Effects of the Treadmill Walking Exercise on the Biochemical Bone Markers

Walaiporn Kitareewan MD*, Jariya Boonhong MD*,
Siriporn Janchai MD*, Sek Aksaranugraha MD**

** Department of Rehabilitation Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Thai Red Cross Rehabilitation Center, Bangkok, Thailand*

Objective: *To study the effects of moderate-intensity treadmill walking exercise on the biochemical bone markers in the menstruating and menopausal women.*
Study design: *Experimental study.*
Setting: *Department of Rehabilitation Medicine, King Chulalongkorn Memorial Hospital.*
Material and Method: *Twenty-two healthy volunteer women at the age of 30-70 were recruited: 11 menstruating women and 11 menopausal women. The exercise consisted of the treadmill walking exercise, intensity of 50% of heart rate reserve, for the duration of 30 minutes, at the frequency of 3 times a week, over a 3-month period. Serum beta CTx, PINP and NMID osteocalcin were measured at the baseline and in the 1st, 2nd, and 3rd months.*
Results: *Twenty women: 11 menstruating women and 9 menopauses completed the exercise protocol. The baseline characteristics including age, body mass index, serum beta CTx, PINP and NMID were statistically different. The serum beta CTx and NMID levels were decreased from the baseline from Month 1 to 3 in both menstruating and menopausal groups. Serum PINP was not significantly changed in the 1st and 2nd months except the significant decreasing in the 3rd month in the menstruating women. There were no significant differences of bone marker changes between the menstruating women and the menopauses. The biochemical bone markers' levels (beta CTx, PINP and NMID) had the strong correlations analyzed by Pearson's correlation coefficients (> 0.8 with p-value < 0.001).*
Conclusion: *The present study clearly demonstrates that the moderate intensity treadmill walking exercise for 30 minutes, 3 times a week reduces bone resorption and bone turnover markers in both the menstruating women and the menopauses after the first month until the third month of the experiment. Although the bone formation markers had a tendency of decreasing after exercising, the significant changes showed only in the 3rd month in the menstruating group. All of the bone markers including beta CTx, NMID osteocalcin and PINP were highly correlated.*

Keywords: *Biochemical bone marker, Beta CTx, NMID osteocalcin, PINP, Treadmill walking exercise*

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Osteoporosis is a disease of bone that leads to an increased risk of fracture and becomes a serious health threat for aging women. Osteoporotic fractures are associated with reductions in the quality of life, deformity and a cause of death in the elderly population[1]. It is estimated that over 200 million people worldwide have an osteoporosis[2]. In the United States, the National Health and Nutritional Survey (NHANES III) estimated that 13% to 18% of women have an osteoporosis and 27% to 50% have an osteopenia[3]. In Thailand, these diseases are increasing due to population's aging. The prevalence of osteopenia and osteoporosis in Thai women was ranging from 13.6% to 24.7%[4,5].

The state of bone can be evaluated by many techniques, including histomorphometry, densitometry and measurement of calcium fluxes. Histomorphometry is invasive, expensive, and limited to a single skeletal site (iliac crest). It also has a long turnaround time. Densitometry is precise and noninvasive but slow to reveal changes. The measurement of calcium fluxes is technically difficult. The biochemical markers of bone remodeling offer the noninvasive means of complimenting these techniques, in other words,

Correspondence to:
Boonhong J, Department of Rehabilitation Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Phone: 0-256-4433, Fax: 0-256-4433
E-mail: Jariya_boonhong@yahoo.com

providing direct information[6]. The bone markers can be used in the clinical investigation of new therapeutic agents to monitor their effects and mechanism of action[7].

Bone markers are categorized into two groups, *i.e.* bone formation marker and bone resorption marker. Procollagen type 1 N-terminal propeptide (P1NP) is a bone formation marker. The serum concentration of total P1NP reflects changes in the synthesis of new type1collagen[8]. In the process of bone resorption, the amino- and corboxy-fragments of collagen released with attached cross-links are called telopeptides. Serum carboxy-terminal telopeptide collagen degradation product (beta-CrossLaps or beta-CTx) is a useful assay for assessing bone resorption state[9]. N-MID osteocalcin is a bone turnover marker which may be released during the bone formation and resorption.

Weight-bearing and strength-training exercises are beneficial to bone development and maintenance[10,11]. The weight-bearing exercise can be as simple as brisk walking. Extreme exercises are not necessary to affect a bone benefit. A mild form of exercise that improves agility and balance can benefit the skeleton[12]. Exercises for women with osteoporosis should not include high-impact aerobics or activities in which a fall is likely.

