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Original Article

Factors Predicting Bone Mineral Density (BMD) Changes in Young Women over A One-year Study: Changes in Body Weight and Bone Metabolic Markers during the Menstrual Cycle and Their Effects on BMD

Tadayuki Iida[a]*, Chiho Chikamura[b], Hiroaki Ishikawa[c], Satomi Aoi[d], Hiromi Ikeda[d], Toshihide Harada[e], Kazuhiro Katada[f], Fumiko Ishizaki[g], Hiroshi Yatsuya[a], and Yuichiro Ono[a]

Departments of [a]Public Health, [c]Medical Technology, [f]Radiology, Fujita Health University School of Medicine, Toyoake, Aichi 470–1192, Japan, [b]Attached Clinic, Department of [d]Nursing, [e]Physical Therapy, [g]Communication Sciences and Disorders, Prefectural University of Hiroshima, Mihara, Hiroshima 723–0053, Japan

Currently, 26% of Japanese women in their twenties are under weight, and therefore at risk of developing various metabolic abnormalities due to an inadequate nutrient intake, which in turn affects the acquisition of a peak bone mineral density (BMD). In this study, we aimed to clarify the effects of menstrual cycle-related changes in body weight and bone metabolic marker levels on the BMD changes. The subjects were 42 women (19.6 ± 0.8 years). The levels of osteocalcin (OC), BAP, s-NTx, u-DPD, and E2 in the menstrual and ovulatory phases were measured. The associations between dependent variables (BMD changes/year in the lumbar spine, femur, femoral neck) and explanatory variables (body weight changes/year, the levels of OC, BAP, s-NTx, u-DPD) were evaluated using multiple regression analysis. Analysis of the correlations between the changes in bone metabolic markers and changes in BMD showed a correlation between the OC level in the menstrual phase and changes in the BMD of the entire femur, suggesting that a high OC level protects against BMD reduction, probably by promoting osteoblast activity, and that bone formation activity suppresses the decrease in BMD. These results suggest that, to predict BMD changes from bone metabolic markers in young women, it is necessary to measure OC levels in the menstrual phase.

Key words: BMD, bone metabolic marker, menstrual cycle

Osteoporosis is an important issue in Japan, which is fast becoming a "super-aging" society. Unfortunately, the current osteoporosis treatment does not lead to reacquisition of sufficient bone mass. At the present time, a physiological increase in bone mass can be expected only in adolescence. Moreover, sufficient bone mass in adolescence can only be assured by adequate osteoporosis education efforts [1, 2]. The acquisition of a high peak bone mass (PBM) in youth is considered necessary [3]. A sufficient bone mass in this period is considered an effective preventive method for osteoporosis. Various studies on changes in the bone mass in young people have been performed [4–7]. The influence on PBM of a recent

Received August 29, 2011; accepted January 10, 2012.
*Corresponding author. Phone:+81–562–93–2453; Fax:+81–562–93–3079
E-mail:iida@fujita-hu.ac.jp (T. Iida)

thinness trend among youth is considered problematic [8], particularly since even short-term extreme diets can result in a long-term reduction of bone mass [9]. According to the 2002 National Nutrition Survey, 26% of Japanese women in their 20s were under weight (BMI<18.5), and the rate was found to have doubled over 2 decades. Many of these women evaluate themselves as "fat" even though their BMI is low. They are in a state of low energy availability, insufficient nutrient intake, and unbalanced eating habits due to extreme diets that often involve skipping meals. Moreover, the intakes of trace nutrients, such as calcium, iron, copper, and zinc, are below the required levels <http://www. mhlw. go. jp/houdou/2003/12/h1224-4. html> (accessed June 21, 2010). Under such conditions, various metabolic abnormalities occur in the body and influence the PBM. Grinspoon *et al.* [10] reported that bone mass was markedly reduced in women with thinness oriented eating habits, and, in addition to estrogen insufficiency, being under weight was an important factor in bone mass reduction. It has been shown that a low body weight affects the bone mineral density (BMD), but no association has been demonstrated between longitudinal changes of body weight and BMD.

