HHS Public Access

Author manuscript

Curr Opin Nephrol Hypertens. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Curr Opin Nephrol Hypertens. 2021 July 01; 30(4): 411–417. doi:10.1097/MNH.0000000000000713.

The Impact of Advanced Glycation End Products on Bone Properties in Chronic Kidney Disease

John G. Damrath[#1], **Amy Creecy**[#2], **Joseph M. Wallace**[2], **Sharon M. Moe**[3,*]

[1]Purdue University Weldon School of Biomedical Engineering, West Lafayette, IN, United States

[2]Indiana University – Purdue University at Indianapolis Department of Biomedical Engineering, Indianapolis, IN, United States

[3]Indiana University School of Medicine, Division of Nephrology, Indianapolis, IN, United States

[#] These authors contributed equally to this work.

Abstract

Purpose of review: Chronic kidney disease (CKD) affects over 15% of Americans and results in an increased risk of skeletal fractures and fracture-related mortality. However, there remain great challenges in estimating fracture risk in CKD patients, as conventional metrics such as bone density assess bone quantity without accounting for the material quality of the bone tissue. The purpose of this review is to highlight the detrimental effects of advanced glycation end products (AGEs) on the structural and mechanical properties of bone, and to demonstrate the importance of including bone quality when assessing fracture risk in CKD patients.

Recent findings: Increased oxidative stress and inflammation drive the production of AGEs in CKD patients which form non-enzymatic crosslinks between type I collagen fibrils in the bone matrix. Non-enzymatic crosslinks stiffen and embrittle the bone, reducing its ability to absorb energy and resist fracture. Clinical measurement of AGEs is typically indirect and fails to distinguish the identity and properties of the various AGEs.

Summary: Accounting for the impact of AGEs on the skeleton in CKD patients may improve our estimation of overall bone quality, fracture risk, and treatments to improve both bone quantity and quality by reducing AGEs in patients with CKD merit investigation in order to improve our understanding of the etiology of increased fracture risk.

Keywords

advanced glycation end products; chronic kidney disease; bone mechanics; collagen; fracture risk

[*]**Correspondence:** Sharon M. Moe, Sharon M. Moe, MD, FASN, Stuart A. Kleit Distinguished Professor of Medicine, Associate Dean for Clinical and Translational Research, Director, Division of Nephrology and Hypertension, Co-Director Indiana CTSI, Indiana University School of Medicine, smoe@iu.edu, 317 278 2868 phone, 317 274-8575 fax.

Conflicts of interest: none

Annotations

*Hunt HB, Torres AM, Palomino PM, et al. Altered Tissue Composition, Microarchitecture, and Mechanical Performance in Cancellous Bone From Men With Type 2 Diabetes Mellitus. Journal of Bone and Mineral Research. 2019;34(7):1191–206. This paper shows increases in AGEs with type 2 diabetic patients and establishes a negative correlation between pentosidine (an AGE) and post-yield properties.

Introduction

Skeletal fractures in patients with chronic kidney disease (CKD) remain a critical clinical problem with few methods for prevention. CKD patients are four times more likely to fracture than their age-matched population and their mortality rate following fracture is over 60% (1, 2) (Figure 1). To date, bone mineral density (BMD), as assessed by dual energy X-ray absorptiometry (DXA), is the only commonly performed assessment of bone health, and this technique has not been optimized for estimating fracture risk in CKD patients(3). While bone mass is related to bone strength from a mechanical perspective, this measure alone does not account for the intrinsic material quality of the bone tissue. In CKD patients, elevated parathyroid hormone (PTH) is linked to pore formation and thinning of cortical bone, as determined by modern high resolution-peripheral quantitative computed tomography (HR-pQCT) studies, and these microarchitectural changes are correlated to fracture risk(4, 5). However, material characteristics of bone quality are only assessed *ex vivo*, as detailed below.

On the nanoscale, bone consists of a type I collagen matrix bound with hydroxyapatite mineral and water. The combination and interaction of these fundamental constituents allow bone to withstand higher loads, absorb more energy before breaking, and resist cracking, allowing repair processes to intervene before a critical fracture(6, 7). Therefore, altering any one of these components has the potential to impact fracture resistance at the level of the whole bone. As an example, collagen in bone undergoes a tightly-controlled process of enzymatic crosslinking. Crosslinking is not only beneficial, but also essential to proper mechanical function (Table 1). Other non-enzymatic crosslinks form as advanced glycation end products (AGEs), which accumulate in tissues over time. These crosslinks prevent proper sliding of collagen molecules, embrittling the bone tissue and reducing its resistance to fracture(8). Therefore, accurately determining fracture risk in CKD patients requires methods to assess bone quantity and quality, and interventions that target both may be the most effective at reducing fractures.

