Bone 82 (2016) 21–27

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review

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

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ARTICLE INFO

Article history:
Received 8 May 2015
Revised 7 July 2015
Accepted 13 July 2015
Available online 23 July 2015

Keywords:
Diabetes
Bone strength
Bone mechanics
Bone quality
Advanced glycation end-products

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.

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http://dx.doi.org/10.1016/j.bone.2015.07.028
8756-3282/

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 that 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 the 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 non-enzymatic 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,” “non-enzymatic 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. Post-translational 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),