Furthermore, estrogen secretion in the female body controls bone metabolism, and a decrease in estrogen secretion leads to reduced levels of bone formation markers and increased levels of bone resorption markers [11–18]. Accordingly, bone metabolism is controlled by female sex hormone release, and the reduction of this release lowers the bone formation marker levels and elevates the bone resorption marker levels, leading to bone destruction surpassing bone formation and subsequent BMD reduction.

The guidelines for the appropriate use of bone metabolic markers were revised in 2004 by the Japan Osteoporosis Society Exploratory Committee [19]. However, our previous study showed that menstrual cycle-related changes of estrogen levels were related to changes in bone formation marker levels in menstruating young women [20]. Therefore, it is necessary to identify the menstrual cycle phase before measuring the bone metabolic markers, and then to investigate the association between the bone metabolic markers and BMD reduction.

For all of these reasons, the acquisition of BMD is crucial in young women who have begun menstruation. And thus it is important to detect BMD changes in young women promptly, in order to promote osteoporosis prevention. In this study, we investigated the association between changes of menstrual cycle-related estrogen levels and bone metabolic marker levels in young women. In addition, we conducted a 1-year longitudinal study to investigate the effects of changes of body weight and bone metabolic markers on BMD changes in the menstrual and ovulatory phases.

Materials and Methods

Subjects. Subjects were young women recruited from among 1st-3rd year university students in Hiroshima Prefecture. The study content and method were sufficiently explained to all subjects, and written consent was obtained before the study. Eighty-two of 84 participants in the explanatory meeting provided consent and underwent the survey. Of the 67 subjects who participated in the survey in 2004 and 2005 and underwent all biochemical tests in both the menstrual and ovulation phases, 42 subjects aged 19.6 ± 0.8 years were classified as having a normal menstrual cycle, defined as a cycle of 23–38 days' duration based on the cumulative menstrual cycle pattern reported by Treloar *et al.* [21]. and Mishell [22], and these subjects were included in analysis. This study was conducted in accordance with the Declaration of Helsinki after approval by the Ethics Committee of Fujita Health University School of Medicine (#07–140).

Measurements. An interview and measurements of physical characteristics, BMD, and biochemical parameters were conducted. The menstrual phase was determined through self-reporting during the interview. The ovulation phase was also determined in the interview, and confirmed by the observation of fern-like crystals in the subject's saliva under a microscope. As physical characteristics, the body weight and height were measured. The BMD (g/cm^2) was measured in the lumbar vertebra (L2–L4), left femoral neck, and entire left femur using an X-ray BMD measurement system (QDR-4500; Hologic, Bedford, MA, USA). Calibration of this BMD system was performed 99 times during the survey period, and the mean BMD, SD, and c.v. were $1.038\,g/cm^2$, $0.003\,g/cm^2$, and 0.37%, respectively, which were in the normal reference range, based on the data pro-

vided by HOLOGIC Inc. As the biochemical parameters we measured 2 bone formation markers, serum osteocalcin (OC) and serum bone-specific alkaline phosphatase (BAP), and 2 bone resorption markers, serum type I collagen-crosslinked peptide (s-NTx) and urinary deoxypyridinoline (u-DPD). We also studied blood estradiol (E2). Blood and urine were collected in the menstrual and ovulation phases. Serum samples for OC, BAP, s-NTx, and E2 measurement were prepared by centrifuging blood at 1,500 × g for 10 min. For u-DPD measurement, the 2nd morning urine was collected and centrifuged at 500 × g for 5 min, and the supernatant was stored at −20℃. These biochemical measurements were performed by SRL Inc. (Tokyo. Japan) using commercially available kits. Table 1 shows the normal ranges of the biochemical parameters.

Outcome. The changes in the BMD of the lumbar spine, femoral neck, and entire femur were obtained by subtracting their BMD values measured in 2004 from those measured in 2005. Data were tested for normal distribution using the Kolmogorov-Smirnov test. The *p*-values for the lumbar spine, femoral head, and entire femur were 0.200, 0.102, and 0.200, respectively, indicating a normal distribution ($p > 0.005$).

