Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : December 9, 2014
Accepted : January 11, 2015
Published online : March 31, 2015

Review Article

New treatment strategy against osteoporosis: Advanced glycation end products as a factor for poor bone quality

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Abstract

Based on the present definition of osteoporosis, both bone density and quality are important factors in the determination of bone strength. Collagen crosslinking is a determinant of bone quality. Collagen cross-link, a major post-translational modification of collagen, plays important roles in the biological and biomechanical features of bone. Collagen cross-links can be divided into lysyl hydroxylase and lysyl oxidase-mediated enzymatic immature divalent cross-links, mature trivalent pyridinoline and pyrrole cross-links, and glycation- or oxidation-induced non-enzymatic cross-links (advanced glycation end products: AGEs) such as glucosepane and pentosidine. These types of cross-links differ in the mechanism of formation and in function. Material properties of newly synthesized collagen matrix may differ in tissue maturity and senescence from older matrix in terms of cross-link formation. Additionally, newly synthesized matrix in osteoporotic patients or diabetic patients may not necessarily be as well-made as age-matched healthy subjects. Data have accumulated that collagen cross-link formation affects not only the mineralization process, but also microdamage formation. Consequently, collagen cross-linking is thought to affect the mechanical properties of bone. Furthermore, recent basic and clinical investigations of collagen cross-links seem to face a new era. For instance, serum or urine pentosidine levels are now being used to estimate future fracture risk in osteoporosis and diabetes. In this review, we describe age-related changes in collagen cross-links in bone and abnormalities of cross-links in osteoporosis and diabetes that have been reported in the literature.

***KEY WORDS*:** osteoporosis, bone quality, collagen cross-links, advanced glycation end products, pentosidine

Introduction

Bone mineral density (BMD) decreases as female sex hormones decrease with menopause and as bone resorption increases and calcium absorption decreases with age, resulting in increased risk of bone fracture. In the 2010 Consensus Development Conference, the National Institute of Health (NIH) proposed the concept of bone quality (BQ) as a factor other than BMD that affects bone strength. At the conference, osteoporosis was defined as a disorder that decreases bone strength and in which bone strength is determined by BMD and BQ [1)]. BQ is determined by bone material properties and structural properties (microstructure: porosity of the cortical bone and trabecular structure of the cancellous bone) [2)].

BMD and microstructure are regulated by bone remodeling, which is bone metabolism (bone formation with bone resorption). Approximately 40% of the cancellous bone and 4-7% of the cortical bone are continually replaced annually due to bone remodeling, enabling bone structure and BMD to be maintained. In the remodeling process, there is minimal reduction in bone mass because the level of bone resorption due to osteoclasts is similar to the level of bone formation due to osteoblasts. However, the level of resorption can exceed the level of formation due to aging, reduced estrogen levels associated with menopause, or reduced androgen levels in men. Bone microstructure deteriorates and BMD decreases in such individuals.

Bone material properties are affected by various factors. Aging bone tissue is repaired in the process of bone remodeling. However, tissue degradation markedly advances due to increases in oxidative stress, glycation stress, reactive oxygen species, and carbonyl stress associated with aging and reduced sex hormone levels. Bone material properties are determined by the quality of the structural components. The structural components of the bone consist of mineral components made of hydroxyapatite and organic components made of collagen proteins. Collagen accounts for approximately 20% of bone by weight but 50% of bone by volume [2)]. Bone is like a reinforced concrete building where collagen is reinforcing bars and minerals are concrete. When reinforced concrete building deteriorates, repair work is done to prevent a collapse. In coastal reinforced concrete structures, reinforcing bars rust severely due to salt air damage and earthquake resistance decreases even if repair work is periodically performed. A similar process has been

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shown to occur in bones [2]. That is, collagen deteriorates with age due to increases in reactive oxygen species, oxidative stress, glycation stress, and carbonyl stress which correspond to salt air damage in reinforced concrete buildings. As a result, bone strength decreases. Approximately 30% of bone strength is determined by BQ in primary osteoporosis. When lifestyle disease-related osteoporosis, secondary osteoporosis, was examined, poor BQ was found to markedly decrease bone strength [3,4]. These lifestyle diseases included diabetes, arteriosclerosis, and renal impairment (to be described later).

In osteoporosis, bone strength is decreased due to excessive collagen deterioration with age. This decreased bone strength cannot be explained by increased bone remodeling. In other words, deterioration of bone collagen cannot be assessed using markers (bone formation and bone resorption markers) that reflect bone remodeling or using calcium-based analysis. Deterioration of collagen is affected by reduction in osteoblast function and by glycation and oxidative stress involving matrix proteins [2]. Therefore, the assessment of bone fracture risk needs to evaluate material properties of collagen as well as structures and calcium-based parameters that are dependent on bone remodeling. Such assessment is necessary to evaluate fracture risk with good accuracy. In recent basic and clinical studies, the authors of the present article found that osteoporosis is not of a single disease type but can be classified into multiple types depending on BMD and BQ (bone collagen) and proposed tailor-made treatment according to this classification [5].

BQ (material property) factor: bone collagen

Collagen maturity and deterioration with age are determined by the formation of intermolecular cross-links in post-translational modification [2]. Collagen is made by strong bonds created from bridges between collagen molecules or "cross-links" (corresponding to beams connecting reinforcing bars in the building structure analogy) (***Fig.1***). Collagen cross-links are classified into two types. One type is an enzyme-dependent cross-link ("beneficial" cross-link). It involves an orderly bonding of molecules due to the actions of enzymes whose secretion is regulated by osteoblasts. This type of cross-link enables appropriate elasticity as mineralization is induced, and the resulting bone has flexibility and strength [6-8]. The other type is an advanced glycation end product (AGE) cross-link, a "detrimental" aged cross-link. It involves disorderly bonding of molecules, and the resulting bone is excessively rigid and becomes brittle like porcelain (***Figs.2 & 3***) [2]. Fragility of bone increases with decreased enzyme-dependent cross-links and increased AGEs [2-4], and intermolecular cross-linking of collagen occurs through a mechanism that is independent of bone remodeling. This mechanism is regulated by the level of cellular function and systemic factors involving bone matrix, such as oxidative stress (high homocysteine levels and arteriosclerosis) and glycation (diabetes) (***Figs.2 & 4***). Based on the aforementioned findings, one can increase the accuracy in the assessment of bone fracture risk by simultaneously measuring BMD and assessing BQ from the perspective of collagen [2,4,9-11]. The amount of AGEs in bone is positively correlated with serum and urinary pentosidine levels. High levels of serum and urinary pentosidine are a factor of fracture risk [9-11]. Pentosidine is one of the AGEs and has been used as a surrogate marker that reflects the total amount of AGEs. Methods are being developed to measure pentosidine levels in body tissues and fluids [2]. In 2012, the Japan Osteoporosis Society published the Japanese Guidelines for the Use of Biomarkers of Bone Turnover in Osteoporosis (Life Science Publishing). This publication described the following as bone matrix markers: serum and urinary pentosidine and homocysteine, which induces abnormal collagen cross-linking. The Japan Osteoporosis Society also published the Japanese 2013 Guidelines for Prevention and Treatment of Osteoporosis and the Clinical Practice Guide on Fracture Risk Associated with Lifestyle-related Diseases. These two publications included the concept involving the mechanism of bone fragility caused by deterioration of bone collagen. Our laboratory was the first in the world to develop this concept. In these publications, our laboratory members were contributing authors in charge of the content related to the aforementioned, and the concept of osteoporosis was greatly changed in Japan.

