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Effect of type 2 diabetes-related non-enzymatic glycation on bone biomechanical properties

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Abstract

There is clear evidence that patients with type 2 diabetes mellitus (T2D) have increased fracture risk, despite having high bone mineral density (BMD) and body mass index (BMI). Thus, poor bone quality has been implicated as a mechanism contributing to diabetic skeletal fragility. Poor bone quality in T2D may result from the accumulation of advanced glycation end-products (AGEs), which are post-translational modifications of collagen resulting from a spontaneous reaction between extracellular sugars and amino acid residues on collagen fibers. This review discusses what is known and what is not known regarding AGE accumulation and diabetic skeletal fragility, examining evidence from *in vitro* experiments to simulate a diabetic state, *ex vivo* studies in normal and diabetic human bone, and diabetic animal models. Key findings in the literature are that AGEs increase with age, affect bone cell behavior, and are altered with changes in bone turnover. Further, they affect bone mechanical properties and microdamage accumulation, and can be inhibited *in vitro* by various inhibitors and breakers (e.g. aminoguanidine, N-Phenacylthiazolium Bromide, vitamin B6). While a few studies report higher AGEs in diabetic animal models, there is little evidence of AGE accumulation in bone from diabetic patients. There are several limitations and inconsistencies in the literature that should be noted and studied in greater depth including understanding the discrepancies between glycation levels across reported studies, clarifying differences in AGEs in cortical versus cancellous bone, and improving the very limited data available regarding glycation content in diabetic animal and human bone, and its corresponding effect on bone material properties in T2D.

Keywords

diabetes; bone strength; bone mechanics; bone quality; advanced glycation end-products

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1. Introduction

Men and women with type 2 diabetes mellitus (T2D) have 20% to 3-fold increased fracture risk, depending on the skeletal site and severity of disease [1-5]. A systematic review of 16 observational studies including over 800,000 participants and 135,000 incident fractures found that T2D was associated with a 2-3 fold increased risk of hip fracture [5]. Whereas T2D is associated with a modest increase in overall fracture risk, the huge and growing number of persons with T2D renders this as a compelling clinical issue. Notably, among those aged 65 years and older, a group already at increased risk of fracture, prevalence of T2D exceeds 25% and is predicted to increase by 4.5-fold by 2050 [6].

The increased risk of fracture in T2D patients is paradoxical, given they tend to have normal to high bone mineral density (BMD) [7] and high body mass index (BMI), two factors which are generally associated with reduced fracture risk. Thus, several mechanisms have been proposed to contribute to the increased fracture risk seen in individuals with T2D, including an increased propensity to fall, deficits in bone microarchitecture, and poor bone quality. Notably, the increased fracture risk persists even after adjustment for a higher incidence of falls [8], implicating altered bone microarchitecture and/or poor quality as key factors. Interestingly, reports to date indicate relatively preserved trabecular bone, but increased cortical porosity in those with T2D (see review in this issue by Farr and Khosla for more detail).

Accumulation of advanced glycation end-products (AGEs) underlies the pathogenesis of many diabetic complications, and thus, we focus here on their possible role in diabetic skeletal fragility. It is now generally reported that AGEs accumulate in bone, stiffen the collagen matrix, and alter biomechanical properties of the bone matrix (see for example, a recent review by Saito and Marumo [9]). Numerous literature reviews have been conducted on the role of nonenzymatic glycation on diabetic skeletal fragility [10-16]. The primary conclusions in these reviews are that AGEs affect various factors including bone mineralization, material properties, microstructure, and microdamage accumulation, and that these factors may ultimately contribute to diabetic skeletal fragility. However, these conclusions should be evaluated in light of limited and some contradicting data in the literature. Our goal was to indicate the areas in which data is lacking and where data is inconsistent so that future work can complete these gaps in knowledge.

