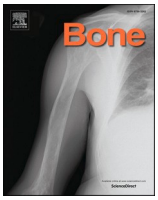
Bone 174 (2023) 116832

Contents lists available at ScienceDirect

Bone

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



Full Length Article

Bone intrinsic material and compositional properties in postmenopausal women diagnosed with long-term Type-1 diabetes

Wen Qian [a,1], Sonja Gamsjaeger [b,1], Eleftherios P. Paschalis [b,1], Laura A. Graeff-Armas [c,1], Sue P. Bare [d,1], Joseph A. Turner [a,1], Joan M. Lappe [d,1], Robert R. Recker [d,1], Mohammed P. Akhter [d,*,1]

[a] University of Nebraska, Lincoln, NE, USA
[b] Ludwig Boltzmann Institute of Osteology, Vienna, Austria
[c] University of Nebraska Medical Center, Omaha, NE, USA
[d] Osteoporosis Research Center, Creighton University, Omaha, NE, USA

ARTICLE INFO

Keywords:
Type-1 diabetes
Transiliac bone biopsies
Postmenopausal women
Mineral properties
Hardness
modulus

ABSTRACT

The incidence of diabetes mellitus and the associated complications are growing worldwide, affecting the patients' quality of life and exerting a considerable burden on health systems. Yet, the increase in fracture risk in type 1 diabetes (T1D) patients is not fully captured by bone mineral density (BMD), leading to the hypothesis that alterations in bone quality are responsible for the increased risk. Material/compositional properties are important aspects of bone quality, yet information on human bone material/compositional properties in T1D is rather sparse. The purpose of the present study is to measure both the intrinsic material behaviour by nanoindentation, and material compositional properties by Raman spectroscopy as a function of tissue age and microanatomical location (cement lines) in bone tissue from iliac crest biopsies from postmenopausal women diagnosed with long-term T1D ($N = 8$), and appropriate sex-, age-, BMD- and clinically-matched controls (postmenopausal women; $N = 5$). The results suggest elevation of advanced glycation endproducts (AGE) content in the T1D and show significant differences in mineral maturity / crystallinity (MMC) and glycosaminoglycan (GAG) content between the T1D and control groups. Furthermore, both hardness and modulus by nanoindentation are greater in T1D. These data suggest a significant deterioration of material strength properties (toughness) and compositional properties in T1D compared with controls.

1. Introduction

In the clinic, bone mineral density (BMD) by dual X-ray absorptiometry (DXA) measurements, complemented by algorithms such as Fracture Risk Assessment Tool (FRAX) are the mainstay in the estimation of fracture risk. The incidence of diabetes mellitus and the associated complications are growing worldwide, affecting the patients' quality of life and exerting a considerable burden on health systems [1,2]. Yet, the increase in fracture risk in type 1 diabetes (T1D) patients is not fully captured by bone mineral density (BMD) [1,3]. This increase in fracture risk occurs at all ages and in both genders and worsens with age [1]. Higher rate of fractures in T1D have also been attributed to increased risk of falls due to diabetes-related complications (diminished balance, reduced muscle strength, and vision problems among others), and compromised bone quality [1], the latter including the structural and material/compositional properties of bone [4].

Quantitative computed tomography investigations indicate that T1D affects mostly the cortical bone structure, while alterations in the cancellous compartment (thinner, wider spaced trabeculae) are affected to a lesser extent [5]. Studies in patients with T1D have also indicated an association between microvascular complications (associated with increased oxidative stress) and bone structural deficits [5], with reports suggesting that microangiopathy may have a more pronounced adverse effect on hip structure [6,7].