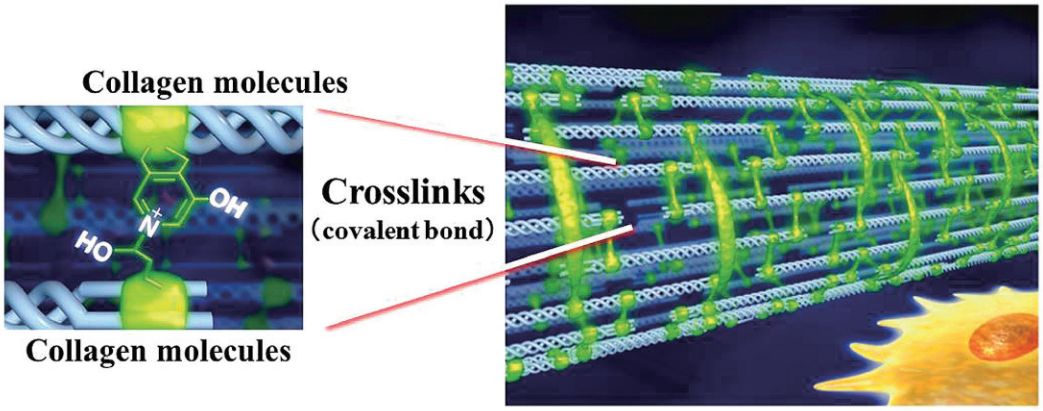
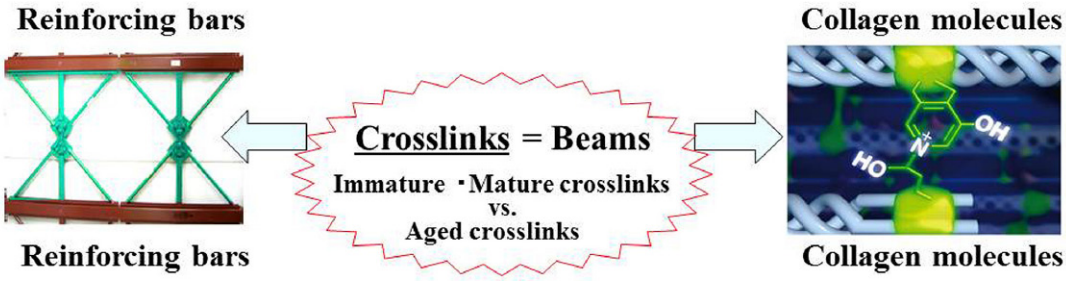


***Fig.1*: Collagen cross-links**

A collagen fiber consists of aggregates of collagen molecules and its strength is dependent on the formation of intermolecular cross-links.



	Enzyme-dependent crosslinks	Non-physiological crosslinks (AGEs)
Triggers	Enzyme reaction ・lysyl oxidase	・Oxidation ・Carbonyl stress ・Glycation
Bone strength	Essential for calcification	Inhibiting calcification
	↑ Flexible ・ Stout	↓ Fragile ・ Chalk-like
	Immature → mature cross links (good)	Aged AGE cross links (bad)

Immature, lysinonorleucine crosslinks; mature, pyridinoline crosslinks

***Fig.2*: Classification and functions of collagen cross-links**

The strength of collagen fibers is determined by collagen cross-links, which bond adjacent molecules. In an analogy with building structures, collagen cross-links correspond to beams that connect reinforcing bars. Collagen cross-links can be classified into two types: beneficial, physiological cross-links that increase bone strength and detrimental, non-physiological cross-links that weaken the bone. Detrimental cross-links are advanced glycation end products (AGEs) known to be products of aging. In the building structure hydroxylysine analogy, detrimental cross-links are like rust that accumulates on reinforcing bars.

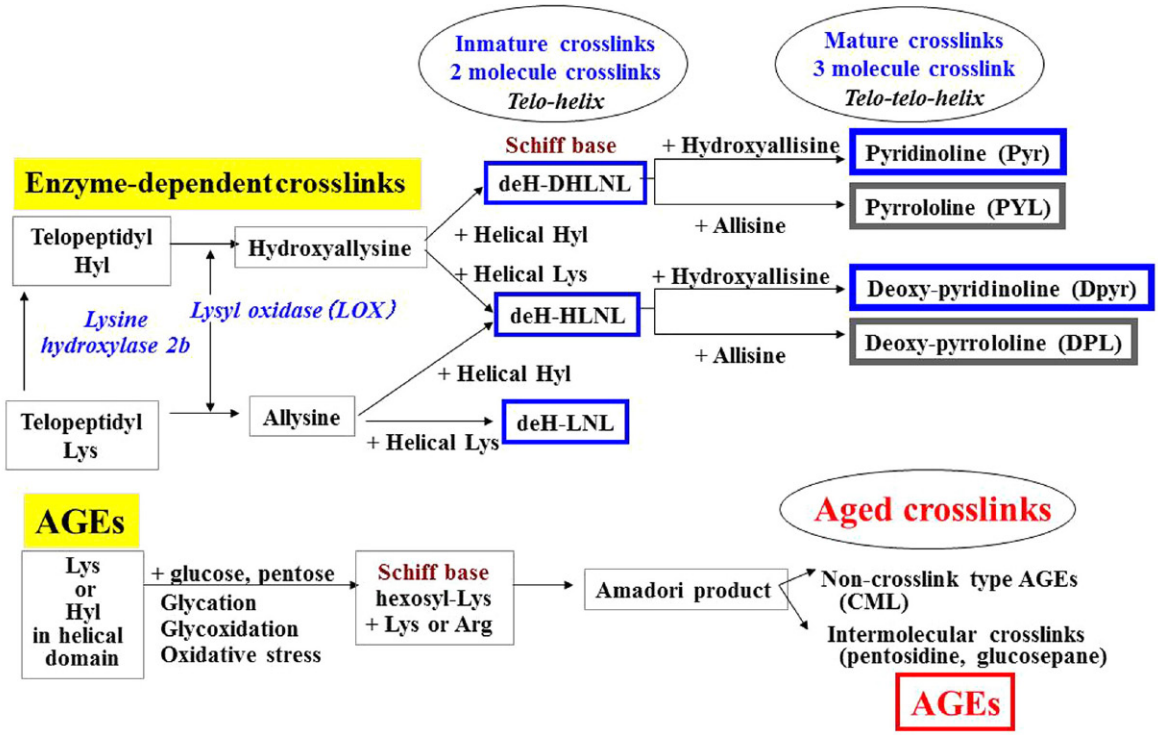


***Fig.3*: Biochemistry of collagen cross-links**

AGEs, advanced glycation end products; CML, N^{ε}-(carboxymethyl)lysine; Lys, lysine; Hyl, hydroxylysin; Arg, arginine; deH-DHLNL, dehydrodihydroxynorleucine; deH-HLNL, dehydro-hydroxylysinonorleucine; deH-LNL, dehydro-lysinonorleucine.

