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ABSTRACT

This study assessed the effects of multidimensional exercises on functional decline, urinary incontinence, and fear of falling in community-dwelling Japanese elderly women with multiple symptoms of geriatric syndrome (MSGS). Sixty-one participants were randomly assigned either to an intervention ($n = 31$) or to a control group ($n = 30$). For 3-month period, the intervention group received multidimensional exercise, twice a week, aiming to increase the muscle strength, walking ability, and pelvic floor muscle (PFM). Outcome variables were measured at baseline, and after intervention and follow-up. The functional decline of the intervention group decreased from 50.0% at baseline to 16.7% after intervention and follow-up ($Q = 16.67$, $p < 0.001$). For urinary incontinence, the intervention group decreased from 66.7% at baseline to 23.3% after intervention and 40.0% at follow-up ($Q = 13.56$, $p = 0.001$), whereas the control group showed no improvement. Intervention group showed greater and significant decrease in the score of MSGS compared to control group ($F = 12.66$, $p = 0.001$). Within the subjects that showed improvement to normal status of MSGS, a significantly higher proportion demonstrated increased maximum walking speed at follow-up ($Q = 6.50$, $p = 0.039$). These results suggest that multidimensional exercise is an effective strategy for reducing geriatric syndromes in elderly population. An increase in walking ability may contribute to the improvement of MSGS.

1. Introduction

The geriatric syndrome such as functional decline, urinary incontinence, and fear of falling are used to capture those clinical conditions that do not fit into discrete disease categories, and are serious problems among the elderly population (Inouye et al., 2007). Many studies have demonstrated that a decline in walking speed, muscle strength and balance ability of the elderly is strongly associated with the development of geriatric syndrome (Vellas et al., 1997; Ishizaki et al., 2000; Maggi et al., 2001).

It is well documented that as age advances, the proportion of people with more than one symptom of geriatric syndrome increases. In addition, people with MSGS have an increased prevalence of functional disability and mortality compared to people with only one or no symptoms present. Several studies have put emphasis on the fact that multidimensional exercises focusing on strength, balance, and mobility improvement, even into advanced age, was helpful in reducing functional decline, urinary incontinence and fear of falling (Nelson et al., 2004; Gitlin et al., 2006; Kim et al., 2007). These previous studies validated the effectiveness of the multidimensional exercises focusing on the improvement of a single geriatric syndrome such as functional decline or urinary incontinence, but did not provide any information on whether the subjects possessed symptoms other than functional decline or urinary incontinence. One study demonstrated (Tinetti et al., 1995) that falls and urinary incontinence were associated with the occurrence of functional decline, and that the identification of shared risk factors associated with falls and urinary incontinence is the key in establishing effective and efficient interventional strategies. However, few multidimensional exercises studies have been performed in community-dwelling elderly persons with MSGS.

In the present study, we hypothesize that deteriorations in muscle strength, walking and balance ability are common risk factors associated with functional decline, urinary incontinence and fear of falling. We conducted a randomized and controlled trial to evaluate the effects of the multidimensional exercises targeted at reducing the symptoms of functional decline, urinary inconti-

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nence, and fear of falling in community-dwelling Japanese elderly women with MSGS.

2. Methods

2.1. Study sample and procedures

Overall health surveys were conducted at the Tokyo Metropolitan Institute of Gerontology (TMIG), aiming at early screening of geriatric syndromes in elderly persons and at developing intervention strategies, which would reduce those geriatric syndromes. As subjects, 1016 women were chosen randomly from the Basic Resident Register as persons aged 70 or older residing in Itabashi ward of Metropolitan Tokyo.

A letter outlining the study and describing its objective, and the way that the personal data would be used was mailed to the elderly women selected, inviting them to participate in the study. The baseline survey was conducted in November 2004, and 669 women aged 70 years and older participated.

The participants were screened based on three geriatric syndromes: functional decline, urinary incontinence, and fear of falling. A person who was reported as having two or more geriatric syndromes present was defined as having MSGS. Out of the 669 women participated, 102 were classified as having MSGS (Fig. 1). A pamphlet containing information on the "Exercise Classes for the Treatment of Geriatric Syndromes" was mailed to the 102 potential participants. A response was obtained from 74 of them, of whom 61 were willing to participate. There were no statistically significant differences in physical fitness, age, and geriatric syndromes between the 61 willing participants and the 41 unwilling ones including those who did not submit any response. The research protocol was approved by the institutional review board, and informed consent was obtained from each participant.

2.2. Randomization

After baseline assessment, subjects were divided into two groups with an allocation ratio of 1:1 according to computer-generated random numbers. There was no attempt to equalize the sizes of the groups based on characteristics or to recruit subjects with specific characteristics. Thereafter, one group was allocated to the intervention ($n = 31$) and the other group to the control ($n = 30$) (Fig. 1).

2.3. Data collection

Data collected by interview and a physical fitness test at baseline, after 3-month exercise, and were reassessed at 6-month follow-up.

2.3.1. Interview survey

A face-to-face interview was conducted to assess the following variables: The functional decline was measured using the TMIG index of competence (Koyano et al., 1991). For each of the 13 items, "yes" was scored as 1 and "no" as 0 (maximum score: 13). A person with a TMIG index score less than 10 was defined as having functional decline. Urinary incontinence was assessed through the question "Have you ever experienced urine leakage during the last 1 year?" If a subject responded with a "yes", we would then ask concerning the frequency of urinary incontinence. The frequency of urinary incontinence was assessed based on a five-point scale through interview (1: several times per year; 2: once or more per month: 3: once or twice per week; 4: once every 2 days; 5: everyday). A person whose response ranged 2–5 was defined as having urinary incontinence (Burgio et al., 1991). The fear of falling was assessed by asking "At this moment, are you afraid of falling?" and classified as "1. not at all", "2. somewhat", "3. very much", and "4. activity restriction due to fear of falling". Subjects who responded within 2 and 4 were assigned to the fear group (Maki et al., 1991).

The effect of the multidimensional exercises on the geriatric syndromes was assessed based on shifts of the responses from the interview, which was conducted at a baseline, completion of the 3-month exercise, and at the 6-month follow-up. The scores of geriatric syndromes were calculated as follows: functional decline, 0 for TMIG index score more than 11, 1 for 10, 2 for 9, and 3 for less than 8; urinary incontinence, 0 for no urine leakage or several times per year, 1 for once or more per month, 2 for once or twice per week, and 3 for once every 2 days or everyday; fear of falling, 0 for not at all, 1 for somewhat, 2 for very much, and 3 for activity restriction due to afraid of falling. The score of MSGS was calculated as add up three geriatric syndrome score (functional decline, urinary incontinence, and fear of falling). And, a participant with a MSGS score less than 1 was defined as improvement of MSGS.

2.3.2. Physical fitness test

Body mass index (BMI) was calculated from body weight (kg) divided by height (m) squared. Physical fitness tests were used for the assessment of muscle strength, walking speed, and balance ability. The following standardized tests were performed: grip strength (Suzuki et al., 2004); adductor muscle strength (Kim et al., 2007); usual and maximum walking speed (Suzuki et al., 2004); one leg standing time with eyes open (Suzuki et al., 2004); tandem walking (Speers et al., 1998); functional reach (Duncan et al., 1990). The staff members who performed the assessments did not know the subjects' group assignments.

2.4. Interventions

2.4.1. Exercise group

The exercise group participated in an intervention comprised of 60-min exercise sessions held at the TMIG Health Promotion Classes, twice per week for 3-month. Weight-bearing exercise: strength training of the thigh, abdominal, and back muscles was performed and included bending the knees, and other similar exercises.

PFM exercise: The exercise regimen was designed to strengthen the fast- and slow-twitch muscle fibers located at the pelvic floor. Participants were initially instructed to perform 10 fast contractions (3-s) with a 5-s relaxation period and 10 sustained contractions (6–8 s) with a 10-s relaxation period in between the contractions. The PFM exercise was performed in sitting, lying, and standing positions with legs apart, emphasizing training of the PFM and relaxation of the other muscles.

Chair exercises: Used in the early stage of the program. The exercises included seated toe and heel raises, seated lift foot and point/flex toes, and others.

Resistance band exercise: Focused on increasing the strength of the muscles of the upper extremities, abdomen, and lower extremities in frail elderly people (arm pull back, leg extension, and others).

Ball exercise: Exercises with a training ball were conducted using a small (diameter: 21 cm) and a large ball (diameter: 45–55 cm), aiming to increment the muscle strength and balance (sitting on the ball and extending legs, and others).

Walking ability training: Focused on maintenance of stability during walking and on the improvement of responses to postural changes during walking (walking with directional changes, gait pattern variations and enhancement, and others).

