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.

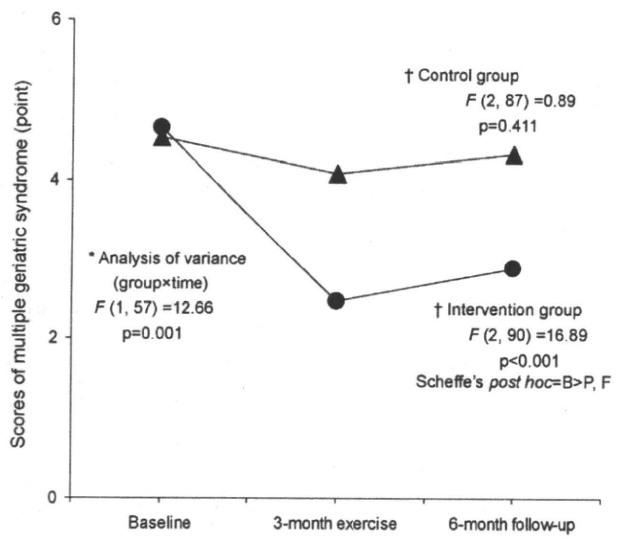

Fig. 2. Change in mean scores of MSGS at baseline, after 3-month exercise, and at 6-month follow-up in intervention (●) and control (▲) group. (*) Comparison of multiple geriatric syndrome scores between intervention and control group. (†) Comparison of within-group multiple geriatric syndrome scores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

Burgio, K.L., Matthews, K.A., Engel, B.T., 1991. Prevalence, incidence and correlates of urinary incontinence in healthy, middle-aged women. J. Urol. 146, 1255–1259.

Duncan, P.W., Weiner, D.K., Chandler, J., Studenski, S., 1990. Functional reach: a new clinical measure of balance. J. Gerontol. Med. Sci. 45, M192–M197.

Gitlin, L.N., Winter, L., Dennis, M.P., Corcoran, M., Schinfeld, S., Hauck, W.W., 2006. A randomized trial of a multicomponent home intervention to reduce functional difficulties in older adults. J. Am. Geriatr. Soc. 54, 809–816.

Howland, J., Peterson, E.W., Levin, W.C., Fried, L., Bak, S., 1993. Fear of falling among the community-dwelling elderly. J. Aging Health 5, 229–243.

Inouye, S.K., Studenski, S., Tinetti, M.E., Kuchel, G.A., 2007. Geriatric syndromes: clinical, research, and policy implications of a core geriatric concept. J. Am. Geriatr. Soc. 55, 780–791.

Ishizaki, T., Watanabe, S., Suzuki, T., Shibata, H., Haga, H., 2000. Predictors for functional decline among nondisabled older Japanese living in a community during a 3-year follow-up. J. Am. Geriatr. Soc. 48, 1424–1429.

Kim, H., Suzuki, T., Yoshida, Y., Yoshida, H., 2007. Effectiveness of multidimensional exercises for the treatment of stress urinary incontinence in elderly community-dwelling Japanese women: a randomized, controlled, crossover trial. J. Am. Geriatr. Soc. 55, 1932–1939.

Koyano, W., Shibata, H., Nakazato, K., Haga, H., Suyama, Y., 1991. Measurement of competence: reliability and validity of the TMIG index of competence. Arch. Gerontol. Geriatr. 13, 103–116.

Maggi, S., Minicuci, N., Langlois, J., Pavan, M., Enzi, G., Crepaldi, G., 2001. Prevalence rate of urinary incontinence in community-dwelling elderly individuals: the Veneto study. J. Gerontol. Med. Sci. 56A, M14–M18.

Maki, B.E., Holliday, P.J., Topper, A.K., 1991. Fear of falling and postural performance in the elderly. J. Gerontol. Med. Sci. 46, M123–M131.

Nelson, M.E., Layne, J.E., Bernstein, M.J., Nuernberger, A., Castaneda, C., Kaliton, D., Hausdorff, J., Judge, J.O., Buchner, D.M., Roubenoff, R., Singn, M.A.F., 2004. The effects of multidimensional home-based exercise on functional performance in elderly people. J. Gerontol. Med. Sci. 59A, 154–160.

Resnick, N.M., Beckett, L.A., Branch, L.G., Scherr, P.A., Wetle, T., 1994. Short-term variability of self report of incontinence in older persons. J. Am. Geriatr. Soc. 42, 202–207.

Shumway-Cook, A., Gruber, W., Baldwin, M., Liao, S., 1997. The effect of multidimensional exercise on balance, mobility, and fall risk in community-dwelling older adults. Phys. Ther. 77, 46–57.

Smith, L.A., Branch, L.G., Scherr, P.A., Wetle, T., Evans, D.A., Hebert, L., Taylor, J.O., 1990. Short-term variability of measures of physical function in older people. J. Am. Geriatr. Soc. 38, 993–998.

Speers, R.A., Ashton-Miller, J.A., Schultz, A.B., Alexander, N.B., 1998. Age differences in abilities to perform tandem stand and walk tasks of graded difficulty. Gait Posture 7, 207–213.

Suzuki, T., Kim, H., Yoshida, H., Ishizaki, T., 2004. Randomized controlled trial of exercise intervention for the prevention of falls in community-dwelling elderly Japanese women. J. Bone Miner. Metab. 22, 602–611.

Tennstedt, S., Howland, J., Lachman, M., Peterson, E., Kasten, L., Jette, A., 1998. A randomized, controlled trial of a group intervention to reduce fear of falling and

associated activity restriction in older adults. J. Gerontol. Psychol. Sci. 53B, P384–P392.
Tinetti, M.E., Baker, D.I., McAvay, G., Claus, E.B., Garrett, P., Gottschalk, M., Koch, M.L., Trainor, K., Horwitz, R.I., 1994. A multifactorial intervention to reduce the risk of falling among elderly people living in the community. N. Engl. J. Med. 331, 821–827.
Tinetti, M.E., Inouye, S.K., Gill, T.M., Doucette, J.T., 1995. Shared risk factors for falls, incontinence, and functional dependence: unifying the approach to geriatric syndromes. J. Am. Med. Assoc. 273, 1348–1353.
Vellas, B.J., Wayne, S.J., Romero, L.J., Baumgartner, R.N., Garry, P.J., 1997. Fear of falling and restriction of mobility in elderly fallers. Age Ageing 26, 189–193.

第52回日本老年医学会学術集会記録
〈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

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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

（日老医誌　2011；48：39–41）

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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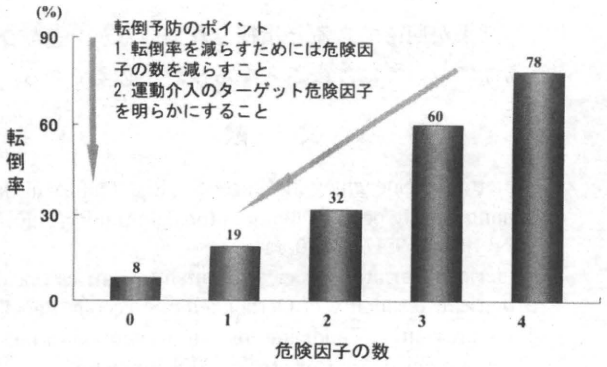

図1　転倒の危険因子の数と転倒率
文献1より改変

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，

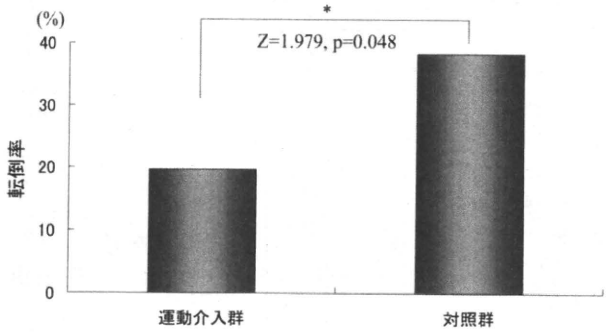

図3　転倒経験者における運動介入後1年間の転倒率
文献14より

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．

文　献

1) Tinetti ME, Speechley M, Ginter SF: Risk factors for falls among elderly persons living in the community. N Engl J Med 1988; 319: 1701–1707.
2) American Geriatrics Society, British Geriatrics Society, American Academy of Orthopaedic Surgeons Panel on Falls Prevention: Guideline for the prevention of falls in older persons. J Am Geriatr Soc 2001; 49: 664–672.
3) Province MA, Hadley EC, Hornbrook MC, Lipsitz LA, Miller JP, Mulrow CD, et al.: The effects of exercise on falls in elderly patients: A prepalnned meta-analysis of the FICSIT trials. JAMA 1995; 273: 1341–1347.
4) Campbell AJ, Robertson MC, Gardner MM, Norton RN, Tilyard MW, Buchner DM: Randomised controlled trial of a general practice programme of home based exercise to prevent falls in elderly women. BMJ 1997; 315: 1065–1069.
5) Suzuki T, Kim H, Yoshida H, Ishizaki T: Randomized controlled intervention for the prevention of falls in community-dwelling elderly Japanese women. J Bone Miner Metab 2004; 22: 602–611.
6) Day L, Fildes B, Gordon I, Fitzharris M, Flamer H, Lord S: Randomized factorial trial of falls prevention among older people living in their own homes. BMJ 2002; 325: 128–131.
7) Mulrow CD, Gerety MB, Kanten D, Cornell JE, DeNino LA, Chiodo L, et al.: A randomized trial of physical rehabilitation for very frail nursing home residents. JAMA 1994; 271: 519–524.
8) Rubenstein LZ, Robbins AS, Josephson KR, Schulman BL, Osterweil D: The value of assessing falls in an elderly population: A randomized clinical trial. Ann Intern Med 1990; 113: 308–316.
9) Lord SL, Ward JA, Williams P, Strudwick M: The effect of a 12-month exercise trial on balance, strength, and falls in older women: A randomized controlled trial. J Am Geriatr Soc 1995; 43: 1198–1206.
10) Reinsch S, MacRae P, Lachenbruch PA, Tobis JS: Attempts to prevent falls and injury: A prospective community study. Gerontologist 1992; 32: 450–456.
11) 金　憲経，吉田英世，鈴木隆雄，石崎達郎，細井孝之，山本精三ほか：高齢者の転倒関連恐怖感と身体機能―転倒外来受診者について―．日老医誌　2001; 38: 805–811.
12) Greenspan SL, Myers ER, Kiel DP, Parker RA, Hayes WC, Resnick NM: Fall direction, bone mineral density, and function: Risk factors for hip fracture in frail nursing home elderly. Am J Med 1998; 104: 539–545.
13) Skelton D, Dinan S, Campbell M, Rutherford O: Tailored group exercise (falls management exercise-FaME) reduces falls in community-dwelling older frequent fallers (an RCT). Age Aging 2005; 34: 636–639.
14) 金　憲経，鈴木隆雄：転倒経験者に対する転倒予防戦略―運動中心プログラムの効果検証―．Geriat　Med 2009; 47: 751–754.