Formation and Measurement of AGEs

Advanced glycation end-products (AGEs) form from the attachment of reducing sugars to a protein or lipid in a non-enzymatic chemical reaction. AGEs are classified as endogenous or exogenous. Endogenous AGEs form in the body whereas exogenous AGEs are derived from the environment(9). For endogenous AGE formation, the initial phase is the nucleophilic attack of glucose or other metabolic intermediate to a lysine group(10). Multiple unstable intermediate products are formed, including Amadori products. A series of reactions involving rearrangement, dehydration, oxidation, and fragmentation of Amadori products results in final AGE products(11). Different fragmentation methods to form the final AGEs include oxidation, isomerization, β-cleavage, and cyclization among others and are reviewed elsewhere(12). In bone, crosslinking AGEs such as pentosidine and glucosepane can form between a lysine and arginine residue if a carbonyl group on an Amadori product on a lysine attaches to an arginine on an adjacent collagen molecule(10). Other AGEs can attach to a single residue such as N^{ε}-carboxymethyl lysine (CML) and N^{ε}-carboxyethyl lysine (CEL).

AGEs are traditionally measured in the blood using enzyme-linked immunosorbent assays (ELISAs) or high-performance liquid chromatography (HPLC), the latter of which is preferred due to the general lack of AGE specificity with ELISAs(13). HPLC can be used to measure both free and protein-bound AGEs including pentosidine, CML, and CEL within the blood and tissues(14). However, a single measurement of AGEs in the blood may be unreliable, as most AGEs are formed intracellularly, and due to the abundance of proteins and lipids in tissues, tend to accumulate outside the blood. Furthermore, clearance of AGEs by the kidney is reduced in CKD, which may increase the apparent level of AGEs in the blood(15). In tissues, extracellular matrix proteins are a common site for AGE formation and accumulation, and the fluorescent properties of some AGEs can be used to approximate total AGE accumulation in these compartments.

In both skin and bone, collagen is a common site for non-enzymatic glycation and subsequent AGE formation(8, 16). Utilizing these properties, non-invasive skin autofluorescence (SAF) and fluorescent bone assays have been used to estimate AGE content. Increasing SAF values moderately correlate with pentosidine, CML, and CEL in the skin despite the lack of fluorescence of CML and CEL, and negatively correlate with estimated glomerular filtration rate (eGFR)(17–19). While SAF is rapid and non-invasive, it varies based on skin tone and does not distinguish which AGEs are present. HPLC and fluorescence assays can each be performed on bone specimens and are normalized by hydroxyproline to estimate the amount of non-enzymatic crosslinks. However, pentosidine is the only reliably distinguished AGE in bone tissue and the crosslinks formed by pentosidine are scarce compared to CML(20). Fluorescence-based bone assays are typically normalized to a quinine standard, which may better approximate the total number of AGEs in bone. However, this method does not distinguish between the fluorescent AGEs and does not account for non-fluorescent AGEs, which are likely more prevalent. Therefore, further work is needed to develop techniques to identify and measure the amount and properties of the various AGEs in bone.

Accumulation of AGEs in CKD

While hyperglycemia is known to drive AGE formation in diabetes, increased oxidative stress may explain the buildup of AGEs in CKD patients without diabetes. In CKD, the balance between pro-oxidant molecules and antioxidant mechanisms becomes altered as antioxidants fail to decrease the pool of reactive oxygen species (ROS) due to chronic inflammation(21, 22). Because oxidation is known to drive the glycoxidation reaction, a critical step in the formation of pentosidine and CML, increased ROS levels increase AGE production(23). To mitigate the effects of ROS, antioxidants including catalase, superoxide dismutase, and glutathione peroxidase transform ROS into stable products. AGEs also increase oxidative stress by increasing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) activity, a major producer of the superoxide radical(24). NOX activity is notably induced by the receptor for AGEs (RAGE) signaling pathway, thereby linking oxidative stress with AGE formation and activity on the cellular level. The pro-oxidant effects of AGEs are mitigated by AGE receptor 1 (AGER1), which neutralizes AGEs by binding and transporting them for degradation(25). However, chronic inflammation and continuously elevated AGE levels reduce AGER1 activity, resulting in increased oxidative

stress(26). Taken together, oxidative stress is an important underlying cause of increased AGEs in CKD patients, and AGEs disrupt the mechanisms responsible for neutralizing oxidative stress and AGEs.

There is some evidence that an increase in exogenous AGEs can lead to increased endogenous AGEs. AGEs are more prevalent in high fat foods and foods cooked with dry heat(27). In one clinical study, diets higher in CML, CEL, and Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) were correlated with higher levels in CML, CEL, and MG-H1 in plasma and urine(28). Others have found similar positive correlations in serum CML with dietary AGEs in patients with renal failure(29, 30). Our group found that autoclaving rat food, a common practice in animal facilities, increased the content of AGEs and accelerated kidney decline in our Cy/+ rat model of progressive CKD(31). Serum AGEs do not necessarily indicate an increase in the AGEs in bone. There is some evidence in diabetic and non-diabetic patients of a weak correlation between serum pentosidine and cortical bone AGEs, though interestingly, not a correlation with total serum AGEs and cortical bone AGEs(32). Higher AGEs have been observed in the bones of diabetic patients(33), aged patients(34), and dialysis patients(35). However, given the frequently concomitant nature of high AGE-diet, inflammation, and metabolic conditions, it is difficult to determine the exact cause of increased bone AGEs.