Information on human bone material and compositional properties in T1D is rather sparse [5]. In a study involving iliac crest biopsies from

* Corresponding author at: Professor of Medicine, Creighton University Osteoporosis Research Center, Immanuel Hospital, 6751 North 72nd Street, Building-2, Suite 104, Omaha, NE 68122-1746.
E-mail address: akhtermp@creighton.edu (M.P. Akhter).
[1] All authors have contributed equally.

https://doi.org/10.1016/j.bone.2023.116832
Received 12 January 2023; Received in revised form 12 June 2023; Accepted 21 June 2023
Available online 27 June 2023

age- and sex-matched non-fracturing T1D patients as well as healthy age- and sex-matched controls, it was shown that trabecular bone from fracturing T1D had higher levels of pentosidine compared to healthy controls, and was more mineralized compared to either nonfracturing T1D patients or healthy controls [3]. On the other hand, no differences were evident between these three groups by either micro- or nano-indentation [3].

In the present study, we used quasi-static nanoindentation to quantify local/intrinsic material properties of modulus (GPa) and hardness (GPa) in the interstitial region and the cement lines of iliac crest biopsies obtained from postmenopausal women diagnosed with T1D ($N = 8$, T1D; duration of disease >10 years), and age- and BMD-matched postmenopausal women controls ($N = 5$, Control). Following this, the biopsies were analysed for compositional / material properties by Raman spectroscopy. The monitored properties are greatly dependent on tissue age [8], such that the measurements were performed at precisely known tissue ages and interstitial bone in both cancellous and cortical compartments, as well as cortical cement lines with prior nanoindentations.

2. Materials & methods

2.1. Patients

The Control group ($N = 5$) consisted of postmenopausal patients, while the T1D group ($N = 8$) of age- and BMD-matched postmenopausal, non-fracturing type 1 diabetics. All participants were Caucasian, >50 years old and 5 years past the onset of menopause and had diabetes for 10–50 years (mean duration of diabetic disease 34 ± 13 yrs., Table-1). All the subjects, T1D and Control, were free of diagnoses (fractures) other than diabetes, and had DXA T-scores at the total hip and lumbar spine between −1.0 and − 2.5. Each Control subject was matched to the T1D with the following criteria:1. DXA measures (BMD, gm/cm) was within +/− 10 % in the spine and hip, 2. Body mass index (BMI) was within +/− 10 %, and 3. Age within +/− 5 years. The clinical characteristics are summarized in Table 1.

Prior to undergoing biopsies, each subject received in vivo double tetracycline labelling. The oral tetracycline HCl schedule was 250 mg four times daily, three days on, 14 days off, three more days on and then biopsy 5–14 days later [9–11]. All analysed biopsies in the present report were embedded in low viscosity quick setting EpoThin plastic material (Buehler, IL, USA) as described elsewhere [12].

2.2. Nanoindentation

The localized mechanical properties (modulus and hardness) were measured using a Hysitron TI 950 Triboindenter. Through quasi-static nanoindentation, we quantified local/intrinsic material properties of modulus (GPa) and hardness (GPa) in interstitial regions (Supplementary Fig. 1-A), and the cement lines (CL, Supplementary Fig. 1-B) of the iliac crest bone biopsies (cortical). Several indentations (with ~10 μm spacing) were made with a Berkovich tip and a targeted maximum force of 6 mN (milliNewtons) at a constant loading rate of 400 μN/s (micro-Newtons per second). The indentation procedure included a linear loading ramp of 15 s, a holding period of 10s at the maximum load and a linear unloading ramp of 15 s. A Poisson's ratio of 0.3 was assumed for bone tissue in calculations for the analysis [12].

Table 1
Clinical characteristics of the two patient groups analysed. Mean ± SD values are shown, along with the p-values of the statistical comparisons.

	Control	T1D	p-Value
Age (yrs)	59 ± 2.9	60.6 ± 4.5	0.73
BMD	0.853 ± 0.033	0.794 ± 0.062	0.295
Hip T-score	−0.7 ± 0.3	−1.2 ± 0.5	0.354
Height (cm)	165.24 ± 3.3	165.13 ± 7.17	0.231
Weight (kg)	68.92 ± 6.97	68.66 ± 5.89	0.517
BMI	25.24 ± 2.43	25.38 ± 3.79	0.546
HgA1c	5.45 ± 0.40	7.41 ± 0.81	0.001

All participants were postmenopausal (at least 5 years) Caucasian women with no history of skeletal fractures. The T1D participant had diabetes for at least at 10 years (34 ± 13 yrs. duration of disease). There are no differences between the two groups in all the clinical data collected (age, BMD, BMI) except hemoglobin-HgA1c, which is greater in T1D group. The comprehensive metabolic panel-CMP and bone-turnover markers were in the normal range for both the T1D and Controls.