Data analysis. The data are presented as the means±standard deviation. The changes over a one-year period were defined as the values for 2005 minus those for 2004. The differences in the OC, BAP, s-NTx, u-DPD, and E2 levels between the menstrual and ovulation phases were analyzed by employing a paired *t*-test. The correlations among the body weight values in 2004, body weight changes over a one-year period, change of lumbar spine BMD, femoral neck BMD, entire femur BMD for a year (g/cm^2), and the levels of OC, BAP, s-NTx, u-DPD and E2 in the menstrual and ovulation phases were tested using Pearson's correlation coefficients. Multiple regression analysis was performed using outcome variables as dependent variables, and the body weight in 2004, body weight changes over a one-year period, and the levels of OC, BAP, s-NTx, u-DPD, and E2 as explanatory variables to examine the associations between the variables. The correlations between the body weight in 2004, body weight changes over a one-year period, and the levels of OC, BAP, s-NTx, u-DPD and E2 in the menstrual and ovulatory phases were investigated. No strong correlation (0.7 or higher, $p < 0.001$) was noted in any combination of these parameters. In addition, the body weight in 2004 was adjusted as a confounding factor of the multiple regression analysis, because we considered that the BMD would likely be increased by the burden of additional body weight [23–26]. Residual errors of the multiple regression analysis were tested for normal distribution using the Kolmogorov-Smirnov test. The *p*-values for the lumbar spine, femoral head, and entire femur were 0.200, 0.072, and 0.200, respectively, indicating a normal distribution in the menstrual phase ($p > 0.005$). The *p*-values for the lumbar spine, femoral head, and entire femur were 0.200, 0.074, and 0.102, respectively, indicating a normal distribution in the ovulation phase ($p > 0.005$). Probability values of 0.05 or lower were regarded as statistically significant in all tests. Statistical analysis was performed using SPSS16.0J software (SPSS Japan Inc., Tokyo, Japan).

Table 1 Normal ranges of biochemical parameters

biochemical parameters	reference value	
OC (ng/ml)		3.1–12.7
BAP (U/l)		9.6–35.4
s-NTx (nmol BCE/l)		7.5–16.5
u-DPD (nmol/mmol · Cr)		2.8–7.6
E2 (pg/ml)	menstrual periods	10–78
	ovulation periods	103–366

Results

The physical characteristics of the subjects are shown in Table 2. The subjects were 157.0 ± 4.9cm in height and 51.8 ± 6.0kg in body weight. These values were not significantly different from those in the 2004 Survey on the Trends of National Health. The body weight and BMD of the femoral neck in 2005 were significantly lower than those in 2004. The BMD of the lumbar spine, femoral neck, and entire femur varied in the range of −0.049–0.05, −0.106–0.023, and −0.078–0.122, respectively.

On comparison of the bone metabolic markers in 2004 between the menstrual and ovulation phases, the u-DPD level was significantly lower in the ovulation phase than in the menstrual phase ($p = 0.002$), while the E2 level was significantly higher in the ovulation

phase ($p < 0.001$), but no significant differences were noted in the OC, BAP, or s-NTx level between the phases (Table 3).

Correlations among the bone metabolic markers in the menstrual and ovulation phases are shown in Table 4. In the menstrual phase, significant positive correlations were detected between the OC and BAP level ($r = 0.424$, $p = 0.005$) and between the OC and s-NTx level ($r = 0.332$, $p = 0.031$), and a significant negative correlation was noted between the u-DPD and E2 level ($r = -0.415$, $p = 0.006$). In the ovulation phase, significant positive correlations were noted between the OC and BAP level ($r = 0.341$, $p = 0.027$) and between the u-DPD and E2 level ($r = 0.349$, $p = 0.023$).

Table 5 shows the results of multiple regression analysis using the body weight changes over a one-year period and levels of OC, BAP, s-NTx, and u-DPD in the menstrual and ovulatory phases as explanatory variables, and BMD changes as dependent variables. In the menstrual phase, the BMD of the femoral neck was significantly correlated with the changes of body weight over a one-year period ($\beta = 0.355$, $p = 0.041$), and that of the entire femur was significantly correlated with the OC level ($\beta = 0.463$, $p = 0.012$). In the ovulation phase, the BMD of the femoral neck was significantly correlated with the changes of body weight over a 1-year period ($\beta = 0.371$, $p = 0.032$).