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Associations between components of the metabolic syndrome versus bone mineral density and vertebral fractures in patients with type 2 diabetes

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ABSTRACT

The association of bone with the metabolic syndrome and its features, visceral fat accumulation or insulin resistance, remains unclear. We determined visceral and subcutaneous fat areas (V and S) by computed tomography on 187 men (28–83 years) and 125 postmenopausal women (46–82 years) with type 2 diabetes. Men whose V was 100 cm^2 or more had significantly lower urinary N-terminal cross-linked telopeptide of type-I collagen ($p=0.005$), higher femoral neck bone mineral density (FN-BMD) ($p=0.004$), and lower prevalence of vertebral fractures (VFs) ($p=0.04$) than controls. Fat mass, V, S, and lean body mass positively correlated with FN-BMD in men and with lumbar (L) and FN-BMD in women. When adjusted for weight, these correlations became negative. Urinary C-peptide positively correlated with FN-BMD in both genders. Multivariate logistic regression analysis adjusted for age, height, weight, L-BMD, duration of diabetes, and diabetes therapies identified V in men and urinary C-peptide in women as factors inversely associated with the presence of VFs [odds ratio (OR) = 0.61 per SD increase, $p=0.04$, and OR = 0.32, $p=0.01$, respectively]. These findings suggest that, of the components of the metabolic syndrome, body fat in gravity and hyperinsulinemia could increase FN-BMD in diabetic subjects. Visceral fat in men and hyperinsulinemia in women may protect against VFs independent of weight, L-BMD, diabetes duration, or therapies.

Introduction

The number of patients with the metabolic syndrome is rapidly increasing in industrialized countries where Western life-style is prevalent. Osteoporosis also becomes a common disease in those aging societies. The metabolic syndrome predisposes patients to atherosclerosis, which may result in coronary heart disease and cerebral stroke [1,2]. Osteoporosis puts patients at an increased risk for fractures. Thus, patients with these two disorders in combination are more likely to become bedridden after suffering from the cardiovascular disease and fractures. However, little is known whether or not the metabolic syndrome and osteoporosis are etiologically related to each other.

The main features of the metabolic syndrome are visceral fat accumulation and insulin resistance, which usually coincide with obesity. Cumulating studies show that obesity is beneficial for bone health, with increasing bone mineral density (BMD) and decreasing fracture rates [3–5]. Circulating insulin concentration was found to be the principal determinant of BMD at femoral neck and lumbar spine [6]. Insulin resistance, which was estimated from an intravenous glucose tolerance test, was significantly and positively correlated with BMD [7]. On the other hand, it is unclear whether patients with the metabolic syndrome have increased or decreased fracture risks. Ahmed at al. reported that an increasing number of the metabolic syndrome features such as increased waist circumference, hypertension, and dyslipidemia was associated with significantly reduced risk of prevalent non-vertebral fractures in both men and women in a cross-sectional study [8]. In contrast, von Muhlen et al. reported that incident clinical fractures were 2.6 times more likely to occur in participants with the metabolic syndrome as compared to those without the syndrome after an average follow up of 2 years in a longitudinal analysis, although there was no association between the syndrome and prevalent non-vertebral fractures in the cross-sectional analysis [9]. Thus, non-vertebral fracture risks in the metabolic syndrome may be different between study designs. On the other hand, to our knowledge, there are few studies on whether the metabolic syndrome would affect vertebral fracture (VF) risk or not.

Abbreviations: BMD, bone mineral density; BAP, bone specific alkaline phosphatase; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; C, cholesterol; CI, confidential interval; CT, computed tomography; Cr, creatinine; DBP, diastolic blood pressure; DXA, dual X-ray absorptiometry; Fat, total fat mass; FN, femoral neck; FPG, fasting plasma glucose; IMT, intima-media thickness; L, lumbar; LBM, lean body mass; OC, osteocalcin; OR, odds ratio; %Fat, percent fat mass; R, radial; S, subcutaneous fat area; SBP, systolic blood pressure; T, total; T-C, total cholesterol; TG, triglyceride; Trunk fat, trunk fat mass; uC-peptide, urinary C-peptide; uNTX, urinary N-terminal cross-linked telopeptide of type-I collagen; V, visceral fat area; VF, vertebral fracture.

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In this study, we assessed visceral fat accumulation by calculating visceral fat area (V) using a computed tomography (CT), which is a hallmark of the diagnosis of the metabolic syndrome. We also measured fat and trunk fat mass by dual X-ray absorptiometry (DXA). We investigated whether or not these fat parameters as well as other components of the metabolic syndrome, in particular urinary C-peptide (uC-peptide) as a surrogate marker for insulin secretion, were associated with BMD or the presence of prevalent VFs in patients with type 2 diabetes in a cross-sectional analysis.

Methods

Subjects

The subjects in this study were 187 men and 125 postmenopausal women with type 2 diabetes mellitus (age range, 28–83 and 46–82 years; mean 59.7 and 64.7, respectively). We consecutively recruited subjects who visited Shimane University Hospital for an education, evaluation, or treatment of diabetes. Baseline characteristics of subjects are shown in Table 1. All women had been without spontaneous menses for more than 1 year. Nobody had hepatic or renal dysfunction or nutritional derangements. Twenty-seven, 65, 26, and 22 men, as well as 34, 38, 29, and 15 women had been taking insulin treatment, sulfonylurea, metformin, and alpha-glucosidase inhibitor, respectively. Subjects treated with thiazolidinedione were excluded in this study because of its possible detrimental effects on bone by reducing BMD and increasing fracture rate [10,11]. Forty-two men and 51 women had taken calcium antagonists, 37 men and 51 women had taken angiotensin converting enzyme inhibitors or angiotensin II receptor blockers for hypertension treatment, and 24 men and 42 women had taken HMG-CoA reductase inhibitors for dyslipidemia treatment, and 29 men and 30 women have taken aspirin for atherosclerosis treatment. Sixty-nine men (36.9%) and 2 (1.6%) women were current smokers. All subjects were free of drugs known to influence bone and calcium metabolism like vitamin D, bisphosphonate, or estrogen replacement therapy until the time of the present study. This study was cross-sectional and approved by the ethical review board of our institution and complied with the Helsinki declaration. All subjects agreed to participate in the study and gave informed consent.

Table 1
Baseline characteristics of diabetic patients.

	Men	Women
Number	187	125
Age (year)	59.7 ± 13.5	64.7 ± 10.9
Diabetes duration (year)	11.6 ± 9.6	11.6 ± 9.2
Height (cm)	165.2 ± 7.5	150.6 ± 5.6
Weight (kg)	64.9 ± 13.8	57.2 ± 12.1
BMI	23.6 ± 4.0	25.2 ± 4.9
Fat (kg)	13.1 ± 5.8	17.8 ± 6.8
%Fat	19.8 ± 5.0	30.4 ± 6.7
Trunk fat (kg)	6.8 ± 3.4	9.3 ± 3.9
LBM (kg)	48.5 ± 7.8	37.2 ± 5.3
V (cm^2)	115.7 ± 70.4	116.6 ± 71.6
S (cm^2)	118.4 ± 86.6	195.0 ± 105.4
T-C (mg/dl)	191.2 ± 41.6	199.7 ± 45.8
TG (mg/dl)	144.8 ± 84.4	112.8 ± 54.5
HDL-C (mg/dl)	52.0 ± 15.7	56.4 ± 16.2
LDL-C (mg/dl)	110.5 ± 35.9	120.7 ± 39.2
SBP (mm Hg)	129.3 ± 17.4	128.3 ± 20.6
DBP (mm Hg)	78.2 ± 12.0	74.3 ± 12.5
FPG (mg/dl)	167.3 ± 60.2	167.0 ± 57.7
HbA1c (%)	9.0 ± 2.3	9.1 ± 2.4
uC-peptide (μg/day)	71.8 ± 41.7	56.9 ± 39.0
Fasting plasma insulin (μIU/ml)	5.7 ± 4.5	7.5 ± 6.6
HOMA-IR	2.3 ± 1.9	3.0 ± 3.0
Cr (mg/dl)	0.8 ± 0.2	0.6 ± 0.3
Right (r)-baPWV (m/s)	14.4 ± 2.8	15.1 ± 2.6
Left (l)-baPWV (m/s)	14.2 ± 2.5	15.1 ± 2.7
IMT-max (mm)	2.2 ± 1.3	2.1 ± 1.2
Smoking (%)	36.9	1.6
BAP (U/l)	26.2 ± 10.9	30.9 ± 12.1
OC (ng/ml)	5.0 ± 2.5	7.0 ± 3.2
uNTX (nmol/mmol Cr)	34.7 ± 27.1	52.2 ± 33.5
L-BMD (g/cm^2)	1.043 ± 0.175	0.886 ± 0.194
(z-score)	0.5 ± 1.1	0.5 ± 1.2
FN-BMD (g/cm^2)	0.785 ± 0.123	0.656 ± 0.131
(z-score)	0.3 ± 1.0	0.5 ± 1.2
R-BMD (g/cm^2)	0.708 ± 0.068	0.538 ± 0.095
(z-score)	−0.6 ± 1.3	0.5 ± 1.5
T-BMD (g/cm^2)	1.083 ± 0.098	0.921 ± 0.121
VF (%)	32.6	32.0

Radiography

Total and trunk fat mass (designated "Fat" and "Trunk fat", respectively) and lean body mass (LBM) were measured by DXA (QDR-4500; Hologic, Waltham, MA) using whole-body absorptiometry software and each value was expressed in kilograms. Percent fat mass (%Fat) was calculated by dividing total fat mass by total body mass. Coefficient of variation (precision) of measurements of fat mass was 2.0% [12].

Abdominal adipose tissue was calculated using commercially available CT (Toshiba medical systems, Tokyo, Japan), which determined adipose tissue area electronically by setting the attenuation values for the region of interest within a range of −150 and −50 Hounsfield units. V and subcutaneous fat area (S) were determined separately with the use of a trace function, which manually defined the boundary between the visceral and subcutaneous fat with a cursor.

BMD values of the total (T), lumbar spine (L), femoral neck (FN), and one-third of the radius (1/3R) were measured by DXA (QDR-4500). The coefficients of variation (precision) of measurements of L-, FN- and 1/3R-BMD by our methods were 0.9, 1.7 and 1.9%, respectively. *Z* score indicates deviation from the normal age- and sex-matched mean in standard deviation (SD).

Lateral X-ray films of the thoracic and lumbar spine were taken, and the anterior, central, and posterior heights of each of the 13 vertebral bodies from Th4-L4 were measured. VF 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 [13]. None of the subjects had a history of serious trauma.