When AGEs are continuously elevated, degradation and clearance mechanisms become less efficient at removing AGEs from the circulation, resulting in increased accumulation in tissues. AGEs are known to develop from glycated proteins, the most abundant of which are collagens. While bone remodeling can remove AGEs bound to collagen, pentosidine was found to reduce bone resorption by osteoclasts when cultured on bone sections(36). AGEs further reduce osteoblast activity and mineralization while promoting apoptosis(37, 38). Osteocytes are also affected by AGEs, resulting in increased sclerostin and decreased Receptor activator of nuclear factor kappa-B ligand (RANKL) expression, resulting in an overall inhibition of osteoblast and osteoclast activity and differentiation(39). Taken together, the cellular effects of AGEs reduce the rate of bone turnover and increase AGE accumulation in the bone, limiting the formation of high-quality bone and potentially leading to microdamage accumulation and reduced mechanical properties(40).

Biomechanics and its Translation to Fracture Risk

Given the important structural role of bone, biomechanics is important to determine the likelihood a disease or condition will result in an increased risk of fracture and to quantify the effects of treatments. Although *in vivo* assessments in humans by HR-pQCT can provide insight, true testing requires *ex vivo* assessments of bone. One type of test involves measuring the force a bone requires to be deformed until it fractures. The resulting force-displacement curve (Figure 2A) has two characteristic regions: the elastic region where the bone can return its original shape, and the plastic region where the sample has been permanently deformed. There is an important distinction between extrinsic and intrinsic material properties. Extrinsic mechanical properties represent mechanical behavior of the entire structure and are influenced by the amount of material and the quality of that material. Intrinsic mechanical properties result when extrinsic properties are normalized by the

amount of material, and therefore reflect mechanical behavior at the tissue-level. Bone quality refers to intrinsic mechanical properties regardless of the geometry and quantity of the bone. For intrinsic mechanical properties, the force-displacement curve is converted to a stress-strain curve (41), where stress is force per area and strain is the change in length relative to the original length (Figure 2B,C). Ultimate force becomes ultimate strength, work becomes toughness, and stiffness becomes modulus. Additional tests include local indentation tests that measure properties on the micro- or nanoscale, fracture toughness and fatigue testing (Table 1).

Overall fracture risk is an interplay between the properties described above. Strength is strongly influenced by the mineralization of the bone(42) while toughness and post-yield properties are primarily driven by the state of the collagen matrix. Strength determines how likely a bone is to withstand a single load event such as a fall(43). Fracture toughness reflects the likelihood of the bone to fail due to the presence of flaws in the tissues (e.g. microdamage, porosities). A bone with low toughness is typically brittle with little ability to dissipate energy through deformation during loading. A strong bone can still break in a single load event if it is brittle. High energies needed for crack propagation would prevent fracture from happening through this mechanism. Fatigue testing determines how likely the bone will fail from damage accumulated over repeated loadings.

Enzymatic crosslinks form in bone between helical and non-helical domains of adjacent collagen molecules (44). The process and types of enzymatic collagen crosslinking is reviewed extensively elsewhere(10). Enzymatic crosslinks are important to bone quality, but data on their contribution to mechanical integrity are inconsistent. Studies have shown that bone mechanical properties are deteriorated when samples are treated with high dose gamma irradiation(45) or β-aminopropionitrile (BAPN)(46) which break or prevent crosslinks, respectively. In other studies with samples machined from human bone, mechanical properties did not correlate with mature collagen enzymatic crosslinks(47). However, collagen can also be crosslinked by AGEs such as pentosidine and glucosepane (Figure 3). AGE crosslinks can negatively impact bone’s mechanical properties. One study found that AGEs impeded fibril sliding of collagen in rat tail tendons incubated in ribose, increasing brittleness(48) and reducing the post-yield mechanical properties of bone(49). Other studies have found that propagation toughness negatively correlated with AGEs and that glycation decreased energy dissipation of collagen molecules as measured by atomic force microscopy(50). In human samples, AGE content measured by fluorescent AGEs negatively correlated with fracture toughness(50). However, others reported no relationship between pentosidine and mechanical properties(47). Increases in AGEs can deteriorate bone’s mechanical properties, but this may occur conditionally based on disease state.

Effects of Skeletal AGEs and Treatments in CKD

While AGEs are known to form readily on collagen-bound Amadori products, few studies have examined the direct effects of bone matrix-bound AGEs on skeletal properties in CKD. Through a combination of *in vitro* and *in vivo* studies using a rodent model of adenine nephropathy, it was determined that AGEs reduce osteoblast LOX expression, disrupting the proper enzymatic crosslinking of collagen fibrils in bone(51). Our group also detected

elevated fluorescent AGEs including pentosidine in a naturally progressive rat model of CKD, the Cy/+ rat, using fluorescent assays and HPLC, and AGEs increase most rapidly late in the disease course(52). Pentosidine levels in Cy/+ rats were reduced by the compound ALT-711, which has previously been shown to reduce AGEs in cardiac tissues(53, 54). Additionally, antioxidant therapy with N-acetylcysteine (NAC) has been used in CKD patients to reduce oxidative stress and is hypothesized to reduce downstream formation of AGEs. Interestingly, NAC did not alter oxidative stress markers but did reduce bone AGE content in Cy/+ rats, as determined by a fluorescent AGE assay(55). HPLC measurements did not indicate changes in skeletal pentosidine levels, and bone mechanical properties failed to improve. Therefore, pentosidine may be more closely related to bone mechanical properties while other fluorescent AGEs play a less significant role in bone quality.