Discussion

We compared the E2 and bone metabolic marker levels between the menstrual and ovulation phases in healthy young women aged about 20 years. The E2 and u-DPD levels were significantly different between the 2 phases. The ovulation phase was identified by observing fern-like crystals in saliva under a microscope. Cervical mucus alters during the ovulation phase and fern-like crystals are observed in saliva [27]. In menstruating women, E2 secretion increases immediately before ovulation, which promotes ovarian follicular development. Secretion slowly decreases thereafter, entering the menstrual phase. The difference in the E2 level between the menstrual and ovulation phases validated the identification of the menstrual phase based on menstrual bleeding reported by the subjects and that of the ovulation phase by observing fern-like crystals in saliva. However, the standard deviation of the E2 level in the ovulation phase was a high value. This was thought to be because identification of the ovulation phase by observation of fern-like crystals is subject to error for several days before and after the date of ovulation [28]. Identification of the date of ovulation thus requires measurement of the basal body temperature and luteinizing hormone. However, these measurements were not performed, since they are generally not included in infertility or pregnancy surveys like those used in the present study. This is a limitation inherent in identification of the ovulation phase based on the observation of fern-like crystals *i.e.*, the method used in this study. The u-DPD level decreases with elevation of the carboxy-terminal propeptide of type I procollagen (PICP), a bone formation marker, 4 days after the E2 level peaks in the ovulation phase [29], because osteoblasts possess estrogen receptors and estrogen promotes collagen synthesis [12, 13]. Zittermann *et al.* [30] reported that ovulation-related elevation of the estrogen level elevated the PICP level and resulted in a low u-DPD level, suggesting that estrogen directly influences bone metabolism by stimulating osteoblasts and inhibiting osteoclasts. No changes in the BAP and OC levels during the menstrual cycle have been reported [13], which is consistent with our findings. Regarding the s-NTx level, we previously reported its usefulness for the evaluation of bone resorption in premenopausal women as an index not influenced by the menstrual cycle [20].

Positive correlations were noted between the BAP and OC levels in the menstrual and ovulation phases. BAP is an enzyme abundant in osteoblasts [31]. It reflects bone growth and its level rises in bone neogenesis. OC is a calcium-binding protein accounting for 25% of non-collagen protein in bone. It is synthesized in osteoblasts and converted to the Gla form in the presence of vitamin K, which serves as an index of bone-forming activity. The positive correlation between the BAP and OC levels may have been due to the fact that both are osteoblast-derived bone formation markers. The E2 and u-DPD levels showed a significant negative correlation in the menstrual phase and significant positive correlation in the ovulation phase. The E2 level starts to decrease 3 days before menstruation and remains low during the menstrual phase [30]. E2 deficiency during this phase may lead to the proliferation of osteoclasts which have escaped from normal cell death in bone tissue, thereby promoting

Table 2 Physical characteristics of the subjects

	in 2004 (n = 42)		in 2005 (n = 42)		Difference (2005–2004) (n = 42)			Exclusion of subjects in 2004 (n = 15)		
	mean SD	min – max	mean SD	min – max	mean SD	min – max	*p* value※1	mean SD	min – max	*p* value※2
Age (y)	19.6 (0.8)	18.0 – 21.0	20.6 (0.8)	19.0 – 22.0	–	–	–	19.3 (0.6)	19.0 – 20.0	–
Hight (cm)	157.0 (4.9)	147.6 – 168.0	157.2 (4.8)	148.5 – 168.3	0.2 (0.5)	−0.9 – 1.0	0.007	159.0 (4.4)	154.9 – 165.9	0.208
body weight (kg)	51.8 (6.0)	40.8 – 67.2	51.2 (6.0)	39.8 – 63.8	−0.5 (2.3)	−5.8 – 4.0	0.125	55.2 (11.3)	47.0 – 74.0	0.209
lumbar spine BMD (g/cm^2)	1.001 (0.084)	0.819 – 1.190	1.000 (0.083)	0.830 – 1.181	−0.001 (0.021)	−0.049 – 0.051	0.669	1.033 (0.085)	0.955 – 1.172	0.194
femoral neck BMD (g/cm^2)	0.826 (0.126)	0.574 – 1.171	0.809 (0.119)	0.554 – 1.071	−0.016 (0.027)	−0.106 – 0.023	<0.001	0.797 (0.087)	0.707 – 0.914	0.722
entire femur BMD (g/cm^2)	0.898 (0.122)	0.649 – 1.216	0.896 (0.109)	0.733 – 1.138	−0.002 (0.048)	−0.078 – 0.122	0.820	0.885 (0.070)	0.806 – 0.963	0.717