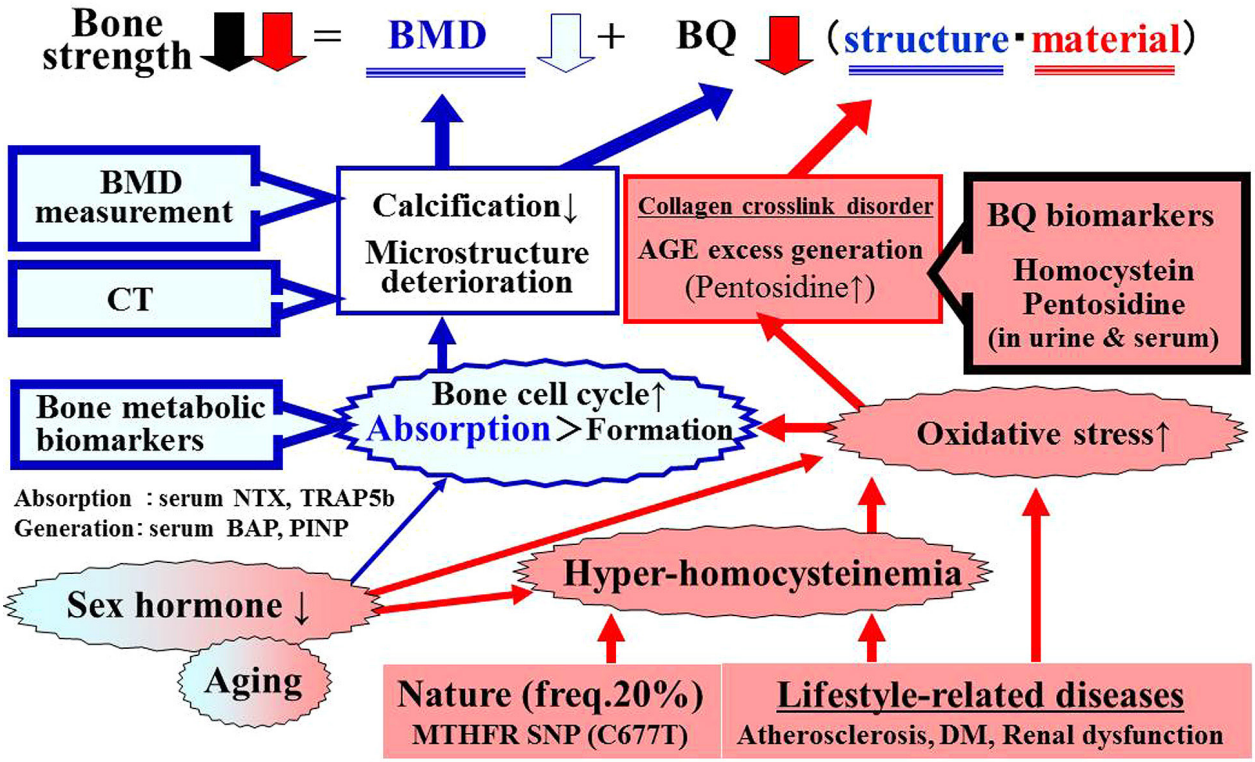


***Fig.4*: Mechanism of reduction in bone strength**

Bone quality (BQ) is determined by bone material qualities and structural properties (microstructure) based on the material make-up. Sex hormone deficiency, aging, and lifestyle-related diseases induce not only reduction in bone mineral density (BMD) but also reduction in BQ, particularly involving an AGE increase in collagen. Thus, bone strength is negatively affected. BMD and bone microstructure are parameters of bone strength that are dependent on bone remodeling. Material properties among bone quality factors are inhibited by the level of cellular function and the environment of the matrix surroundings (oxidative stress and glycation level). CT, computed tomography; AGE, advanced glycation end product; NTX, collagen type 1 cross-linked N-telopeptide; TRAP5b, band 5 tartrate-resistant acid phosphatase; BAP, bone alkaline phosphatase; PINP, procollagen type I N-terminal propeptide; freq.,frequency; MTHFR, methylenetetrahydrofolate reductase; SNP, single nucleotide polymorphism; DM, diabetes mellitus.

Necessity of markers for poor BQ

Oxidation and glycation increase when there is primary osteoporosis (*Fig.5a*)[12-14], diabetes (*Fig.5b*)[15], or renal failure (*Fig.5c*)[16]. In these diseases, our laboratory has shown that reduced formation of enzyme-dependent cross-links and excessive AGE formation are induced in bone collagen, resulting in reduced bone strength. It has also been reported that AGEs increase in bone collagen with aging and bone strength decreases[2-4,17]. Bone remodeling is increased due to aging and reduced sex hormones in both men and women. When bone remodeling is increased, collagen metabolism greatly increases. Therefore, it was unexpected that AGEs increase in bone collagen because AGEs are formed in proteins with long lifespans. However, there are factors related to AGE formation other than the lifespan of matrix. If there is an environment that increases oxidation, glycation, or carbonyl stress (such as aging and lifestyle-related diseases), AGE formation is easily induced even with increased remodeling and shortened collagen lifespan (Figs.2 & 4)[2]. Not surprisingly, if bone remodeling is decreased and oxidation and glycation are increased, AGE formation is markedly increased in bone collagen. Diabetes corresponds to this type of pathological condition[15]. The aforementioned findings indicate that the assessment of bone fragility needs not only to measure calcium-based parameters and bone metabolism markers reflecting bone remodeling but also to simultaneously evaluate the deterioration of BQ. From such a perspective, one needs to understand the mechanism of detrimental AGE cross-linking and beneficial enzyme-dependent cross-linking in collagen[2-4].

Enzyme-dependent (beneficial) cross-links and bone strength (Fig.2)

Enzyme-dependent cross-links are formed in an orderly manner during collagen maturation and promote mineralization. The total number of such cross-links is dependent on the activity of lysyl oxidase, an enzyme secreted by osteoblasts themselves[6-8]. This cross-link formation plateaus before osteoid mineralizes[8]. If the enzyme activity

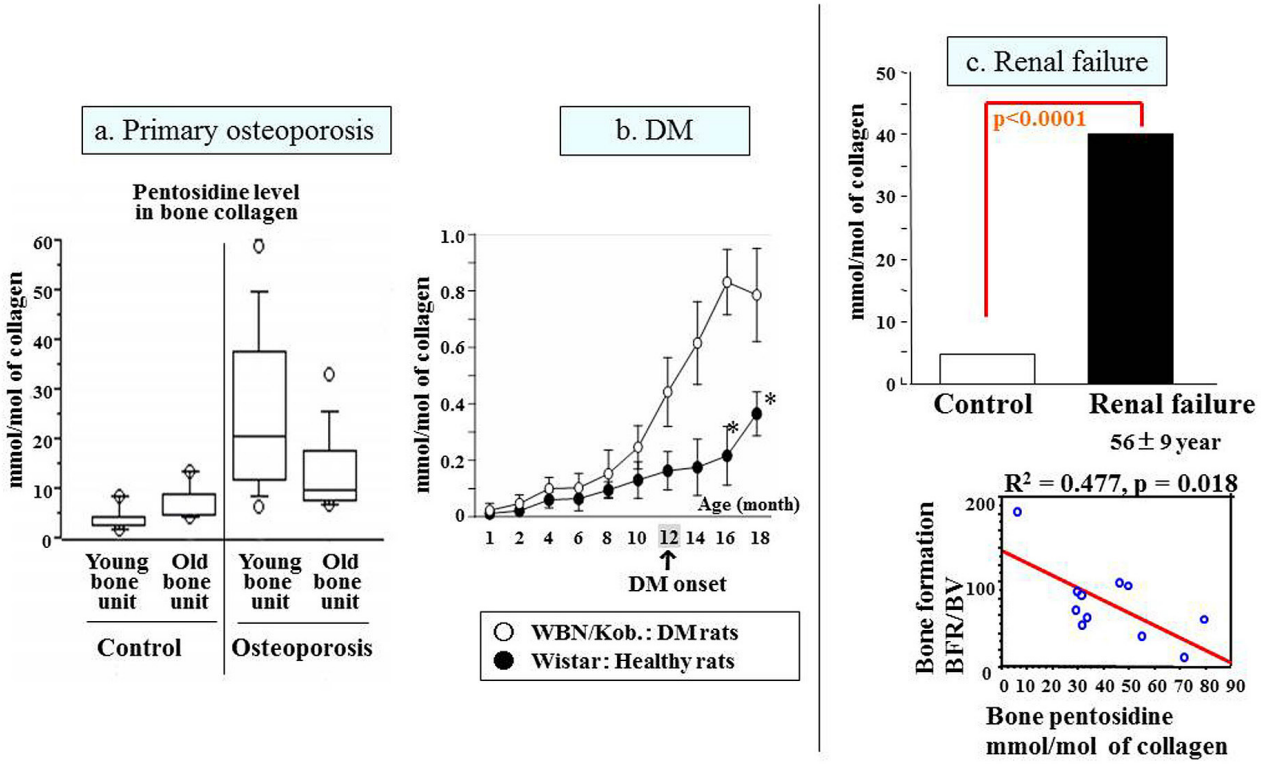


***Fig.5*: AGE cross-link pentosidine in bone collagen**

a. Excessive pentosidine formation in bone collagen in patients with primary osteoporosis. Bones were analyzed by dividing them into those with young osteons and those with old osteons. In a patient group with bone fractures, pentosidine was also increased even in young osteons, and AGE formation was seen from early stages of bone formation (references 12 and 13).

b. Collagen cross-links and bone strength in spontaneously diabetic rats. Pentosidine increased with progression of diabetes (DM) (-○-: WBN/Kob rats, -●-: Wistar rats). *p<0.05: Comparison of age-matched controls and Wistar rats (reference 15).