We conducted a literature search for English language articles in the PubMed database using the following keywords in various combinations: “diabetes,” “bone,” “aging,” “fracture,” “fracture risk,” “skeletal fragility,” “collagen,” “advanced glycation end-products,” “nonenzymatic glycation,” “*in vitro*,” “*ex vivo*,” “strength,” “mechanics,” “mechanical properties,” “cancellous,” “trabecular,” “cortical,” “microdamage,” “turnover,” “remodeling,” “breaker,” “inhibitor,” “review”. Approximately 100 relevant articles were reviewed to discuss the experimental evidence for a relationship between AGEs in bone and bone's biomechanical properties as investigated through *in vitro* experiments to simulate a diabetic state, and comparing these findings to *ex vivo* studies conducted in normal and diabetic human bone as well as in diabetic animal models.

2. Posttranslational modifications of collagen: enzymatic and non-enzymatic crosslinks

The main organic constituent of bone is type I collagen, comprised of two non-helical domains and a triple helical region. This structural protein is composed of three polypeptide chains with a very specific sequence of amino acids that allows the chains to wind into a triple helical structure (e.g. glycine-X-hydroxyproline with X representing an amino acid such as lysine). Amino acids that lie on the surface of the helix are involved in collagen crosslink formation [17].

Crosslinking, a prominent post-translational modification of collagen, occurs by two distinct processes: 1) enzymatic crosslinking, mediated by lysyl hydroxylase and lysyl oxidase; and 2) non-enzymatic crosslinking, mediated by glycation and/or oxidation. The enzymatic and non-enzymatic crosslinks differentially affect collagen stability and mechanical properties.

Enzymatic crosslinking requires an enzyme (e.g. lysyl oxidase) to create intra- or inter-fibrillar crosslinks [18]. During the process, bivalent crosslinks transform into trivalent and stable crosslinks. Two commonly assessed enzymatic crosslinks, deoxypyridinoline and pyridinoline, represent collagen maturity and are bone resorption markers [19]. These crosslinks increase collagen fibril stiffness and contribute to increased tissue strength [20, 21]. Enzymatic crosslinks are typically characterized by high performance liquid chromatography (HPLC) [22], mass spectrometry [23], and Fourier transform infrared spectroscopy [24].

AGEs are produced by non-enzymatic glycation (NEG), which is an irreversible and spontaneous biochemical reaction that occurs between free-floating sugars and exposed amino acid residues on proteins [21, 25]. This process occurs in various proteins including hemoglobin, albumin, osteocalcin, and collagen, among others [26, 27]. NEG incorporates a biochemical reaction between the ε-amino group of lysine or hydroxylysine and an aldehyde group of a sugar such as glucose. This reaction forms glucosyl-lysine, a product that then experiences additional reactions to form a Schiff base adduct or an Amadori product. These intermediate products endure further biochemical reactions to eventually create post-translational modifications of collagen (AGEs) that accumulate in numerous tissues including tendons, skin, cartilage, and bone [28]. AGEs include both crosslinking modifications that form within or across collagen fibers (e.g. pentosidine, vesperlysines, crossline) and non-crosslinking modifications (e.g. carboxymethyllysine, carboxyethyllysine, pyrraline) [21].

3. Assessment of AGEs in bone

The two methods available for quantifying AGEs in bone are based on measuring AGE fluorescence, and thus require a specimen of bone. Pentosidine is the single AGE that has been isolated and measured in bone specimens, and is quantified by HPLC [28, 29]. Current HPLC methods use lyophilized and acid-hydrolyzed bone samples in which pentosidine is separated from enzymatic crosslinks via a solid phase extraction column and is then subsequently quantified with a fluorescence detector [28]. Pentosidine amounts are

normalized to the amount of collagen present in the sample, which is estimated from hydroxyproline content. Thus pentosidine content is typically expressed in units of mmol / mol collagen. However, pentosidine composes <1% of total fluorescent AGEs in bone [30], and is only weakly correlated to the amount of total fluorescent AGEs in human cortical and cancellous bone [31]. Thus, it is important to also measure total fluorescent AGEs rather than pentosidine alone.