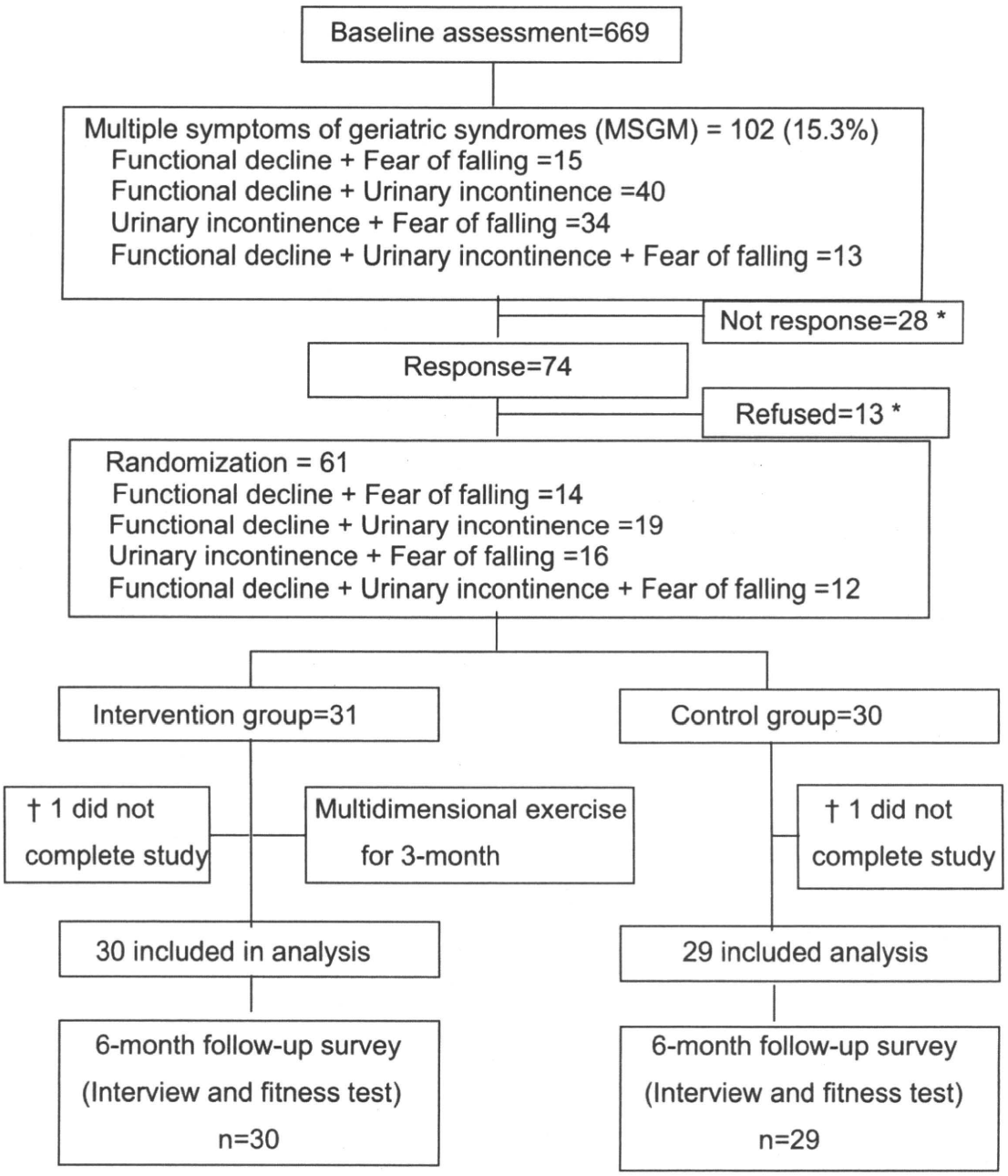

Fig. 1. Flow chart of participants through the randomized controlled trial of the exercise program and analysis. (*) Forty-one of MSGM (n = 102) were excluded due to the not response (n = 28) and refusal (n = 13). (†) Two subjects could not complete the study because of hospitalization (n = 1), and fracture (n = 1).

Balance training: Focused on the improvement of the static, dynamic, and lateral balancing ability (multidirectional weight shifts, tandem walking, and others).

2.4.2. Control group

The control group attended a general health education class (albumin, osteoporosis, and prevention of malnutrition) held at the TMIG once a month for a 3-month period.

2.5. Follow-up and compliance

During the 6-month follow-up period, subjects of the intervention group attended group exercise classes (60 min) once per month in addition to receiving a home-based exercise program. The home-based exercise program consisted of two to three sets of the 15 exercises and PFM exercise that they had learned during the group exercise session. They were also advised to do the home-based exercises at least three times or more per week for about 30-min per day. In order to accurately monitor the exercise times and the number of sets performed at home during the follow-up period, a pamphlet illustrating the PFM and strengthening exercises and a recording sheet were distributed to the participants, who were instructed to record the time and sets of exercises performed at home everyday. The record sheets were collected once a month at the group exercise class and analyzed in order to calculate the mean exercise frequency per week, and the mean exercise time per day.

2.6. Statistical analysis

Both the mean and standard deviation were calculated for each variable. The differences in the baseline data between the

Table 1
Selected variable characteristics of participants at baseline by study group, mean ± S.D.

Variables	Intervention group	Control group	$p^{\dagger}$
Number	31	30	
Age (year)	79.0 ± 3.9	78.1 ± 4.4	0.424
Height (cm)	146.9 ± 5.4	147.0 ± 5.8	0.940
Body weight (kg)	47.4 ± 6.4	50.7 ± 9.1	0.108
BMI (kg/m^2)	22.0 ± 2.6	23.4 ± 3.6	0.084
One leg standing time (s)	29.2 ± 23.5	34.6 ± 22.8	0.367
Tandem walking (step)	7.2 ± 4.7	7.8 ± 4.7	0.631
Functional reach (cm)	31.0 ± 7.1	33.2 ± 4.9	0.167
Grip strength (kg)	16.5 ± 4.3	17.9 ± 4.7	0.239
Adductor muscle strength (kg)	17.3 ± 4.0	18.0 ± 5.1	0.740
Usual walking speed (m/s)	1.1 ± 0.3	1.2 ± 0.2	0.685
Maximal walking speed (m/s)	1.7 ± 0.4	1.7 ± 0.4	0.979
TMIG index score (point)	10.6 ± 1.6	10.4 ± 1.5	0.654
Urinary incontinence, yes (%)	64.5	50.0	0.252
Functional decline, yes (%)	51.6	43.3	0.517
Fear of falling, yes (%)	67.7	76.7	0.390
Chronic medical conditions, yes (%)			
Hypertension	58.1	60.0	0.902
Stroke	13.2	13.3	0.988
Diabetes	19.4	20.0	0.948

† Two group t-test for continuous variables and the χ^2-test for categorical variables.

exercise and control group were analyzed using t-test for the continuous variables and Chi-square test for the categorical variables. The changes in dependent variables pre-intervention, post-intervention and follow-up in the exercise and control group were analyzed using an analysis of variance (ANOVA) with repeated measures. Significant interactions were analyzed to determine whether or not the effects were greater in the intervention than the control group. Cochran's Q-test was used to evaluate within-group differences of the effect of the exercise on the categorical variables for pre-intervention, post-intervention, and follow-up data. In the case of items which were showing significant differences, a post hoc analysis was performed using McNumar's test. One-way ANOVA was performed to evaluate the within-subgroup effect of the intervention on multiple geriatric syndrome scores at baseline, after the 3-month exercise, and at 6-month follow-up. For the subgroup showing significant differences, a post hoc analysis was performed using Scheffe's method. The percentage improvement in physical fitness was calculated using the following formula: % improvement = {(after 3-month exercise or at 6-month follow-up values − baseline value)/baseline value × 100}. The percentage improvement was divided into tertiles. The power of the current study was calculated at 80% to demonstrate a difference in the outcome variable of at least 20% at a significance level of alpha = 0.05. All the analyses were performed using the SPSS software package for Windows version 15.0 (SPSS, Inc., Tokyo, Japan).

3. Results

There were no significant differences between the groups in any of the baseline characteristics such as age, BMI, walking speed, adductor muscle strength, functional decline, urinary incontinence, fear of falling, and chronic medical conditions (Table 1).

Attendance 15 (62.5%) or more than of the exercise sessions (24) was defined as trial completion. Two participants (3.3%) could not complete the trial after the randomization because of hospitalization ($n = 1$) and fracture ($n = 1$) (Fig. 1). The mean attendance rate was 77.4% (61.3–90.3%) during the intervention period and 74.2% during the follow-up. In the exercise group, 32.3% of the subjects attended the exercise sessions 24 times, 22.6% attended 20–23 times, 35.5% attended 16–19 times, 6.5% attended 15 times, and 3.3% attended 14 or less of the exercise sessions. During the follow-up, the mean frequency of performing the

Table 2
Comparison of physical fitness and geriatric syndrome variables between intervention = I ($n = 30$) and control = C ($n = 29$) groups after 3-month exercise and at 6-month follow-up, mean ± S.D.