2.3. Raman analysis

Raman spectra were obtained with a confocal Raman spectrometer (Renishaw InVia Qontor, www.renishaw.de). These spectra were collected at the interstitial (Fig. 1A), cement lines (Fig. 1B), and actively bone forming osteons with evident fluorescent labels (Fig. 1C). A continuous laser beam with an excitation of 785 nm and power of 10 mW was focused through a Raman microscope (Leica DM2700M), using the 50× objective, down to a micrometer-sized spot on the sample. The instrument was operated in a temperature-controlled room (constant temperature of 20° C), to minimize any potential performance variability due to ambient temperature fluctuations. All Raman spectra were obtained in confocal mode (1 μm below the biopsy surface). The integration time was 10s and co-additions were 10 to improve the signal-to-noise ratio (SNR; minimum SNR for a peak to be considered acceptable was 3 [13]). Spectra of pure embedding material from every biopsy block were also obtained to check the consistency of the instrument between the different biopsies. Spectra were cut (350–1800 cm^{-1}) and baseline corrected (5-point rubber band) to account for fluorescence background. No further spectra manipulation was performed. If cosmic spikes were evident in any of the collected spectra, these were rejected from further consideration rather than applying smoothing or spike removal algorithms. The following parameters were calculated (Wire5.4 software Renishaw and Opus 8.5 software Bruker) as described elsewhere [14]:

i) Mineral/matrix ratio (MM). The mineral/matrix ratio (MM) from the integrated areas of the ν_2PO_4 (410–460 cm^{-1}) and the amide III (1215–1300 cm^{-1}) bands, which, is independent of tissue organization / orientation [15], takes into account the amount of organic matrix content in the microvolume analysed.
ii) Mineral maturity/crystallinity (MMC). The mineral maturity/crystallinity (MMC) of the bone mineral apatite crystallites, approximated from the inverse of the full width at half height (FWHH) of the ν_1PO_4 (930–980 cm^{-1}) band
iii) Tissue water content. Sub-micron pore tissue water content (TW; nanoporosity), approximated by the ratio of the integrated areas of the spectral slice 494–509 cm^{-1} (embedding material) to Amide III band.
iv) Relative lipid content. The relative lipid content was expressed as the ratio of the integrated area of the lipids band ~1298 cm^{-1}/ amide III.
v) Glycosaminoglycan. The glycosaminoglycan (GAG) content expressed as the GAG / matrix ratio (the ratio of the integrated areas of the proteoglycan/CH_3 [1365–1390 cm^{-1}] band [representative of mucopolysaccharides] to the Amide III [1215–1300 cm^{-1}] band).
vi) Pyridinoline. The pyridinoline (Pyd; enzymatic trivalent collagen cross-link) content was calculated as the absorbance height at 1660 cm^{-1} / area of the amide I (1620–1700 cm^{-1}).
vii) Advanced glycation endproducts. The content of two advanced glycation endproducts (AGEs), namely CML (ε-*N*-Carboxymethyl-L-lysine) and PEN (Pentosidine) from the integrated area ratio of bands at 1150 (representative of NH_2 groups present in CML) cm^{-1} or 1495 (PEN) cm^{-1} / 1450 cm^{-1} (methylene side chains (CH_2)).