Biochemical measurements

After overnight fasting, serum was collected. Biochemical markers were measured by standard biochemical methods. Hemoglobin A_{1c} (HbA_{1c}) was determined by high performance liquid chromatography. uC-peptide pooled for 24 h was measured by Chemiluminescent EIA. Total cholesterol (T-C), triglyceride (TG), and HDL-Cholesterol (C) were evaluated using an enzymatic method. LDL-C was calculated by Friedewald's formula [T-C − (HDL-C + TG/5)] [14]. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by the following formula [Fasting plasma glucose (FPG) × fasting plasma insulin/405] [15]. Bone specific alkaline phosphatase (BAP) and serum osteocalcin (OC) were measured by Enzyme Immuno Assay and radioimmunoassay, respectively. Urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX) was measured by enzyme linked immunosorbent assay.

Arterial stiffness measurement

Brachial-ankle pulse wave velocity (baPWV) was measured using the VaSera VS-1000 (Fukuda Denshi, Tokyo, Japan), an automated recording device that calculates the time delay between two pulse waves recorded simultaneously, as previously described [16].

Ultrasonographic measurement of carotid intima-media thickness

B-mode ultrasonographic imaging of the carotid artery was performed using HDI 5000 (Philips, Tokyo, Japan), a high-resolution, real-time ultrasonograph with a 7.5-MHz transducer, as previously described [16]. Intima-media thickness (IMT) was measured as the distance between the lumen–intima interface and the media–adventitia interface on the B-mode image. To quantify carotid artery wall thickness, we used the maximum of IMT (IMT-max) in the present study.

Diagnosis of the metabolic syndrome

We diagnosed patients as the metabolic syndrome based on the guideline in Japan [17], when they have visceral obesity with at least two of the following three conditions: hypertension, dyslipidemia, and impaired fasting glucose. Although waist circumference is generally used for evaluating visceral obesity in epidemiological studies, we precisely defined visceral obesity as V equal to or more than 100 cm^2, which was calculated using CT. Hypertension was defined as systolic blood pressure (SBP) equal to or more than 130 mm Hg, diastolic blood pressure (DBP) equal to or more than 85 mm Hg, or previous treatment for hypertension. Dyslipidemia was defined as TG concentrations equal to or more than 150 mg/dl, HDL-C less than 40 mg/dl, or current treatment for dyslipidemia. Impaired fasting glucose was defined as a FPG level equal to or more than 110 mg/dl or previous treatment for diabetes mellitus.

Statistical analysis

Data were expressed as mean ± SD. An unpaired *t*-test was used to compare continuous variables between two groups. Comparisons of categorical variables were made using chi-square test. Simple, multiple, and logistic regression analyses were performed using the statistical computer program Statview (Abacus Concepts, Berkely, CA). $P<0.05$ was considered to be significant.

Results

Comparison of atherosclerosis- and osteoporosis-related parameters between diabetic patients with and without the metabolic syndrome, or those with and without visceral fat accumulation were shown in Table 2. Diabetic men with the metabolic syndrome had a lower uNTX value ($p=0.08$) and a higher FN-BMD value ($p=0.05$), and tended to have a lower prevalence of VF ($p=0.07$) than those without the metabolic syndrome. These findings became more significant when compared between diabetic men with and without visceral fat accumulation (V equal to or more than 100 cm^2) ($p=0.005$, $p=0.004$, and $p=0.04$, respectively), although age was not significantly different between the two groups. On the other hand,

Table 2
Comparison of atherosclerosis- and osteoporosis-related parameters between diabetic patients with and without the metabolic syndrome, or those with and without visceral fat accumulation.

	Metabolic syndrome			Visceral fat area≧100 cm^2		
	Yes	No	*p* value	Yes	No	*p* value
Men						
Number	79	108		103	84	
Age (year)	59.0 ± 13.7	60.1 ± 13.4	0.59	58.8 ± 13.8	60.7 ± 13.1	0.33
Height (cm)	166.8 ± 6.7	164.1 ± 7.8	0.01	166.3 ± 6.7	163.9 ± 8.1	0.03
Weight (kg)	70.6 ± 14.6	60.7 ± 11.6	<0.0001	70.3 ± 14.2	58.3 ± 9.8	<0.0001
BMI	25.2 ± 4.1	22.5 ± 3.5	<0.0001	25.3 ± 4.0	21.6 ± 2.9	<0.0001
r-baPWV (m/s)	14.6 ± 3.4	14.2 ± 2.4	0.13	14.5 ± 3.1	14.4 ± 2.5	0.81
l-baPWV (m/s)	14.4 ± 2.8	14.1 ± 2.3	0.42	14.2 ± 2.6	14.2 ± 2.3	0.97
IMT-max (mm)	2.2 ± 1.1	2.2 ± 1.4	0.96	2.1 ± 1.1	2.3 ± 1.5	0.29
BAP (U/l)	25.2 ± 8.7	26.9 ± 12.2	0.28	25.2 ± 8.1	27.4 ± 13.4	0.16
OC (ng/ml)	5.0 ± 2.5	5.0 ± 2.5	0.99	5.0 ± 2.3	5.0 ± 2.7	0.88
uNTX (nmol/mmol Cr)	30.5 ± 14.8	37.6 ± 32.9	0.08	29.5 ± 13.7	40.8 ± 36.3	0.005
L-BMD (g/cm^2)	1.037 ± 0.178	1.047 ± 0.174	0.68	1.044 ± 0.175	1.041 ± 0.176	0.90
(z-score)	0.4 ± 1.1	0.5 ± 1.1	0.56	0.4 ± 1.1	0.5 ± 1.1	0.73
FN-BMD (g/cm^2)	0.806 ± 0.121	0.770 ± 0.122	0.05	0.808 ± 0.118	0.757 ± 0.124	0.004
(z-score)	0.5 ± 1.0	0.2 ± 1.0	0.06	0.5 ± 1.0	0.1 ± 1.0	0.008
R-BMD (g/cm^2)	0.706 ± 0.073	0.709 ± 0.066	0.80	0.707 ± 0.070	0.709 ± 0.067	0.87
(z-score)	−0.6 ± 1.5	−0.7 ± 1.2	0.90	−0.7 ± 1.4	−0.6 ± 1.2	0.85
T-BMD (g/cm^2)	1.083 ± 0.096	1.083 ± 0.099	0.97	1.082 ± 0.092	1.084 ± 0.104	0.86
VF (%)	25.3	38.0	0.07	26.2	40.4	0.04
Women						
Number	59	66		73	52	
Age (year)	65.1 ± 10.2	64.6 ± 11.2	0.80	64.2 ± 10.8	65.3 ± 11.0	0.57
Height (cm)	150.4 ± 5.3	150.8 ± 5.9	0.69	150.7 ± 5.2	150.5 ± 6.2	0.83
Weight (kg)	62.8 ± 11.8	52.1 ± 10.1	<0.0001	62.7 ± 11.5	49.8 ± 8.5	<0.0001
BMI	27.7 ± 4.6	22.9 ± 4.0	<0.0001	27.5 ± 4.4	22.0 ± 3.6	<0.0001
r-baPWV (m/s)	15.2 ± 2.7	15.0 ± 2.6	0.65	15.0 ± 2.7	15.2 ± 2.5	0.59
l-baPWV (m/s)	15.3 ± 2.8	15.0 ± 2.7	0.56	15.0 ± 2.9	15.3 ± 2.5	0.46
IMT-max (mm)	2.3 ± 1.5	1.9 ± 1.0	0.16	2.1 ± 1.4	2.0 ± 1.0	0.83
BAP (U/l)	30.6 ± 10.6	31.2 ± 13.5	0.78	30.1 ± 10.0	32.0 ± 14.8	0.40
OC (ng/ml)	6.6 ± 3.3	7.4 ± 3.0	0.20	6.8 ± 3.3	7.3 ± 3.0	0.41
uNTX (nmol/mmol Cr)	49.5 ± 28.8	54.6 ± 37.2	0.41	47.6 ± 26.8	58.6 ± 40.4	0.07
L-BMD (g/cm^2)	0.915 ± 0.182	0.857 ± 0.201	0.09	0.905 ± 0.184	0.860 ± 0.207	0.20
(z-score)	0.8 ± 1.2	0.3 ± 1.2	0.05	0.7 ± 1.1	0.4 ± 1.3	0.24
FN-BMD (g/cm^2)	0.672 ± 0.135	0.639 ± 0.125	0.16	0.672 ± 0.130	0.632 ± 0.129	0.09
(z-score)	0.7 ± 1.3	0.3 ± 1.2	0.10	0.6 ± 1.2	0.3 ± 1.2	0.15
R-BMD (g/cm^2)	0.535 ± 0.092	0.539 ± 0.098	0.83	0.539 ± 0.100	0.557 ± 0.088	0.91
(z-score)	0.5 ± 1.6	0.6 ± 1.5	0.73	0.4 ± 1.7	0.6 ± 1.4	0.57
T-BMD (g/cm^2)	0.929 ± 0.117	0.913 ± 0.124	0.47	0.926 ± 0.119	0.915 ± 0.124	0.61
VF (%)	35.6	29.2	0.45	30.1	34.6	0.60

comparison of diabetic women with and without the metabolic syndrome, or with and without visceral fat accumulation showed no significant difference in uNTX, BMD or VF prevalence. Atherosclerosis-related parameters (baPWV or IMT-max) were not significantly different between the groups in either gender.

Next, we examined correlation between BMD and features of the metabolic syndrome in diabetic patients (Table 3). FN-BMD in diabetic men and L- and FN-BMD in diabetic women were potently and significantly correlated with each of Fat, %Fat, Trunk fat, V, and S (at least $p<0.01$). BMD at any site in both genders were potently and significantly correlated with LBM (at least $p<0.01$). When the mechanical loading effect of body weight on BMD was adjusted for, the positive correlations between BMD and body fat became negative, while those between BMD and LBM remained positive. uC-peptide was also significantly and positively correlated with FN-BMD in both genders (at least $p<0.01$). Features of the metabolic syndrome other than body fat or hyperinsulinemia were not significantly or only weakly correlated with BMD, except for a positive correlation between HbA1c and R-BMD in diabetic women ($p<0.001$).

Table 3
Correlation between BMD and features of the metabolic syndrome in diabetic patients.