c. AGE pentosidine concentrations in bone collagen and bone morphometry in hemodialysis patients. In hemodialysis patients, pentosidine levels increased markedly in bone, and the rate of bone formation (BFR) decreased with such increase (reference 16).
AGE, advanced glycation end product; BV, bone volume.

is not sufficiently increased before osteoid mineralization, formation of beneficial cross-links is decreased and collagen fibers with sufficient strength cannot be formed [15]. Our laboratory has shown that beneficial enzyme-dependent cross-links were decreased 25% when there was deficiency of vitamin B6, which is essential for lysyl oxidase activity, and that bone strength was reduced without a decrease in BMD in healthy rats [18] and diabetic rats [15]. In another study, our laboratory examined a rat model of steroid-induced osteoporosis in which bone fracture risk increases before BMD decreases. Enzymatic cross-link formation decreased due to the inhibitory effect of glucocorticoid on lysyl oxidase, and bone strength decreased despite high BMD [19]. These results indicate that enzymatic cross-links are beneficial cross-links that positively affect bone strength. In a physiological environment, the total number of enzymatic cross-links peaks in human bone from childhood to 30 years of age, and there is no excessive induction of such cross-linking [20,21].

AGEs (detrimental cross-links) and bone strength (Fig.2)

AGE formation is induced by increased oxidative stress and carbonyl stress and persistent hyperglycemia. Pentosidine and glucosepane are common AGE crosslinks [2]. Bone tissue analysis has shown that the amount of pentosidine formed is positively correlated with the total amount of AGEs and that pentosidine can be used as a surrogate marker of overall AGEs [2]. AGE cross-links promote glycation and oxidation. They form in proteins with long lifespans because AGEs form in a time-dependent manner in a physiological environment [2,12,13,20]. Pentosidine increases in bone collagen with aging, and bone strength decreases [2]. If patients have diseases that increase glycation or oxidation, excessive AGE formation occurs, which greatly exceeds time-dependent AGE formation [2-4,14-16]. AGEs decrease bone strength in two ways. One way is by directly affecting cross-link formation [2-4]. The other way is by decreasing osteoblast function via cell surface receptors for AGE (RAGE) and by decreasing biological

function through induction of apoptosis [22,23]. When excised bone blocks were incubated at 37C and AGE cross-linking was induced, the strength of these bone blocks was reduced. Thus, bone strength is decreased not only with decreased cell function but also with excessive cross-linking alone due to AGEs. This result is important in better understanding deterioration of BQ [24].

Correlation of age with bone, serum, and urinary pentosidine levels and issues (Fig.6)

Urinary [10] and serum [25] pentosidine levels increase with age (***Figs.6a, b,&c***) just as pentosidine levels in bone collagen increase with age. The age-related AGE increase in bone is a phenomenon observed in both men and women [12,13,17,20,26].

Currently, the established methods of pentosidine measurement are precision instrumental analysis using high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) using antibodies against pentosidine. Measurements using ELISA are covered by the Japanese national health insurance as a routine test for patients with renal impairment (measured at SRL, Shinjuku-ku, Tokyo, Japan). We examined the correlation between pentosidine levels in bone collagen and serum and urinary pentosidine levels. In this study, samples from orthopedic surgery patients (n=100) were used, and correlation was examined between measurements by HPLC and measurements by ELISA [27]. The results showed that pentosidine levels measured by HPLC were positively correlated among samples of bone collagen, blood, and urine. However, plasma pentosidine levels measured by ELISA were not significantly correlated with urinary or bone pentosidine levels measured by HPLC. One reason is that current ELISA method uses heat treatment as pretreatment for pentosidine measurement [28]. It has been indicated that AGEs such as pentosidine and carboxymethyllysine are increased as artifacts when heat treatment is used. Artifacts do not occur in acidic conditions even when heat treatment is used, and improvement in the pretreatment method should be considered based on such a finding [28]. Improved ELISA methods are currently being developed that enable measurement without heat treatment, and there is a potential in this method.

BQ (matrix) markers: pentosidine and homocysteine

It is known that decreased enzymatic cross-linking and increased pentosidine formation occur based on bone collagen analysis in primary osteoporotic patients (15-25 patients) with femoral neck fractures (Fig.5a) [2-4]. This result was consistent with the finding in our collaborative study with Shiraki et al. in which a high urinary pentosidine concentration was a

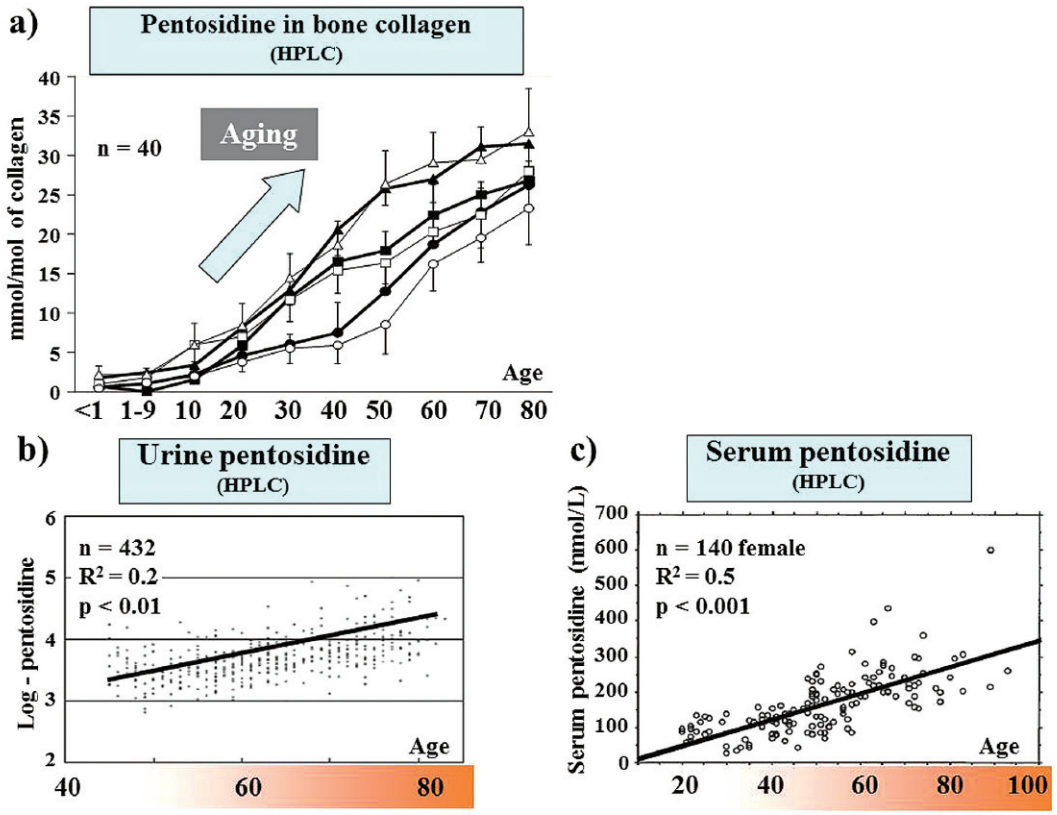


***Fig.6*: Bone, serum, and urinary pentosidine changes with age in humans**

a. Pentosidine in bone collagen:
(-■-: proximal humerus, -●-: distal radius, -▲-: central ilium, -□-: femur -○-: central tibia -△-: lumbar vertebral body (reference 17)

b. Urinary pentosidine concentrations (reference 10)

c. Serum pentosidine concentrations (creatinine correction) (reference 25)

All samples were hydrolyzed, and then high performance liquid chromatography (HPLC) was used for measurement.

fracture risk factor independent of BMD (*Fig.7*) [10]. In the study of Shiraki et al., 432 postmenopausal women untreated for osteoporosis were examined longitudinally with the endpoint of incident fracture. The results showed that the highest quartile group of urinary pentosidine (creatinine correction: 47.5 pM/mg Cr) was a fracture risk factor (odds ratio: 1.3) independent of BMD, age, bone metabolism markers, prevalent fracture, and renal function (creatinine clearance). This risk was higher than that for BMD, a traditional fracture risk factor. In another report, we showed that serum homocysteine levels were high in patients with high pentosidine levels in bone collagen [13]. Homocysteine inhibits lysyl oxidase activity and causes decreased formation of beneficial cross-links. Simultaneously, homocysteine increases oxidative stress, which promotes AGE formation in collagen [14]. Mild hyperhomocysteinemia has been shown to be a fracture risk factor independent of BMD in studies such as the Rotterdam study [29], Framingham study [30], and Women's Health Initiative (WHI) cohort study [31]. Thus, hyperhomocysteinemia began to be considered a factor that reduces BQ [2,4,32]. In a recent study, meta-analysis showed that hyperhomocysteinemia is a fracture risk factor independent of BMD in both men and women [33].