Variables	Gr	Baseline	3-Month exercise	6-Month follow-up	ANOVA F=	p=
Body weight (kg)	I	46.6 ± 5.4	47.4 ± 5.4	47.1 ± 5.4	(1,57) = 2.74	0.105
	C	51.0 ± 9.5	51.0 ± 9.4	50.6 ± 9.1		
BMI (kg/m^2)	I	21.5 ± 2.2	21.9 ± 2.2	21.8 ± 2.2	(1,57) = 2.82	0.100
	C	23.4 ± 3.9	23.4 ± 3.8	23.3 ± 3.6		
One leg standing time (s)	I	34.0 ± 24.2	28.2 ± 20.4	32.4 ± 22.6	(1,57) = 0.01	0.920
	C	33.4 ± 23.4	28.8 ± 23.5	32.4 ± 24.6		
Tandem walking (step)	I	7.2 ± 4.7	6.1 ± 4.5	5.9 ± 3.3	(1,57) = 4.70	0.036
	C	7.8 ± 4.7	5.2 ± 3.8	3.5 ± 2.0		
Functional reach (cm)	I	31.7 ± 6.8	33.5 ± 5.13	3.5 ± 4.4	(1,56) = 4.18	0.046
	C	33.7 ± 4.7	32.7 ± 5.3	31.6 ± 8.8		
Grip strength (kg)	I	17.2 ± 4.0	20.9 ± 5.2	17.9 ± 4.7	(1,57) = 0.02	0.874
	C	18.0 ± 4.6	21.5 ± 5.1	18.6 ± 4.8		
Adductor muscle strength (kg)	I	17.2 ± 4.0	18.9 ± 5.1	19.3 ± 4.7	(1,57) = 4.18	0.045
	C	17.9 ± 5.0	18.2 ± 4.01	17.8 ± 3.7		
Usual walking speed (m/s)	I	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	(1,57) = 13.03	0.001
	C	1.2 ± 0.2	1.1 ± 0.3	1.1 ± 0.3		
Maximal walking speed (m/s)	I	1.7 ± 0.4	1.8 ± 0.5	1.8 ± 0.4	(1,56) = 4.24	0.044
	C	1.7 ± 0.4	1.6 ± 0.4	1.6 ± 0.4		
Functional decline, yes (%)	I	50.0	16.7	16.7	16.67[a]	<0.001
	C	41.4	31.0	27.6	4.10[a]	0.109
Urinary incontinence, yes (%)	I	66.7	23.3	40.0	13.56[a]	0.001
	C	51.7	44.8	44.8	4.00[a]	0.135
Fear of falling, yes (%)	I	66.7	70.0	70.0	0.17[a]	0.920
	C	75.9	62.1	75.9	2.91[a]	0.234

[a] Cochran's Q-value.

Table 3
Improvement of MSGS according to maximum walking speed and adductor muscle strength tertiles in intervention group.

Survey variable	Changes compared to baseline[a]	Improvement of MSGS[†] *n* (%)	Cochran's *Q*-value	*p*	Post hoc[‡]
3-Month exercise ($n=8$)					
Maximum walking speed	Increased	3 (37.5)	2.80	0.247	
	No change	4 (50.0)			
	Decreased	1 (12.5)			
Adductor muscle strength	Increased	3 (37.5)	0.50	0.779	
	No change	3 (37.5)			
	Decreased	2 (25.0)			
6-Month follow-up ($n=7$)					
Maximum walking speed	Increased	5 (71.4)	6.50	0.039	In > De
	No change	1 (14.3)			
	Decreased	1 (14.3)			
Adductor muscle strength	Increased	3 (42.8)	0.57	0.713	
	No change	2 (28.6)			
	Decreased	2 (28.6)			

[a] Decreased (De) means lower range (0.0–33.3%), no change (no) means medium range (33.4–66.6%), and increased (In) means upper range (66.7–100%) of tertile.

exercise series at home was 3.8 times per week (23.3% performed everyday, 50.0% 2-3 times per week, 26.7% once or less per week), while the mean exercise time was 29.0 min.

The exercise group showed significant improvement compared with the control group in muscle strength, walking speed and balance. There was a significant group by time interaction for tandem walking ($F=4.70$, $p=0.036$), functional reach ($F=4.18$, $p=0.046$), adductor muscle strength ($F=4.18$, $p=0.045$), usual walking speed ($F=13.03$, $p=0.001$), and maximum walking speed ($F=4.24$, $p=0.044$) with significantly greater increases in the exercise group. The functional decline decreased significantly from 50.0% at baseline to 16.7% after the intervention and follow-up in the exercise group ($Q=16.67$, $p<0.001$), whereas the changes were not significant in the control group. Urinary incontinence was decreased significantly from 66.7% at baseline to 23.3% after the intervention and to 40.0% at the follow-up ($Q=13.56$, $p=0.001$) in the exercise group. However, no significant changes observed in the control group. There were no significant changes concerning fear of falling in either group (Table 2).

Fig. 2 shows the changes in the scores of multiple geriatric syndromes. As shown in Fig. 2, the intervention group showed greater and significant decrease compared with the control group ($F=12.66$, $p=0.001$). Within-group scores were compared, and significant changes were observed in intervention group, with the score of multiple geriatric syndromes decreasing significantly after 3-month exercise and at 6-month follow-up ($F=16.89$, $p<0.001$).

Eight subjects after 3-month intervention and seven subjects after 6-month follow-up were improved to normal status of multiple symptoms in the intervention group. Table 3 shows the distribution of the subjects who showed improvement to normal status of multiple symptoms according to the tertiles of maximum walking speed and adductor muscle strength. Within the subjects that showed improvement to normal status of multiple symptoms, a significantly higher proportion had an improved maximum walking speed at the 6-month follow-up ($Q=6.50$, $p=0.039$) compared with those having maintained or decreased walking speed. There was no difference at either time point in the proportion of the improved subjects with increased adductor muscle strength.

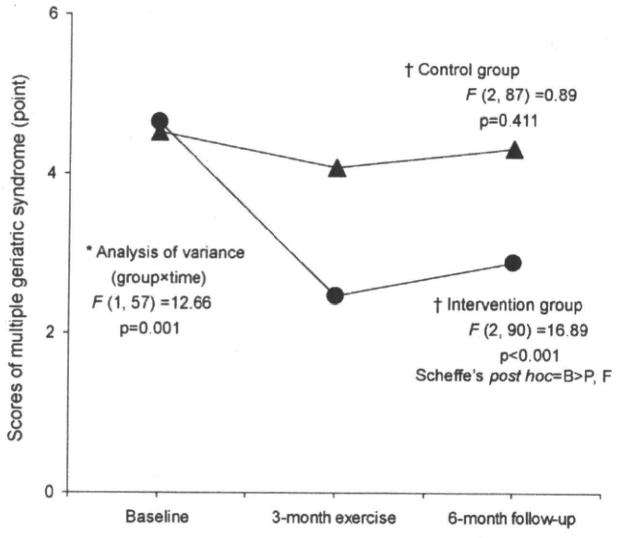

Fig. 2. Change in mean scores of MSGS at baseline, after 3-month exercise, and at 6-month follow-up in intervention (●) and control (▲) group. (*) Comparison of multiple geriatric syndrome scores between intervention and control group. (†) Comparison of within-group multiple geriatric syndrome scores at baseline (B), after the 3-month exercise (P), and at 6-month follow-up (F).

4. Discussion

This study demonstrates that the 3-month, multidimensional exercises, consisting of progressive strength training, balance and walking ability exercises along with PFM exercises, improved the usual walking speed, maximum walking speed, abductor muscle strength, tandem walking and functional reach in community-dwelling elderly women with MSGS. Furthermore, the increment of the physical fitness components appeared to contribute greatly to the improvement of the functional decline, urinary incontinence, and multiple symptoms. Therefore, the results of this study suggest that the improvements of the muscle strength, walking speed, and balance, which have been reported as risk factors for geriatric syndromes, may be effective in the improvement of geriatric syndrome.

Several studies of multidimensional intervention trials have reported beneficial effects (Tinetti et al., 1994; Shumway-Cook et al., 1997; Nelson et al., 2004; Gitlin et al., 2006; Kim et al., 2007). In a recent study, Gitlin et al. (2006) conducted a multidimensional home-based intervention in elder adults with functional difficulties, and confirmed that activity of daily living (ADL), instrumental ADL, self-efficacy, fear of falling, and home hazards were all improved and that the effects were sustained even after 6-month. Kim et al. (2007) assessed the effect of PFM and fitness exercises in improving urinary incontinence in elderly community-dwelling Japanese with stress urinary incontinence, and confirmed that

decrease in BMI and increase in walking speed may contribute to the treatment of urinary incontinence.

In this study, the prevalence of the functional decline decreased significantly from 50.0% before the intervention to 16.7% after intervention and follow-up. The cure rate of urinary incontinence was 43.3% after the 3-month exercise and 26.7% at 6-month follow-up for the intervention group. On the other hand, no significant improvement was observed in the control group. The effects of this multidimensional exercise affecting only a single symptom of urinary incontinence or functional decline were consistent with previously reported studies. Although the previous studies using multidimensional intervention were targeted to treat only a single geriatric syndrome, the current study was aiming to treat MSGS. Our findings suggest that the multidimensional intervention was significantly effective in the improvement of geriatric syndrome.

We analyzed the relationship between the increment of the physical fitness components and the improvement of the multiple symptoms, despite the small sample size. We found an increment rate of 9.6% in adductor muscle strength after the 3-month exercise and a rate of 12.3% after the follow-up in the intervention group, whereas the changes were not significant for the control group. This difference in the increment rate of muscle strength is not considered to account for the difference in geriatric syndrome improvement rate. However, the proportion of the subjects with improved to normal status of multiple symptoms was significantly higher among those who demonstrated an increase in maximum walking speed at 6-month follow-up ($Q = 6.50$, $p = 0.039$). These results suggest that the increment of walking speed is a major factor for the improvement of the multiple symptoms present in this population. The increased walking ability probably allowed the subjects to increase their physical activity and consequently contributed to the improvement of their functional capacity. But, the current study's results were obtained based on a small sample size. The above relationships need to be further researched in a population study which would contain a larger number of subjects and for a longer follow-up period.