The second technique quantifies the bulk fluorescence of AGEs from papain-digested or acid-hydrolyzed bone samples relative to a quinine sulfate standard [32, 33]. The amount of quinine-based fluorescence is normalized to the amount of collagen present in the sample, which is estimated from hydroxyproline content [34], and thus total fluorescent AGE content is usually expressed in units of ng quinine / mg collagen. The fluorometric assay utilizes wavelengths (370/440 nm excitation/emission) that encompass the excitation and emission spectra of several crosslinking and non-crosslinking AGEs such as pentosidine (335/385 nm excitation/emission), crossline (379/400 nm excitation/emission), vesperlysines A and B (366/442 nm excitation/emission), vesperlysine C (345/405 nm excitation/emission), carboxymethyllysine (345/455 nm excitation/emission), and carboxyethyllysine (345/455 nm excitation/emission) [29, 35-38]. However, the contributions of each of these crosslinks to the total fluorescence cannot be determined from this assay and are currently unknown. Furthermore, although the fluorescence spectra for enzymatic crosslinks, pyridinoline and deoxypyridinoline, are known (297/395 nm excitation/emission), to our knowledge no work has been conducted to clarify the extent to which these crosslinks may be captured using the fluorometric assay.

4. Differences in AGEs in human cortical versus cancellous bone

Two studies have compared differences in AGE levels between cancellous and cortical bone and showed differing results. Odetti *et al*. reported greater pentosidine content in cortical compared to cancellous bone, possibly due to higher turnover rates in cancellous bone that results in increased removal of AGEs [39]. In contrast, Karim *et al*. reported that there were more total fluorescent AGEs and pentosidine in cancellous than in cortical bone, and this was attributed to the greater surface-to-volume ratio in cancellous bone that allows for increased access of sugars to form AGEs [31]. Further studies are needed to determine whether there are differences in glycation content between cortical and cancellous bone.

5. Age-related changes in AGEs in human bone

AGE contents reported in the literature are highly variable (Table 1). For instance, two studies report extremely low concentrations of cortical bone pentosidine: ~0.44-1.39 mmol / mol collagen (19-89 years) [40] and ~56.7×10^{-8}-62.0×10^{-8} mmol / mol collagen (34-92 years) [39], while another reports much higher values ranging from ~0.1-60 mmol / mol collagen (18-97 years) [31]. One study reported a very low concentration of cancellous bone pentosidine ranging from ~4.5×10^{-8}-16.3×10^{-8} mmol / mol collagen (34-92 years) [39], while two others report higher values ranging from ~0.1-70 mmol / mol collagen (18-97 years) [31, 33]. Only two of these studies reported total fluorescent AGE content ranging from ~0.1-330 ng quinine / mg collagen in cortical bone and ~0.1-2000 ng quinine / mg

collagen in cancellous bone (18-97 years) [31, 33], and these values are on the same order of magnitude as several other reports in bovine, murine, and human bone [32, 41, 42]. The reasons for vastly different ranges in glycation content among the various studies are not completely clear.

Despite the vastly different values reported, all of these studies indicate that there is an increase in AGEs with age within a very wide age range (~20-100 years) [31, 33, 39, 40]. However, only 2 of these studies investigated how the age-related changes in AGEs vary between cancellous and cortical bone. Odetti *et al*. reported that pentosidine increased exponentially with age from ~56.7×10^{-8} to 62.0×10^{-8} mmol / mol collagen in human cortical bone from femurs and tibias, but this trend was not evident in cancellous bone [39]. The authors suggested that the lack of age-related changes in cancellous bone may be attributed to the more variable levels of pentosidine in cancellous than in cortical bone, which may make it difficult to observe relationships. In comparison, Karim *et al*. reported that total fluorescent AGEs but not pentosidine increased with age in cortical bone, from ~0.1-330 ng quinine / mg collagen, whereas AGEs did not increase with age in cancellous bone [31].

