathyroid hormone (PTH), $1,25(OH)_2D_3$, bone type of alkaline phosphatase (b-ALP), intact bone gla protein (I-BGP), tartrate-resistant acid phosphatase (TRAP) in serum. Bone mineral density (BMD) of lumbar spine was measured by dual energy X-ray absorptiometry (DEXA). Two atherogenic indices (AIs) were calculated: AIc equals [TC−HDLC]/HDLC, and AIap equals (apolipoprotein B)/ (apolipoprotein A1).** ***Results:*** **TC increased in ≃10 % within 2 years after menopause with increased LDLC (≃20 %) and decreased HDLC (≃10 %), and atherogenic indices were both elevated. In HRT, HDLC increased, while TC and LDLC and TG showed no significant change; lumbar BMD increased by 3 % after 12 month, while bone formation markers decreased; PTH increased and $1,25(OH)_2D_3$ decreased.** ***Conclusion:*** **We provided the natural changes of lipid and bone metabolism after menopause and how extent an estrogen replacement can reset these changes.**

Key words : Menopause, lipids, osteoporosis, HRT, aging, sex hormones, atherogenic index

INTRODUCTION

Hyperlipidemia and osteoporosis are the major targets of medical treatment for elder women, since reduction of those susceptibility is believed to improve the quality of life of our society especially in the context of increasing female elder generation. In women, incidence of both hyperlipidemia and osteoporosis significantly increases after menopause [1, 2] and beneficial effects of estrogen replacement on postmenopausal events are also reported separately [3, 4]. Arteriosclerosis also increases in elder women, and thought to be an effect of changed metabolism of lipid after menopause [5].

In the present study, we tried to investigate the effect of menopause on lipid and bone metabolism simultaneously and to elucidate the benefit of hormone replacement therapy (HRT) on those metabolic changes.

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SUBJECTS AND METHODS

Effect of menopause

The subjects were 89 naturally postmenopausal women enrolled in the outpatient clinic of our department with the written consent of their participation of this study. Volunteers of 30 premenopausal women with regular mensruation are enrolled into the regular menstrual group (RM) as a control group. None of them had diabetes mellitus, hypertension, thromboembolic disease, a history of cancer, or other chronic illnesses. Postmenopausal women were classified according to the duration of postmenopausal period (PMP) as follows: Group PM1 ($1 \leqq PMP < 2$ years, N = 18), PM2 ($2 \leqq PMP < 5$ years, N = 26), PM5 ($5 \leqq PMP < 10$ years, N = 28), and PM10 ($10 \leqq PMP <$ 15 years, N = 17). Fasting blood was obtained in the morning to measure the parameters of serum lipid, i.e. total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), triglycerides (TG), lipoproteins, and apolipoproteins as well as those about bone metabolism, i.e. calcium ion (Ca), inorganic phosphorus (IP), parathyroid hormone (PTH), $1,25(OH)_2D_3$, bone type of alkaline phosphatase (b-ALP), intact bone gla protein (I-BGP), tartrate-resistant acid phosphatase (TRAP) in serum.

Moreover, bone mineral density (BMD) of lumbar spine was measured by a dual energy X-ray absorptiometry (DEXA) using QDR2000 (Hologic, Waltham, MA). Two atherogenic indices (AIs) were calculated from lipid parameter [6]. An AI from cholesterol in lipoprotein-fraction, AIc, equals [TC − HDLC]/HDLC. Another AI from apolipoprotein, AIap, equals (apolipoprotein B)/ (apolipoprotein A1).

Effect of hormone replacement

To assess the effect of hormone replacement therapy, 35 postmenopausal women of more than 2 years after menopause were enrolled to this study under their informed consent. Eighteen women (HRT group) received a conjugated equine estrogen (CEE, 0.625 mg/day) and medroxyprogesterone acetate (MPA, 2.5 mg/day). Other 17 women (Control group) did not want HRT and received oral calcium drug, calcium aspartate 800 mg/day. The parameters of lipid and bone metabolsm listeded above were measured at 6th and 12th month after the treatment.

Statistical analysis

One way ANOVA was used with Bonferoni/ Dunn's test as post hoc comparison when necessary. The p value less than 0.05 was considered as statistically significant.

RESULTS

Postmenopausal change

The results from the postmenopausal groups (PM1, PM2, PM5, PM10) were compared with the data from the regular menstruation group (RM). As in Table 1, where the profile of each group is summarized, there was no statistical difference on BMI among these groups.

Serum levels of estrone (E1) and estradiol (E2) were significantly depressed (Fig. 1a), while LH and FSH levels were significantly elevated in PM groups in a FSH-dominant manner (Fig. 1b).

Serum levels of TC and TG increased only with statistical significance of TG in PM1 ($p < 0.05$ vs. RM, Fig. 2a). LDLC and HDLC showed increasing and decreasing tendency respectively after menopause (Fig. 2b). Both atherogenic indices (AIc and AIap) were also increased after menopause (Fig. 2c).