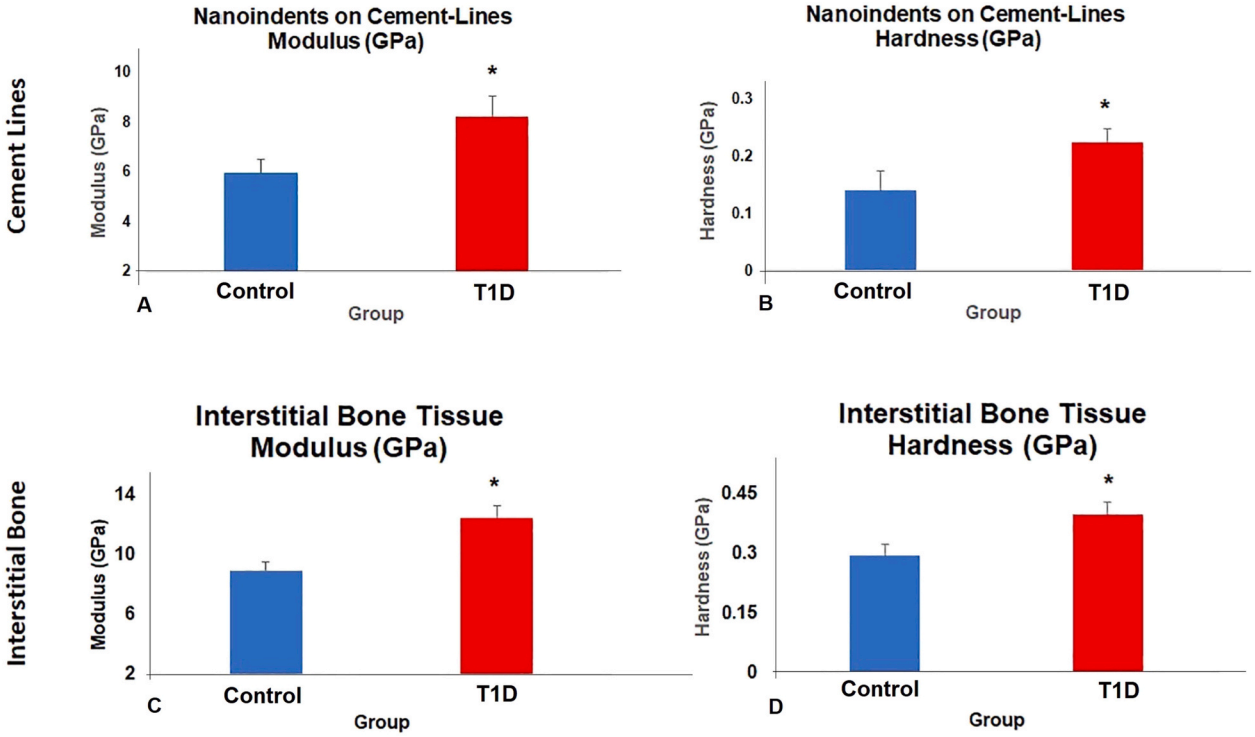


Fig. 1. Results of nanoindentation experiments at cement lines (top row) and in interstitial bone (bottom row). At both anatomical locations, long-term T1D postmenopausal patients had significantly higher modulus and hardness values compared to age-matched controls (* $p < 0.05$).

viii) Glucose (Glu) content. The tissue preparation and embedding protocols utilized in the present study allowed for the determination of glucose content based on the ratio of the integrated areas of a peak ~1345 cm^{-1} [18,19], to Amide III.

2.4. Raman area of analysis selection criteria

The following microanatomical areas were analysed: i) osteoid: a surface with evident tetracycline labels, 1 μm distance from the mineralizing front, and for which the Raman spectra showed the presence of organic matrix but not mineral [20], ii) TA1: mid-distance between the second fluorescent label and the mineralizing front, iii) TA2: mid-distance between the two fluorescent labels, iv) 1 μm behind the first fluorescent label, v) Interstitial bone: geometrical centers in cortical and cancellous compartment, away from surfaces with evident fluorescent labels, and vi) Cement lines that were previously indented. In these areas, we obtained Raman spectra on the cement line, 2 μm away from the indents, as well as 2 μm from the indents of both inside (towards the Haversian canal) and outside of the cement line. Typical pictures of the areas of analysis are shown in Supplementary Fig. 1. For each iliac crest biopsy considered, three areas were considered, the average value calculated at equivalent ones and treated as a single statistical unit.