6. Influence of AGEs on bone cells

Studies indicate that AGEs may alter the behavior of bone cells, which in turn may influence bone biomechanical properties. Specifically, *in vitro* studies indicate that AGEs decrease osteoblast activity by impairing adhesion of osteoblasts to the collagen matrix [43], inhibiting osteoblast proliferation and differentiation [44], and decreasing mRNA expression of key osteoblastic products [45]. Additionally, *in vitro* studies have shown that AGEs can affect osteoclast behavior, although there are contradictory findings. Specifically, one study showed that resorption was significantly inhibited in bone treated with pentosidine *in vitro* [46], while another found that there were more and larger resorption pits in bone areas containing high *in vivo* AGE levels [47]. Further, only a single study has examined the effect of glycation on osteocyte behavior. This study showed that treatment of an osteocytic cell line (MLO-Y4-A2) with laboratory-synthesized AGEs increased *SOST* and decreased *RANKL* expression [48]. Further work is needed to characterize the effect of naturally occurring AGEs versus chemically-induced AGEs on bone cell behavior and the implications for bone biomechanical properties.

7. Effect of bone turnover on AGE accumulation

Alterations in bone turnover may influence accumulation of AGEs. In adult beagles after one year treatment with bisphosphonates, cortical bone AGE content was unchanged using doses equivalent to those used in postmenopausal women, but was increased following treatment at doses 5-fold higher than the standard clinical dose [49]. In comparison, increased bone turnover due to once-weekly parathyroid hormone treatment for 18 months in ovariectomized monkeys led to a decrease in pentosidine content [50]. However, to our knowledge, no studies have been conducted investigating the effect of bone turnover modulation via anabolic or anti-resorptive treatment on AGEs in humans.

8. Effect of AGEs on bone's biomechanical behavior

8.1. A review of basic bone biomechanics

Key biomechanical properties describing bone's monotonic behavior are based on the relationship between applied loads and the resulting deformation in bone. Two important measures are stress and strain where stress is defined as force per unit area (intensity of the applied force) and strain is defined as the change in length divided by the initial length (normalized deformation). Many important mechanical variables are calculated from the load-deformation or stress-strain curves acquired from a mechanical test (Figure 1). In performing a test of a whole bone, structural properties are assessed as follows: load and deformation are linearly related until the yield point is reached, and the slope of the curve in the pre-yield region represents bone stiffness. Loading beyond the yield point results in permanent deformation upon removal of the load. In the post-yield region the ultimate or failure load for bone is reached, and the energy needed to result in failure is calculated as the area under the load-displacement curve. Materials that are brittle undergo little deformation prior to failure, while materials that are ductile undergo significant deformation prior to failure. In comparison, the intrinsic properties of bone material are derived from a stress-strain curve that is generated by conducting a mechanical test on a specimen that has a standardized shape. The slope of the stress-strain curve up until the yield point is known as the elastic modulus. After this point, the bone undergoes irrecoverable plastic strain. The maximum stress (or strength) occurs when the bone nears the ultimate or failure point, and finally the ultimate stress and strain are the points at which the bone breaks. The total area under the load-displacement curve represents the toughness of the specimen, or its ability to absorb energy until failure.

8.2. Influence of AGEs on bone biomechanics

The pre-yield mechanical behavior of bone tissue is largely influenced by its mineral composition while post-yield mechanical properties are influenced by the organic matrix [51]. Because NEG directly modifies the organic matrix, it is likely that primarily the post-yield properties of bone will be affected by NEG, although there is evidence that NEG can affect pre-yield mechanical behavior as well.

A few *in vitro* studies have incubated bones in a sugar solution to simulate a diabetic state. The incubation process incorporates a sugar in the presence of protease inhibitors in a buffer solution, at physiological temperature and pH [41]. Very few *in vitro* studies have been conducted, and they suggest the mechanical responses to *in vitro* glycation may be different in cancellous and cortical bone. Specifically, *in vitro* glycation of bovine cortical bone specimens for 38 days resulted in approximately 17-fold greater AGE content in glycated versus vehicle bone (vehicle: 75.9 ± 14.8 ng quinine / mg collagen, glycated: 1274 ± 141.3 ng quinine / mg collagen). *In vitro* glycation resulted in stiffer collagen fibers (i.e. equilibrium modulus assessed in demineralized bone), greater yield stress and strain, less post-yield displacement, but similar post-yield strain energy compared to vehicle-treated specimens [32]. The AGE levels in the vehicle group were on the same order of magnitude as reported in a study conducted in human bone, but the *in vitro* induced levels were much

greater than that observed in human bone [31], and thus the physiologic relevance of these results are unclear.