PTH level was elevated after menopause in contrast with decreased $1,25(OH)_2D_3$ (Fig. 3a). Bone formation markers, I-BGP and b-Alp, were increasing after menopause (Fig. 3b); I-BGP showed stepwise increase with statistically significance according to postmenopausal period (PM1; $p < 0.05$, PM2; $p < 0.001$), while b-Alp showed slower increase with statistical significance on PM10 ($P < 0.05$).

Bone resorption marker, TRAP, was also increased soon after menopause (Fig. 3c) with statistical significance. BMD showed slow and stepwise decrease after menopause (Fig. 3c) with statistical significance (PM2; $p < 0.05$, PM5 and PM10; $p < 0.01$). These values are the level of "osteopenia: deceased BMD" diagnosed by "criteria for bone loss by silver-science team of Japan Ministry of Health and Welfare" and the BMD range considerable for active treatment.

HRT effect

At the beginning of treatment, there was no statistical difference on parameters compared between control and HRT group, except an atherogenic index (AIap), as summarized in Table 2. Hormonal changes after HRT are shown in Fig. 4. Serum estrone levels were significantly ($p < 0.01$) increased in

Table 1 Analyzed groups for postmenopausal changes of lipid and bone metabolism.

postmenopausal year(s)	0	1 ～ 2	2 ～ 5	5 ～ 10	10 ～
group name	RM	PM1	PM2	PM5	PM10
n	30	18	26	28	17
age	40.8 ± 0.9	48.7 ± 0.6	52.1 ± 0.8	57.3 ± 1.2	62.9 ± 1.7
BMI	21.9 ± 0.4	22.1 ± 0.6	21.9 ± 0.5	22.1 ± 0.5	23.3 ± 1.1
postmenopausal years	0	1.5 ± 0.1	3.9 ± 0.3	8.1 ± 0.6	14.7 ± 1.1

Postmenopausal women were classified according to the duration of postmenopausal period and compared with premenopausal volunteers with regular menstruation. Data were designated as mean ± SEM.

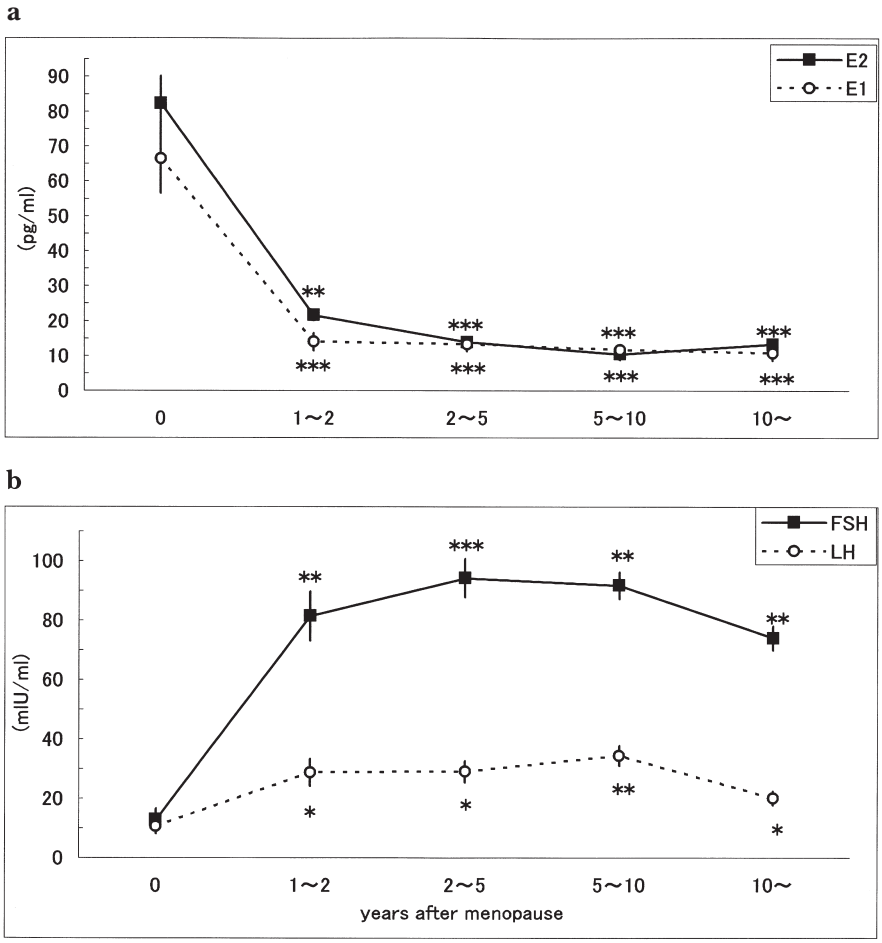

Fig. 1 Postmenopausal change of hormonal data.
Postmenopausal women were classified according to the duration of postmenopausal period and compared with premenopausal volunteers with regular menstruation (for precise description, see table 1 and text). Changes of estrone (E1) and estradiol (E2) were designated as mean ± SEM (a), as well as LH and FSH (b). *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ vs. RM group (data of 0 years) respectively.