In dialysis patients, bone pentosidine is significantly increased and negatively correlated with bone formation and mineralization(35), consistent with the effects of AGEs *in vitro* and in preclinical models. While measuring AGEs in the bone may be the most direct way to assess their effects on strength, most human studies to date have assayed the skin and blood. SAF measurements positively correlate with mortality in dialysis patients and circulating AGE levels, namely pentosidine, correlate with vascular disease and chronic inflammation(44, 56, 57). While these results are an important start to unraveling the systemic and skeletal effects of AGEs, further work is needed to determine how skin and serum AGE measurements compare to those in the bone, and which AGEs are responsible for increasing bone fragility in CKD patients.

Conclusion

In summary, AGEs are elevated in CKD patients and directly impact bone matrix collagen by forming non-enzymatic crosslinks. The accumulation of non-enzymatic crosslinks contributes to the elevated fracture risk in CKD patients by decreasing bone quality. To date, however, there are few clinical studies that have directly measured AGEs in bone, and current measurement techniques do not provide a comprehensive view of the amount and identities of AGEs in bone. Therefore, additional preclinical and clinical studies are needed to determine the effects of specific AGEs on bone mechanical properties and to better characterize the impact of treatments to reduce AGEs on fracture risk and fracture-related mortality.

Acknowledgements

Financial support and scholarship: JGD: DK121399, AC: AR065971, JMW: AR072609, SMM: AR072581, DK110871, UL1TR002529, DK12054.

References

1. Alem AM, Sherrard DJ, Gillen DL, et al. Increased risk of hip fracture among patients with end-stage renal disease. Kidney Int 2000;58(1):396–9. [PubMed: 10886587]
2. Coco M, Rush H. Increased incidence of hip fractures in dialysis patients with low serum parathyroid hormone. Am J Kidney Dis 2000;36(6):1115–21. [PubMed: 11096034]
3. Moe SM, Nickolas TL. Fractures in Patients with CKD: Time for Action. Clin J Am Soc Nephrol 2016;11(11):1929–31. [PubMed: 27797903]

4. Nickolas TL, Stein EM, Dworakowski E, et al. Rapid cortical bone loss in patients with chronic kidney disease. J Bone Miner Res 2013;28(8):1811–20. [PubMed: 23456850]
5. Nickolas TL, Stein E, Cohen A, et al. Bone mass and microarchitecture in CKD patients with fracture. J Am Soc Nephrol 2010;21(8):1371–80. [PubMed: 20395370]
6. Wang X, Shen X, Li X, Agrawal CM. Age-related changes in the collagen network and toughness of bone. Bone. 2002;31(1):1–7. [PubMed: 12110404]
7. Zimmermann EA, Schaible E, Bale H, et al. Age-related changes in the plasticity and toughness of human cortical bone at multiple length scales. Proc Natl Acad Sci U S A. 2011;108(35):14416–21. [PubMed: 21873221]
8. Saito M, Fujii K, Soshi S, Tanaka T. Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation-induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. Osteoporos Int 2006;17(7):986–95. [PubMed: 16552468]
9. Ruiz HH, Ramasamy R, Schmidt AM. Advanced Glycation End Products: Building on the Concept of the “Common Soil” in Metabolic Disease. Endocrinology. 2020;161(1).
10. Saito M, Marumo K. Effects of Collagen Crosslinking on Bone Material Properties in Health and Disease. Calcified Tissue International. 2015;97(3):242–61. [PubMed: 25791570]
11. Henning C, Smuda M, Girndt M, et al. Molecular basis of maillard amide-advanced glycation end product (AGE) formation in vivo. The Journal of biological chemistry. 2011;286(52):44350–6. [PubMed: 22069309]
12. Henning C, Glomb MA. Pathways of the Maillard reaction under physiological conditions. Glycoconjugate Journal. 2016;33(4):499–512. [PubMed: 27291759]
13. Scheijen JL, van de Waarenburg MP, Stehouwer CD, Schalkwijk CG. Measurement of pentosidine in human plasma protein by a single-column high-performance liquid chromatography method with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877(7):610–4.
14. Takahashi M, Hoshino H, Kushida K, Inoue T. Direct measurement of crosslinks, pyridinoline, deoxypyridinoline, and pentosidine, in the hydrolysate of tissues using high-performance liquid chromatography. Anal Biochem 1995;232(2):158–62. [PubMed: 8747470]
15. Makita Z, Radoff S, Rayfield EJ, et al. Advanced glycosylation end products in patients with diabetic nephropathy. N Engl J Med 1991;325(12):836–42. [PubMed: 1875967]
16. Dyer DG, Dunn JA, Thorpe SR, et al. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. J Clin Invest 1993;91(6):2463–9. [PubMed: 8514858]
17. McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Skin autofluorescence and the association with renal and cardiovascular risk factors in chronic kidney disease stage 3. Clin J Am Soc Nephrol 2011;6(10):2356–63. [PubMed: 21885790]
18. Tanaka K, Nakayama M, Kanno M, et al. Skin autofluorescence is associated with the progression of chronic kidney disease: a prospective observational study. PLoS One. 2013;8(12):e83799. [PubMed: 24349550]
19. Meerwaldt R, Graaff R, Oomen PHN, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia. 2004;47(7):1324–30. [PubMed: 15243705]
20. Thomas CJ, Cleland TP, Sroga GE, Vashishth D. Accumulation of carboxymethyl-lysine (CML) in human cortical bone. Bone. 2018;110:128–33. [PubMed: 29408699]
21. Gupta J, Mitra N, Kanetsky PA, et al. Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. Clin J Am Soc Nephrol 2012;7(12):1938–46. [PubMed: 23024164]
22. Daenen K, Andries A, Mekahli D, et al. Oxidative stress in chronic kidney disease. Pediatr Nephrol 2019;34(6):975–91. [PubMed: 30105414]
23. Vistoli G, De Maddis D, Cipak A, et al. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic Res 2013;47 Suppl 1:3–27. [PubMed: 23767955]
24. Wautier MP, Chappey O, Corda S, et al. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol Endocrinol Metab 2001;280(5):E685–94. [PubMed: 11287350]