In one study, we used ovariectomized rabbits to examine whether hyperhomocysteinemia induces AGE formation in bone collagen [14]. The results showed that when hyperhomocysteinemia was induced using a 1% methionine diet, decreased enzymatic cross-linking in bone collagen and increased pentosidine formation were induced and bone strength decreased without a decrease in BMD. Even in a general population, high serum homocysteine levels induce cross-link abnormalities in bone collagen and are thought to increase the fracture risk by reducing BQ [2]. Recently, the OFELY study indicated that many fracture events occurred in patients with high urinary pentosidine levels and a replication study was performed to investigate the importance of BQ assessment [34].

Other factors for poor bone quality: diabetes and renal impairment

In meta-analysis, Vestergaard showed that fractures occur in patients with type 2 diabetes and high BMD, and deterioration of BQ began to be considered as the cause of fractures [35]. The authors of the present article examined spontaneously diabetic WBN/Kob rats. The results showed that when persistent hyperglycemia and vitamin B6 deficiency due to impaired insulin action develop, enzymatic cross-linking decreases and AGE cross-linking increases in bone collagen, resulting in decreased bone strength without a decrease in BMD (Fig.5b) [15]. Other studies have reported that high serum pentosidine levels [36] and high urinary pentosidine levels [37] become independent fracture risk factors in patients with type 2 diabetes. These results were consistent with the

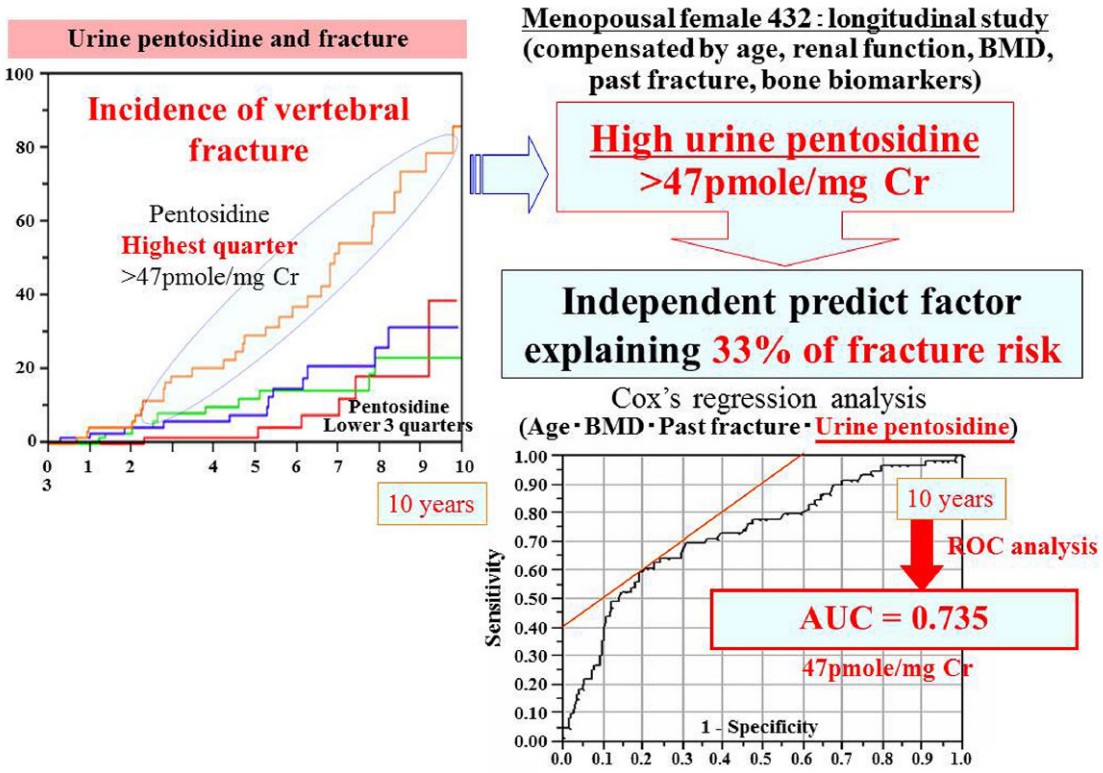


***Fig.7*: Urinary pentosidine levels and fracture risk**

A prospective study was conducted on 432 postmenopausal women using incident fracture as an endpoint (reference 34). The results showed that the highest quartile group of urinary pentosidine (creatinine correction: 47.5 pM/mg Cr) was a fracture risk factor independent of bone mineral density (BMD), age, bone metabolism markers, prevalent fracture, and renal function (creatinine clearance). In addition, 93% of incident vertebral fractures could be explained by BMD, prevalent fracture, age, and urinary pentosidine concentration, and a high urinary pentosidine level was shown to be a predictor of fracture, which was attributed 33% of the fracture risk (AUC = 0.735) (reference 10). Cr, creatinine; AUC, area under curve; ROC, receiver operating characteristic.

aforementioned relationship between pentosidine increase in bone collagen and decrease in bone strength. The authors of the present article found that pentosidine levels increase in bone collagen when oxidative stress increases with increased AGE formation in collagen and when renal impairment occurs, which increases carbonyl stress (Fig.5c)[16]. In addition, the authors showed that osteoblast function decreased as AGE formation increased in bone collagen[16]. Clinical application of BQ markers can also be expected in patients with diabetes and renal impairment[2-4].

Classification of osteoporosis by BMD and BQ markers (Fig.8)

The increased fracture risk associated with aging cannot be explained by decreased BMD alone or by decreased BQ alone. The degrees to which BMD and BQ decrease vary by individual. In one study, our laboratory examined 502 postmenopausal women (Nagano cohort study) and assessed the fracture risk by type of bone fragility. Bone fragility of each woman was classified into one of the following three types based on BMD and BQ (***Fig.8***)[6] : osteoporosis with low BMD, osteoporosis with low BQ, and osteoporosis with a combination of both types (osteoporosis with low BMD + low BQ). Patients with low BMD osteoporosis have BMD of <70% of young adult mean (YAM). Patients with low BQ osteoporosis have high serum levels of homocysteine or urinary pentosidine. When the fracture risk was compared with patients who had BMD of >80% of YAM, the fracture risk was increased 3.6-fold in patients with low BMD osteoporosis and was increased 1.5-fold in patients with low BQ osteoporosis. It was increased markedly at 7.2-fold in patients with low BMD + low BQ osteoporosis due to their synergistic effect. The ratio of patients by type was 5:3:2 for low BMD type: low BQ type: low BMD + low BQ type, showing that the low BQ type was not rare.