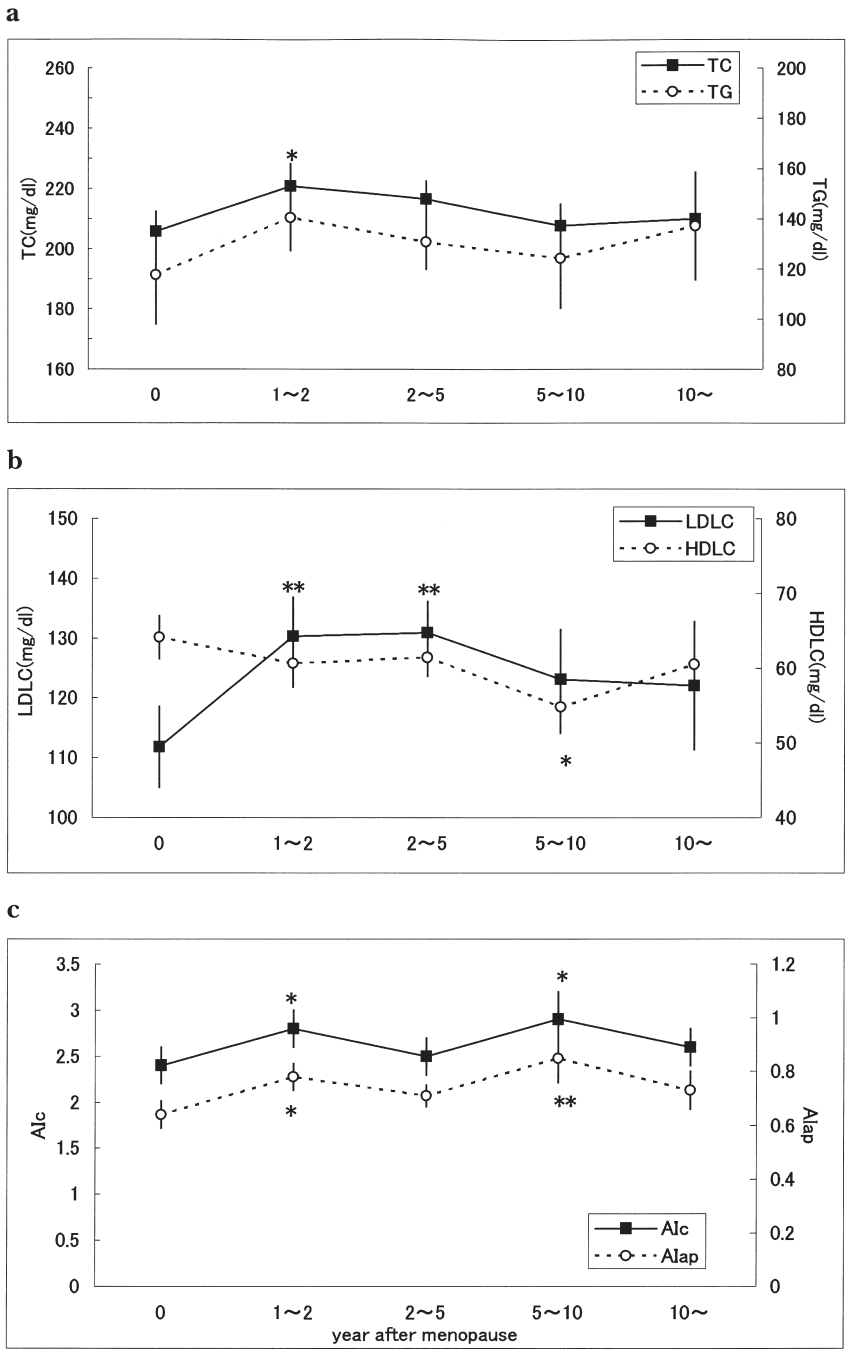

Fig. 2 Postmenopausal change of parameters of lipid metabolism.
Changes of parameters of lipid metabolism were designated as mean ± SEM; TC and TG (a), LDLC and HDLC (b), AIc and AIap (c). *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ vs. RM group (data of 0 years) respectively.