	L-BMD	FN-BMD	R-BMD	T-BMD
Men				
Age	0.03	−0.34[d]	−0.42[d]	−0.26[c]
Diabetes duration	0.15[a]	−0.07	−0.09	−0.01
Height	0.10	0.31[d]	0.27[c]	0.19[a]
Weight	0.19[b]	0.52[d]	0.27[c]	0.31[d]
BMI	0.19[b]	0.48[d]	0.19[a]	0.29[d]
Fat	0.12 (−0.26)	0.39[d] (−0.36[b])	0.09 (−0.71[d])	0.14 (−0.67[d])
%Fat	0.06 (−0.13)	0.25[c] (−0.15)	−0.10 (−0.47[d])	−0.04 (−0.41[d])
Trunk fat	0.13 (−0.14)	0.38[d] (−0.26[a])	0.11 (−0.49[c])	0.15[a](−0.46[c])
LBM	0.24[b] (0.35[a])	0.55[d] (0.41[b])	0.35[d] (0.61[c])	0.39[d] (0.58[c])
V	0.06 (−0.05)	0.24[c] (−0.04)	0.05 (−0.13)	0.06 (−0.15)
S	0.07 (−0.17)	0.28[c] (−0.25[c])	0.14 (−0.15)	0.11 (−0.29[b])
T-C	0.15[a]	0.11	0.13	0.12
TG	0.08	0.21[b]	0.09	0.12
HDL-C	−0.03	−0.10	0.12	0.03
LDL-C	0.14	0.08	0.05	0.07
FPG	0.003	−0.04	0.19a	0.03
HbA1c	−0.007	−0.04	0.13	0.03
uC-peptide	0.01	0.21[b]	0.08	0.11
Fasting plasma insulin	−0.01	0.17[a]	−0.08	−0.02
HOMA-IR	0.001	0.14	0.02	0.02
SBP	0.05	0.06	−0.03	0.02
DBP	−0.05	0.13	0.15[a]	0.08
Women				
Age	−0.41[d]	−0.56[d]	−0.63[d]	−0.60[d]
Diabetes duration	−0.16	−0.24[b]	−0.29[b]	−0.24[b]
Height	0.30[c]	0.38[d]	0.43[d]	0.36[d]
Weight	0.48[d]	0.48[d]	0.35[d]	0.40[d]
BMI	0.40[d]	0.37[d]	0.23[a]	0.30[c]
Fat	0.39[d] (−0.39)	0.39[d] (−0.37)	0.28[b] (−0.33)	0.31[c] (−0.43)
%Fat	0.23[b] (−0.27[a])	0.26[b] (−0.20)	0.14 (−0.25[a])	0.17 (−0.29[a])
Trunk fat	0.35[d] (−0.51[b])	0.38[d] (−0.35)	0.25[b] (−0.44[a])	0.27[b] (−0.56[b])
LBM	0.52[d] (0.52[b])	0.54[d] (0.56[b])	0.38[d] (0.33)	0.44[d] (0.43[a])
V	0.23[b] (−0.15)	0.27[b] (−0.09)	0.08 (−0.26[a])	0.14 (−0.22[a])
S	0.32[c] (−0.15)	0.33[c] (−0.14)	0.18 (−0.28[a])	0.23[b] (−0.23)
T-C	0.12	0.10	0.24[b]	0.21[a]
TG	0.10	0.16	0.05	0.09
HDL-C	0.05	−0.06	0.19[a]	0.10
LDL-C	0.10	0.09	0.19[a]	0.17
FPG	−0.006	0.04	0.17	0.07
HbA1c	0.12	0.11	0.31[c]	0.23[b]
uC-peptide	0.11	0.36[d]	0.14	0.14
Fasting plasma insulin	0.06	0.008	0.02	−0.02
HOMA-IR	0.001	0.05	0.04	0.05
SBP	0.007	0.04	−0.05	−0.004
DBP	−0.003	0.17	0.06	0.04

Values in parentheses were the standardized regression coefficients when BMD was adjusted for body weight.
[a] $p<0.05$.
[b] $p<0.01$.
[c] $p<0.001$.
[d] $p<0.0001$.

Next, we compared features of the metabolic syndrome between diabetic patients with and without VFs (Table 4). Diabetic men with VFs were significantly older ($p=0.02$) and tended to be shorter in height ($p=0.08$), and tended to have less Trunk fat and V ($p=0.07$ and $p=0.05$, respectively) than those without VFs. Diabetic women with VFs were significantly older ($p<0.0001$) and shorter in height ($p=0.05$), and had significantly lower uC-peptide excretion ($p=0.003$) than those without VFs. They also tended to have less LBM ($p=0.06$) and higher FPG ($p=0.07$) than those without VFs. When multivariate logistic regression analysis adjusted for age, body height, weight, L-BMD, duration of diabetes, diabetes therapies was performed (Table 5), V in men and uC-peptide in women were selected as indices significantly and inversely associated with the presence of VFs ($p=0.04$ and $p=0.01$, respectively).

Discussion

In this cross-sectional study, we examined whether or not each component of the metabolic syndrome (visceral fat accumulation, dyslipidemia, hypertension, and hyperglycemia) is related to parameters of osteoporosis (bone markers, BMD, and prevalent VFs) in patients with type 2 diabetes. We also evaluated the effect of insulin resistance using uC-peptide as a surrogate marker for endogenous insulin secretion. We found that body fat positively correlated with FN-BMD in men and with L- and FN-BMD in women, and that u-C-peptide positively correlated with FN-BMD in both genders (Table 3). In diabetic men, subjects with the metabolic syndrome or visceral fat accumulation had lower uNTX, higher FN-BMD, and lower prevalence of VF than those without them (Table 2). V in men and u-C-peptide in women were significantly and inversely associated with the presence of prevalent VFs independent of body weight, L-BMD, diabetes duration, or therapies (Table 5). On the other hand, dyslipidemia or hypertension were not significantly or only weakly associated with BMD or VF in either gender (Tables 3 and 4). Thus, of the components of the metabolic syndrome, body fat accumulation and insulin resistance may increase BMD, especially at the femoral neck, and reduce the risk of VFs in patients with type 2 diabetes.

Other researchers also reported the protective effect of the metabolic syndrome on BMD and fractures. Kinjo et al. found that after multivariable adjustment, FN-BMD was higher among subjects with the metabolic syndrome than those without it in a large cohort of American population. They also found that adjusted FN-BMD increased with additional components of the metabolic syndrome, and there was a significant positive association with abdominal obesity ($p<0.0001$) [18]. A cross-sectional study by Ahmed at al. showed that increasing number of the metabolic syndrome features was associated with significantly reduced risk of prevalent non-VFs in both men and women [8]. von Muhlen et al. reported that there was no association between the syndrome features and prevalent non-VFs in a cross-sectional analysis [9]. Taken together, these findings as well as ours from cross-sectional analyses suggest that the metabolic syndrome has the potential to increase FN-BMD and reduce both VF and non-VF risks, and thus is beneficial for or at least not detrimental to bone. In contrast, von Muhlen et al. reported that incident clinical fractures were 2.6

Table 4
Comparison of features of the metabolic syndrome or osteoporosis-related parameters between diabetic patients with and without vertebral fractures.

	Men			Women		
	Fracture (+)	Fracture (−)	*p* value	Fracture (+)	Fracture (−)	*p* value
Number	61	126		40	85	
Age (year)	62.9 ± 13.7	58.1 ± 13.2	0.02	71.2 ± 8.3	61.8 ± 10.7	<0.0001
Diabetes duration (year)	12.6 ± 9.2	11.2 ± 9.8	0.36	13.2 ± 8.9	10.9 ± 9.4	0.21
Height (cm)	164.0 ± 7.7	166.0 ± 7.1	0.08	149.1 ± 5.7	151.2 ± 5.4	0.05
Weight (kg)	63.0 ± 13.8	66.0 ± 13.6	0.17	55.5 ± 14.3	58.1 ± 11.0	0.25
BMI	23.3 ± 3.9	23.8 ± 4.0	0.35	24.9 ± 6.0	25.4 ± 4.4	0.60
Fat (kg)	12.2 ± 4.9	13.6 ± 6.2	0.12	17.3 ± 8.1	18.2 ± 6.1	0.53
%Fat	19.1 ± 4.4	20.2 ± 5.3	0.18	30.1 ± 8.5	30.6 ± 5.6	0.68
Trunk fat (kg)	6.2 ± 2.8	7.1 ± 3.6	0.07	9.0 ± 4.7	9.5 ± 3.4	0.49
LBM (kg)	47.3 ± 8.3	49.2 ± 7.4	0.12	36.0 ± 5.9	37.9 ± 5.0	0.06
V (cm^2)	101.8 ± 63.4	123.2 ± 72.5	0.05	116.0 ± 83.0	117.9 ± 65.8	0.89
S (cm^2)	104.2 ± 66.1	126.2 ± 94.2	0.10	188.2 ± 127.4	200.1 ± 92.9	0.10
T-C (mg/dl)	189.3 ± 38.9	192.7 ± 42.7	0.61	192.6 ± 40.9	202.9 ± 48.0	0.24
TG (mg/dl)	137.0 ± 94.1	149.2 ± 79.2	0.36	116.3 ± 67.0	111.1 ± 48.2	0.62
HDL-C (mg/dl)	54.4 ± 16.2	50.7 ± 15.4	0.14	116.3 ± 67.0	111.1 ± 48.2	0.62
LDL-C (mg/dl)	107.5 ± 31.7	112.4 ± 37.6	0.38	113.6 ± 33.4	124.2 ± 41.6	0.16
FPG (mg/dl)	163.1 ± 57.1	169.1 ± 62.0	0.53	180.4 ± 62.0	160.3 ± 54.9	0.07
HbA1c (%)	8.9 ± 2.0	9.0 ± 2.5	0.64	9.3 ± 2.2	9.0 ± 2.4	0.56
uC-peptide (μg/day)	74.3 ± 39.5	71.0 ± 43.0	0.61	39.9 ± 28.2	63.5 ± 40.8	0.003
Fasting plasma insulin (μIU/ml)	5.9 ± 5.7	5.7 ± 3.9	0.78	8.3 ± 7.5	7.2 ± 6.3	0.40
HOMA-IR	2.3 ± 2.2	2.3 ± 1.8	0.98	3.5 ± 3.3	2.8 ± 2.8	0.22
SBP (mm Hg)	130.1 ± 18.8	128.5 ± 16.5	0.56	127.4 ± 24.3	128.7 ± 18.9	0.73
DBP (mm Hg)	77.7 ± 10.9	78.3 ± 12.5	0.75	72.9 ± 16.1	74.9 ± 10.5	0.41
BAP (U/l)	28.0 ± 13.0	25.4 ± 9.7	0.14	33.4 ± 15.0	29.8 ± 10.7	0.14
OC (ng/ml)	5.0 ± 2.5	5.0 ± 2.5	0.97	7.3 ± 3.1	6.9 ± 3.2	0.47
uNTX (nmol/mmol Cr)	34.3 ± 17.7	34.9 ± 30.6	0.89	61.2 ± 43.8	48.0 ± 26.8	0.04
L-BMD (g/cm^2)	1.026 ± 0.146	1.052 ± 0.188	0.35	0.847 ± 0.220	0.902 ± 0.179	0.14
(*z*-score)	0.4 ± 0.9	0.5 ± 1.2	0.54	0.6 ± 1.4	0.5 ± 1.1	0.72
FN-BMD (g/cm^2)	0.767 ± 0.125	0.795 ± 0.120	0.15	0.603 ± 0.121	0.678 ± 0.129	0.003
(*z*-score)	0.3 ± 1.0	0.4 ± 1.0	0.65	0.4 ± 1.2	0.5 ± 1.2	0.41
R-BMD (g/cm^2)	0.695 ± 0.066	0.715 ± 0.06	0.07	0.503 ± 0.084	0.554 ± 0.096	0.006
(*z*-score)	−0.7 ± 1.1	−0.6 ± 1.3	0.52	0.5 ± 1.4	0.5 ± 1.6	0.88
T-BMD (g/cm^2)	1.064 ± 0.094	1.092 ± 0.099	0.07	0.858 ± 0.095	0.949 ± 0.119	<0.0001

times more likely to occur in participants with the metabolic syndrome as compared to those without the syndrome after an average follow up of 2 years in a longitudinal analysis [9]. Further studies seem to be needed to confirm their observation, and to clarify whether or not fracture risk of the metabolic syndrome is different between study designs.