Potential of therapeutic drug use based on BMD and BQ markers

In a recent study, the authors of the present article have shown that drugs for osteoporosis can be used based on osteoporosis type classified by BMD and BQ[38]. Bisphosphonate, a bone resorption inhibitor, was administered to 251 postmenopausal patients with osteoporosis, and factors were analyzed which affected incident bone fractures after drug administration began. The study examined BMD, bone resorption and formation markers, presence or absence of prevalent fracture, age, and BQ markers (plasma homocysteine and urinary pentosidine). The results showed that independent risk factors for incident fracture were a high plasma homocysteine level and a high urinary pentosidine level at the commencement of treatment. Even when BMD was increased, the risk for incident fracture was increased 1.6-fold in patients with low BMD + low BQ osteoporosis compared to normal BMD and BQ (***Fig.9***). Bisphosphonates inhibit bone resorption and increase BMD but do not affect

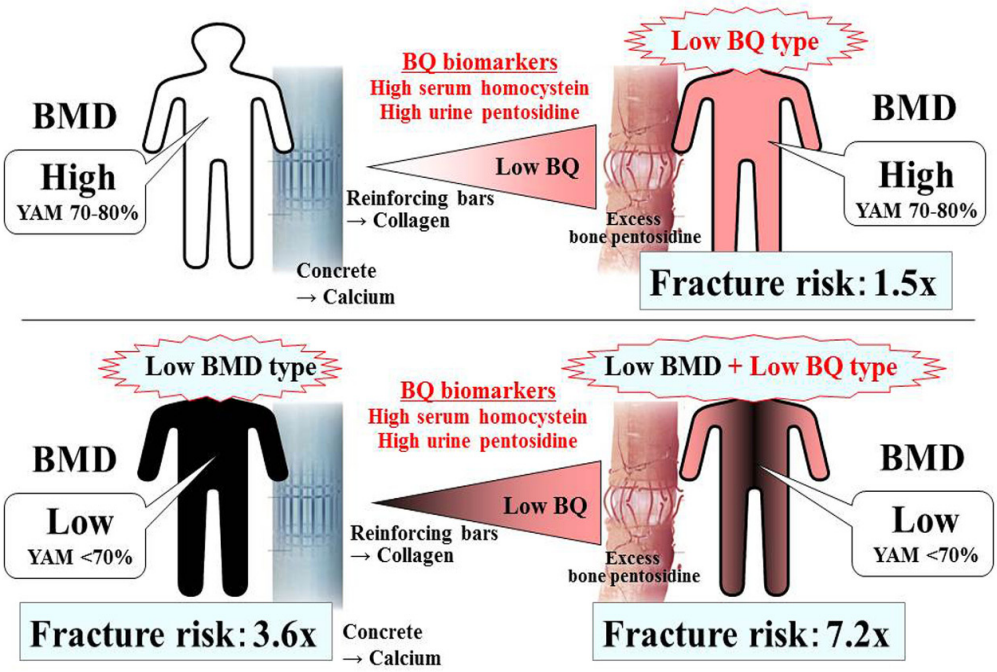


***Fig.8*: Classification of osteoporosis by BMD and BQ markers (homocysteine and pentosidine)**

Increases in fracture risk in osteoporosis were divided into three patterns using the finding that hyperhomocysteinemia causes AGE (pentosidine) accumulation in bone. "Low BQ osteoporosis" is a condition in which fracture risk increases with the presence of abnormal homocysteine metabolism alone, even if the BMD is >70% of young adult mean (YAM). "Low BMD osteoporosis" is a condition in which fracture risk increases due to low BMD even if homocysteine metabolism is satisfactory. "Low BMD + low BQ osteoporosis" is a condition in which both BMD and homocysteine metabolism decrease. The fracture risk was increased 1.5-fold in patients with low BQ osteoporosis, 3.6-fold in patients with low BMD osteoporosis, and 7.2-fold in patients with low BMD + low BQ osteoporosis. YAM: young adult mean BMD (modified from reference 9). BMD, bone mineral density; BQ, bone quality; AGE, advanced glycation end product.

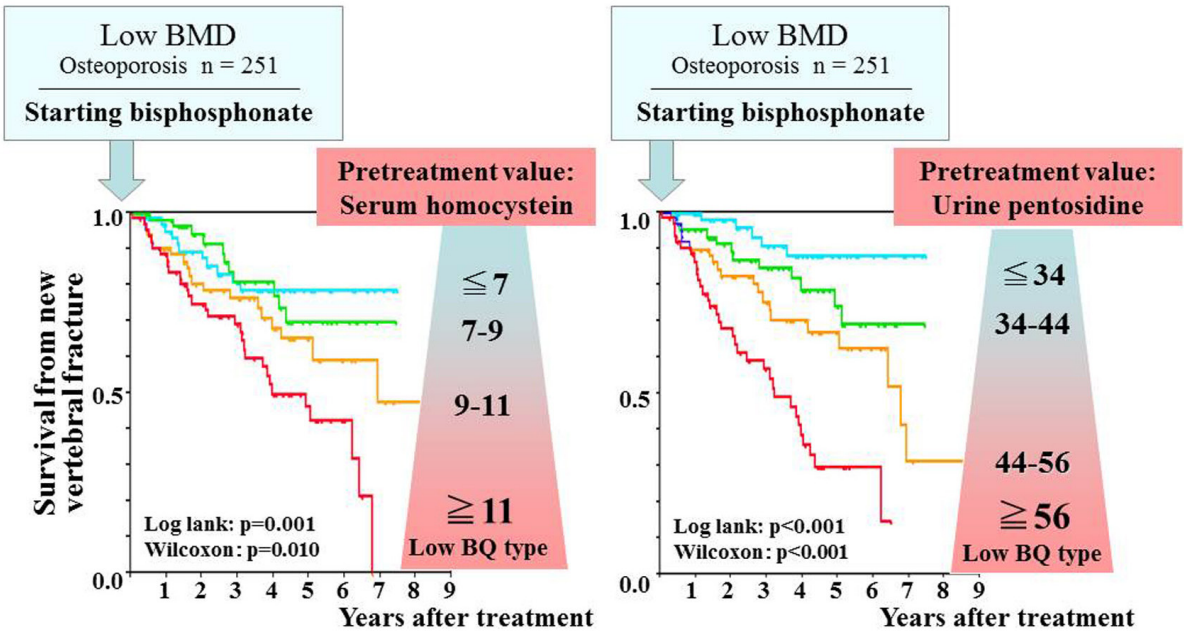


***Fig.9*: Usefulness of preliminary measurements of BQ markers in patients with resistance to bisphosphonates**

Bisphosphonates were administered to 251 postmenopausal patients with osteoporosis (low BMD). A longitudinal study was performed on incident fractures in these patients after bisphosphonate administration was begun. Even when bone metabolism markers improved and BMD increased, there was a lower preventive effect on fracture in patients with higher BQ marker levels (serum homocysteine and urinary pentosidine levels) at the beginning of treatment (reference 38). BQ, bone quality; BMD, bone mineral density.

the formation of enzymatic cross-links in bone collagen [38]. In addition, bone collagen renewal is inhibited when metabolism is excessively inhibited long term by bisphosphonates. Then AGE cross-links increases in a time-dependent manner and microcracks develop in bone. Thus, it is important to monitor the level of bone metabolism over time using bone resorption and formation markers in clinical practice. The aforementioned findings indicate the need to improve the quality of bone collagen in addition to the importance of increased BMD in patients with low BMD + low BQ osteoporosis [5].

Usefulness of BQ marker level as a risk factor for severe vertebral collapse

In the Nagano cohort study, the authors of the present article examined 1475 postmenopausal women and found that BQ markers can be an independent risk factor for severe vertebral collapse (collapse of vertebral height of >40%) (*Fig.10*) [39]. Low BMD was also extracted as a risk factor for severe vertebral collapse, but the frequency of severe vertebral collapse markedly increased when there was a high level of urinary pentosidine, a BQ marker. Thus, one must be mindful that when patients have both low BMD and high urinary pentosidine levels, the risk for incident fracture increases and simultaneously the risk for severe vertebral collapse increases. Such patients with severe collapse are in a high risk group for incident fracture (vertebral or proximal femoral fracture). Therefore, it is necessary to assess both BMD and BQ and to provide proper therapeutic intervention.

