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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