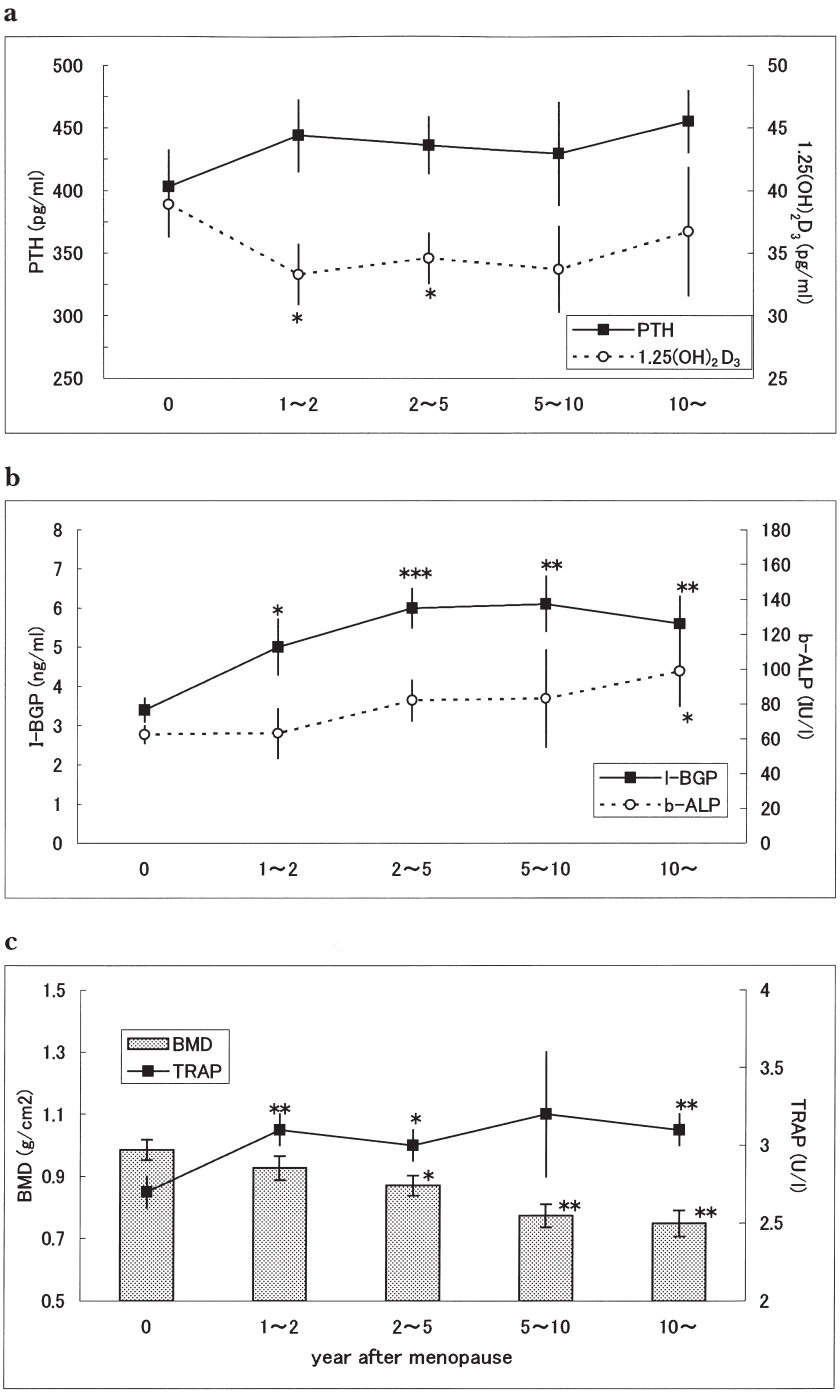

Fig. 3 Postmenopausal change of parameters of bone metabolism.
Changes of parameters of bone metabolism were designated as mean ± SEM; PTH and $1,25(OH)_2D_3$ (a), I-BGP and b-ALP (b), TRAP and BMD (c). *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ vs. RM group (data of 0 years) respectively.

Table 2 Analyzed groups in HRT.

	control	HRT
n	17	18
age	49.6 ± 1.1	51.1 ± 0.7
BMI	21.6 ± 0.5	21.7 ± 0.7
E1 (pg/ml)	17.5 ± 3.0	19.1 ± 4.5
E2 (pg/ml)	17.8 ± 3.4	19.0 ± 4.1
LH (mlU/ml)	34.1 ± 3.5	26.9 ± 3.0
FSH (mlU/ml)	94.3 ± 8.9	80.8 ± 9.3
TC (mg/dl)	210.4 ± 9.1	213.4 ± 6.4
TG (mg/dl)	98.9 ± 10.9	131.2 ± 15.4
HDLC (mg/dl)	66.1 ± 2.8	62.0 ± 3.5
LDLC (mg/dl)	124.5 ± 9.9	125.2 ± 6.4
AIc[#]	2.3 ± 0.2	2.6 ± 0.2*
AI ap[$]	0.63 ± 0.05	0.75 ± 0.05*
PTH (pg/ml)	415.6 ± 26.6	399.3 ± 23.3
$1.25(OH)_2D_3$(pg/ml)	33.1 ± 3.1	36.3 ± 2.5
I-BGP (ng/ml)	4.4 ± 0.6	4.6 ± 0.4
b-ALP (IU/l)	63.9 ± 9.6	66.1 ± 5.9
TRAP (U/l)	3.0 ± 0.2	3.1 ± 0.2
BMD (g/cm^2)	0.910 ± 0.039	0.886 ± 0.033

Postmenopausal women were divided into two groups to investigate the effect of HRT. Two groups were compared on the listed parameters and the data were designated as mean ± SEM.