Drugs and vitamins that improve BQ from the perspective of bone collagen (Table 1)

Vitamins B6 and K2

Vitamin B6 is an essential co-enzyme for lysyl oxidase, an enzyme involved in the formation of enzymatic cross-links. It is also a vitamin with an anti-AGE effect. The authors of the present article used diabetic WBN/Kob rats that develop increased AGE cross-links associated with increased oxidative stress and hyperglycemia and that develop decreased enzymatic cross-links due to B6 deficiency. The rats received pyridoxal 5'-phosphate as vitamin B6, and its effects on bone strength, BMD, and BQ were examined [40]. Instead of receiving vitamin B6, some diabetic WBN/Kob rats received menatetrenone as vitamin K2, which can improve BQ by acting on osteoblasts. Its effects on the aforementioned items were examined [40]. The results showed that no significant improvement in bone collagen or bone strength was observed with two-month administration of vitamin B6 and vitamin K2. However, enzymatic cross-links significantly increased and AGE cross-links significantly decreased with four-month administration. Bone strength was found to significantly increase even without an increase in BMD. It can be said that vitamin B6 has the potential as a drug that improves BQ. In Japan, menatetrenone can be used as a drug for osteoporosis and can be expected to show effectiveness in low BQ osteoporosis.

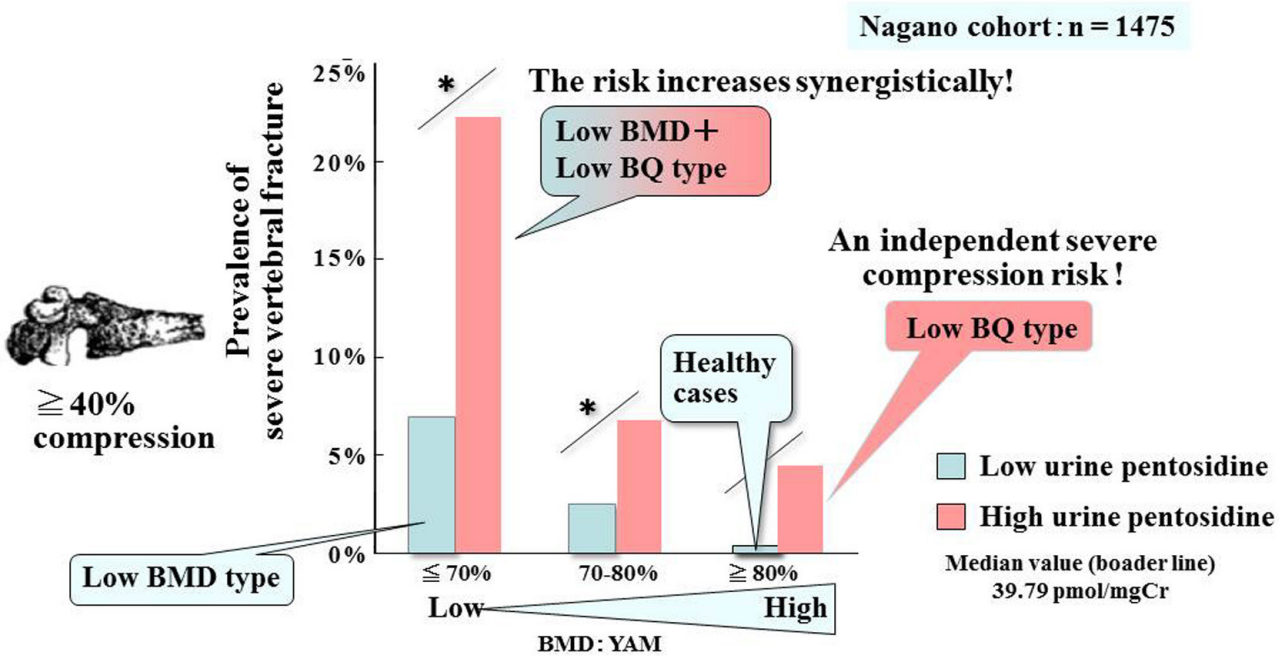


***Fig.10*: High levels of BQ marker s as a risk factor for severe vertebral collapse**

Since severe vertebral collapse is an independent risk factor for subsequent incident fracture, it can be considered a "severe type" of osteoporosis. BMD and BQ are each an independent risk factor for severe vertebral collapse. The Nagano cohort study showed that the risk for severe collapse is greatly increased in patients with "low BMD + low BQ osteoporosis". In the building structure analogy, concrete corresponds to BMD and reinforcing bars correspond to collagen. When concrete and reinforcing bars deteriorate, the likelihood of building collapse becomes higher. Once the building begins to collapse, it can completely collapse easily (reference 39). BQ, bone quality; BMD, bone mineral density; YAM, young adult mean.

Table 1. Osteoporosis remedy : Effects on BMD and quality

		Bone resorption inhibition Matrix maturation	Anti-oxidation Homocysteine reduction	Osteoblast function improvement	Osteoblast function improvement	Osteoblast function improvement Nascent collagen↑
Bone strength		Bisphosphonate	SERM	Active Vit. D3 (Alfacalcidol)	Vit. K2 Vit.B6	PTH drugs (Teriparatide)
BMD	Calcification Density	↑↑	↗	→	→	↑
BQ	Enzymatic crosslink (immature・mature)	→	↑	↑	↑	↑
	AGE crosslinks (pentosidine)	→~↗	↓	→	↘	↓
	Reference	21	14	19,42	40	45

Active form of vitamin D3

The active form of vitamin D3 acts as the nutrient vitamin D, which promotes calcium absorption from the intestine. It also has a pharmacological effect by binding to intranuclear receptors in osteoblasts, the vitamin D receptors (VDRs). The active form of vitamin D3 has been shown to improve bone collagen cross-link formation by acting on osteoblasts. To an osteoblastic cell culture system, Nagaoka et al. added native vitamin D or alfacalcidol as an active form of vitamin D3, and the effect on collagen cross-links was examined. They showed that only alfacalcidol increased lysyl oxidase activity and promoted the formation of enzymatic cross-links [41]. The authors of the present article administered alfacalcidol to ovariectomized rats of an osteoporosis model and found that bone strength increased due to increased enzymatic cross-links in bone collagen [42]. In other words, the active form of vitamin D3 can be thought to be a drug that improves BQ.

Selective estrogen receptor modulators (SERMs)

Oxidative stress and serum homocysteine concentration cause deterioration in BQ, and they are decreased by SERMs [43,44]. The authors of the present article induced hyperhomocysteinemia in ovariectomized rabbits and administered raloxifene to them. The bone mineralization levels and collagen cross-links were analyzed, and the increase in bone strength was examined [14]. In this rabbit model, cross-link abnormalities were observed that were similar to those observed in human osteoporosis with low BQ, which the authors reported in another report. In other words, there was induction of decreased enzymatic cross-link formation and increased AGE cross-link pentosidine formation, and bone strength was decreased without a decrease in BMD. When raloxifene was used as a SERM in the same model, BMD and bone metabolism markers did not show clear changes after 4-month administration. However, the serum homocysteine concentration was reduced approximately 40%. When collagen cross-links were analyzed, enzymatic cross-links were significantly increased and AGE cross-links were decreased (65% decrease) due to raloxifene administration. Based on the aforementioned results, raloxifene was thought to be suitable for patients without markedly decreased BMD and with high urinary and serum pentosidine levels or high serum homocysteine levels, markers for BQ.

Teriparatide (parathyroid hormone)

Parathyroid hormone regulates calcium and promotes both bone formation and resorption. Hyperparathyroidism occurs when there is continuous and excessive action of parathyroid hormone. It is a condition in which bone remodeling is markedly increased (bone resorption > bone formation), resulting in osteoporosis. When continuous administration or intermittent administration of once a week is used, bone mass is increased where an increase in bone formation exceeds an increase in bone resorption and BQ improves. Consequently, fracture risk decreases. Teriparatide is an agent that promotes bone formation and contains a sequence of 34 N-terminal amino acids identical to the biologically active region of parathyroid hormone. The authors of the present article used ovariectomized monkeys with a BQ abnormality similar to human osteoporosis. Teriparatide was administered to said monkeys for 18 months, and analysis was performed on the following parameters which determine bone strength: bone mass, bone mineralization level, bone microstructure, bone collagen content, and collagen cross-links [45]. The results showed that parathyroid hormone (PTH) administration not only increased BMD but also significantly increased collagen content and enzyme-dependent cross-links and decreased AGE cross-link pentosidine. This improvement in bone collagen cross-links was shown to be an independent factor that increases bone strength. These results indicate that teriparatide is suitable for patients who have osteoporosis with low BMD + low BQ. There are patients who develop incident fractures despite receiving oral drugs for osteoporosis (including bisphosphonates) for at least one year. In such patients, clinicians should consider changing the drug to teriparatide because some patients could also have low BQ.