Table 5
Associations between the presence of vertebral fractures and features of the metabolic syndrome in diabetic patients.

Independent variables	OR	Presence of vertebral fractures	
		(95% CI)	*p* value
Men			
Trunk fat (DXA)[a]	0.81	(0.55–1.16)	0.29
V (CT)[a]	0.74	(0.52–1.04)	0.08
V (CT)[b]	0.64	(0.41–0.99)	0.04
V (CT)[c]	0.62	(0.40–0.97)	0.04
V (CT)[d]	0.61	(0.38–0.99)	0.04
Women			
LBM (DXA)[a]	0.95	(0.58–1.55)	0.82
FPG[a]	1.50	(0.97–2.31)	0.07
FPG[b]	1.54	(0.99–2.39)	0.06
FPG[c]	1.54	(0.99–2.40)	0.06
FPG[d]	1.35	(0.80–2.26)	0.26
uC-peptide[a]	0.64	(0.37–1.12)	0.12
uC-peptide[b]	0.59	(0.33–1.06)	0.08
uC-peptide[c]	0.57	(0.31–1.04)	0.07
uC-peptide[d]	0.32	(0.13–0.80)	0.01

Unit of change; SD per increase.
[a] Each independent variable was adjusted for age and body height.
[b] Adjusted for age, body height, and body weight.
[c] Adjusted for age, body height, body weight, and L-BMD.
[d] Adjusted for age, body height, body weight, L-BMD, diabetes duration, and diabetes therapies.

Obesity is known to be linked to increased bone mass. Several studies showed that body weight or BMI were positively correlated with BMD, and that body weight loss lowered BMD values [3-5]. We also found that each of body weight, BMI, Fat, %Fat, Trunk fat, V, S, and LBM positively correlated with BMD in both sexes in this study (Table 3). However, the positive correlations between fat mass and BMD became negative after additionally adjusting for body weight. Zhao et al. reported that the correlation between fat mass and BMD was negative when adjusted for body weight in both Chinese and Caucasian subjects [19]. Another large-scale Chinese study also showed that fat mass was inversely associated with bone mineral content in the whole body and total hip when comparing the highest quartile with the lowest quartile of percentage fat mass across 5-kg strata of body weight in men and women [20]. These findings suggest that, when the mechanical loading effect of body weight on bone mass was adjusted for, fat mass may have a negative effect on bone mass in contrast with the positive effect of weight-bearing itself. Thus, body fat, the main component of the metabolic syndrome, may act on bone in the opposite direction between the circumstances in gravity on earth and in outer space.

In this study, we found that V, but not S, Fat, %Fat, or Trunk fat, was inversely associated with the presence of VFs in diabetic men independent of body weight, L-BMD, diabetes duration or therapies (Table 5). In contrast, V, albeit not significant, was negatively correlated with BMD after being adjusted for body weight in men (Table 3). These findings suggest that visceral fat, which plays a central role in the pathogenesis of the metabolic syndrome, has a protective effect on VF, while it has a possible negative effect against BMD independent of its weight-bearing effect on bone. We have recently reported that VF incidence in patients with type 2 diabetes is less dependent on BMD than non-diabetic controls [21]. Such

inconsistency between BMD and VF rate in the present study in the view of visceral fat might partly explain the pathogenesis underlying this observation.

In diabetic postmenopausal women, we found that uC-peptide was significantly and inversely associated with the presence of prevalent VFs independent of body weight, L-BMD, diabetes duration or therapies (Table 5). These findings suggest that insulin resistance might reduce the risk of VFs through hyperinsulinemia in this population. Insulin stimulates proliferation of osteoblasts [22], and increases indices of bone formation such as insulin-like growth factor-I [23] and bone morphogenetic protein [24] when administered locally over bone [25]. Although obese diabetic patients have insulin resistance that blunts hypoglycemic effect of the hormone and causes its compensatory over-secretion, other hormonal actions are kept intact, and increased circulating insulin may exert anabolic actions on bone [26]. Thus, high BMD is a very consistent finding across a wide range of hyperinsulinemic states [6,7], including obesity and type 2 diabetes [27]. This incremental effect of hyperinsulinemia on BMD may partly explain the positive correlation between uC-peptide and FN-BMD in the present study (Table 3). However, our analysis showed that the association of higher uC-peptide with reduced VF rate in women was independent of L-BMD (Table 5), suggesting that hyperinsulinemia might exert a preventive effect on VFs through the mechanism not related to BMD increase, possibly through improving bone quality not reflected by BMD.

This study has some limitations. First, the sample size was not large enough to make definite conclusions. Second, we analyzed only subjects who visited Shimane University Hospital, a tertiary center, for the evaluation or treatment of type 2 diabetes. Therefore, the patients enrolled in this study seem to have relatively severe states of hyperglycemia and may not be representative of Japanese men and postmenopausal women with the metabolic syndrome. Third, the subjects in this study were only Japanese. Capacity of insulin secretion and degree of obesity in Asian are known to be different from those in Caucasian [28]. Ethnic groups such as the Hispanics and the Asians, who are more prone to develop abdominal obesity, have more insulin resistance than the African-Americans or White-Americans, who develop less abdominal obesity for a similar degree of generalized adiposity [29]. Ethnic differences in fat distribution have been considered a major contributor to the observed excessive prevalence of insulin resistance and diabetes in the Asian Indians, Japanese and Hispanics, and Native Americans. In fact, mean BMI of our patients whose V was 100 cm^2 or more was 25.3 (Table 2), showing that they were less obese by Western standards. Thus, our findings might not be universal and only applicable to specific ethnic groups. Fourth, our study group was heterogeneous in treatments for diabetes, including those who had been taking insulin treatment. This seems the reason why HOMA-IR, a surrogate marker for insulin resistance as well as uC-peptide, was not associated with BMD or VFs in the present study, because insulin injection affects its value by raising plasma insulin levels. On the contrary, a strength of our study is that we precisely evaluated visceral fat accumulation by CT, and examined its relationship to bone parameters in separate genders. We also for the first time investigated the association between each component of the metabolic syndrome and the presence of prevalent VFs, which was assessed by X-ray films.

In conclusion, we found that visceral fat and insulin resistance were more strongly associated with bone than any other component of the metabolic syndrome in patients with type 2 diabetes. These two features were beneficial for bone by reducing VF risk in men and women, respectively. We also found that body fat in gravity and insulin resistance could also increase BMD, especially at the femoral neck, in the population.

Disclosure statement
The authors have nothing to declare.

References

[1] Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683–9.
[2] Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, et al. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. Circulation 2004;110:1245–50.
[3] Edelstein SL, Barrett-Connor E. Relation between body size and bone mineral density in elderly men and women. Am J Epidemiol 1993;138:160–9.
[4] Hannan MT, Felson DT, Anderson JJ. Bone mineral density in elderly men and women: results from the Framingham osteoporosis study. J Bone Miner Res 1992;7:547–53.
[5] Nguyen TV, Sambrook PN, Eisman JA. Bone loss, physical activity, and weight change in elderly women: the Dubbo Osteoporosis Epidemiology Study. J Bone Miner Res 1998;13:1458–67.
[6] Reid IR, Evans MC, Cooper GJ, Ames RW, Stapleton J. Circulating insulin levels are related to bone density in normal postmenopausal women. Am J Physiol 1993;265: E655–9.
[7] Abrahamsen B, Rohold A, Henriksen JE, Beck-Nielsen H. Correlations between insulin sensitivity and bone mineral density in non-diabetic men. Diabet Med 2000;17:124–9.
[8] Ahmed LA, Schirmer H, Berntsen GK, Fonnebo V, Joakimsen RM. Features of the metabolic syndrome and the risk of non-vertebral fractures: the Tromso study. Osteoporos Int 2006;17:426–32.
[9] von Muhlen D, Safii S, Jassal SK, Svartberg J, Barrett-Connor E. Associations between the metabolic syndrome and bone health in older men and women: the Rancho Bernardo Study. Osteoporos Int 2007;18:1337–44.
[10] Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, et al. ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med 2006;355:2427–43.
[11] Yaturu S, Bryant B, Jain SK. Thiazolidinedione treatment decreases bone mineral density in type 2 diabetic men. Diabetes Care 2007:1574–6.
[12] Kaji H, Tobimatsu T, Naito J, Iu MF, Yamauchi M, Sugimoto T, et al. Body composition and vertebral fracture risk in female patients treated with glucocorticoid. Osteoporos Int 2006;17:627–33.
[13] Genant HK, Jergas M, Palermo L, Nevitt M, Valentin RS, Black D, et al. Comparison of semiquantitative visual and quantitative morphometric assessment of prevalent and incident vertebral fractures in osteoporosis The Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1996;11:984–96.
[14] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[15] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28: 412–9.
[16] Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, Yano S, et al. Serum DHEA-S level is associated with the presence of atherosclerosis in postmenopausal women with type 2 diabetes mellitus. Endocr J 2008;55:667–76.
[17] Committee to Evaluate Diagnostic Standards for Metabolic Syndrome. Definition and the diagnostic standard for metabolic syndrome (in Japanese). Nippon Naika Gakkai Zassi 2005;94:794–809.
[18] Kinjo M, Setoguchi S, Solomon DH. Bone mineral density in adults with the metabolic syndrome: analysis in a population-based U.S. sample. J Clin Endocrinol Metab 2007;92:4161–4.
[19] Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. J Clin Endocrinol Metab 2007;92:1640–6.
[20] Hsu YH, Venners SA, Terwedow HA, Feng Y, Niu T, Li Z, et al. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. Am J Clin Nutr 2006;83:146–54.
[21] Yamamoto M, Yamaguchi T, Yamauchi M, Kaji H, Sugimoto T. Diabetic patients have an increased risk of vertebral fractures independent of bone mineral density or diabetic complications. J Bone Miner Res 2009;24:702–9.
[22] Hickman J, McElduff A. Insulin promotes growth of the cultured rat osteosarcoma cell line UMR-106-01: an osteoblast-like cell. Endocrinology 1989;124:701–6.
[23] Mccarthy TL, Centrella M, Canalis E. Insulin-like growth factor (IGF) and bone. Connect Tissue Res 1989;20:277–82.
[24] Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation in vitro and in rodents by statins. Science 1999;286:1946–9.
[25] Cornish J, Callon KE, Reid IR. Insulin increases histomorphometric indices of bone formation in vivo. Calcif Tissue Int 1996;59:492–5.
[26] Reid IR. Relationships among body mass, its components, and bone. Bone 2002;31: 547–55.
[27] Leidig-Bruckner G, Ziegler R. Diabetes mellitus a risk for osteoporosis? Exp Clin Endocrinol Diabetes 2001;109:S493–514.
[28] Fujimoto WY. Overview of non-insulin-dependent diabetes mellitus (NIDDM) in different population groups. Diabet Med 1996;13:S7–S10.
[29] Abate N, Chandalia M. The impact of ethnicity on type 2 diabetes. J Diabetes Complications 2003;17:39–58.