* indicates $p < 0.05$ vs. control

[#]AIc, calculated from cholesterol in lipoprotein-fraction, equals [TC − HDLC]/HDLC.

[$]AIap, calculated from apolipoprotein, equals (apolipoprotein B)/ (apolipoprotein A1).

HRT groups (Fig. 4a), as well as estradiol (Fig. 4b). LH and FSH levels were depressed after HRT (Fig. 4c, d); compared with the value at the beginning of each group, FSH at 12 month had only a statistical significance ($p < 0.05$).

For comparison of the parameters of lipid or bone, the data are expressed % of the beginning value of each group in figures of HRT study (Figs. 5, 6).

Serum TG fluctuated after HRT without statistical significance (Fig. 5b) comparing with control group. Serum TC decreased in HRT group but without statistical significance (Fig. 5a). HDLC increased at 12 month after HRT ($p < 0.01$, Fig. 5c), while LDLC decreased without significance (Fig. 5d). Both atherogenic indices (AIc and AIap) were depressed only with significance at 6 month after HRT ($p < 0.05$, Fig. 5e, f).

In HRT group, PTH level was stable with significantly elevated $1,25(OH)_2D_3$ ($p < 0.05$ in 12 month vs. 0 month), while in Control group, PTH level was depressed ($p < 0.05$ in 6 month) with elevated $1,25(OH)_2D_3$ ($p < 0.05$ in 6 month). Hence, comparing with Control group, HRT increased PTH levels ($p < 0.01$ in 6 month) without an effect on $1,25(OH)_2D_3$ (Fig. 6a, b). Both bone formation markers, I-BGP and b-Alp, decreased (Fig. 6c, d) significantly at 12 month ($p < 0.01$). Bone resorption marker, TRAP, was depressed after HRT (Fig. 6e) with statistical significance after 6 month ($p < 0.01$; HRT versus Control group). BMD showed slow decrease in Control group with increase in HRT group (Fig. 6f). Hence, HRT