In comparison, *in vitro* glycation of human cancellous bone specimens for 7 days resulted in approximately 2-fold greater AGE content in glycated versus vehicle bone (vehicle: 169.9 ± 120.7 ng quinine / mg collagen, glycated: 322.4 ± 256.6 ng quinine / mg collagen). This study suggests that a 7-day incubation of human cancellous bone specimens in a 0.6 M ribose solution produces AGE levels equivalent to that measured after approximately 2-3 decades of natural aging. Specifically, the authors report that the *in vitro* induced AGE content in bone specimens from a 42 year old donor had similar AGE levels as in vehicle-incubated bone specimens from a 74 year old donor. Glycated trabecular bone specimens had similar yield stress and strain, but lower post-yield strain energy than vehicle-treated specimens [41]. These two investigations were conducted in different models (i.e. bovine versus human), making it difficult to compare results. Furthermore, the clinical relevance of the *in vitro* induced AGE levels is difficult to deduce as these values cannot be compared to glycation content in diabetic human bone, as the only study that measured glycation in diabetic bone assessed pentosidine content but not total fluorescent AGEs [52].

Ex vivo studies using normal human bone report a negative relationship between AGE levels and post-yield bone mechanical properties. For example, total fluorescent AGEs and pentosidine content in human cancellous bone specimens from the tibial plateau (age range 18-97 years) is negatively associated with ultimate strain ($r = -0.38$ and $r = -0.44$, respectively) [33], while pentosidine content in human cortical bone specimens from the femoral midshaft (age range 19-89 years) and from the tibial midshaft (age range 51-90 years) are negatively associated with ultimate stress and fracture toughness [40, 53]. Another investigation similarly reports that pentosidine content is weakly correlated (r = -−. 3) with ultimate strain of individual trabeculae extracted from human vertebrae (age range 54-94 years) [54]. However, one *ex vivo* study using human vertebral bone (age range 54-94 years) shows contradictory results, reporting that glycation content does not influence bone biomechanical properties as suggested by previously described studies (i.e. no relationship between pentosidine and compressive biomechanical properties) [55].

8.3. Effect of AGE content on microdamage accumulation

A few studies have reported that AGEs influence microdamage accumulation. For instance, in dogs treated with the bisphosphonate incadronate for 3 years, pentosidine content was positively associated with microdamage accumulation in ribs (e.g. crack density $r^2 = 40\%$, crack length $r^2 = 22\%$) [56]. *In vitro* glycation of human cancellous bone leads to increased microcracks following compressive loading [57], and higher levels of AGEs are reported in regions with increased amounts of crack-like microdamage [33]. Although increased NEG appears to be associated with increased microdamage content, further work is needed to elucidate the relevance of this microdamage accumulation to overall skeletal fragility.

8.4. AGE inhibitor and reversal of mechanical property degradation

Work is now being done to investigate therapeutic interventions to inhibit AGE formation or to cleave existing AGEs, as several AGE inhibitors and breakers have been described,

including aminoguanidine, N-Phenacylthiazolium Bromide (PTB), and pyridoxamine (vitamin B6) [58]. However, very few studies have been conducted using these agents in bone tissue. One study showed that whereas aminoguanidine treatment reduced the pentosidine content induced by *in vitro* glycation of bovine cortical bone, it did not influence hardness assessed by microindentation or mechanical properties assessed by 3-point bending [59]. In contrast, a recent study showed that human cancellous bone specimens treated with PTB *in vitro* resulted in decreased AGE content and a corresponding increase in ductility (e.g., post-yield strain) [60]. It has also been shown that vitamin B6 inhibits AGEs [61, 62]. Specifically, there is decreased pentosidine content in diabetic rats treated with vitamin B6 with a corresponding increase in elastic modulus and toughness [62]. Further work needs to be done to determine whether AGE inhibitors and breakers can alter bone mechanical properties in animal models and/or in human bone.