25. Cai W, He JC, Zhu L, et al. AGE-receptor-1 counteracts cellular oxidant stress induced by AGEs via negative regulation of p66shc-dependent FKHRL1 phosphorylation. Am J Physiol Cell Physiol 2008;294(1):C145–52. [PubMed: 18032526]
26. He CJ, Koschinsky T, Buenting C, Vlassara H. Presence of diabetic complications in type 1 diabetic patients correlates with low expression of mononuclear cell AGE-receptor-1 and elevated serum AGE. Mol Med 2001;7(3):159–68. [PubMed: 11471552]
27. Uribarri J, Woodruff S, Goodman S, et al. Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet. Journal of the American Dietetic Association. 2010;110(6):911–6.e12. [PubMed: 20497781]
28. Scheijen JLJM, Hanssen NMJ, van Greevenbroek MM, et al. Dietary intake of advanced glycation endproducts is associated with higher levels of advanced glycation endproducts in plasma and urine: The CODAM study. Clinical Nutrition. 2018;37(3):919–25. [PubMed: 29381139]
29. Uribarri J, Peppa M, Cai W, et al. Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. American Journal of Kidney Diseases. 2003;42(3):532–8. [PubMed: 12955681]
30. Uribarri J, Peppa M, Cai W, et al. Restriction of Dietary Glycotoxins Reduces Excessive Advanced Glycation End Products in Renal Failure Patients. Journal of the American Society of Nephrology. 2003;14(3):728. [PubMed: 12595509]
31. Biruete A, Srinivasan S, O'Neill KD, et al. Adverse Effects of Autoclaved Diets on the Progression of Chronic Kidney Disease and Chronic Kidney Disease-Mineral Bone Disorder in Rats. Am J Nephrol 2020;51(5):381–9. [PubMed: 32146472]
32. Karim L, Moulton J, Van Vliet M, et al. Bone microarchitecture, biomechanical properties, and advanced glycation end-products in the proximal femur of adults with type 2 diabetes. Bone. 2018;114:32–9. [PubMed: 29857063] * This paper demonstrates a correlation of serum AGEs with skeletal AGEs. This connection is important to investigate, as serum AGEs can be measured clinically.
33. Heveran CM, Schurman CA, Acevedo C, et al. Chronic kidney disease and aging differentially diminish bone material and microarchitecture in C57Bl/6 mice. Bone. 2019;127:91–103. [PubMed: 31055118]
34. Odetti P, Rossi S, Monacelli F, Poggi A, Cirnigliaro M, Federici M, Federici A Advanced Glycation End Products and Bone Loss during Aging. Annals of the New York Academy of Sciences. 2005;1043(1):710–7. [PubMed: 16037297]
35. Mitome J, Yamamoto H, Saito M, et al. Nonenzymatic cross-linking pentosidine increase in bone collagen and are associated with disorders of bone mineralization in dialysis patients. Calcif Tissue Int 2011;88(6):521–9. [PubMed: 21499867]
36. Valcourt U, Merle B, Gineyts E, et al. Non-enzymatic glycation of bone collagen modifies osteoclastic activity and differentiation. J Biol Chem 2007;282(8):5691–703. [PubMed: 17142454]
37. Sanguineti R, Storace D, Monacelli F, et al. Pentosidine effects on human osteoblasts in vitro. Ann N Y Acad Sci 2008;1126:166–72. [PubMed: 18448811]
38. Alikhani M, Alikhani Z, Boyd C, et al. Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. Bone. 2007;40(2):345–53. [PubMed: 17064973]
39. Tanaka K, Yamaguchi T, Kanazawa I, Sugimoto T. Effects of high glucose and advanced glycation end products on the expressions of sclerostin and RANKL as well as apoptosis in osteocyte-like MLO-Y4-A2 cells. Biochem Biophys Res Commun 2015;461(2):193–9. [PubMed: 25721666]
40. Kume S, Kato S, Yamagishi S, et al. Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. J Bone Miner Res 2005;20(9):1647–58. [PubMed: 16059636]
41. Turner CH, Burr DB Basic Biomechanical Measurements of Bone: A Tutorial. Bone. 1993;14:595–608. [PubMed: 8274302]
42. Currey J. Effects of differences in mineralization on the mechanical properties of bone. Philosophical Transactions of the Royal Society B. 1984;304(1121):509–18.
43. Hernandez C, van der Meulen MCH. Understanding Bone Strength Is Not Enough. Journal of Bone and Mineral Research. 2017;32(6):1157–62. [PubMed: 28067411]