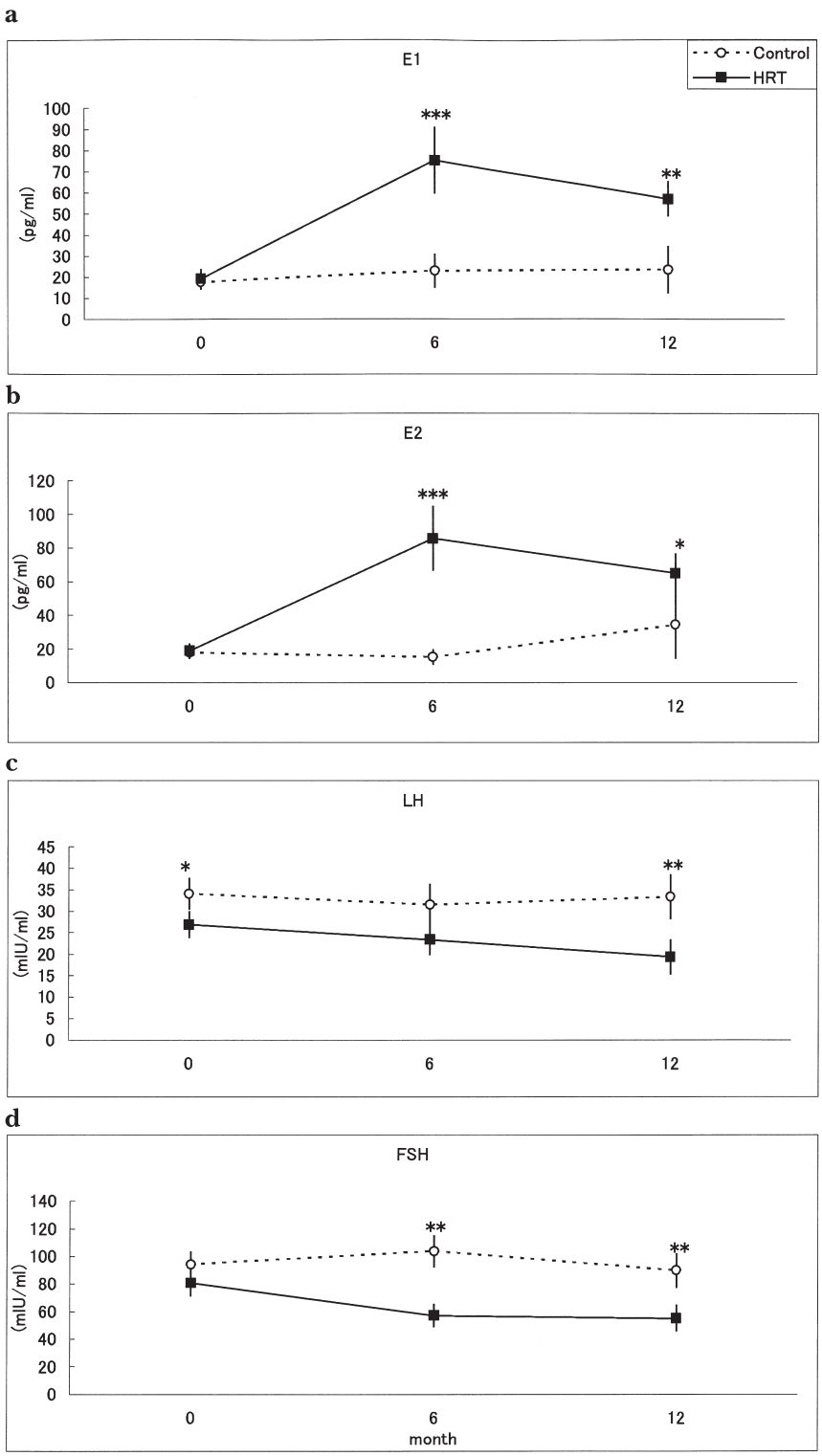

Fig. 4 Change of hormonal data during HRT.
Comparing with control group (dashed line), change of estrone (E1; a), estradiol (E2; b), LH (c), and FSH (d) were designated by solid line. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ (HRT versus Control group) respectively.

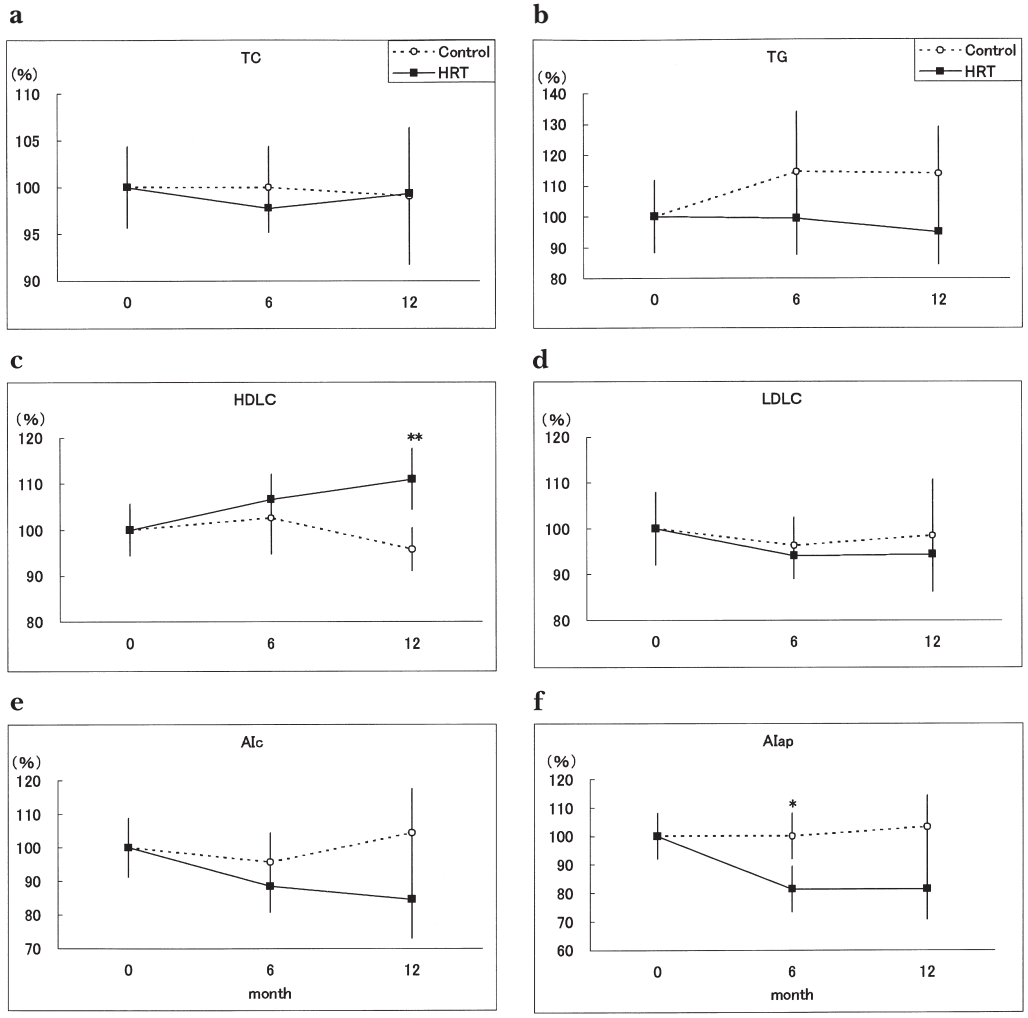

Fig. 5 Change of parameters of lipid metabolism during HRT.
Comparing with control group (dashed line), change during HRT of TC (a), TG (b), HDLC (c), LDLC (d), AIc (e), or AIap (f) is designated by solid line. The data are expressed % of the beginning value of each group. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ (HRT versus Control group) respectively.

significantly increased BMD after 6 month ($p < 0.05$; HRT versus Control group).