※1 2004 VS 2005

※2 2004 VS Exclusion of subjects in 2004

Table 4 Correlation among bone metabolic markers in the menstrual and ovulatory phases

Menstrual phases / Ovulatory phases		Body weight in 2004 (kg)	Change of body weight for a year (kg/y)	OC (ng/ml)	BAP (U/l)	s-NTx (nmolBCE/l)	u-DPD (nmol/mmol・CRE)	E2 (pg/ml)	Change of lumbar spine BMD (g/cm^2)	Change of femoral neck BMD (g/cm^2)	Change of entire femur BMD (g/cm^2)
Body weight in 2004 (kg)	r		−0.192	0.189	0.262	−0.172	0.086	0.021	−0.273	−0.200	−0.026
	p		0.222	0.229	0.094	0.277	0.591	0.893	0.080	0.203	0.872
Change of body weight for a year (kg/y)	r	−0.192		0.112	0.039	0.189	−0.123	−0.264	0.211	0.411	0.164
	p	0.222		0.481	0.807	0.232	0.445	0.091	0.180	0.007	0.300
OC (ng/ml)	r	0.228	0.115		0.424	0.332	0.143	0.047	0.044	0.039	0.428
	p	0.147	0.468		0.005	0.031	0.365	0.766	0.784	0.805	0.005
BAP (U/l)	r	0.239	−0.029	0.341		−0.052	−0.059	0.182	−0.227	0.039	0.123
	p	0.128	0.855	0.027		0.748	0.712	0.249	0.148	0.804	0.440
s-NTx (nmolBCE/l)	r	−0.095	0.079	0.115	−0.139		−0.101	−0.148	−0.055	−0.137	0.066
	p	0.550	0.619	0.467	0.379		0.528	0.349	0.735	0.393	0.682
u-DPD (nmol/mmol・CRE)	r	0.035	−0.257	0.040	−0.056	−0.119		−0.415	0.112	0.116	0.142
	p	0.825	0.100	0.799	0.726	0.451		0.006	0.481	0.465	0.369
E2 (pg/ml)	r	0.247	0.049	−0.149	−0.136	−0.135	0.349		−0.054	−0.226	−0.096
	p	0.114	0.756	0.345	0.389	0.394	0.023		0.735	0.151	0.545
Change of lumbar spine BMD (g/cm^2)	r	−0.273	0.211	−0.171	−0.118	−0.120	0.019	−0.187		0.061	0.012
	p	0.080	0.180	0.279	0.458	0.451	0.904	0.236		0.701	0.937
Change of femoral neck BMD (g/cm^2)	r	−0.200	0.411	−0.017	−0.012	−0.142	0.116	0.127	0.061		0.140
	p	0.203	0.007	0.913	0.942	0.371	0.464	0.424	0.701		0.378
Change of entire femur BMD (g/cm^2)	r	−0.026	0.164	0.028	0.026	−0.150	0.281	−0.071	0.012	0.140	
	p	0.872	0.300	0.859	0.872	0.342	0.071	0.654	0.937	0.378	

Table 3 Relationship between the menstrual cycle and bone metabolic markers in 2004