2.5. Statistics

Osteoid data between groups were compared with either unpaired *t*-tests or Mann-Whitney test depending on whether the values were normally distributed or not (Kolmogorov Smirnov test).

Data obtained in anatomical areas ii – v were compared by 2-way ANOVA with patient group and tissue age as the two factors, followed by Tukey's multiple comparison test.

Data obtained at cement lines were compared by 2-way ANOVA with patient group and location with respect to the cement line as the two factors, followed by Tukey's multiple comparison test. Nanoindentation variables (modulus and hardness) were compared using one-way ANOVA. Correlations between nanoindentation and Raman outcomes in the anatomical area of cement lines were explored by Pearson or Spearman test (depending on whether data were normally distributed or not, by Kolmogorov-Smirnov test). In all instances, significance was assigned to $p < 0.05$.

3. Results

Nanoindentation experiments indicated that T1D at both cement lines (Fig. 1, top row) and in interstitial bone (Fig. 1, bottom row) had significantly ($p < 0.05$) higher modulus (Fig. 1A and C, respectively) and hardness (Fig. 1B and D, respectively).

No differences were evident in interlabel distance (IrLD) between T1D and Controls (data not shown), indicating similar rates of new bone formation at actively forming intracortical and trabecular (cancellous) surfaces.

Raman analysis indicated differences in the osteoid composition between the two groups. Specifically, T1D had significantly lower GAG content ($p < 0.05$) in the osteoid of both intracortical (Fig. 2A) and trabecular (Fig. C) surfaces, and reduced TW ($p < 0.05$) at the trabecular forming surfaces (Fig. 2B).

MM was similar between the two patient groups in both anatomical compartments (data not shown). While MMC between the two groups was similar in the cortical compartment (data not shown), T1D had significantly higher values in the cancellous bone ($p < 0.0001$), while Tukey's post-hoc tests showed that T1D had higher MMC compared to Controls at TA1 ($p < 0.0001$) (Fig. 3B). T1D had significantly lower tissue water content compared to Controls in both anatomical compartments considered ($p = 0.03$ for cortical, and 0.002 for trabecular) (Fig. 3A and C, respectively). There were no differences in lipids content in either intracortical or cancellous compartments (data not shown). Finally, T1D had significantly lower ($p = 0.0004$) GAG content in trabecular bone (Fig. 3D), but not in the intracortical (data not shown).

Pyd enzymatic collagen cross-links were similar between the two patient groups in both anatomical compartments (data not shown). On