DISCUSSION

We tried to investigate the effect of menopause on lipids and bone metabolism simultaneously. The impact of menopause overlaps with aging effect, which is common for both genders. In this context, our additional study of HRT trial reveals the estrogenic recovery of impaired condition in postmenopause, hence uncovers the specific effect of estrogen deficiency independent from aging effect, and provides the useful information about the nature of sex steroid.

As an impact of menopause, serum level of TC increased in approximately 10 % within 2 years after menopause as well as increased LDLC (≃20 %) and decreased HDLC (≃10 %). Atherogenic indices (AIc and AIap) were both elevated after menopause. While atherosclerosis is partly induced by the depleted estrogenic action via estrogen receptor of vascular wall [7], the changed lipid metabolism is accepted as a major risk factor. In our study we utilized the two indices for evaluating the atherogenic as-

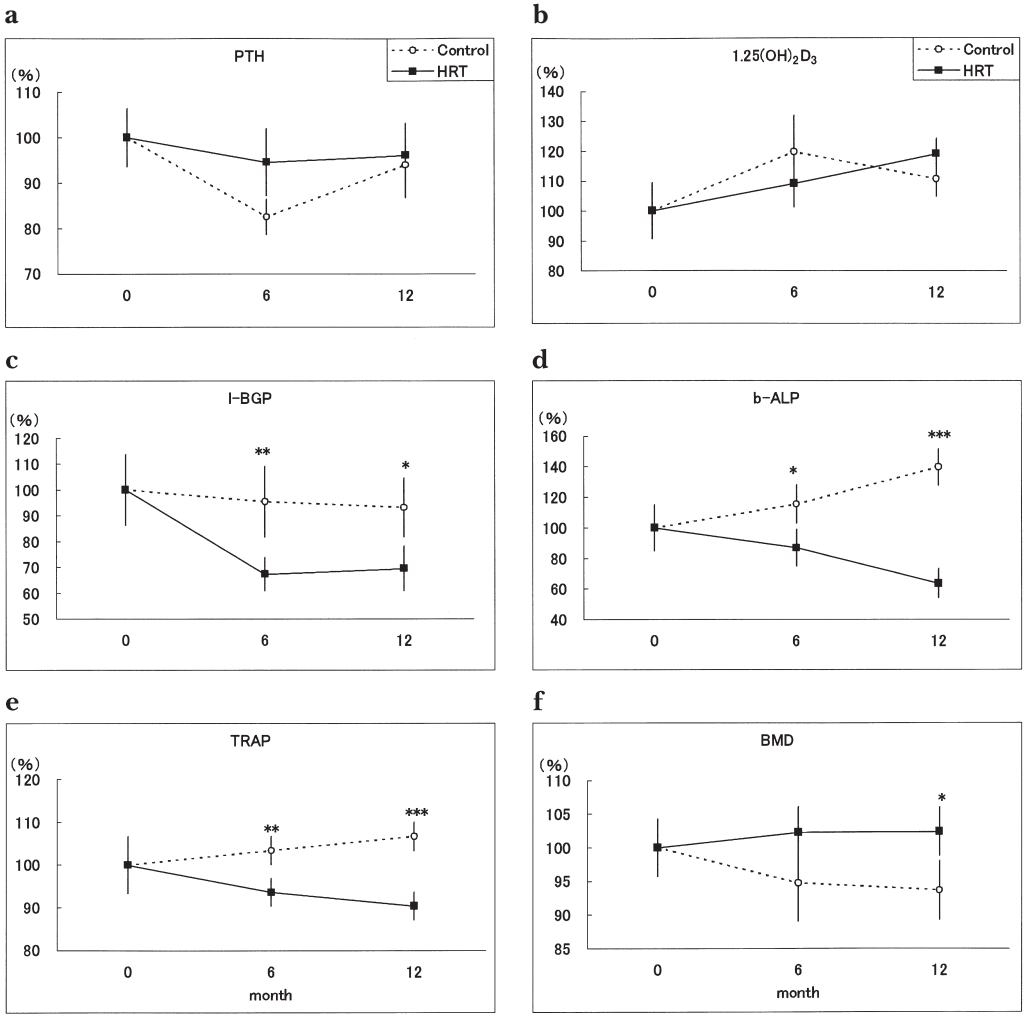

Fig. 6 Change of parameters of bone metabolism during HRT.
Comparing with control group (dashed line), change during HRT of PTH (a), $1,25(OH)_2D_3$ (b), I-BGP (c), b-ALP (d), TRAP (e), and BMD (f) is designated by solid line. The data are expressed % of the beginning value of each group. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ (HRT versus Control group) respectively.