In the previous studies of the effects of exercises on bone marker levels[8,13-16], both bone formation and bone resorption are inconclusive. Some[8,13,14] have reported that the markers of bone formation increase and some markers of bone resorption decrease. Others[15,16] have reported that there were no changes or other decreases in bone turnover. These inconsistent findings may concern with the age of the subjects, the type and the intensity of exercises.

Thorsen et al[17] studied the effects of a single bout of brisk walking (50% of VO2 max) for 90 minutes in twelve postmenopausal women by measuring the bone formation marker of type I collagen formation (P1CP) and bone resorption marker (ICTP) at the time of the exercise as follows: before the exercise and at the 1st-24th and 72nd hours. They reported that a significant increasing of PICP was noted at the 24th and 72nd hours ($p < 0.01$) after exertion as well as a significant decreasing in the concentration of serum ICTP was noted at the 1st hour ($p < 0.05$) and followed by an increase at the 72nd hours ($p < 0.001$). The results of the present study are the effects of a single exercise that may not be identical with the longer period of walking exercise.

Yamazaki et al[18] studied the effect of walking exercise on bone metabolism in fifty postmenopausal women, aged 49-75 years old, with osteopenia or osteoporosis. The exercise consisted of daily outdoor walking, the intensity of which was 50% of maximum oxygen consumption, with the duration of at least 1 hour with more than 8,000 steps, at the frequency of 4 days a week, over a 12-month period. They reported that the urinary NTx level (bone resorption) rapidly responded to the walking exercise from the 3rd month and this reduction was sustained until the 12th month. P1NP was increased after the first month and reduced from the 3rd month until the 12th month. The present study used the urinary bone resorption marker (urinary NTx) that may not have the same result as the serum marker. Furthermore, the results of P1NP that increased in the first month and reduced from the 3rd month until the 12th month should be confirmed by another clinical trial.

The present study was designed to study the effects of moderate-intensity treadmill walking exercise for 30 minutes, 3 times a week over 3-month period on bone markers in the healthy volunteers consisting of both menstruating and menopausal women.

Material and Method

Subjects

Twenty-two healthy volunteer women who were 30 to 70 years old and did not take part in a regular physical training or exercise more than 3 months were included into the study. All of them were underwent medical screening by the doctor who is one of the present study's author. The subjects having no disease that would affect the serum level of bone markers were recruited into the study. The exclusion criteria were the subjects who had an underlying disease of cardiovascular, hepatobiliary, renal, or musculoskeletal system and were on medication of anti-resorptive drug. The present study was approved by Ethics Committee of Faculty of Medicine, Chulalongkorn University and all subjects submitted the written informed consent to participate.

Interventions

All of the subjects did the treadmill walking exercise under supervision of a doctor who is one of our authors. They were instructed to begin treadmill walking with a 5-minute warm-up period. Later, the walking speed was increased until their pulse rates were at about the target heart rate zone. This speed was maintained for 20 minutes and slowly decreased for a 5-minute cool-down period. The target heart rate was

calculated at 50% of heart rate reserve by using Karvonen method[19] in each subject. The subjects were asked to do the exercise 3 times a week for 3 months. The one who had participated less than 80% of the total time would be dropped from the present study.

Biochemical bone marker measurement

After the subjects had an 8-hour overnight fast, the blood samples were drawn at the laboratory at 8:00 am for the analysis of bone markers. The biochemical bone markers included beta-CrossLaps or beta-CTx (bone resorption marker), PINP (bone formation marker) and NMID (bone turnover marker) that tested by enzyme immunoassay (EIA). The blood samples were taken at the beginning of the reseach and after the exercises in the 1st, 2nd and 3rd months.

The subjects were recorded the demographic data, including age, body weight, height and menstruation history.

The statistical analysis was conducted by using SPSS version 13.0 for Windows. The demographic data and the bone marker levels were analyzed and presented as the mean and SD. The blood biochemical data were separately analyzed into two groups: menstruating and menopausal subjects. Each bone marker was compared between before starting the program and after exercising in the 1st, 2nd and 3rd months by paired t-test. The modified Bonferroni adjustment was performed to account for the three-time point's measurement for reducing a Type I error from the multiple t-test (significant level at p-value < 0.02). The baseline data of each bone marker were compared between the groups of menstruation and menopause by the unpaired t-test at the significant level at $p < 0.05$. The correlation between beta CTx, PINP and NMID levels were analyzed by Pearson's correlation coefficient test.

The simple linear regression model was used to construct the equation for predicting the serum beta CTx after the 3-month exercise.