Conclusion

In a journal associated with Nature, a Professor Emeritus of Yale University commented about “low BQ osteoporosis,” a concept developed first in the world by the Department of Orthopaedic Surgery of Jikei University [46]. Subsequently, its validity was examined in replication studies in Japan and overseas (***Fig. 11***) [2,31,34,37,47]. As a result, this concept was included in three Japanese guidelines: the Japanese 2013 Guidelines for Prevention and Treatment of Osteoporosis, the Clinical Practice Guide on Fracture Risk Associated with Lifestyle-related Diseases, and the Japanese Guidelines for the Use of Biomarkers of Bone Turnover in Osteoporosis. Our laboratory was responsible for writing chapters in these three guidelines.

In summary, when physiological, enzymatic-dependent cross-links decrease between bone collagen molecules and AGEs (aged cross-links) increase, bone strength decreases and fracture risk increases. Pentosidine is a surrogate marker whose level reflects the total amount of AGEs. A pentosidine increase in bone collagen occurs by a mechanism independent of an increase in bone resorption. The pentosidine increase is caused by (1) hyperhomocysteinemia, (2) increased oxidative stress, (3) increased carbonyl stress, or (4) increased glycation. Thus, existing bone resorption markers cannot be used to assess excessive collagen deterioration associated with aging. Serum homocysteine and urinary and serum pentosidine can potentially be BQ (matrix) markers for predicting fracture risk caused by decreased BQ, which cannot be assessed by BMD alone. Pentosidine and homocysteine measurements are presently not covered by the Japanese national insurance for the evaluation of osteoporosis. However, the 2012 Japanese Guidelines for the Use of Biomarkers of Bone Turnover in Osteoporosis mentioned that pentosidine and homocysteine might be used in future clinical practice as bone matrix markers to evaluate fracture risks, if further evidence is accumulated. It should be noted that there are some issues related to measurement. When an ELISA method is used to measure serum pentosidine (analysis that can be currently outsourced), heat treatment is used as a pretreatment. Heat treatment has been shown to cause AGE artifact formation and greatly lowers the accuracy at low pentosidine concentrations. Thus, it is necessary to improve the ELISA method and to

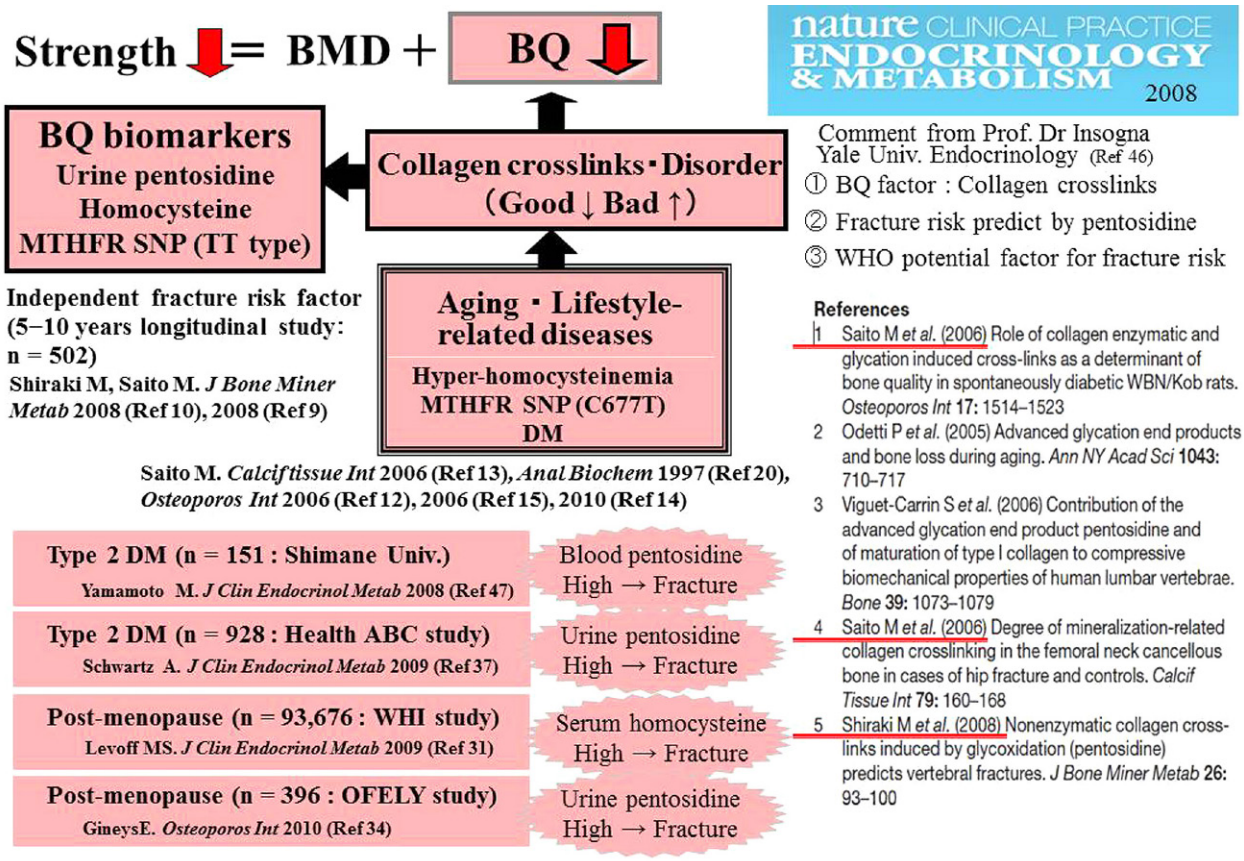


***Fig. 11*: "Poor BQ osteoporosis," a concept developed first in the world by the Department of Orthopaedic Surgery of Jikei University (reference 2)**

In a journal associated with Nature (reference 46), a Professor Emeritus of Yale University commented about this concept, "There is much anticipation about its future development." Subsequently, its validity was examined in replication studies in Japan and overseas. As a result, this concept was included in three Japanese guidelines: the Japanese 2013 Guidelines for Prevention and Treatment of Osteoporosis, the Clinical Practice Guide on Fracture Risk Associated with Lifestyle-related Diseases, and the Japanese Guidelines for the Use of Biomarkers of Bone Turnover in Osteoporosis. Our laboratory was responsible for writing chapters in these three guidelines.

BQ, bone quality; BMD, bone mineral density; MTHFR, methylenetetrahydrofolate reductase; SNP, single nucleotide polymorphism; DM, diabetes mellitus; Univ., university; WHO, World Health Organization; Health ABC study, Health, Aging, and Body Composition Study; WHI, Women's Health Initiative; Ref, reference.

confirm its correlation with HPLC method. In addition, blood and urinary pentosidine concentrations are affected by renal function, and pentosidine levels increase with age not only in bone but also in blood vessels, cartilage, and skin. In the Nagano cohort study, the authors of the present article found that a high urinary pentosidine level was an independent fracture risk factor even after correction for renal function (creatinine clearance). Therefore, pentosidine is not a marker that merely reflects decreased BQ due to renal impairment. In addition, formation of AGE increases in collagen in a "population with excessive aging" having increased systemic oxidative stress. Therefore, arteriosclerosis can develop and simultaneously bone fractures can occur due to deterioration in BQ. In other words, serum pentosidine and urinary pentosidine can be considered "markers for excessive aging" that predict cardiovascular events and bone fractures due to BQ deterioration. Osteoporosis has various manifestations depending on the combinations of different levels of BMD and BQ. Thus, it is necessary to simultaneously assess both BMD and BQ and to select a more effective drug or a combination of drugs.

Conflict of interest

The authors declare no conflict of interest regarding this review article.

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