9. AGEs and diabetic skeletal fragility

Studies conducted in diabetic animal models play an important role in the goal of identifying the underlying mechanisms of diabetic skeletal fragility. However, diabetic rodent models have some limitations such as having low bone mass and/or the onset of diabetes occurring before reaching skeletal maturity, which is in contrast to what occurs in adult humans [63]. With these limitations in mind, Saito *et al.* reported that diabetic WBN/Kob rats have a sharp increase in bone pentosidine content at the onset of diabetes, associated with a lower femoral bending stiffness (pre-yield mechanical property), energy absorption, and maximum load compared to controls, despite having no difference in BMD [64]. In Tallyho mice, a model of early onset T2D, total fluorescent AGEs were increased in femoral cortical bone, while femoral maximum load was higher and post-yield deformation lower (suggesting increased brittleness) than non-diabetic controls [42]. Additionally, Tallyho mice had increased indentation distance as measured by cyclic reference point indentation, suggesting that the diabetic bone matrix is more susceptible to cracks propagating into the bone after multiple loading cycles.

Detailed characterization of AGEs in bone from patients with diabetes is currently lacking. To our knowledge, only one study has reported AGE levels in bone patients with T2D, finding ~30% higher pentosidine levels in cortico-cancellous bone specimens from men with T2D versus non-diabetics (9 ± 3 vs 7 ± 2 mmol / mol collagen).

10. Comparison of AGE levels in bone specimens exposed to *in vitro* glycation, diabetic animal models, and bone tissues from T2D patients

It is important to compare AGE levels measured in animal studies and *in vitro* glycation studies to quantities in diabetic human bone. As previously mentioned, total fluorescent AGEs and pentosidine content reported in the literature (Table 1) are highly variable. These differences are likely the result of several factors including: animal model, bone type, location of specimen within the bone, gender, and age range, but further work needs to be done to explain these differences.

In vitro induced glycation in human cancellous bone (7-day incubation) shows AGE levels similar to that measured after 2-3 decades of natural aging [41], but *in vitro* induced AGE levels in bovine cortical bone (38-day incubation) results in AGE values beyond that observed in human bone [31, 32]. These values are higher than that observed in human bone even after only ~7 days of the total 38-day incubation. Further, the *in vitro* induced quantities are difficult to compare to that in diabetic human bone because the single study that assessed glycation in diabetic human bone measured only pentosidine, but not total fluorescent AGEs [52]. Given the weak relationship between pentosidine and total fluorescent AGEs [31], it is difficult to extrapolate and compare the extent of glycation across these studies.

Of the two studies reported here on glycation content in T2D animal models, one assessed total fluorescent AGEs [42]. The other assessed pentosidine in cortical bone, which reported ~23-fold lower pentosidine content than that measured in diabetic human bone [52, 64]. Furthermore, in the study on diabetic human bone specimens, findings are difficult to interpret as it is unclear whether the specimens were cortical or cancellous bone or a mixture of both. [52].

11. Summary and Recommendations for Future Research

Overall there are several important points that are well-known regarding non-enzymatic glycation in bone: 1) AGEs increase with age, 2) glycation is associated with altered osteoblast activity, 3) glycation content is not affected by treatment with a clinically relevant dose of bisphosphonate, but increases with high bisphosphonate doses and decreases with parathyroid hormone treatment as shown in animal models, 4) *in vitro* glycation alters bone biomechanical properties and influences the type and extent of microdamage formed, 5) AGE-inhibitors tested *in vitro* can decrease AGE accumulation and restore biomechanical properties as shown in animal models, and 6) bone from diabetic rodents has higher AGE content than non-diabetic controls with corresponding changes in mechanical properties.

However, our review indicates there are still many gaps in knowledge and inconsistencies in reported results that require further investigation. Specifically, the following points are still unknown and/or need to be clarified: 1) the reasons why there are drastic differences in glycation levels across the various published studies, 2) whether glycation content differs between cancellous and cortical bone as the two studies that have compared bone types show opposite findings, 3) how glycation affects osteoclast and osteocyte behavior, 4) how bone cell behavior is affected by chemically-induced versus naturally-produced AGEs, 5) whether AGE levels change in patients treated with osteoporosis drugs (as this has only been assessed in animal models), 6) whether AGEs are increased in patients with T2D and how AGE levels are influenced by T2D duration and/or severity, and 7) how glycation levels induced *in vitro* or in diabetic animal models relate to levels in human diabetic bone.