44. Suliman ME, Heimbürger O, Bárány P, et al. Plasma pentosidine is associated with inflammation and malnutrition in end-stage renal disease patients starting on dialysis therapy. J Am Soc Nephrol 2003;14(6):1614–22. [PubMed: 12761263]
45. Burton B, Gaspar A, Josey D, Tupy J, Grynpas MD, Willett TL. Bone embrittlement and collagen modifications due to high-dose gamma-irradiation sterilization. Bone. 2014;61(C):71–81. [PubMed: 24440514]
46. McNerny EMB, Gong B, Morris MD, Kohn DH. Bone Fracture Toughness and Strength Correlate With Collagen Cross-Link Maturity in a Dose-Controlled Lathyrism Mouse Model. Journal of Bone and Mineral Research. 2015;30(3):455–64. [PubMed: 25213475]
47. Willett TL, Dapaah DY, Uppuganti S, et al. Bone collagen network integrity and transverse fracture toughness of human cortical bone. Bone. 2019;120:187–93. [PubMed: 30394355]
48. Gautieri A, Passini FS, Silván U, et al. Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. Matrix Biology. 2017;59:95–108. [PubMed: 27616134]
49. Willett TL, Sutty S, Gaspar A, et al. In vitro non-enzymatic ribation reduces post-yield strain accommodation in cortical bone. Bone. 2013;52(2):611–22. [PubMed: 23178516]
50. Poundarik A, Wu PC, Evis Z, Sroga GE, Ural A, Rubin M. A direct role of collagen glycation in bone fracture. Journal of Mechanical Behavior of Biomedical Materials. 2015;52:120–30.
51. Aoki C, Uto K, Honda K, et al. Advanced glycation end products suppress lysyl oxidase and induce bone collagen degradation in a rat model of renal osteodystrophy. Lab Invest 2013;93(11):1170–83. [PubMed: 23979426]
52. Allen MR, Newman CL, Chen N, et al. Changes in skeletal collagen cross-links and matrix hydration in high- and low-turnover chronic kidney disease. Osteoporos Int 2015;26(3):977–85. [PubMed: 25466530]
53. Chen NX, Srinivasan S, O'Neill K, et al. Effect of Advanced Glycation End-Products (AGE) Lowering Drug ALT-711 on Biochemical, Vascular, and Bone Parameters in a Rat Model of CKD-MBD. J Bone Miner Res 2020;35(3):608–17. [PubMed: 31743501] * This is one of the first preclinical studies examining a compound that cleaves AGEs *in vitro*. This article demonstrates that both calcium and ALT-711 reduce AGEs in the bone, although ALT-711 did not improve bone mechanical properties.
54. Candido R, Forbes JM, Thomas MC, et al. A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. Circ Res 2003;92(7):785–92. [PubMed: 12623881]
55. Allen MR, Wallace J, McNerney E, et al. N-acetylcysteine (NAC), an anti-oxidant, does not improve bone mechanical properties in a rat model of progressive chronic kidney disease-mineral bone disorder. PLoS One. 2020;15(3):e0230379. [PubMed: 32203558]
56. Tanaka K, Tani Y, Asai J, et al. Skin autofluorescence is associated with renal function and cardiovascular diseases in pre-dialysis chronic kidney disease patients. Nephrol Dial Transplant 2011;26(1):214–20. [PubMed: 20591816]
57. Nishizawa Y, Koyama H, Inaba M. AGEs and cardiovascular diseases in patients with end-stage renal diseases. J Ren Nutr 2012;22(1):128–33. [PubMed: 22200429]

Key Points

- CKD patients have elevated AGEs due to oxidative stress and inflammation, which activate cell signaling through RAGE and AGER1 while accumulating in the extracellular matrix
- AGEs form non-enzymatic crosslinks between bone collagen fibrils, reducing bone toughness and increasing brittleness
- Measuring AGEs in CKD patients is typically indirect, and does not identify or determine the properties of the detected AGEs
- Improving bone strength in CKD requires interventions that target bone mass and quality