Results

Twenty-two healthy volunteer women were recruited into the present study. Only twenty women could complete the exercise protocol whereas two subjects were dropouts because of influenza and minor trauma. There were eleven menstruating women and nine menopauses. The demographic data and the baseline levels of bone markers were shown in Table 1. The baseline levels of beta-CTx, PINP and NMID osteocalcin were significantly higher in the menopausal group.

The mean of the serum beta-CTx (bone resorption marker) in the menstruating women was significantly decreased after one month of the treadmill walking exercise and maintained this decreasing after the 2nd and 3rd months of the exercise. The menopausal group also had the similar beta-CTx result to the menstruating women's except the decreased result in the 2nd month that was not statistically significant (Table 2,3).

In the menstruating group, the mean of the serum PINP (bone formation marker) was not significantly changed after the 1st and 2nd months of exercise, but after the 3rd month it showed significant decreasing. However, the decreasing of PINP level in the 3rd month was not significantly changed in the menopausal group (Table 2,3).

The mean of the serum NMID (bone turnover marker) in the menstruating women was significantly decreased in the 2nd and 3rd months after exercising while the menopausal group was earlier significantly reduced in the 1st month after the exercise (Table 2,3).

The mean difference between the before and

Table 1. Demographic data

Characteristics	Menstruating group (n = 11)	Menopausal group (n = 9)	p-value*
Age (yr) (Mean ± SD)	36.90 ± 6.3	56.90 ± 3.6	< 0.001
BMI	27.80 ± 4.4	23.30 ± 2.9	0.015
Baseline level of bone markers (Mean ± SD)			
Beta-CTx	0.27 ± 0.14	0.53 ± 0.15	0.001
PINP	36.40 ± 14.73	60.76 ± 21.55	0.008
N-MID	17.44 ± 5.06	29.10 ± 9.46	0.002

* Comparison of the baseline between the groups by the unpaired t-test, significance at p-value < 0.05

Table 2. Biochemical bone markers of the menstruating women (n = 11)

Bone markers	Beta CTx (Mean ± SD)	PINP (Mean ± SD)	N-MID (Mean ± SD)
Before Exercise	0.27 ± 0.14	35.60 ± 10.94	17.44 ± 5.06
After ex 1st month	0.22 ± 0.15	36.40 ± 14.73	16.35 ± 4.73
p-value*	< 0.001	0.747	0.09
95% CI	(0.03, 0.07)	(-6.17, 4.57)	(-0.21, 2.39)
After ex 2nd months	0.23 ± 0.12	36.84 ± 12.15	14.52 ± 4.10
p-value*	0.01	0.567	< 0.001
95% CI	(0.01, 0.06)	(-5.94, 3.46)	(1.66, 4.19)
After ex 3rd months	0.18 ± 0.11	29.06 ± 9.76	13.70 ± 4.25
p-value*	< 0.001	0.01	0.001
95% CI	(0.05, 0.1)	(1.73, 11.35)	(2.00, 5.49)

* Comparison with the baseline data by the paired t-test, significance at p-value < 0.02

Table 3. Biochemical bone markers of the menopausal women (n = 9)

Bone markers	Beta CTx (Mean ± SD)	PINP (Mean ± SD)	N-MID (Mean ± SD)
Before Exercise	0.53 ± 0.15	60.76 ± 21.55	29.10 ± 9.46
After ex 1st month	0.43 ± 0.18	58.68 ± 22.24	26.73 ± 8.08
p-value*	0.002	0.378	0.012
95% CI	(0.05, 0.15)	(-3.09, 7.28)	(0.67, 4.05)
After ex 2nd months	0.51 ± 0.20	62.61 ± 20.04	26.05 ± 9.64
p-value*	0.670	0.581	0.010
95% CI	(-0.06, 0.09)	(-9.18, 5.52)	(0.97, 5.13)
After ex 3rd months	0.43 ± 0.17	55.56 ± 17.83	23.96 ± 8.15
p-value*	0.006	0.165	< 0.001
95% CI	(0.04, 0.17)	(-2.64, 13.07)	(3.91, 6.37)

* Comparison with the baseline data by the paired t-test, significance at p-value < 0.02

Table 4. Mean difference of the bone marker levels between the pre-and post-exercises of the 1st, 2nd and 3rd months