Low Serum Level of the Endogenous Secretory Receptor for Advanced Glycation End Products (esRAGE) Is a Risk Factor for Prevalent Vertebral Fractures Independent of Bone Mineral Density in Patients With Type 2 Diabetes

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OBJECTIVE — Patients with type 2 diabetes are known to have an increased risk for fracture compared with non–type 2 diabetic control subjects, despite having higher bone mineral density (BMD). We previously showed that serum pentosidine, one of the advanced glycation end products (AGEs), was associated with prevalent vertebral fractures (VFs) in those with type 2 diabetes. The involvement of the endogenous secretory receptor for AGEs (esRAGE) in VFs in those with type 2 diabetes, however, is still unknown.

RESEARCH DESIGN AND METHODS — We compared parameters including esRAGE, pentosidine, and BMD in Japanese type 2 diabetic patients (137 men >50 years old and 140 postmenopausal women) with and without VFs.

RESULTS — The esRAGE-to-pentosidine ratio in type 2 diabetic patients with VFs was significantly lower than in those without VFs (men: 7.1 ± 2.8 vs. 9.4 ± 6.2, $P = 0.013$, respectively; women: 4.7 ± 2.7 vs. 8.2 ± 5.4, $P < 0.001$, respectively). Multivariate logistic regression analysis adjusted for age, BMI, A1C, serum creatinine, duration of diabetes, therapeutic agents, diabetes complications, osteoporotic risk factors, and lumbar BMD identified the serum esRAGE level and esRAGE-to-pentosidine ratio as factors associated with the presence of VFs, independent of BMD in men (odds ratio [OR] 0.46 [95% CI 0.25–0.84], $P = 0.012$; and OR 0.34 [0.15–0.76], $P = 0.009$, respectively) and in women (OR 0.32 [0.16–0.67], $P = 0.002$; and OR 0.14 [0.04–0.43], $P = 0.001$, respectively).

CONCLUSIONS — These results show that serum esRAGE level and esRAGE-to-pentosidine ratio are more useful than BMD for assessing the risk of VFs in type 2 diabetic patients.

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The association between diabetes and osteoporosis has been investigated in many studies because these two disorders affect a large proportion of the elderly population. Recent meta-analyses of accumulating studies have shown that patients with type 2 diabetes have an increased risk for hip fracture compared with non–type 2 diabetic control subjects, despite their higher bone mineral density (BMD) (1,2). We have also shown that patients with type 2 diabetes have an increased risk for vertebral fractures (VFs) and that BMD at any site fails to assess the risk of VF (3). Because bone strength reflects integration of bone density and bone quality (4), these findings suggest that bone quality may be more important than bone density in defining bone strength in type 2 diabetic patients.

Bone quality is known to be determined by bone architecture, turnover, accumulation of microdamage, mineralization, and properties of bone matrix proteins such as collagen (4). In diabetic patients, advanced glycation end products (AGEs) are generated by sequential nonenzymatic glycosylation of protein amino groups (5). Pentosidine is one of the well-known AGEs, and its bone content in spontaneous diabetic rats has been shown to increase concurrently with the onset of diabetes, resulting in impaired mechanical properties of the bone despite normal BMD (6). We have shown clinically that the serum pentosidine level is associated with the presence of VFs in postmenopausal diabetic women independent of BMD (7). These findings suggest that AGEs, including pentosidine, may act as causative factors for poor bone quality in type 2 diabetic patients.

The receptor for AGEs (RAGE) belongs to the immunoglobulin superfamily of cell surface receptors and is capable of interacting with multiple ligands, including AGEs (8). When transgenic mice overexpressing human RAGE in vascular cells were crossbred with a transgenic line that develops insulin-dependent diabetes shortly after birth, a more progressive histological change of diabetic nephropathy was observed compared with controls (9), confirming that RAGE is associated with the development of diabetes complications. Endogenous secretory RAGE (esRAGE), a splice variant of one of the naturally occurring secretory forms, is known to carry all the extracellular domains but lacks the transmembrane and cytoplasmic domains (10). Secreted esRAGE in the extracellular space is thought to act as a decoy receptor that binds AGEs and results in reducing the activity of intercellular signal pathways via RAGE (10). Indeed, administration of a genetically engineered murine-soluble RAGE

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suppressed the development of diabetic atherosclerosis in a dose-dependent manner in streptozotocin-induced apoE-null diabetic mice (11). Recently, RAGE-knockout mice have been shown to increase BMD and biomechanical bone strength by decreasing osteoclast formation as well as serum levels of interleukin-6 and pyridinoline (12). We have also shown that the combination of high glucose with AGEs inhibits osteoblastic mineralization through glucose-induced increases in the expression of RAGE in vitro (13). These experimental findings suggest that enhanced RAGE activity may also be clinically linked to reduced bone strength in diabetic patients. Given the neutralizing nature of esRAGE, it is possible that the ratio of serum esRAGE-to-AGE levels could be linked to clinical bone problems, such as fractures, more prominently than either parameter alone.

To examine this issue, we compared serum levels of esRAGE and pentosidine as well as the esRAGE-to-pentosidine ratio between type 2 diabetic patients with and without VFs and evaluated the usefulness of these markers for assessing the risk of VFs in the population.

RESEARCH DESIGN AND METHODS

— We consecutively enrolled 277 Japanese patients with type 2 diabetes (137 men [age range 50–82 years] and 140 postmenopausal women [age range 46–87 years]) who underwent BMD measurements at the outpatient clinic of Shimane University Hospital. The patients were referred to our hospital from community clinics for the treatment of diabetes. We excluded patients who had higher-than-normal range of serum creatinine (normal range for women, 0.44–0.83 mg/dl; men, 0.56–1.23 mg/dl) or >300 mg albumin/g urine creatinine for urinary albumin excretion because serum esRAGE levels are known to be influenced by decreased renal function (14). We also excluded patients with an abnormality of calcium metabolism such as primary hyperparathyroidism or a history of falls or traffic accidents to eliminate the possibility of injury-associated fractures. We defined the onset of type 2 diabetes as the first time when glucosuria or hyperglycemia was noticed. None of the patients were taking any drugs or hormones that affected bone metabolism, including sex steroids, warfarin, and bisphosphonates. Baseline characteristics of the subjects are shown in Table 1. Serum esRAGE levels in men (0.294 ± 0.102 ng/ml) and women (0.257 ± 0.161 ng/ml) were equivalent to those previously reported in subjects with metabolic syndrome (0.253 ± 0.111 ng/ml) (15) and lower than those in normal control subjects (0.436 ± 0.121 ng/ml) (16). Serum pentosidine levels in men (0.0413 ± 0.0194 μg/ml) and women (0.0400 ± 0.0159 μg/ml) were higher than those previously reported in normal control subjects (0.0261 ± 0.0007 μg/ml) (17). There were 48 men (35%) and 61 women (44%) with diabetic retinopathy, whereas 83 men (61%) and 93 women (66%) had diabetic neuropathy. There were 95 men (69%) and 6 women (4%) who smoked >20 cigarettes/day and 86 (63%) men and 12 (9%) women with habitual alcohol drinking. This study was cross-sectional and was approved by the ethics review board of our institution, in compliance with the Declaration of Helsinki. All subjects agreed to participate in the study and provided written informed consent.

Table 1—*Background data of men and postmenopausal women with type 2 diabetes*

	Men	Women
n	137	140
VFs	52 (37.9)	41 (29.2)
VFs grade 2 or more	19 (13.9)	16 (11.4)
≥2 VFs	24 (17.5)	15 (10.7)
Age (years)	65.0 ± 7.9	66.9 ± 10.1
BMI (kg/m^2)	23.3 ± 3.3	24.5 ± 4.5
L-BMD (g/cm^2)	1.047 ± 0.198	0.883 ± 0.201
t score	−0.02 ± 1.65	−1.18 ± 1.80
Z score	0.53 ± 1.14	0.65 ± 1.38
FN-BMD (g/cm^2)	0.765 ± 0.128	0.646 ± 0.130
t score	−0.78 ± 0.95	−1.29 ± 1.19
Z score	0.31 ± 1.10	0.51 ± 1.23
R-BMD (g/cm^2)	0.691 ± 0.062	0.529 ± 0.088
t score	−1.66 ± 1.34	−2.57 ± 1.71
Z score	−0.51 ± 1.25	0.52 ± 1.56
Serum creatinine (mg/dl)	0.75 ± 0.15	0.60 ± 0.15
Urinary albumin excretion (mg alb/g urine Cr)	39.7 ± 54.4	30.4 ± 41.6
Fasting plasma glucose (mg/dl)	167 ± 62	168 ± 59
A1C (%)	8.9 ± 2.4	8.7 ± 2.1
Duration of diabetes (years)	11.8 ± 9.0	12.5 ± 9.8
Pentosidine (μg/ml)	0.0413 ± 0.0194	0.0400 ± 0.0159
esRAGE (ng/ml)	0.294 ± 0.102	0.257 ± 0.161
esRAGE-to-pentosidine ratio	8.5 ± 5.3	7.2 ± 5.1
BAP (units/l)	26.0 ± 7.6	31.6 ± 12.9
uNTX (nmol BCE/mmol Cr)	31.6 ± 15.5	52.6 ± 34.2
Use of insulin secretagogue	50 (36)	51 (36)
Use of metformin	28 (20)	38 (27)
Use of pioglitazone	18 (13)	14 (10)
Use of insulin	25 (18)	38 (27)
Diabetic retinopathy	48 (35)	61 (44)
Diabetic neuropathy	83 (61)	93 (66)
Smoking	95 (69)	6 (4)
Alcohol	83 (61)	12 (9)

The data are expressed as *n*, *n* (%), or means ± SD. alb, albumin; BCE, bone collagen equivalents; Cr, creatinine.

Biochemical measurements

Fasting blood was obtained, and fasting plasma glucose (FPG), A1C, and serum creatinine were measured by automated techniques at the central laboratory of our hospital. Serum bone-specific alkaline phosphatase (BAP) and urinary N-telopeptide (uNTX) were commercially measured using enzyme-linked immunosorbent assay (ELISA).