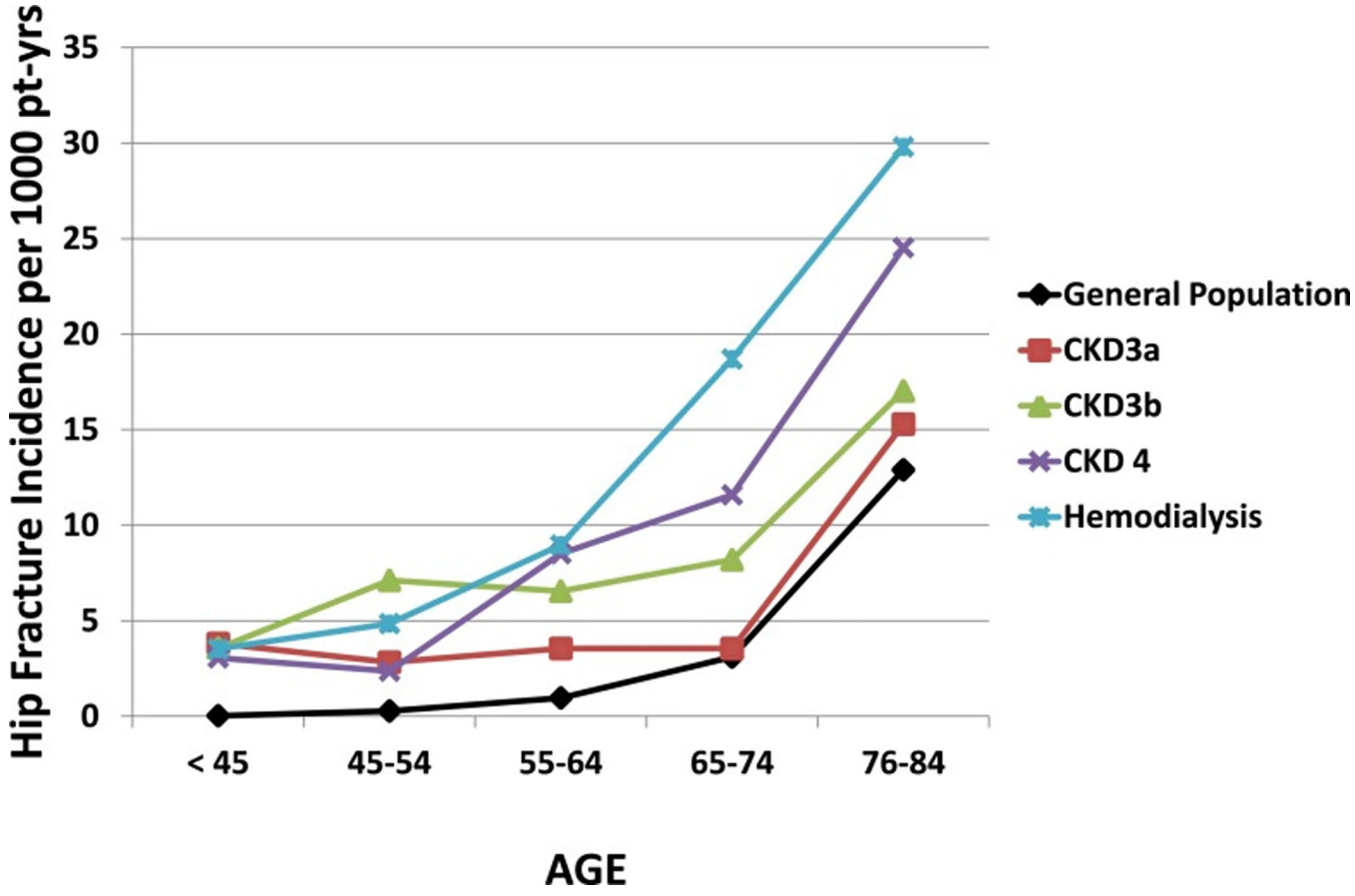

Figure 1: Fracture Risk Increases with CKD Progression.

Hip fracture incidence increases as renal function worsens. While fracture risk increases with age in the general population, fracture incidence is further elevated in aging CKD patients(3). Previously published: Moe & Nickolas, Clin J Am Soc Nephrol, 2016 (3).