Bone markers	Post-ex duration	Menstruation (n = 11) (Mean ± SD)*	Menopause (n = 9) (Mean ± SD)*	p-value**
Beta CTx	1st month	-0.05 ± 0.03	-0.10 ± 0.06	0.067
	2nd month	-0.04 ± 0.04	-0.02 ± 0.10	0.542
	3rd month	-0.08 ± 0.05	-0.10 ± 0.08	0.551
PINP	1st month	0.80 ± 8.00	-2.10 ± 6.74	0.399
	2nd month	1.25 ± 6.98	1.83 ± 9.56	0.876
	3rd month	-6.54 ± 7.16	-5.21 ± 10.22	0.737
NMID	1st month	-1.09 ± 1.94	-2.36 ± 2.20	0.186
	2nd month	-2.93 ± 1.88	-3.05 ± 2.71	0.908
	3rd month	-3.75 ± 2.60	-5.14 ± 1.60	0.179

* The minus value means that decreased from the baseline
** Comparison between the groups by the unpaired t-test, significance at p-value < 0.02

Table 5. The Pearson's correlation coefficient between each bone marker

Bone markers	Pre-ex	Post-ex 1 mo	Post-ex 2 mos	Post-ex 3 mos	p-value*
Beta CTx & NMID	0.84	0.88	0.88	0.91	< 0.001
Beta CTx & PINP	0.86	0.89	0.84	0.86	< 0.001
N-MID & PINP	0.94	0.94	0.91	0.86	< 0.001

* Significance at p-value < 0.05

after exercises at the duration of the 1st, 2nd and 3rd months in each bone marker was shown in Table 4. There was no significant difference of changing between the menstruating and the menopausal women (Table 4).

All of the biochemical bone marker levels (beta CTx, PINP and N-MID) had a strong correlation with Pearson's correlation coefficients (> 0.8) of every pair of markers and period of time (Table 5).

The mathematical equation for predicting the serum levels of beta CTx (bone resorption marker) after the 3-month treadmill walking exercise by using the baseline or the before exercise level, was constructed by the simple linear regression model that is: beta $CTx_{after\ ex}$ = 0.85 (beta $CTx_{before\ ex}$) -0.02, with adjusted R^2 = 0.87.

Discussion

The baseline levels of bone markers: both bone formation and bone resorption were higher in the menopausal group which indicated that the overall rates of bone turnover remained high in the menopausal women. When each mean marker level of the menstruating group was compared with the menopausal women's, the results showed that both bone resorption and bone formation in the menopausal subjects more increased than those of the menstruating women. These evidences supported the bone loss in aging and played a major role in osteoporosis. Many studies[20-22] have reported the similar results that really support the author findings.

The present study found that the beta CTx was decreased after the first month of treadmill walking exercise. This decreasing was still on in the second and third months in both the menstruating and the menopausal women. The decreasing in these two groups was not significantly different. Yamazaki et al[18] studied the effect of moderate walking exercise on bone metabolism in the postmenopausal women. They reported that the decreasing of urinary NTx (bone resorption marker) was obviously detected after the 3-month exercise, but not after the first month. The earlier detection in the present study can explain why and confirm that the serum bone marker is more sensitive than the urinary examination.

The response of serum osteocalcin (N-MID) in the present study showed the results that correlated to the beta CTx's. The reduction was detected from the first month after the exercise and this was continuously on until the third month in both research groups. These evidences confirm that the decreasing of bone turnover after the treadmill exercise was certainly found.

After the 3-month exercise, a decreasing of serum PINP (bone formation marker) in the menstruating group was significantly changed while the decreasing in the menopausal group did not reach the significant level. The present study's results were similar to the report of Yamazaki et al[18]. They reported that the decreasing of serum bone specific alkaline phosphatase (BAP) that is a bone formation marker after the 3-month exercise significantly changed after the 12-month exercise. Both decreasing may be the biochemical responses to the decreasing of bone resorption and bone turnover. However, further studies are needed to more explore the effects of exercise to the bone formation metabolism in order to confirm the results.

The present study also found the high correlation of between each bone marker with significance at p < 0.001. Bunyaratavej et al[21] studied the level of NMID osteocalcin and Beta CTx in 700 Thai women and reported that the NMID osteocalcin had a high correlation with beta crosslap (r = 0.789, p = 0.0001). This evidence shows that the bone resorption and bone formation mechanism are related.

The data were used to construct the equation that could predict the beta CTx level after the 3-month exercise by using the beta CTx level at the time before the exercise started. This equation may be useful in the clinical practice for estimating the exercise effects before prescribing the program. From the value of slope (= 0.85) in the linear equation, the reduction of beta CTx is about 15% from the baseline level.