Serum concentrations of human esRAGE were measured using an esRAGE

Table 2—*Simple regression analysis between esRAGE level and various parameters*

	Men		Women	
	r	*P*	*r*	*P*
Age (years)	0.164	0.053	−0.003	0.976
BMI (kg/m^2)	−0.086	0.323	−0.046	0.590
L-BMD (g/cm^2)	−0.023	0.791	0.044	0.615
t score	−0.016	0.849	0.040	0.647
Z score	−0.015	0.864	0.042	0.627
FN-BMD (g/cm^2)	−0.147	0.087	−0.085	0.325
t score	−0.136	0.113	−0.078	0.369
Z score	−0.064	0.459	0.068	0.466
R-BMD (g/cm^2)	−0.126	0.143	0.077	0.383
t score	−0.186	0.029*	0.069	0.435
Z score	−0.129	0.134	0.086	0.324
Serum creatinine (mg/dl)	0.377	<0.001†	0.048	0.571
Urinary albumin excretion (mg alb/g urine Cr)	0.039	0.654	−0.104	0.257
Fasting plasma glucose (mg/dl)	−0.106	0.219	−0.109	0.199
A1C (%)	−0.077	0.371	−0.119	0.162
Duration of diabetes (years)	0.086	0.319	0.093	0.278
Pentosidine (μg/ml)	0.102	0.235	0.097	0.255
BAP (units/l)	0.101	0.242	−0.033	0.699
uNTX (nmol BCE/mmol Cr)	0.026	0.766	−0.077	0.371

*$P < 0.05$; †$P < 0.01$. alb, albumin; BCE, bone collagen equivalents; Cr, creatinine.

ELISA kit (Daiichi Fine Chemicals, Takaoka, Japan) as reported previously (10,14). The interassay coefficient of variation (CV) for repeated measurements ranged from 2.5 to 5.1%. Serum pentosidine levels were detected using a competitive ELISA kit (FSK pentosidine ELISA kit; Fushimi Pharmaceutical, Kagawa, Japan) as described previously (7,18). The inter- and intra-assay CVs of absorbance were 6.6 and 8.0%, respectively. This ELISA was highly correlated with the conventional high-performance liquid chromatography method ($r = 0.9356$) (18).

Assessment of fractures

In all subjects VFs were identified on lateral X-ray films of the thoracic and lumbar spine according to the semiquantitative method (19) by two investigators who were blinded to the other's readings. VFs were classified as follows: mild (grade 1), a reduction of 20–25%; moderate (grade 2), 25–40%; severe (grade 3), >40%. If judgment of VFs was not in agreement, the film was independently reassessed. If the reevaluated findings were still different, we regarded the case as a milder grade.

Densitometry

BMD values of the lumbar spine (L), the femoral neck (FN), and one-third the radius (R) were measured by dual-energy X-ray absorptiometry using the QDR-4500 system (Hologic, Waltham, MA). Values were expressed relative to the SD of age- and sex-matched normal Japanese mean values of BMD provided by the manufacturer (Z score). The coefficients of variation of measurement of the L-, FN-, and R-BMD were 1.0, 1.0, and <1.0%, respectively.

Statistical analysis

All data are expressed as the means ± SD for each index. An unpaired *t* test was used to compare parameters between subjects with and without VFs. Comparisons of categorical variables were made using a χ^2 test. Statistical analyses were performed using the computer program StatView for Windows, version 5.0 (SAS Institute, Cary, NC).

RESULTS— Simple regression analysis revealed that serum esRAGE level in diabetic men was significantly and positively correlated with creatinine ($r = 0.377$; $P < 0.001$) and significantly and negatively correlated with *t* score of R-BMD ($r = -0.186$; $P = 0.029$). There were no significant correlations between serum esRAGE levels and age, BMI, other BMD values, A1C, duration of diabetes, serum pentosidine level, or bone metabolic markers in either sex (Table 2).

We compared biochemical parameters, including serum esRAGE level and esRAGE-to-pentosidine ratio, between type 2 diabetic patients with and without VFs for each sex (Table 3). Diabetic men with VFs were significantly older ($P < 0.001$) and shorter in height ($P < 0.001$), and they had a significantly lower esRAGE-to-pentosidine ratio ($P = 0.013$) than those without VFs. Diabetic women with VFs were significantly older ($P < 0.001$) and shorter in height ($P = 0.021$), and they had a significantly higher serum pentosidine level ($P = 0.006$) and significantly lower values for the R-BMD *t* score ($P = 0.047$), serum esRAGE ($P = 0.007$), and esRAGE-to-pentosidine ratio ($P < 0.001$) than those without VFs (Table 4). There were no significant differences in other BMD values, bone metabolic markers, or demographic confounders such as therapeutic agents, diabetes complications, smoking status, or alcohol consumption between those with and without VFs for either sex.

To determine the association between the presence of VFs and serum esRAGE, pentosidine, and esRAGE/pentosidine ratio, logistic analyses were performed (Table 4). When no adjustment was made (model 1), esRAGE-to-pentosidine ratio in men and serum esRAGE, pentosidine, and esRAGE-to-pentosidine ratio in women were significantly associated with VFs (men: esRAGE-to-pentosidine OR per SD increase 0.53 [95% CI 0.31–0.88], $P = 0.015$; and women: esRAGE OR 0.53 [0.33–0.85], $P = 0.009$; pentosidine OR 1.65 [1.13–2.41], $P = 0.010$; esRAGE-to-pentosidine OR 0.34 [0.19–0.62], $P < 0.001$). In contrast, no associations between VFs and BMD at any site were found in either sex. esRAGE level in men became significantly associated with VFs after adjustments for age, BMI, A1C, and creatinine level (model 2). These observations remained significant when multivariate logistic regression analysis was performed after the addition of therapeutic agents, the presence of diabetes complications, and risk factors for osteoporosis (e.g., smoking and habitual alcohol drinking) (model 3). However, esRAGE, pentosidine, or esRAGE-to-pentosidine ratio was not associated with severe VFs, including grade 2/3 or multiple ones (data not shown).

CONCLUSIONS— This is the first clinical study to show that serum esRAGE level is significantly and negatively associated with the presence of prevalent VFs in patients with type 2 diabetes. This association was independent of BMD, bone

Table 3—*Comparison of various parameters between type 2 diabetic patients with and without VFs*

	Men			Women		
	VFs			VFs		
	No	Yes	*P*	No	Yes	*P*
n	85	52		99	41	
Age (years)	62.2 ± 8.0	68.0 ± 6.8	<0.001*	64.5 ± 9.5	73.0 ± 3.7	<0.001*
Body height (cm)	165.8 ± 6.3	162.1 ± 5.9	<0.001*	151.0 ± 5.6	148.6 ± 5.3	0.021†
Body weight (kg)	63.8 ± 10.8	62.0 ± 9.5	0.337	55.4 ± 10.0	55.5 ± 12.3	0.956
BMI (kg/m^2)	23.1 ± 3.5	23.5 ± 3.2	0.514	24.3 ± 3.9	25.2 ± 5.6	0.276
L-BMD (g/cm^2)	1.072 ± 0.217	1.006 ± 0.155	0.056	0.892 ± 0.197	0.853 ± 0.207	0.306
t score	0.19 ± 1.81	−0.35 ± 1.30	0.062	−1.10 ± 1.78	−1.43 ± 1.86	0.345
Z score	0.64 ± 1.28	0.35 ± 0.86	0.142	0.62 ± 1.38	0.65 ± 1.40	0.935
FN-BMD (g/cm^2)	0.772 ± 0.127	0.754 ± 0.101	0.383	0.657 ± 0.138	0.616 ± 0.104	0.097
t score	−0.73 ± 1.04	−0.85 ± 0.80	0.473	−1.19 ± 1.24	−1.56 ± 0.96	0.095
Z score	0.28 ± 1.21	0.40 ± 0.90	0.690	0.52 ± 1.25	0.48 ± 1.22	0.846
R-BMD (g/cm^2)	0.697 ± 0.062	0.682 ± 0.063	0.180	0.537 ± 0.092	0.507 ± 0.074	0.067
t score	−1.52 ± 1.36	−1.88 ± 1.28	0.123	−2.39 ± 1.78	−3.04 ± 1.45	0.047†
Z score	−0.48 ± 1.30	−0.57 ± 1.19	0.682	0.50 ± 1.60	0.58 ± 1.50	0.794
Serum creatinine (mg/dl)	0.75 ± 0.16	0.77 ± 0.14	0.396	0.59 ± 0.14	0.64 ± 0.15	0.063
Urinary albumin excretion (mg alb/g urine Cr)	34.3 ± 46.7	48.0 ± 64.2	0.161	29.4 ± 41.2	33.1 ± 40.0	0.647
Fasting plasma glucose (mg/dl)	172 ± 68	158 ± 49	0.214	166 ± 57	173 ± 64	0.550
A1C (%)	9.1 ± 2.5	8.5 ± 2.0	0.144	8.8 ± 2.1	8.5 ± 2.1	0.557
Duration of diabetes (years)	12.0 ± 9.0	11.5 ± 9.1	0.757	11.1 ± 9.0	16.0 ± 10.6	0.007*
Pentosidine (μg/ml)	0.0388 ± 0.0162	0.0453 ± 0.0233	0.059	0.0377 ± 0.0145	0.0458 ± 0.0181	0.006*
esRAGE (ng/ml)	0.303 ± 0.112	0.280 ± 0.083	0.205	0.282 ± 0.173	0.202 ± 0.109	0.007*
esRAGE-to-pentosidine ratio	9.4 ± 6.2	7.1 ± 2.8	0.013†	8.2 ± 5.5	4.7 ± 2.7	<0.001*
BAP (U/l)	26.3 ± 7.7	25.6 ± 7.5	0.635	31.4 ± 13.1	31.9 ± 12.8	0.860
uNTX (nmol BCE/mmol Cr)	32.5 ± 17.6	30.3 ± 11.3	0.428	50.3 ± 29.8	56.1 ± 41.3	0.360
Use of insulin secretagogue	27 (32)	23 (44)	0.197	34 (34)	17 (41)	0.573
Use of metformin	17 (20)	11 (21)	0.999	28 (28)	10 (24)	0.766
Use of pioglitazone	12 (14)	6 (12)	0.842	8 (8)	6 (15)	0.423
Use of insulin	17 (20)	8 (15)	0.630	27 (27)	11 (27)	0.999
Diabetic retinopathy	30 (35)	18 (35)	0.999	43 (43)	18 (44)	0.718
Diabetic neuropathy	50 (59)	33 (63)	0.915	64 (64)	39 (95)	0.627
Smoking	62 (73)	33 (63)	0.412	4 (4)	2 (5)	0.999
Alcohol	49 (58)	34 (65)	0.579	10 (10)	2 (5)	0.490

Data are expressed as *n*, *n* (%), or means ± SD. Unpaired *t* test: *$P < 0.01$; †$P < 0.05$. Comparisons of categorical variables were made using χ^2 test. alb, albumin; BCE, bone collagen euivalents; Cr, creatinine.

metabolic markers, therapeutic agents, diabetes complications, and risk factors for osteoporosis. esRAGE is known to bind various ligands, including AGEs, in the extracellular space and to inhibit the connection between cell surface RAGE and ligands (10). Thus, an insufficient amount of esRAGE to counteract AGEs could intensify the binding of AGEs to RAGE and exert harmful effects on organs via RAGE-transmitted signals in diabetic patients. We found that esRAGE was negatively associated with VFs, whereas pentosidine showed a positive association. esRAGE-to-pentosidine ratio was thought to be more suitable to assess the risk of VFs than serum esRAGE level alone because of its more significant *P* values. These findings support the concept that esRAGE could be beneficial to bones in those with type 2 diabetes by neutralizing the harmful effects of pentosidine or other AGEs rather than by direct activity.