Despite the fact that many studies have reported that exercise is effective in reducing the fear of falling in the elderly (Tennstedt et al., 1998), our intervention had no effect on the fear of falling in both groups. This may be explained by the characteristics of the intervention provided in the present study. Our multidimensional exercises focused on increasing the physical function and did not provide measures such as psychological care. These findings indicate that the comprehensive strategy designed to reduce MSGS in community-dwelling elderly women should include not only exercises addressing to the improvement of the physical functions, but should also incorporate psychological care focusing on reducing the fear of falling.

This study has several limitations. Firstly, the functional decline, urinary incontinence, and fear of falling were assessed using self-reported data obtained through a face-to-face interview, and they were not confirmed by objective and clinical methods. However, several previous studies have indicated that self-reported data have high validity, reliability and objectivity in the analyses of the functional decline, urinary incontinence, and fear of falling (Smith et al., 1990; Howland et al., 1993; Resnick et al., 1994). Therefore, the use of data collected from interviews or self-recording in analyses has minor influence on the interpretation of the results of this study. Secondly, although this study indicates that improvement of physical fitness components such as muscle strength and walking ability contributes to the treatment of geriatric syndrome, it provides no explanation of the mechanism of how increasing functional fitness component improves multiple geriatric symptoms.

5. Conclusions

This study assessed the effects of multidimensional exercises on functional decline, urinary incontinence, and fear of falling in community-dwelling Japanese elderly women with MSGS. The intervention program targeted modification of physical fitness may contribute to a reduction of the functional decline and urinary incontinence, but was not a diminishing symptom over time concerning the fear of falling. Therefore, the intervention strategies designed to reduce MSGS in elderly persons should include not only exercises aiming to the improvement of the physical functions, but should also incorporate psychological care focusing on the reduction of the fear of falling.

Conflict of interest statement

The authors have no conflict of interest to disclose.

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5. 転倒予防のための運動介入の効果と課題

金　憲経

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〈パネルディスカッション2：高齢者の転倒―その成因の解明と予防対策―〉

5. 転倒予防のための運動介入の効果と課題

金　憲経

Key words：転倒予防，運動介入，身体的要素，可変因子，転倒経験者

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はじめに

転倒予防戦略を効率的に構築するためには，転倒は転倒関連危険因子（fall-related risk factor）の数と深く関連し，転倒率は危険因子の数とほぼ直線的に増加することへの考察が必要である[1]．つまり，転倒率を下げるためには危険因子の数を減らすことがポイントである（図1）．転倒の抑制策として今日まで提案されている戦略は，服薬管理，教育，環境改善，ヒッププロテクター着用，ビタミンD補充，運動などが挙げられる．

転倒予防のための運動介入の意義

転倒を予防するためには，多くの内的要因のうちの可変要因および外的要因に当てはまる因子を一つ一つ改善していく方法しかない．転倒の危険因子を総合的にまとめた先行研究によれば，転倒の相対的な危険度は筋力低下（RR＝4.4），転倒歴（RR＝3.0），歩行機能低下（RR＝2.9），バランス低下（RR＝2.9）が高く，他に視力障害，関節炎，ADL障害，認知機能障害，年齢80歳以上と関連すると指摘している[2]．なかでも，筋力，歩行，バランスなど身体的要素に関連した要因は，トレーニングや普段からの訓練によって低下を予防し，機能の強化が可能である．すなわち，高齢者の転倒原因の大きな割合を占めている身体的要因は可変因子であることに運動介入の重要な意味がある（図2）．

転倒予防を目的とした運動介入の成果については実に数多く報告されているが，その結果は必ずしも一致せず異なる成果が散見される．転倒予防効果が検証された代表的な介入は，1990年に全米8つの地域で2,400人以上を対象に3年以上行ったFICSIT研究であり[3]，その結果によれば，太極拳を中心としたバランス訓練と筋力トレーニングが最も有効な手法であることが確認されている．さらに，Campbellら[4]は，80歳以上の地域高齢者に筋力，バランス能力改善を目的とした個別処方の在宅運動プログラムを提供した場合でも，転倒予防に有効であったと報告している．一方，Suzukiら[5]は，74～89歳の地域在住高齢者を対象に，2週1回の頻度での集団指導に加えて在宅実践用の個人プログラムを提供する指導を6カ月間行った後，22カ月間の追跡期間中の累積危険度は，対照群0.545，介入群0.136であり，相対危険度は0.25であったことを報告し，監視型に在宅用運動プログラムを加える介入も転倒予防に有効であることを指摘している．一方，Dayら[6]は，70歳以上の高齢者1,090名を対象に，運動，家庭内障害物整備，視力補正の3手法による転倒予防効果を検証した．その結果によれば，単独介入では運動がRR＝0.82（95%CI＝0.70～0.97）と最も効果的であるが，運動に家庭内障害物整備，視力補正を加えるとRR＝0.67（95%CI＝0.51～0.88）に改善することを検証し，多面的支援が転倒予防により効果的であることを提案している．

しかし，Mulrowら[7]は，ADL2つ以上の障害を有するのナーシングホーム入所者194名を対象に4カ月間の運動指導後，1年間の追跡調査を行った結果，移動能力には効果が検証されたが（15.5%改善），転倒率の抑制効果は見られなかった（運動群＝79転倒，対照群＝60転倒，P＝0.11）ことを，Rubensteinら[8]は，7日以内に転倒経験を有する施設長期入所者160名を対象に行った運動指導の結果を分析したところ，介入群の転倒は9%低いものの有意差はなかった．Lordら[9]も，運動介入後に介入群と対照群との間で転倒率には差が見られなかったが（RR＝0.99，95%CI＝0.65～1.50），参加率75%以上のグループでは，転倒率が低くなる傾向が観察された．さらに，Reinschら[10]は，高齢者を対象に行った介入に

The effects of exercise for the prevention of falls
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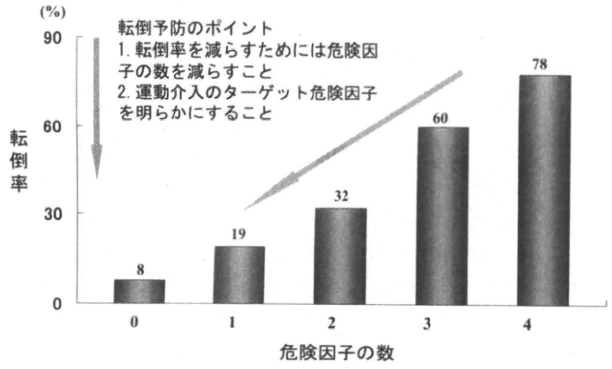

図1　転倒の危険因子の数と転倒率
文献1より改変

転倒危険因子の相対的危険度

危険因子	相対危険度
筋力低下	4.4
転倒歴	3.0
歩行機能低下	2.9
バランス低下	2.9
補助器具の使用	2.6
視力障害	2.5
関節炎	2.4
ADL障害	2.3
うつ病	2.2
認知機能障害	1.8
年齢８０歳以上	1.7

身体的要因は可変要因（運動介入のターゲット危険因子）

運動介入によって転倒率は低下する

図2　転倒予防のための運動介入戦略
文献2より改変

よって転倒率，初回転倒までの時間，複数回転倒，転倒負傷のみならずバランス能力や筋力，転倒恐怖感，健康度自己評価においても効果が見られなかったことを指摘した上で，介入効果がみられなかった理由としては，運動強度が弱いことや介入頻度が少なかったことであると指摘している．

運動介入のポイント

転倒予防のための運動介入の成果について今日まで報告されている先行研究をまとめると，運動介入効果がないとの研究，身体機能の改善には有効であるが転倒率の減少効果はないとの研究，転倒率の低下のみならず転倒恐怖感の改善効果も得られるとの研究など様々である．これらの結果は，運動介入の際には対象者の諸特性を詳細に把握し，対象者特有の危険因子の改善を目的とした介入になってない場合には，効果が期待できない可能性を示唆するものである．運動介入の時の考慮すべき点は，運動種目，運動強度，運動時間，指導頻度，指導期間，指導形式などである．これらに加えてもう一つ重要なポイントがある．高齢者の転倒原因について調べた結果によれば[11]，高齢者転倒の多くは「歩行中のつまずき」によって発生することである．つまり，高齢者の歩行機能と転倒とは密接に関わり，歩行機能の改善は転倒率抑制に有効であることを示唆するものである．よって，運動介入の際には「歩行機能の改善」および「つまずき防止」を目的とした指導を取り入れるべきであると考える．歩行機能を改善するためには，大腿四頭筋，ハムストリングス，腸腰筋，下腿三頭筋，大殿筋，中殿筋などの重点的な鍛えが必要であり，すり足の改善には前脛骨筋の鍛えが必要不可欠である．次に考慮すべき点は，大腿骨頸部骨折予防である．大腿骨頸部骨折の危険因子は，側面転倒（OR＝3.9），骨密度低下（OR＝1.8），移動障害（OR＝6.4）が指摘され[12]，大腿骨頸部骨折を予防するためには側面バランス機能向上が大切であり，運動指導に当たっては，側面バランス機能の向上を目的とした運動指導が必要であるといえる．