Conclusion

The present study clearly demonstrates that a moderate intensity treadmill walking exercise for 30 minutes, 3 times a week could reduce bone resorption and bone turnover markers in both menstruating and menopausal women after the first month until the third months. Although the bone formation markers have a tendency of decreasing after the exercise, the significant changes show only at the third months after exercising in the menstruating women. Further studies are suggested to elucidate this metabolism. All of the bone markers including beta CTx, NMID osteocalcin and PINP are highly correlated.

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Potential conflicts of interest

The authors wish to express appreciation to the Ratchadapiseksompotch Fund of the Faculty of Medicine, Chulalongkorn University for supporting the present study.

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ผลการออกกำลังกายบนลู่เดินต่อค่าชีวเคมีกระดูก

วลัยภรณ์ กิจอารีวรรณ, จริยา บุญหงษ์, ศิริพร จันทร์ฉาย, เสก อักษรานุเคราะห์

วัตถุประสงค์*: เพื่อศึกษาผลของการเดินออกกำลังกายบนลู่เดินที่ความหนักระดับปานกลางต่อค่าชีวเคมีกระดูกในหญิงวัยมีระดูและวัยหมดระดู*

การออกแบบการศึกษา*: การศึกษาชนิดทดลอง*

สถานที่*: ฝ่ายเวชศาสตร์ฟื้นฟูโรงพยาบาลจุฬาลงกรณ์*

วัสดุและวิธีการ*: อาสาสมัครหญิงสุขภาพดีมีอายุ 30 ถึง 70 ปี จำนวน 22 คน รับคัดเลือกสู่การศึกษาแบ่งเป็นหญิงวัยมีระดู 11 คน และวัยหมดระดู 11 คน โดยให้เดินออกกำลังกายบนลู่เดินที่ความหนักระดับ 50 เปอร์เซ็นต์ของ heart rate reserve ครั้งละ 30 นาที 3 ครั้งต่อสัปดาห์ นาน 3 เดือน และตรวจเลือดหาระดับชีวเคมีกระดูกของ beta CTx, PINP และ NMID ที่เวลาเริ่มต้นการศึกษา และเดือนที่ 1, 2 และ 3 หลังออกกำลังกาย*

ผลการศึกษา*: อาสาสมัครหญิง 20 คน ที่ออกกำลังกายจนครบ เป็นหญิงวัยมีระดู 11 คน และหญิงวัยหมดระดู 9 คน พบว่าข้อมูลพื้นฐานเริ่มต้น คือ อายุ, ดัชนีมวลกาย, ระดับ beta CTx, PINP และ NMIDในเลือด ของทั้ง 2 กลุ่มแตกต่างกันอย่างมีนัยสำคัญ หลังออกกำลังกายระดับ beta CTx และ NMIDในเลือดลดลงตั้งแต่ เดือนที่ 1 จนถึงเดือนที่3 หลังออกกำลังกายในหญิงอาสาสมัครทั้ง 2 กลุ่ม ระดับ PINP ไม่พบว่ามีการเปลี่ยนแปลงอย่างมีนัยสำคัญในเดือนที่ 1 และ 2 ของทั้ง 2 กลุ่ม แต่พบว่าลดลงในเดือนที่ 3 ทั้ง 2 กลุ่ม โดยมีนัยสำคัญทางสถิติเฉพาะกลุ่มหญิงวัยมีระดูการเปลี่ยนแปลงของค่าชีวเคมีกระดูกไม่มีความแตกต่างกันเมื่อเปรียบเทียบกันระหว่างหญิง 2 กลุ่ม ค่าชีวเคมีกระดูกของ beta CTx, PINP และ NMID มีค่าสัมประสิทธิ์สหสัมพันธ์ระหว่างกันค่อนข้างสูงที่มากกว่า 0.8 และ p-value น้อยกว่า 0.001*

สรุป*: การศึกษานี้แสดงให้เห็นว่าการออกกำลังกายด้วยลู่เดินที่ความหนักระดับปานกลาง ครั้งละ 30 นาที 3 ครั้งต่อสัปดาห์ สามารถลดกระบวนการสลายและการผลัดเปลี่ยนเซลล์กระดูก ได้ตั้งแต่เดือนแรกจนถึงเดือนที่ 3 หลังการออกกำลังกาย ขณะที่กระบวนการสร้างเซลล์กระดูกมีแนวโน้มที่จะลดลงภายหลังออกกำลังกาย แต่มีนัยสำคัญเฉพาะกลุ่มหญิงมีระดูในเดือนที่ 3 เท่านั้น และพบว่า ระดับค่าชีวเคมีกระดูก beta CTx, PINP และ NMID มีความสัมพันธ์ระหว่างกันค่อนข้างสูง*