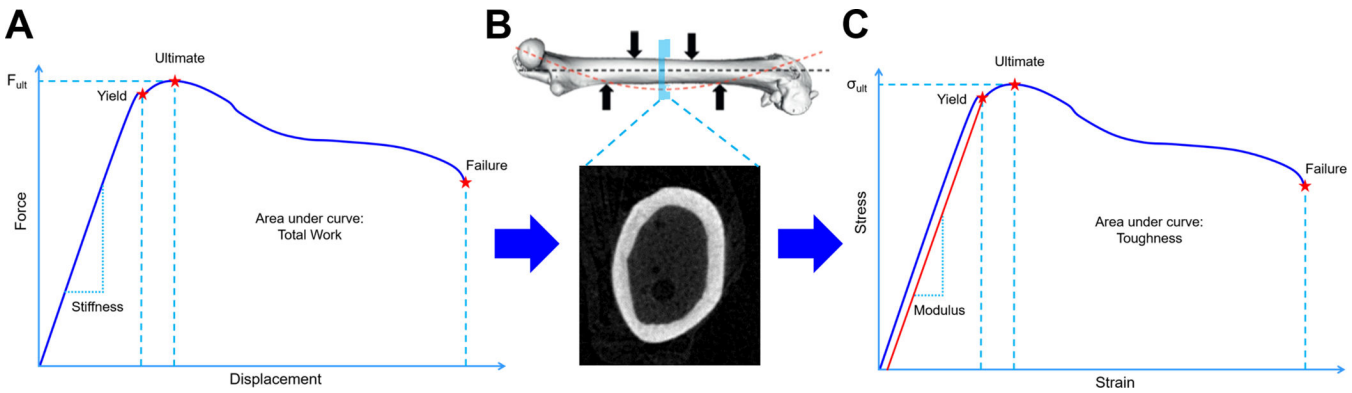

Figure 2: Workflow for Determining Extrinsic and Intrinsic Bone Mechanical Properties.

Force-displacement curves generated during mechanical tests are used to calculate extrinsic mechanical properties of the bone (A). The data is then normalized by the cross-sectional area at the fracture site (B), resulting in normalized force (stress) and displacement (strain). The resulting stress-strain curve is used to calculate intrinsic material properties (C). F_{ult} = ultimate force; σ_{ult} = ultimate stress. Original figure.

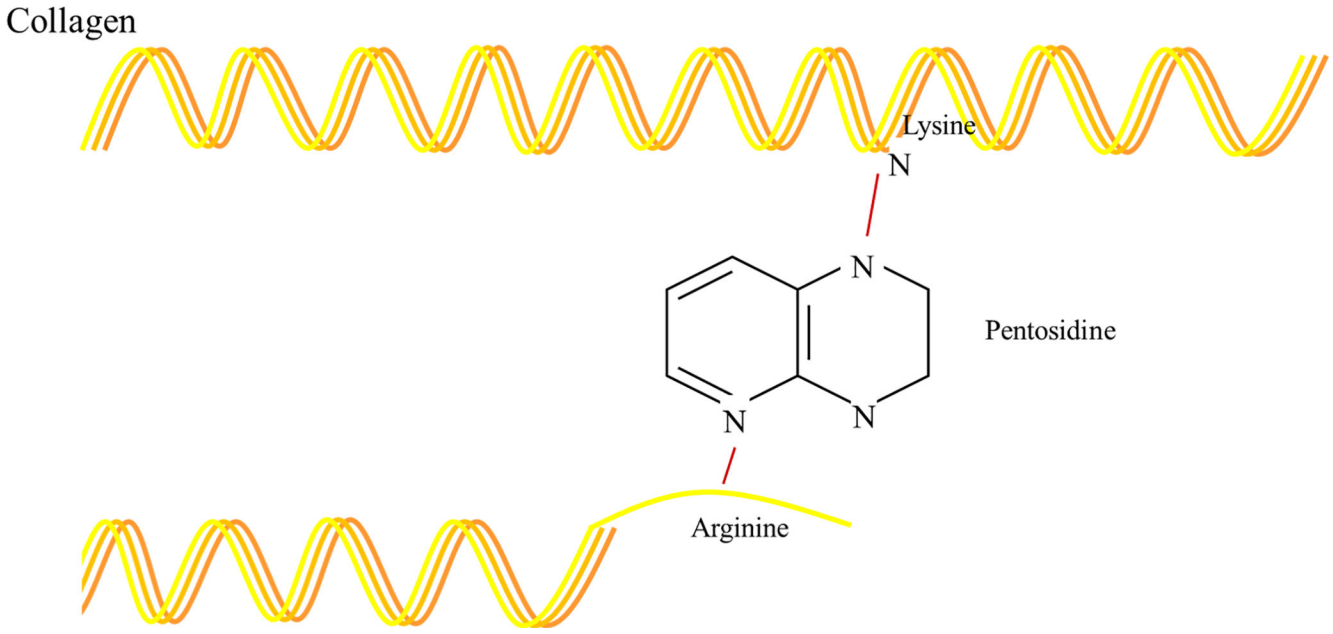

Figure 3: Schematic of Pentosidine Crosslinking two Collagen Molecules.

Pentosidine forms as a crosslink between the arginine and lysine residues on two different collagen proteins. Original figure.

Organ-level Property	Tissue-level Property	Related Bone Property	Mechanical Definition	Clinical Interpretation
Stiffness	Modulus	Mineralization	Slope of elastic region	Ability to resist elastic deformation
Ultimate force	Ultimate stress	Mineralization	Highest point on the curve	Maximum load before loss of mechanical integrity
Work	Toughness	Collagen alignment and crosslinking, hydration	Area under the curve	Ability to dissipate energy that can cause damage
Post-yield displacement	Post-yield strain	Collagen alignment and crosslinking, hydration	Difference on X-axis between the yield and failure points	Ability to withstand fracture after damage and begin repair
Fracture Toughness		Collagen quality, microdamage	Resistance to crack propagation	Ability to resist fracture from existing flaws
Fatigue Life		Porosity, microdamage	Cycles to failure below yield force	Ability to resist fracture from small, repetitive loads