転倒経験者の転倒予防のための運動介入

転倒経験者は転倒経験がない人に比べて身体機能が劣っているとの報告が多く，さらには再転倒の危険因子（RR＝3.0）として指摘されているが，転倒経験者に対する転倒予防戦略の成果についての検討は極めて少ないのが現状である．Skelton ら[13]は，過去1年間で3回以上転倒した65以上の在宅高齢女性81名を運動群50名，対照群31名に分け運動群に週1回，1回当たり60分間の集団指導に家庭用運動プログラムを提供しながら36週間指導したところ，運動指導期間中に発生した転倒数は運動群が対照群に比べて31％も減ったことを指摘し，運動介入は転倒経験者にも有効であると指摘している．筆者らも，2007年度大都市在住70歳以上の男女1,483名を調査し，過去1年間で1回以上転倒者241名（16.3％）に運動介入参加希望者を募集したところ，参加希望者125（51.9％），不参加者116名（48.1％）であった．参加希望者に運動介入を3カ月間実施し，1年間の追跡期間中に発生した転倒率は介入群19.6％，対照群38.3％（Z＝1.979，P＝0.048）であった（図3）[14]．以上のように，再転倒の危険性が高い転倒経験者であっても運動介入へ参加することによって，転倒率の減少効果が得られ，Selton らの効果が追認されたと言える．

運動介入の課題

1．施設入所者に対する効果検証

施設入所者を対象とした研究結果によれば，バランス，

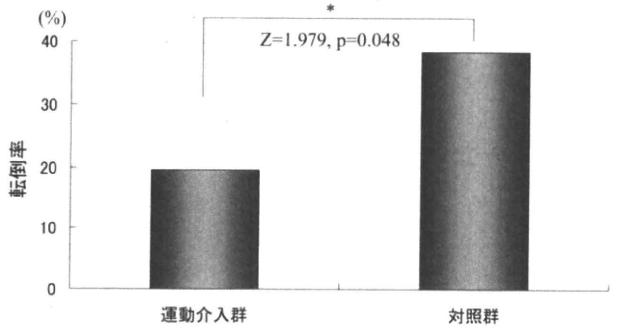

図3　転倒経験者における運動介入後1年間の転倒率
文献14より

筋力，歩行速度などの身体機能や転倒率，転倒恐怖感に改善がみられないとの報告が多く，部分的な改善効果がみられたとの報告はわずかにみられる程度である．長期施設入所者に対する運動介入の有効性については今後さらなる検討が必要といえよう．

2．介入不参加者に対する対応策の確立

前述した通り，転倒経験者でも運動介入への不参加者が48.1％と多いことが問題点である．確かに運動介入に参加し指導を受ければ転倒率は下がることが多くの研究で検証され，筆者も確かめている．しかし，運動介入不参加者の転倒率が上昇した場合には運動介入によって減少した転倒率は不参加者の上昇によって相殺されてしまい，地域全体から見たときの運動介入効果は見えにくくなることも推測される．従って，介入不参加者の特徴を詳細に把握し，不参加者への対応策の確立が最大の課題ともいえる．不参加者への対応策の一つとして「転倒予防手帳」を配布し，間接的介入効果を検討するのも1つの案であると考える．

おわりに

要介護状態になる主な原因として知られている転倒を予防するためには，転倒の可変的な因子を解消していく介入が有効である．中でも，身体的要素の減衰に基づく筋力低下，バランス機能低下，歩行機能低下は普段からの訓練によって低下を最小限に食い止め，機能強化が可能である．すなわち，高齢者の転倒原因の大きな割合を占めている身体的要因は可変因子であることに転倒予防における運動介入の位置づけである．運動介入には，集団指導型，個別処方の在宅介入型が考えられるが，いずれの介入においても，転倒予防効果を認めている．しかし，運動介入には不参加者の割合が高く，不参加者への対策の確立が課題と言える．さらには，施設入所虚弱高齢者の場合は，チームアプローチによる多面的介入によって効果が期待できると指摘されているが，運動介入の有効性については今後さらなる検討が必要である．

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Parathyroid Hormone Upregulates BMP-2 mRNA Expression Through Mevalonate Kinase and Rho Kinase Inhibition in Osteoblastic MC3T3-E1 Cells

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Key words
- osteoblast
- parathyroid hormone
- BMP-2
- Rho kinase

Abstract

It is well known that parathyroid hormone (PTH) possesses an anabolic effect on bone. However, the mechanisms are not fully elucidated. So far, it is unclear whether or not PTH could stimulate the expression of bone morphogenetic protein-2 (BMP-2), a strong mediator for bone formation. Growing evidence suggests that BMP-2 expression is regulated by the mevalonate pathway and Rho-associated protein kinase (ROK) activity. This study was performed to examine if PTH affects BMP-2 expression and to clarify its involvement of the mevalonate pathway. Osteoblastic MC3T3-E1 cells were treated with human PTH-(1–34) to determine BMP-2 mRNA expression levels by real-time PCR and to measure the ROK activity by the kinase assay. Incubation with 10^{-9}–10^{-8} M of hPTH-(1–34) for 6 h induced significant upregulation of BMP-2 mRNA levels in MC3T3-E1 cells. Short-term treatment of hPTH-(1–34) suppressed Rho kinase activity and mevalonate kinase mRNA levels. PTH-induced BMP-2 mRNA upregulation was selectively reversed by geranylgeranyl pyrophosphate (GGPP) pretreatment, but not by mevalonate pretreatment. These findings suggest that BMP-2 mRNA expression was upregulated by PTH in MC3T3-E1 cells mediated by mevalonate pathway suppression followed by ROK inhibition. We have now demonstrated for the first time that PTH stimulated BMP-2 mRNA expression via the mevalonate pathway and ROK in osteoblastic MC3T3-E1 cells.

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Introduction

Parathyroid hormone (PTH) has clinically been introduced in many countries to treat osteoporosis. PTH elevates bone formation markers within a month before an increase in bone resorption markers [1]. The early elevation of bone formation marker has a positive correlation followed with increase in the bone mineral density [2,3]. Although it is well-known that PTH possesses anabolic action on bone, the mechanisms have not been fully understood. The anabolic action has been reported to be mediated by PTH/PTH-related protein receptor (PTH1R) followed by stimulating differentiation and mineralization of osteoblasts, suppressing mature osteoblastic apoptosis, activating canonical Wnt-β-catenin signal, and stimulating IGF-I production [4–11]. However, little is known about PTH effect on bone morphogenetic proteins (BMPs), strong mediators for bone formation.

BMPs, which belong to TGF-β superfamily, bind to BMP type II receptor to activate Smad signaling. BMP-2, BMP-4, and BMP-7, which accelerate bone formation and fracture repair, play critical roles in osteoblastic differentiation as well as bone formation and could be good candidates for mediating the osteogenic signalings of PTH [12–16]. We have reported that dexamethasone (Dex) suppressed osteoblastic differentiation by inhibiting the Wnt and BMP pathways, and that PTH restored the effect of Dex, suggesting that PTH might augment BMP action [17].

On the other hand, accumulating evidence shows that the mevalonate pathway is involved in the augmentation of BMP-2 action. Mundy et al. showed that statins such as lovastatin and simvastatin, and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, increased bone formation when injected subcutaneously over the calvaria of mice and increased cancellous bone volume when orally administered to rats, via increased expression of BMP-2 [18]. Another study had shown that statins were able to activate Akt and to stabilize eNOS mRNA, which resulted in stimulating BMP-2 transcription and

osteoblastic differentiation [19–21]. Effects of simvastatin were selectively reversed by either mevalonate or its metabolite geranylgeranyl pyrophosphate (GGPP), but not by cholesterol or farnesyl pyrophosphate [22]. These results suggest that the effects were mainly derived from depletion of intracellular pools of GGPP, the substrate required for the geranylgeranylation. In addition, simvastatin suppressed the ROK activity, and this effect was reversed by addition of GGPP [23]. We also showed that the AMPK activator as well as the ROK inhibitor was able to stimulate the mineralization of osteoblasts through modulating the mevalonate pathway and enhancing endothelial NOS and BMP-2 expression [24]. These findings suggest that PTH might affect BMP-2 expression in osteoblasts through modulating the mevalonate pathway, although there are no studies investigating this possibility until now.

In this study, to clarify this issue, we used osteoblastic MC3T3-E1 cells and examined if PTH affects BMP-2 expression, or if the mevalonate pathway is involved in its process.

Materials and Methods

Materials

Cell culture medium and supplements were purchased from GIBCO-BRL (Rockville, MD, USA). Human PTH-(1–34) was kindly gifted from Asahi-kasei corporation (Tokyo, Japan). Mevalonate and geranylgeranyl pyrophosphate (GGPP) were purchased from Sigma (St. Louis, MO, USA). For Rho kinase activity analysis, a Rho kinase assay ELISA kit was purchased from Cyclex (Nagano, Japan). All other chemicals were of the highest grade available commercially. PCR primers for BMP-2 and mevalonate kinase were obtained from Sigma Aldrich (St. Louis, MO, USA). Vehicle for PTH peptide was 10 mM acetic acid (100 nM final concentration).