In summary, despite a few reports of altered cortical bone microarchitecture in T2D, there are still several open questions regarding the possible mechanisms underlying skeletal fragility in T2D. While altered bone quality has been suggested as a possible contributor to diabetic skeletal fragility, additional research is needed. In particular, further studies of bone

tissue from patients with T2D and assessment of the biomechanical consequences of accumulation of physiologically-relevant levels of AGEs are warranted. Our current limited understanding of mechanisms underlying diabetic skeletal fragility impedes development of guidelines to assess fracture risk and to determine optimal treatments to prevent fractures in this population.

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Highlights

- Diabetics have increased fracture risk despite having high BMD, implicating poor bone quality as one mechanism of diabetic skeletal fragility.
- Poor bone quality in diabetics may result from the accumulation of advanced glycation end-products (AGEs).
- It is generally reported that AGEs accumulate in bone, stiffen the collagen matrix, and alter bone's biomechanical properties.
- The effect of AGEs on bone mechanics should be assessed in light of limited and some contradicting data in literature.

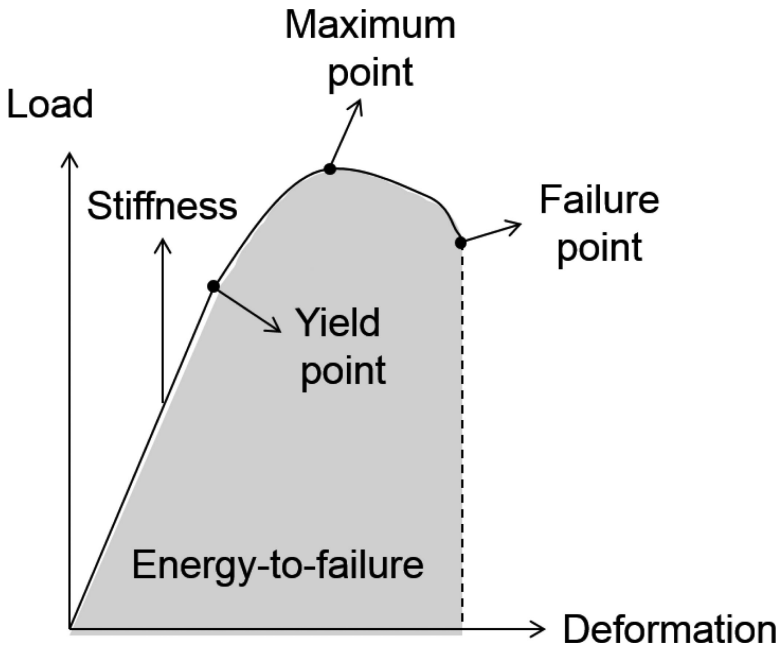

Figure 1.
Schematic diagram of a load-deformation curve acquired from a mechanical test.

Table 1

Pentosidine and/or total fluorescent advanced glycation end-product (AGEs) quantities measured in various models. Quantities are reported as average ± standard deviation or range of values unless otherwise noted. Values estimated from figures are preceded by (~).