pect of lipid parameters; one (AIc) is calculated from cholesterol data in lipoprotein-fraction; another (AIap) from values of apolipoproteins. We preferred these calculated indices to the law values because the two AIs clearly represent the atherogenic risk in changed profile of lipid metabolism. Our data showed that these AIs increase within 2 years after menopause, and HRT for 1 year decreased the AIs. Present data indicated the impairment of lipid metabolism in postmenopausal women, which is mainly attributable not to aging but to the estrogen deficiency. As actions of estrogen to lipid metabolism, estrogen (1) increases HDL2 and decreases of LDL via reducing activity of hepatic triglyceride lipase (HTGL), (2) decreases serum TC via increasing LDL uptake by up-regulating receptor number, (3) reduces cholesterol synthesis as a negative feedback by increased cholesterol storage in hepatic cells [8]. Additionally estrogen is thought to increase serum TG, which might be clinical problems in HRT for treatment of hyperlipidemia [9]. Such effect was not clear in our HRT study, while the postmenopausal women had increased TG level by approximately 10-20 % compared

with RM group. Adverse effect of HRT on TG might be considered in relation with races and in further study of Asian population.

Concerning the hormonal levels in postmenopausal changes, both gonadotropins increased already within 2 years, responding to estrogen decrease. Out of these gonadotropin changes, HRT suppressed FSH dominantly at 6 month, in contrast of slow response of LH. In our data, women have hyperlipidemia after menopause within 2 years, and as a total effect from altered lipid metabolism, their AIs were elevated, indicating increased risk of atherosclerosis. In HRT, compared with control, HDLC increased, while TC and LDLC and TG showed no significant change. Since the enrolled subjects had uteri in our HRT study, our regimen includes MPA as a progestin to protect their endometrium [10]. Though MPA was suggested to reduce the beneficial effect of estrogen to lipid metabolism [11], we could not distinguish this effect of MPA in our protocol. Anyhow, as a total effect on the lipid metabolism, two AIs were depressed in HRT, which might suggest the beneficial effect of HRT to reduce the risk of cardiovascular events.

Calcium metabolism is complex and finely regulated by bio-feedback system [12]. In the calcium metabolism, bone works as a resovoir. Estrogen deficiency triggers the bone resorption, thus reduced BMD and the resulted elevation of serum calcium suppresses the PHT secretion. This, in turn, depresses renal production of $1,25(OH)_2D_3$, which decreases calcium resorption in gut, then keeps the lower level of BMD. In another pathway, aging affects an activity of 1 α -hydroxylase, which reduces production of $1,25(OH)_2D_3$, resulted reduced antagonizatio to PTH, which secondarily causes the bone resorption.

In our study, the bone markers indicated high rate of bone turnover after menopause. TRAP increased drastically just after menopause, while both bone formation markers, I-BGP and b-Alp, changed gradually, implicating that the bone resorption proceeds the bone formation. This time lag between bone formation and resorption might cause delayed appearance of BMD decrease, which became apparent after 2 years, and significant after 5 years of postmenopause. These data indicate the bone markers are more sensitive and predictive for BMD change.

In general, estrogen inhibits PTH-induced bone resorption and to produces $1,25(OH)_2D_3$ in kidney. In our study, comparing with control group, PTH increased and $1,25(OH)_2D_3$ decreased in HRT group. There seems to be some discrepancy between the generally accepted action and our data. However, our actual finding might be a sum of the primary, secondary, and tertiary action of estrogen. This way of comprehension is also applicable to the relation between bone markers and BMD, where HRT increased BMD under the suppressed codition of both bone resorption and formation markers.

Bone formation markers decreased after HRT compared with control group ($p < 0.01$ in 12 month); I-BGP reached nadior after 6 month, b-Alp is still decreasing at 12 month. This behavioral difference between the markers may reflect its sensitivity and usefulness originated from their own nature. TRAP, a bone resorption marker, decreased significantly after 6 month ($p < 0.01$). From this observation, HRT apparently reduces bone turnover rate. Lumbar BMD increased by 3 % after 12 month of HRT. Assuming the measurement variance of DEXA is within 2 %, HRT is evaluated as useful for prevention of osteoporosis. Bone markers responded earlier than BMD in the subjects of medical responders, and their changing magnitude is more than BMD's. Not only BMD but also bone markers are important for clinical evaluation, while, in some cases, some marker changed in the different directions, which might make the evaluation difficult. Anyhow, in our study the knowledge on nature of the specific bone marker is suggested important in clinical practice of managing the BMD.

CONCLUSION

In the clinical management of peri-menopausal women, we should consider not only climacteric symptoms but also other aspects of background of the patient, and a type/rout of drug to be selected. We should accumulate more evidence for effects of HRT for the best clinical practice. In our study, we provided the natural changes of lipid and bone metabolism after menopause and how extent an estrogen replacement can reset these changes.

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