Cell Culture

MC3T3-E1 cells, a clonal osteoblastic cell line isolated from calvariae of late stage mouse embryo, were kindly provided by Dr. H. Kodama (Ohu Dental College, Japan). This cell line has been widely used as a cell culture model for osteoblastic differentiation. MC3T3-E1 cells were cultured in α-MEM (containing 50 µg/ml of ascorbic acid) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (GIBCO-BRL) in 5% CO_2 at 37°C. The medium was changed twice a week for general culture.

Real-time PCR

SYBR green chemistry was used to perform quantitative determinations of the mRNAs for BMP-2, mevalonate kinase (MVK), and a house keeping gene, 36B4, according to an optimized protocol [25,26]. Total RNA was collected from cultured MC3T3-E1 cells using Trizol reagent (Invitrogen, San Diego, CA) according to the manufacturer's recommended protocol. Two µg total RNA was employed for the synthesis of single-stranded cDNA (cDNA synthesis kit; Invitrogen). The double-stranded DNA-specific dye SYBR Green I was incorporated into the PCR buffer provided in the QuantiTech SYBR PCR kit (QIAGEN, Valencia, CA) to allow for quantitative detection of the PCR product. The sense and antisense primers were designed using the Primer Express Version 2.0.0 (Applied Biosystems Inc.) based on published cDNA sequences. Real-time PCR was performed using ABI PRISM 7000 (PE Applied Biosystems Inc.). The PCR primers (listed as forward primer and reverse primer) were as follows:

BMP-2, 5′-CGTCAAGCCAAACACAAACAGCG-3′ and 5′-CACCCACA-ACCCTCCACAACCAT-3′; MVK, 5′-GGGACGATGTCTTCCTTGAA-3′ and 5′-GAACTTGGTCAGCCTGCTTC-3′; 36B4, 5′-AAGCGCGTCCT-GGCATTGTCT-3′ and 5′-CCGCAGGGGCAGCAGTGGT-3′.

Rho kinase activity assay

Cells were rinsed with ice-cold PBS and scraped on ice into a lysis buffer (Cell Signaling Technology) that contained 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EGTA, 1 mM Na_2EDTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 µg/ml leupeptin. The cell lysates were then sonicated for 30 s. After the cell lysates were centrifuged at 15 000 × *g* for 10 min, supernatants were collected for assessing Rho kinase activities using the Rho kinase assay kit (Cyclex) as indicated by the manufacturer. Briefly, 10 µl of the supernatants of lysed cells were added into 96-well plates precoated with a substrate corresponding to the C terminus of the recombinant myosin-binding subunit of myosin phosphate (MSB), which contains a threonine residue that may be phosphorylated by Rho kinase. Subsequently, 90 µl of a kinase reaction buffer (containing 0.1 mM ATP) was added, incubated for 30 min at room temperature, washed five times with a washing buffer provided by the kit, and incubated with 100 µl of a horseradish peroxidase-conjugated monoclonal antiphospho-specific MSB antibody, which specifically detects the phosphorylated form of threonine 697 on MSB (provided by the kit). The colored products were developed by incubating with 100 µl of a horseradish peroxidase substrate tetramethylbenzidine at room temperature for 10 min. The reaction was stopped by adding 100 µl of stop solution containing 0.5 M H_2SO_4, and the absorbance was read at 450 nm. Each value was normalized to the protein concentration.

Statistics

Each experiment was repeated at least three times. Data were shown as means ± SEM. Statistical evaluations for differences between groups were carried out using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD). We used paired *t*-test to analyze statistical evaluations, if it was applicable. For all statistical tests, a value of $p < 0.05$ was considered to be a statistically significant difference.

Results

Time course of BMP-2 mRNA expression treated by hPTH-(1–34)

Time course of BMP-2 mRNA expression was examined after treatment of osteoblastic MC3T3-E1 cells with 10^{-8} M hPTH-(1–34). The expression of BMP-2 mRNA was increased in a time dependent manner up to 6 h and was decreased thereafter (**Fig. 1A**).

Dose response of PTH-induced upregulation of BMP-2 mRNA expression

Dose response of PTH-induced upregulation of BMP-2 mRNA expression was examined under 6-hour treatment with hPTH-(1–34). BMP-2 mRNA expression was significantly upregulated by 10^{-8} M and 10^{-9} M of hPTH-(1–34), compared with the control treated with vehicle alone (100 nM of acetic acid) (**Fig. 1B**).

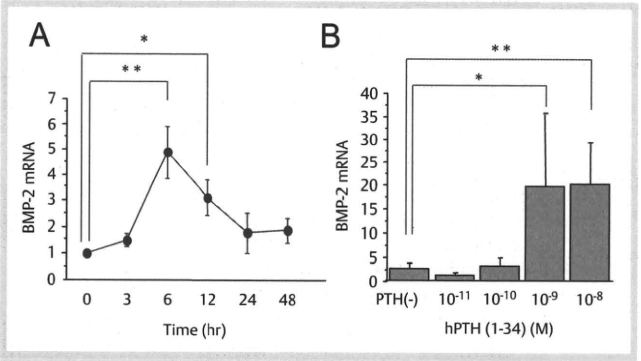

Fig. 1 Effects of PTH on BMP-2 mRNA expression in osteoblastic MC3T3-E1 cells. Time course of BMP-2 mRNA expression was shown after treatment with 10^{-8} M hPTH-(1–34) (**A**), and dose response of PTH-induced upregulation of BMP-2 mRNA expression was demonstrated (**B**). Results were expressed as the mean ± SEM fold increase over control values from more than 3 independent experiments. **p<0.01, *p<0.05.

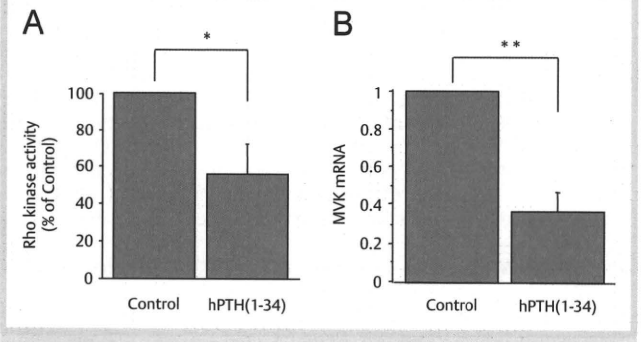

Fig. 2 Effects of PTH on ROK and MVK activities in osteoblastic MC3T3-E1 cells. **A.** ROK activities were measured in whole cell lysate extracted from MC3T3-E1 cells 10 min after treatment of the cells with or without 10^{-8} M hPTH-(1–34). **B.** Total RNA was extracted from MC3T3-E1 cells after 3-h treatment of the cells with or without 10^{-8} M hPTH-(1–34). MVK mRNA expression was quantified using real-time PCR and corrected with 36B4, house-keeping gene expression. Results were expressed as the mean ± SEM-fold increase over control values from more than 3 independent experiments. **p<0.01, *p <0.05.

Rho kinase activity was suppressed by PTH

Next, ROK activity in whole cell lysate was measured to address whether or not PTH would affect the ROK activity. We observed that 10 min treatment of MC3T3-E1 cells with 10^{-8} M hPTH-(1–34) significantly decreased the ROK activity compared with the vehicle-treated control ($p<0.05$) (◉ **Fig. 2A**).

Mevalonate kinase mRNA was decreased by PTH

We determined mevalonate kinase (MVK) mRNA expression by real-time PCR, which is located upstream of ROK. MVK mRNA level was significantly decreased by 3-hour treatment of the cells with 10^{-8} M hPTH-(1–34) compared with the control (◉ **Fig. 2B**). Significant inhibition of MVK mRNA expression was also observed at 6 and 12 h after PTH treatment (data not shown). These findings indicate that PTH might rapidly suppress the MVK activity.

Pretreatment with GGPP inhibited PTH-induced BMP-2 upregulation

We investigated whether or not additions of GGPP, downstream of MVK, or mevalonate, upstream of MVK, were able to reverse

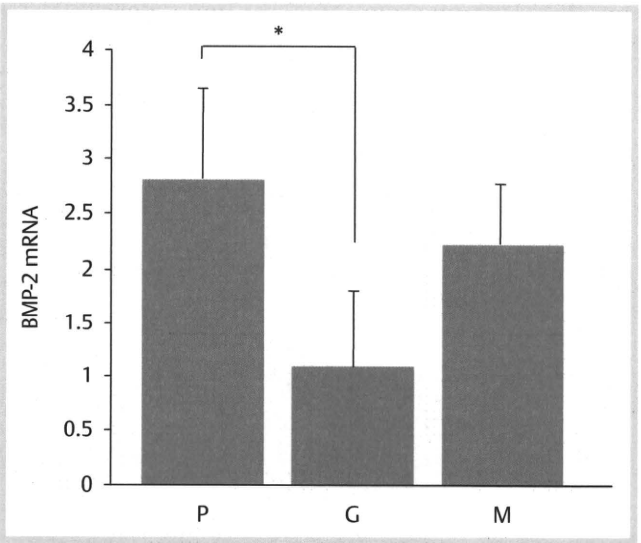

Fig. 3 Pretreatment with GGPP inhibited PTH-induced BMP-2 upregulation in osteoblastic MC3T3-E1 cells. GGPP and mevalonate were preincubated overnight before PTH treatment. Total RNA was extracted from MC3T3-E1 cells after 6-hour treatment of the cells with 10^{-8} M hPTH-(1–34). BMP-2 mRNA expression was quantified using real-time PCR and corrected with 36B4. Results were expressed as the mean ± SEM-fold increase over control values from more than 3 independent experiments. P: 10^{-8} M hPTH-(1–34); G: 5 μM GGPP + 10^{-8} M hPTH-(1–34); M: 1 mM mevalonate + 10^{-8} M hPTH-(1–34). *p<0.05.

the PTH-induced BMP-2 upregulation in MC3T3-E1 cells. PTH-induced upregulation of BMP-2 mRNA in the cells was inhibited by overnight pretreatment with 5 μM GGPP, but not with 1 mM mevalonate (◉ **Fig. 3**), indicating that PTH directly interacts with MVK, and not with its upstream molecules.