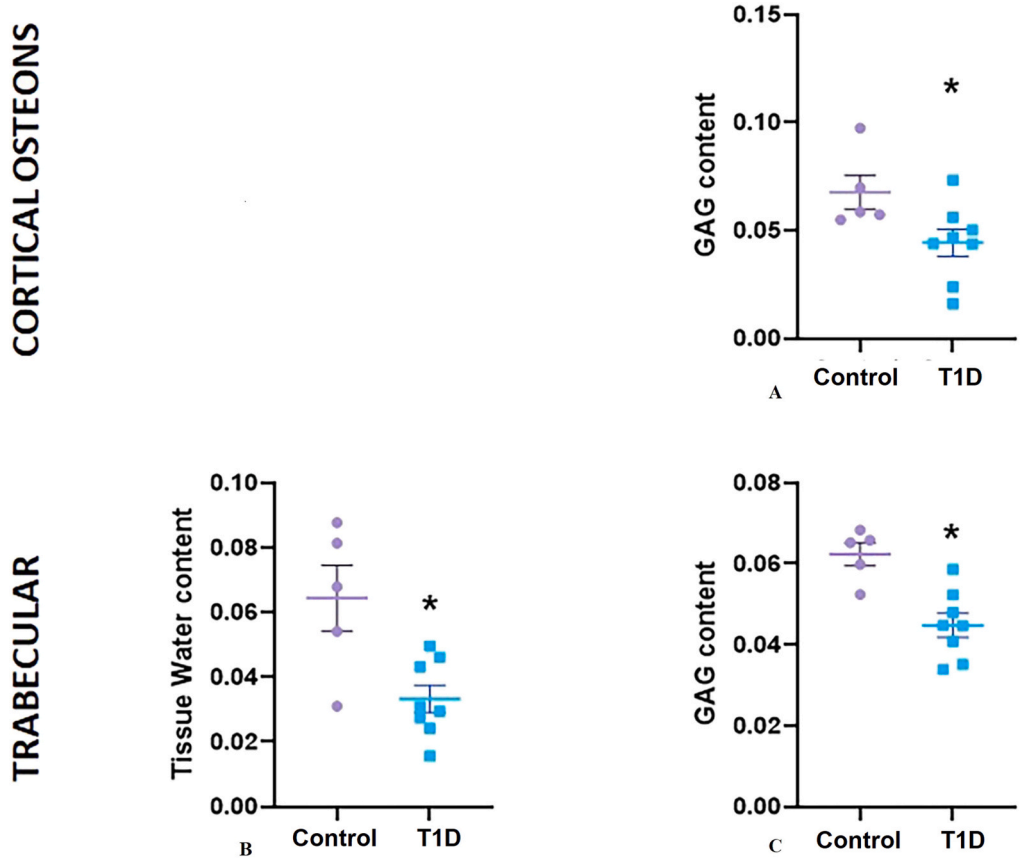


Fig. 2. Raman analysis of the osteoid composition at intracortical (osteons; top row) and trabecular (bottom row) forming surfaces. Long-term T1D postmenopausal patients had lower glycosaminoglycan (GAG) content at both surfaces and reduced sub-micron pores tissue water content at trabecular surfaces, compared to age-matched controls (* $p < 0.05$).

the other hand, significant differences were evident between the two groups when non-enzymatic collagen cross-links were considered. Specifically, T1D had significantly elevated CML values in the trabecular (cancellous) bone ($p = 0.0003$) (Fig. 4C) but not in the intracortical region (data not shown), and PEN content greater in both cortical ($p = 0.048$) and trabecular bone ($p < 0.0001$) (Fig. 4 A and D, respectively). Finally, T1D had lower glucose content compared to Controls in both cortical and trabecular bone ($p = 0.019$, and < 0.0001, respectively) (Fig. 4 B and E, respectively).

Comparisons in the area of indented cement lines (Fig. 5) revealed that T1D had different MMC (Fig. 5A), as well as elevated CML and PEN content (Fig. 5 D and E, respectively), and reduced Pyd (Fig. 5C) and glucose (Fig. 5B) content compared to Controls.

There were no significant correlations between nanoindentation and Raman outcomes right on the cement lines. On the other hand, when the slopes of the lines defined by the three-point Raman analysis were performed, significant correlations existed (Table 2). In particular, significant direct correlations were evident between both Controls' and T1D's MMC and Hardness, and negative ones between TW and T1D patients Hardness values.

4. Discussion

The present study analysed iliac crest bone biopsies from postmenopausal women with long term T1D, and age- and BMD-matched controls, using nanoindentation and Raman microspectroscopy. The results indicate that T1D had higher modulus and hardness suggesting that T1D bone tissue is more brittle and prone to fracture, along with altered MMC, and non-enzymatic cross-links and glucose and GAG content compared to Controls. Moreover, the results indicate significant differences in mechanical and compositional/quality indices at cement lines.

Bone's resistance to fracture is determined by three mechanical attributes: stiffness (often measured by elastic modulus), strength and toughness (Wagermaier, Klaushofer et al. 2015) [26]. While stiffness is determined by the mineral content as well as collagen fiber orientation and size and shape of mineral crystallites, the toughness is dependent on imperfections within the bone material, especially at interfaces, and incorporates properties of the organic matrix [26]. Fragility fractures are mostly due to compromised toughness [25,26], whose role may not be fully captured by BMD considerations alone. In the present work we measured localized modulus and hardness, both of which positively correlate with mineral content [68]. However, there is a 7 % (non-significant) difference in BMD between the two groups which could be clinically meaningful.