	Menstrual phases		Ovulatory phases		
	mean SD	min－max	mean SD	min－max	*p* value
OC (ng/ml)	6.1 (1.9)	2.7－9.1	6.0 (2.2)	1.0－10.9	0.731
BAP (U/l)	24.1 (8.0)	12.5－46.4	24.0 (8.1)	13.3－51.5	0.954
s-NTX (nmolBCE/l)	10.0 (2.4)	7.1－18.7	10.9 (3.0)	6.9－19.4	0.134
u-DPD (nmol/mmol・CRE)	7.7 (2.2)	3.3－13.5	6.8 (1.7)	3.6－12.7	0.002
E2 (pg/ml)	24.5 (15.4)	8.0－76.0	87.2 (66.4)	24.0－331.0	＜0.001

Table 5 Multivariate linear regression analysis of the change of BMD over one year (lumbar spine, femoral neck, entire femur)

〔A〕menstrual phases

	Change of lumbar spine BMD			Change of femoral neck BMD			Change of entire femur BMD		
	β	p	R^2	β	p	R^2	β	p	R^2
Change of body weight for a year (kg/y)	0.166	0.345	0.158	0.355	0.041	0.223	0.076	0.651	0.230
OC (ng/ml)	0.171	0.359		0.025	0.889		0.463	0.012	
BAP (U/l)	−0.253	0.180		0.104	0.561		−0.015	0.935	
s-NTX (nmolBCE/l)	−0.023	0.890		−0.072	0.646		0.110	0.484	
u-DPD (nmol/mmol・CRE)	0.014	0.943		−0.075	0.681		−0.003	0.986	
E2 (pg/ml)	0.033	0.864		−0.199	0.287		−0.112	0.544	

〔B〕ovulatory phases

	Change of lumbar spine BMD			Change of femoral neck BMD			Change of entire femur BMD		
	β	p	R^2	β	p	R^2	β	p	R^2
Change of body weight for a year (kg/y)	0.193	0.267	0.162	0.371	0.032	0.210	0.145	0.406	0.140
OC (ng/ml)	−0.169	0.354		−0.024	0.892		−0.041	0.825	
BAP (U/l)	−0.065	0.716		0.070	0.684		−0.003	0.986	
s-NTX (nmolBCE/l)	−0.080	0.641		−0.003	0.985		−0.101	0.562	
u-DPD (nmol/mmol・CRE)	0.042	0.803		0.038	0.816		0.319	0.068	
E2 (pg/ml)	−0.222	0.221		0.147	0.402		−0.202	0.271	

※adjustment for the body weight in 2004

bone resorption [32]. Blumsohn *et al.* observed a strong negative correlation between E2 and bone metabolic markers in adolescent girls, which was similar to our findings in the menstrual phase [33]. The u-DPD level decreases with elevation of the PICP level, a bone formation marker, 4 days after the E2 level peaks in the ovulation phase [28]. In the above-mentioned study of Zittermann *et al.* [30], an ovulation-related elevation of the estrogen level elevated the PICP level and resulted in a low u-DPD level. Immediately after the rapid rise and peak of the E2 in the ovulation phase, the E2 level decreased. And the u-DPD level decreased by a short time later. Therefore, this study thought by positive correlation was shown as a result that the u-DPD level reduction by E2 level rise takes place in a time lag at the same time it decreases, after E2 level reaches a peak. Based on these findings, identification of the menstrual cycle phase is necessary to investigate the usefulness of the u-DPD level in young women.

Multiple regression analysis showed that the changes of body weight over a one-year period were correlated with the BMD of the femoral neck in the menstrual and ovulation phases. This is also supported by the report of Lueken *et al.* [34] that tetraplegia, postoperative bed rest, and weightlessness in space flight were associated with a BMD decrease. We consider that the load of the body weight on different parts of the body mechanically stimulates bones, thereby strengthening the microstructure of bone tissue. In particular, the results of this study suggest that the BMD changes are at least partly induced by