Model/bone	Genders included (n)	*In vitro* induced?	Age	Group description	Cortical Pentosidine *(mmol / mol collagen)*	Cortical AGEs *(ng quinine / mg collagen)*	Cancellous pentosidine *(mmol / mol collagen)*	Cancellous AGEs *(ng quinine / mg collagen)*	References
In vitro glycation levels									
Human/femur	Female (n=8)	Yes	42—97 yr	Vehicle	--	--	--	169.9 ± 120.7	[41]
				Glycated	--	--	--	322.4 ± 256.5	
Bovine/femur	? (n=22)	Yes	18 mo	Vehicle	--	75.9 ± 14.8	--	--	[32]
				Glycated	--	1274 ± 141.3	--	--	
In vivo glycation levels in human bone									
Human/femur	Female (n=32 cortical, n=50 cancellous)	No	7—86 yr	Hip fracture, low bone density	24.1 ± 16.7	--	19.1 ± 12.5	--	[65, 66]
				No fracture, low bone density	4.1 ± 2.5	--	4.7 ± 1.8	--	
				Hip fracture, high bone density	13.3 ± 8.0	--	15.8 ± 10.3	--	
				No fracture, high bone density	7.8 ± 3.5	--	7.7 ± 2.4	--	
Human/femur	Both (n=30)	No	19—89 yr	Young	0.44 ± 0.21	--	--	--	[40]
				Middle aged	0.90 ± 0.23	--	--	--	
				Elderly	1.39 ± 0.29	--	--	--	
Human/femur,tibia	Both (n=104)	No	34—92 yr	--	$59.42\times10^{-8} \pm 2.63\times10^{-9}$	--	$10.41\times10^{-8} \pm 5.9\times10^{-10}$	--	[39]
Human/femur,tibia	Both (n=170)	No	18—97 yr	--	~0.1—60	~0.1—330	~0.5—70	~0.1—2000	[31]
Human/tibia	Both (n=17)	No	51—90 yr	Middle aged	0.3 ± 0.1	--	--	--	[53]
				Elderly	0.5 ± 0.1	--	--	--	
Human/tibia	Both (n=42)	No	18—97 yr	--	--	--	~0.1—50	~0.1—1500	[33]
Human/tibia	Male (n=20)	No	45—80 yr	Non-diabetic	--	--	~7 ± 2*	--	[52]
				Diabetic	--	--	~9 ± 3*	--	

Model/bone	Genders included (n)	*In vitro* induced?	Age	Group description	Cortical Pentosidine *(mmol / mol collagen)*	Cortical AGEs *(ng quinine / mg collagen)*	Cancellous pentosidine *(mmol / mol collagen)*	Cancellous AGEs *(ng quinine / mg collagen)*	References
Human/vertebrae	Both (n=49)	No	54—95 yr	--	--	--	20.4 ± 9.4	--	[55]
Human/vertebrae	Both (n=19)	No	26—93 yr	--	75 ± 126	--	30 ± 42	--	[28]
Human/vertebrae (individual trabeculae)	Both (n=32)	No	54—94 yr	--	--	--	2.25 (median)	--	[54]
In vivo glycation levels in animal bone									
Monkey/vertebrae	Female (n=76)	No	12 ± 1.5 yr	Sham	--	--	1.1 ± 0.4	--	[50]
				OVX	--	--	1.8 ± 1.1	--	
				Low-PTH treatment	--	--	1.0 ± 0.5	--	
				High-PTH treatment	--	--	0.8 ± 0.3	--	
Mouse/femur	Male (n=36)	No	8 wk	Non-diabetic SWR mouse	--	~225 ± 50	--	--	[42]
				Diabetic Tallyho mouse	--	~275 ± 75	--	--	
			17 wk	Non-diabetic SWR mouse	--	~213 ± 50	--	--	
				Diabetic Tallyho mouse	--	~225 ± 50	--	--	
Rat/femur	Male (n=30)	No	?	Non-diabetic Sprague-Dawley rat	--	14.8 ± 0.7[×]	--	--	[67]
				Type 1 diabetic Sprague-Dawley rat	--	27.3 ± 4.8[×]	--	--	
Rat/femur	Male (n=41)	No	11—12 wk	Non-diabetic Sprague-Dawley rat	44 ± 12	--	--	--	[68]
				Type 1 diabetic Sprague-Dawley rat	65 ± 38	--	--	--	
Rat/femur	Male (n=70)	No	0—18 mo	Non-diabetic Wistar rat	~0.01—0.35	--	--	--	[64]
				Diabetic WBN/Kob rat	~0.01—0.8	--	--	--	

[*] Study does not explicitly state whether pentosidine content is measured from cortical or cancellous bone, only that it is measured in a specimen from the tibial plateau.

[×] Units are expressed in fluorescence units / mg collagen