Taken together, these results suggest that PTH possibly inhibited MVK activity, which in turn suppressed ROK activity and induced BMP-2 expression (◉ **Fig. 4**).

Discussion

In this study, we demonstrated for the first time that PTH stimulated BMP-2 mRNA expression via the mevalonate pathway and the ROK activity in osteoblastic MC3T3-E1 cells. This might be one of the mechanisms by which PTH can accelerate bone formation, although it seems necessary to confirm the present observation by using other experimental system and by protein expression of BMP-2.

Mechanisms of the PTH anabolic action on the bone have mainly been explained as follows:

i PTH increases osteoblastic differentiation by suppressing proliferation [4,5].

ii PTH has an effect of anti-apoptotic action in osteoblastic cells [6,7].

iii The replication, differentiation and survival of osteoblast progenitors are controlled by locally produced autocrine/paracrine factors that are supplied by PTH treatment. Members of Wnt and insulin-like growth factor-I (IGF-I) are included in the local factors [8–11]. Hedgehog and bone morphogenetic protein (BMP) families, as well as transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF-2), and interleukin-6 (IL-6) type cytokines can be the candidates. Moreover, many of these growth factors are deposited into

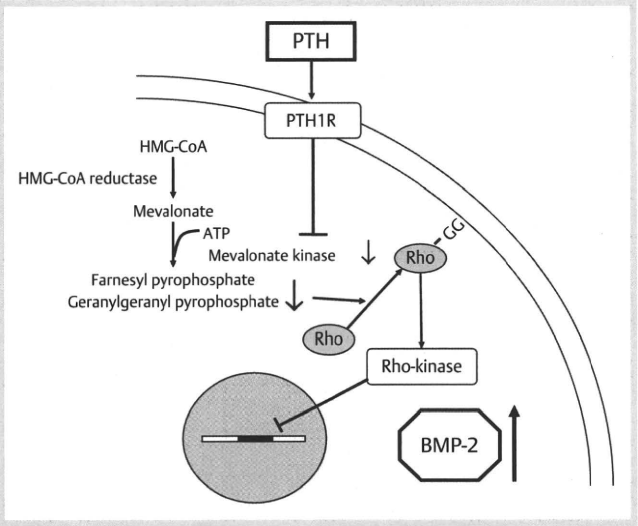

Fig. 4 Scheme of PTH-induced BMP-2 mRNA expression in osteoblasts. PTH binds to a G protein-coupled, 7-transmembrane receptor, PTH1R, located on the cell surface of osteoblasts. PTH1R activation would lead to suppression of MVK activity through undetermined signaling pathways, which results in a decrease in farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP). GGPP deprivation causes inhibition of isoprenylation of Rho protein, which in turn reduces ROK activity [22,23]. Reduction in ROK activity has been reported to stimulate BMP-2 mRNA transcription probably via activation of Akt and stabilization of eNOS mRNA [19–21,24]. In the present study, we have demonstrated for the first time that PTH upregulated BMP-2 mRNA presumably via the mevalonate and Rho-associated kinase pathway.

the bone matrix by osteoblasts and are thought to be released in active form during osteoclastic bone resorption.

iv PTH stimulates activating protein-1 (AP-1), which is a transcription regulatory factor constructed by heterodimer of Fos related proteins (c-fos, fos B, fra-1, fra-2) and Jun related proteins (c-Jun, Jun B, Jun-D) [27].

v PTH inhibits the production of sclerostin, which is made exclusively by osteocytes and prevents binding of Wnt ligands to their receptors and plays an important role in the regulation of bone formation [28].

Our present findings seem to be related to the category (iii), an enhancement of local factor activities. In addition, we have newly clarified the participation of the mevalonate pathway in PTH-induced BMP-2 mRNA upregulation.

In the present study, when MVK mRNA level was quantified by real-time PCR, a significant decrease was observed. This finding was consistent with microarray data reported by Qin et al., where 3-hour treatment of osteoblastic cells with PTH downregulated MVK mRNA expression by approximately 2.5-fold [29]. In this study, addition of GGPP selectively inhibited the BMP-2 upregulation, while that of mevalonate did not. Thus, PTH may suppress the activity of the mevalonate pathway at the level of MVK, but not at the levels of its upstream molecules. Further studies on the interaction between PTH and MVK would more clearly disclose one of the mechanisms by which PTH exerts anabolic action in osteoblasts, and would lead to the discovery of candidate drugs that promote bone formation for the treatment of osteoporosis.

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Serum Osteocalcin/Bone-Specific Alkaline Phosphatase Ratio Is a Predictor for the Presence of Vertebral Fractures in Men with Type 2 Diabetes

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Abstract We examined whether or not BMD or bone markers were useful for assessing the risk of vertebral fractures in 248 Japanese men with type 2 diabetes. We analyzed the relationships between bone markers (osteocalcin [OC], bone-specific alkaline phosphatase [BAP], urinary N-terminal cross-linked telopeptide of type-I collagen) or BMD and HbA_{1c}, urinary C-peptide, insulin-like growth factor-I (IGF-I), parathyroid hormone, $1,25(OH)_2$ vitamin D, and the presence of prevalent vertebral fractures. Multiple regression analysis adjusted for age, body height, weight, duration of diabetes, and serum creatinine showed that serum OC and OC/BAP ratio were correlated negatively with HbA_{1c} ($P < 0.01$) and positively with IGF-I ($P < 0.01$). Multivariate logistic regression analysis adjusted for the above parameters showed that serum OC/BAP ratio was inversely associated with the presence of vertebral fractures (odds ratio = 0.695, $P < 0.05$). This association was still significant after additional adjustment for lumbar or femoral neck BMD. Our results suggest that poor diabetic control and lower IGF-I level are linked to impaired bone formation and resultant reduction in OC/BAP ratio in men with type 2 diabetes. The OC/BAP ratio could be clinically useful for assessing the risk of vertebral fractures independent of BMD in diabetic men.

Keywords Osteocalcin · Bone-specific alkaline phosphatase · Type 2 diabetes mellitus · Vertebral fracture · Bone fragility

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The number of patients with diabetes mellitus and osteoporosis is rapidly increasing in industrialized countries, where Western-style aging societies are prevalent. A relationship between diabetes and osteoporotic fractures is becoming increasingly recognized [1]. Vertebral and hip fractures are the most important osteoporotic fractures because they frequently occur and increase the mortality of elderly people as high as six- to ninefold [2, 3]. Although patients with type 2 diabetes show no bone mineral density (BMD) reduction, fracture risks are known to increase approximately up to 1.5-fold at the hip, proximal humerus, forearm, and foot [4–6]. Moreover, our recent study revealed that Japanese patients with type 2 diabetes have an increased risk of vertebral fractures independent of BMD [7].

Bone fragility in patients with type 2 diabetes may be caused by low bone turnover [8]. Hyperglycemia in type 2 diabetes might be associated with factors that influence bone strength and quality independently of BMD [9–11]. Several studies have indicated that hyperglycemia induces a low turnover bone with osteoblast dysfunction [12, 13]. Hyperglycemia and advanced glycation end products (AGEs) promote the apoptosis of osteoblastic cells [14, 15] and restrain the differentiation of cells [16–19]. These findings suggest that hyperglycemia may cause diminished

bone formation. A previous clinical study has indicated that serum osteocalcin (OC) was low before treatments and elevated after treatments of diabetes, while bone-specific alkaline phosphatase (BAP) was reduced [20]. Previous in vitro studies have shown that chronic hyperglycemia increased the activity and expression of BAP and decreased OC expression and cellular calcium uptake [10]. It is well-known that BAP is expressed in the early period of osteoblastic differentiation, whereas OC is expressed in the later period [21]. Thus, hyperglycemia could cause impaired osteoblastic maturation, resulting in bone fragility in patients with type 2 diabetes.