We found that esRAGE was more significantly associated with VFs than pentosidine in diabetic patients. The association between esRAGE and VFs was significant in both sexes after adjustments for multivariate, whereas the association between pentosidine and VFs was only significant in women. Thus, esRAGE seems to be more useful than pentosidine to assess the risk of VFs irrespective of sex in type 2 diabetes. Several studies have shown that not only pentosidine but also other AGEs are harmful to bone. It has been documented that RAGE is expressed in human bone-derived cells (20) and that AGE-BSA inhibits the synthesis of type I collagen and osteocalcin in primary human osteoblasts and the secretion of parathyroid hormones from human parathyroid cells (21). We have also shown that combination of high glucose with either AGE2 or AGE3 inhibits osteocalcin expression and mineralization through glucose-induced increases in RAGE expression in cultured osteoblasts (13). Thus, various kinds of AGEs seem to impair bone matrix production and mineralization of osteoblasts, which may lead to the bone fragility seen in diabetic patients. Furthermore, esRAGE is known to interact with nonglycated proteins such as

Table 4—*Associations between serum esRAGE level, serum pentosidine level, esRAGE-to-pentosidine ratio, and BMD versus the presence of VFs in type 2 diabetic patients*

	Men		Women	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Model 1				
esRAGE	0.79 (0.55–1.14)	0.206	0.53 (0.33–0.85)	0.009*
Pentosidine	1.39 (0.98–1.99)	0.067	1.65 (1.13–2.41)	0.010†
esRAGE-to-pentosidine ratio	0.53 (0.31–0.88)	0.015†	0.34 (0.19–0.62)	<0.001*
L-BMD	0.70 (0.49–1.01)	0.059	0.81 (0.55–1.21)	0.304
FN-BMD	0.85 (0.59–1.22)	0.381	0.72 (0.48–1.07)	0.099
R-BMD	0.79 (0.55–1.12)	0.181	0.70 (0.47–1.03)	0.069
Model 2				
esRAGE	0.61 (0.38–0.96)	0.032†	0.47 (0.27–0.80)	0.006*
Pentosidine	1.34 (0.89–2.03)	0.164	1.80 (1.08–2.98)	0.023†
esRAGE-to-pentosidine ratio	0.47 (0.25–0.85)	0.013†	0.28 (0.13–0.60)	0.001*
Model 3				
esRAGE	0.46 (0.25–0.84)	0.012†	0.32 (0.16–0.67)	0.002*
Pentosidine	1.49 (0.91–2.42)	0.111	1.82 (1.05–3.15)	0.034†
esRAGE-to-pentosidine ratio	0.34 (0.15–0.76)	0.009*	0.14 (0.04–0.43)	0.001*

Model 1: no adjustment (crude risk for vertebral fractures). Model 2: independent variables were adjusted for age, BMI, A1C, and creatinine. Model 3:model 2 additionally adjusted for duration of diabetes, L-BMD, therapeutic agents, the presence of diabetic complications, and risk factors for osteoporosis (smoking and habitual alcohol drinking). *$P < 0.01$; †$P < 0.05$.

proinflammatory calcium-binding S100/calgranulins proteins and nuclear high-mobility group protein box-1 (HMGB1), which are released by cellular stress through RAGE (22). Given that esRAGE has extensive neutralizing effects against various AGEs that are harmful to bone as well as pentosidine, it seems reasonable that esRAGE was more highly associated with the presence of VFs than pentosidine in type 2 diabetic patients in this study.

This study has some limitations. First, it was not population-based, and the sample size was not large enough to make definitive conclusions. Second, the patients enrolled in this study might have had relatively severe cases of type 2 diabetes and might not be representative of standard Japanese diabetic patients, given that the diabetic conditions of the patients who attended Shimane University Hospital, a tertiary care center, were considered to be more serious than those of other diabetic patients. Third, we did not exclude patients with confounders known to affect bone strength to avoid a considerable reduction in the study population. Diabetic complications such as diabetic retinopathy, longer diabetes duration, and a history of insulin treatment are known to be associated with non-VFs in diabetic patients (23). A recent meta-analysis showed that long-term use of thiazolidinediones was associated with an increased risk of fractures and reduced L- and FN-BMD in female type 2 diabetic patients (24). Multiple logistic regression analysis in this study, however, revealed that both serum esRAGE levels and the esRAGE-to-pentosidine ratio were associated with the presence of VFs independent of diabetes complications, duration of diabetes, or therapeutic agents including insulin and pioglitazone, suggesting that none of these factors affected our observations. Finally, BMI of our patients was 23.3 in men and 24.5 in women, showing that they were less obese by Western standards. It is well known that low BMI increases risk for all kind of fractures (25). Although the significant results were still obtained after adjustment for BMI in the present study, our findings need to be confirmed in other ethnic groups.

In conclusion, we found that serum esRAGE level as well as esRAGE-to-pentosidine ratio were significantly and inversely associated with the presence of prevalent VFs in type 2 diabetic patients. These associations were independent of BMD and diabetes-related confounders and were stronger than serum pentosidine level alone. These findings suggest that the AGE-RAGE system as a whole may affect bone quality, which is not determined by BMD. The present study as well as our previous studies (3) suggest that BMD may not be sensitive enough to assess the risk of VFs, given that BMD was not associated with the presence of VFs in type 2 diabetic patients. Serum esRAGE level and esRAGE-to-pentosidine ratio seem to be useful surrogate markers, which could compensate for the ineffectiveness of BMD in evaluating the risk of VFs in type 2 diabetic patients. However, our study was cross-sectional, and we did not investigate non-VFs in the subjects. Longitudinal studies on both VFs and non-VFs are needed to prove the importance of the AGE-RAGE system in assessing the fracture risk of type 2 diabetic patients.

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References

1. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes: a meta-analysis. Osteoporos Int 2007;18:427–444
2. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol 2007;166:495–505
3. Yamamoto M, Yamaguchi T, Yamauchi M, Kaji H, Sugimoto T. Diabetic patients have an increased risk of vertebral fractures independent of BMD or diabetic complications. J Bone Miner Res 2009;24:702–709
4. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, March 7–29, 2000: highlights of the conference. South Med J 2001;94:569–573
5. Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev Med 1995;46:223–234
6. Saito M, Fujii K, Mori Y, Marumo K. Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. Osteoporos Int 2006;17:1514–1523
7. Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T. Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 2008;93:1013–1019
8. Kislinger T, Fu C, Huber B, Qu W, Taguchi A, Du Yan S, Hofmann M, Yan SF, Pischetsrieder M, Stern D, Schmidt AM.

N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. J Biol Chem 1999; 274:31740–31749
9. Yamamoto Y, Kato I, Doi T, Yonekura H, Ohashi S, Takeuchi M, Watanabe T, Yamagishi S, Sakurai S, Takasawa S, Okamoto H, Yamamoto H. Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. J Clin Invest 2001;108:261–268
10. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, Yamamoto H. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem J 2003;370:1097–1109
11. Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ Jr, Chow WS, Stern D, Schmidt AM. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. Nat Med 1998;4:1025–1031
12. Ding KH, Wang ZZ, Hamrick MW, Deng ZB, Zhou L, Kang B, Yan SL, She JX, Stern DM, Isales CM, Mi QS. Disordered osteoclast formation in RAGE-deficient mouse establishes an essential role for RAGE in diabetes related bone loss. Biochem Biophys Res Commun 2006;340: 1091–1097
13. Ogawa N, Yamaguchi T, Yano S, Yamauchi M, Yamamoto M, Sugimoto T. The combination of high glucose and advanced glycation end-products (AGEs) inhibits the mineralization of osteoblastic MC3T3–E1 cells through glucose-induced increase in the receptor for AGEs. Horm Metab Res 2007;39:871–875
14. Koyama H, Yamamoto H, Nishizawa Y. RAGE and soluble RAGE: potential therapeutic targets for cardiovascular diseases. Mol Med 2007;13:625–635
15. Koyama H, Shoji T, Yokoyama H, Motoyama K, Mori K, Fukumoto S, Emoto M, Shoji T, Tamei H, Matsuki H, Sakurai S, Yamamoto Y, Yonekura H, Watanabe T, Yamamoto H, Nishizawa Y. Plasma level of endogenous secretory RAGE is associated with components of the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25: 2587–2593
16. Katakami N, Matsuhisa M, Kaneto H, Matsuoka TA, Sakamoto K, Nakatani Y, Ohtoshi K, Hayaishi-Okano R, Kosugi K, Hori M, Yamasaki Y. Decreased endogenous secretory advanced glycation end product receptor in type 1 diabetic patients: its possible association with diabetic vascular complications. Diabetes Care 2005;28:2716–2721
17. Yoshida N, Okumura K, Aso Y. High serum pentosidine concentrations are associated with increased arterial stiffness and thickness in patients with type 2 diabetes. Metabolism 2005;54:345–350
18. Sanaka T, Funaki T, Tanaka T, Hoshi S, Niwayama J, Taitoh T, Nishimura H, Higuchi C. Plasma pentosidine levels measured by a newly developed method using ELISA in patients with chronic renal failure. Nephron 2002;91:64–73
19. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res 1993;8:1137–1148
20. Takagi M, Kasayama S, Yamamoto T, Motomura T, Hashimoto K, Yamamoto H, Sato B, Okada S, Kishimoto T. Advanced glycation endproducts stimulate interleukin-6 production by human bone-derived cells. J Bone Miner Res 1997;12:439–446
21. Yamamoto T, Ozono K, Miyauchi A, Kasayama S, Kojima Y, Shima M, Okada S. Role of advanced glycation end products in adynamic bone disease in patients with diabetic nephropathy. Am J Kidney Dis 2001;38:S161–S164
22. Yan SF, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab 2008;4: 285–293
23. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ, Blue Mountains Eye Study. Diabetes and risk of fracture: The Blue Mountains Eye Study. Diabetes Care 2001;24:1198–1203
24. Loke YK, Singh S, Furberg CD. Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. CMaj 2009;180:32–39
25. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey EV, Mellstrom D, Melton LJ 3rd, Meunier PJ, Pols HA, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int 2005;16:1330–1338

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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Bibliography
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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