the body weight change in daily life, and that the loss of body weight decreases the BMD. Accordingly, the maintenance of an appropriate body weight greatly aids in the prevention of osteoporosis in young women who are prone to prioritize their desire for slimness. Nonetheless, further research in this area will be needed, since the present work had a study period of only one year and the statistical significance was particularly strong ($p = 0.025 - 0.028$). Meanwhile, based on the results of mouse studies in which BMD changes were accompanied by a body weight change, it has been speculated that leptin regulates the bone mass via a balance between an indirect action through the nervous system [35, 36] and a direct action on osteoclasts [37]. The epidemiological survey performed a cross-sectional study for men and women reported a positive correlation between blood leptin levels and the BMD [38-40]. Therefore, the present finding of a BMD change accompanied by a body weight change was tentatively attributed to changes in BMD due to the load of the body weight, as well as the regulation of bone mass by leptin. In order to clarify this result, it is necessary to measure leptin levels on a continuous basis in a longer-term study.

We studied the bone metabolic markers in 2004 and BMD in the following year to investigate the influence of the markers on the BMD. In the menstrual phase, the OC level was correlated with the BMD of the entire femur, suggesting that a high OC level in the menstrual phase is associated with an increase in the BMD. It has been shown that the BAP level increases earlier than the OC level in the first menstruation because it reflects bone growth more markedly than OC [41]. However, its variation tends to decrease after the first menstruation [42], and it is unclear whether it reflects the condition of bone formation in adult women. This study suggested that the OC level serves as a preventive factor against BMD reduction and reflects osteoblast activity, and the bone-forming activity inhibits BMD reduction. In contrast, no significant association was observed between the bone metabolic markers and BMD in the ovulation phase, which may have been due to the fact that bone metabolic turnover is influenced several days after the female sex hormone level rises [29]. Therefore, to predict BMD changes by measuring bone metabolic markers in young women, the OC level in the menstrual phase should be measured.

The physiques of the subjects were close to the age-matched means [43] in other surveys in Japan as well as those in subjects excluded from this survey, suggesting that the findings are valid for application to young women in general. The limitations of this study are that the subjects were female college students who participated in a health survey including BMD measurement, and therefore might have been biased toward those who were in good health or had marked health awareness. Furthermore, although this study was performed over a short period of 1 year, some young subjects showed a BMD change of about 10% during that period. Although the cv (= 0.3%) of the equipment suggests the presence of errors, we consider that the BMD changes during the year were accurately detected. However, longitudinal investigation involving broader populations will be needed before our results can be generalized to the population at large.

In conclusion, we examined the association between the changes in female hormone levels and bone metabolic markers during the menstrual cycle in 42 healthy young women, aiming to clarify the effects of menstrual cycle-related changes in the body weight and bone metabolic marker levels on the changes in BMD. The E2 level was significantly higher in the ovulation than in the menstrual phase, while the u-DPD level was significantly lower in the ovulation phase. The OC and BAP levels showed significant positive correlations in both the menstrual and ovulation phases. The E2 and u-DPD levels were negatively correlated in the menstrual phase, and positively correlated in the ovulation phase. The E2 levels in the menstrual phases suggested that estrogen directly influences bone metabolism by stimulating osteoblasts and inhibiting osteoclasts. In addition, this study thought by positive correlation in ovulation phases was shown as a result that the u-DPD level reduction by the increase in E2 levels takes place in a time lag at the same time it decreases, after E2 level reaches a peak. Analysis of the correlations between bone metabolic markers and BMD changes showed a correlation between the OC level and changes in the BMD of the entire femur in the menstrual phase. Based on these findings, to predict BMD changes by measuring bone metabolic markers in young women, the OC level in the menstrual phase should be employed. The changes in the body weight over 1 year were correlated with

the changes in the BMD of the femoral neck in the menstrual and ovulatory phases, suggesting that a higher body weight leads to a heavier load on the femoral neck, thereby inhibiting the decrease in BMD. However, our results did not provide evidence of a change in the bone resorption by estrogen, since there was no correlation between E2 and BMD in the multivariate analysis. This study was limited in that it was performed over a short period of 1 year, and because the subject group was biased toward female college students who had high-level health awareness. Thus, a long-term longitudinal study of a larger population of subjects is still needed.

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