It is thought that bone metabolism in type 2 diabetes is affected by abnormal hormonal actions. Patients with type 2 diabetes appear to have increased BMD, possibly due in part to an anabolic effect of hyperinsulinemia [22, 23] and in part to obesity [24]. In addition, patients with type 2 diabetes have reduced bone turnover and may have reduced levels of parathyroid hormone (PTH) [25]. These factors may protect patients from reduction of BMD and fracture risks. On the other hand, insulin-like growth factor-I (IGF-I), which is anabolic for bone, may also be reduced in patients with type 2 diabetes [26, 27]. However, it is still unclear how these factors are associated with BMD, bone markers, or bone fragility in patients with type 2 diabetes.

In this study, to examine these issues, we investigated the relationships between bone markers (OC, BAP, and urinary N-terminal cross-linked telopeptide of type-I collagen [uNTX]) or BMD and HbA_{1c}, urinary C-peptide (uC-peptide), IGF-I, PTH, $1,25(OH)_2$ vitamin D, and the presence of vertebral fractures in Japanese men with type 2 diabetes.

Subjects and Methods

Subjects

The subjects in this study were 248 Japanese men with type 2 diabetes aged 20–83 years (mean 59.0). We consecutively recruited subjects who visited Shimane University Hospital for education, evaluation, or treatment of diabetes. Subjects agreed to participate in this study and gave informed consent. This study was approved by the institutional review board of our institution. None had hepatic or renal dysfunction or nutritional derangements that might cause changes in bone metabolism. We excluded patients with histories of falls and traffic accidents in order to eliminate the possibility of injury-associated fractures. Forty-two patients had received insulin treatment, 95 patients had taken oral hypoglycemic agents (sulfonylurea, 82; metformin, 28; alpha-glucosidase inhibitor, 28), and 121 patients had not previously been under any medications for diabetes. All subjects were free of drugs known to influence bone and calcium metabolism like vitamin D and bisphosphonate as well as thiazolidinedione until the time of the present study.

Radiography

Lateral X-ray films of the thoracic and lumbar spine were taken in the same week as the serum collection. The anterior, central, and posterior heights of each of the 13 vertebral bodies from Th4 to L4 were measured. A vertebral fracture was diagnosed if at least one of three height measurements along the length of the same vertebrae had decreased by >20% compared to the height of the nearest uncompressed vertebral body [28]. None of the subjects had a history of serious trauma.

BMD and Biochemical Measurements

BMD values of the lumbar spine (L), femoral neck (F), and one-third of the radius (1/3R) were measured by dual-energy X-ray absorptiometry (QDR-4500; Hologic, Waltham, MA). The same operator tested all of the subjects during the study to eliminate operator discrepancies. The coefficients of variation (precision) of measurements of the lumbar spine, femoral neck, and mid-radius by our methods were 0.9, 1.7, and 1.9%, respectively. Values were also expressed relative to the standard deviation (SD) of age- and sex-matched normal Japanese mean values provided by the manufacturer (Z score).

After overnight fasting, serum and first void urine samples were collected. Biochemical markers were measured by standard biochemical methods, as previously described [29, 30]. Hemoglobin A_{1c} (HbA_{1c}) was determined by high-performance liquid chromatography (HPLC). BAP in serum and uC-peptide pooled for 24 h were measured by enzyme immunoassay and chemiluminescent enzyme immunoassay, respectively. Intact PTH was measured by electrochemiluminescent immunoassay. $1,25(OH)_2$ vitamin D, OC, and IGF-I were measured by radioimmunoassay. uNTX was measured by enzyme linked immunosorbent assay.

Statistical Analysis

Data were expressed as mean ± SD. Because uC-peptide and intact PTH showed a markedly skewed distribution, logarithmic (log) transformation of these values was carried out before performing correlation and regression analyses. Statistical significance between the groups was determined using Student's *t*-test. Simple, multiple, and logistic regression analyses were performed using the statistical computer program StatView (Abacus Concepts, Berkeley, CA). $P < 0.05$ was considered significant.

Results

Relationships between BMD or Bone Markers Versus HbA_{1c}, uC-Peptide, IGF-I, Intact PTH, and $1,25(OH)_2$ Vitamin D

Baseline characteristics of subjects are shown in Table 1. Since our simple regression analysis showed that HbA_{1c}, uC-peptide, IGF-I, intact PTH, and $1,25(OH)_2$ vitamin D were affected by age, body stature, and renal function (data not shown), multiple regression analyses were performed with each of these parameters adjusted for age, body height, weight, duration of diabetes, and serum creatinine as an independent variable versus BMD at each skeletal site or bone markers as a dependent variable (Table 2). OC and OC/BAP ratio were correlated significantly and negatively with HbA_{1c} ($P = 0.0057$ and $P < 0.0001$, respectively) and positively with IGF-I ($P = 0.0095$). BAP was correlated significantly and negatively with IGF-I ($P = 0.0304$) and positively with log(intact PTH) ($P = 0.0247$). Although L- and F-BMD were not significantly correlated with HbA_{1c} or any hormonal parameters, 1/3R-BMD was correlated positively with HbA_{1c} ($P = 0.0416$) and negatively with log(intact PTH) ($P = 0.0324$).

Table 1 Baseline characteristics of subjects

Characteristic	Normal range	
Number of subjects		248
Age (years)		59.0 ± 13.7
Duration of diabetes (years)		10.7 ± 9.1
Body height (cm)		165.4 ± 7.0
Body weight (kg)		64.9 ± 16.0
BMI (kg/m^2)		23.6 ± 4.7
Serum creatinine (mg/dl)	0.44–1.23	0.77 ± 0.15
Fasting plasma glucose (mg/dl)	60–110	171 ± 60
HbA_{1c} (%)	4.3–5.8	9.1 ± 2.5
uC-peptide (μg/day)	60–120	70.9 ± 49.6
IGF-I (ng/ml)	59–215	151 ± 60
Intact PTH (pg/ml)	10–65	38.4 ± 16.2
$1,25(OH)_2$ vitamin D (pg/ml)	20–60	49.2 ± 19.4
BAP (U/L)	9.6–35.4	26.3 ± 9.4
OC (ng/ml)	2.5–13.0	5.1 ± 2.4
uNTX (nMBCE/mM-Cr)	13.0–66.2	34.8 ± 24.3
L2–L4 BMD (g/cm^2)		1.042 ± 0.181
T score		−0.04 ± 1.152
Z score		0.47 ± 1.12
F-BMD (g/cm^2)		0.776 ± 0.132
T score		−0.69 ± 1.06
Z score		0.25 ± 1.05
1/3R-BMD (g/cm^2)		0.711 ± 0.070
T score		−1.62 ± 1.32
Z score		−0.66 ± 1.14
Vertebral fracture (yes/no)		76/172 (30.6%)

BMI body mass index, *PTH* parathyroid hormone, *NTX* N-terminal cross-linked telopeptide of type-I collagen, *L* lumbar, *F* femoral neck, *1/3R* one-third of the radius

Comparison of Demographic and Biochemical Parameters, Bone Markers, and BMD Between Patients with and Without Vertebral Fractures

Next, we compared various parameters including HbA_{1c}, uC-peptide, IGF-I, intact PTH, $1,25(OH)_2$ vitamin D, bone markers, and BMD values at each site between patients with and without vertebral fractures (Table 3). Patients with vertebral fractures were significantly older ($P = 0.0071$), were shorter ($P = 0.0203$), and had lower absolute L-BMD ($P = 0.0441$) than patients without vertebral fractures. IGF-I and OC/BAP ratio in patients with vertebral fractures tended to be lower than in patients without them ($P = 0.0620$ and $P = 0.0940$, respectively). On the other hand, no significant differences in the levels of HbA_{1c}, uC-peptide, IGF-I, intact PTH, $1,25(OH)_2$ vitamin D, or bone markers were observed between subjects with and without fractures.

When multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and levels of HbA_{1c}, uC-peptide, IGF-I, intact PTH, $1,25(OH)_2$ vitamin D, bone markers, and BMD adjusted for age, body weight, height, duration of diabetes,

Table 2 Correlations between bone markers or BMD versus HbA_{1c}, uC-peptide, IGF-I, intact PTH, and $1,25(OH)_2$ vitamin D

	HbA_{1c}		Log (uC-peptide)		IGF-I		Log (intact PTH)		$1,25(OH)_2D$	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
BAP	0.063	0.3365	0.011	0.8776	−0.154	0.0304	0.154	0.0247	−0.092	0.2267
OC	−0.184	0.0057	–	0.9984	0.180	0.0095	0.033	0.6379	−0.098	0.2241
OC/BAP ratio	−0.250	<0.0001	−0.135	0.0618	0.255	0.0002	−0.042	0.5385	−0.049	0.5289
uNTX	0.040	0.5437	0.088	0.2165	−0.042	0.5487	–	0.9954	−0.095	0.2133
L2–L4 BMD	−0.074	0.2650	−0.005	0.9409	−0.002	0.9757	−0.134	0.0513	0.005	0.9499
F-BMD	−0.075	0.1973	0.071	0.2498	−0.002	0.9798	−0.101	0.0877	−0.031	0.6316
1/3R-BMD	0.127	0.0416	0.032	0.6437	0.062	0.2392	−0.142	0.0324	0.072	0.3392

Multiple regression analysis adjusted for age, body height, weight, duration of diabetes, and serum creatinine

PTH parathyroid hormone, *NTX* N-terminal cross-linked telopeptide of type-I collagen, *L* lumber, *F* femoral neck, *1/3R* one-third of the radius