The findings of increased modulus and hardness in T1D despite similar BMD values with Controls, strongly advocate differences in bone quality, and are congruent with stiffening of collagen resulting from higher AGEs content. In addition, the lower GAG contents in the osteoid suggest a lower water content of bone tissue [24], and therefore, further stiffening or hardness in T1D (Fig. 2). Additional, research and data will help to confirm the cause of stiffness increase and toughness decline in T1D bone tissue. Differences in bone compositional/material properties between T1D and Controls were also observed. It has been previously reported that activation frequency is decreased in T1D patients [6]. In the present study, Raman analysis was performed in bone areas of similar tissue age, thus any differences are in addition to potential differences due to altered activation frequency. This is supported by the observation that there were no significant differences in interlabel (IrLD) distance at either osteonal or trabecular (cancellous) surfaces

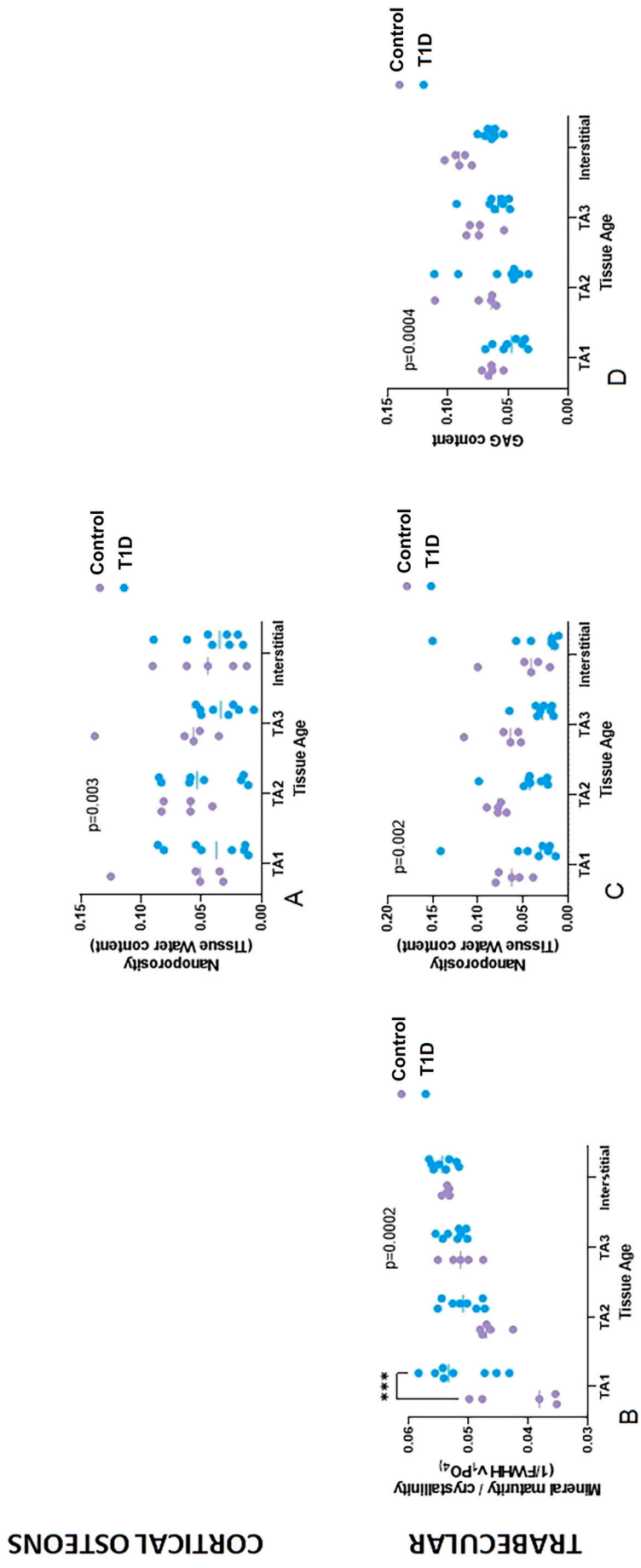


Fig. 3. 2-way ANOVA of Raman data at osteonal (top row) and trabecular surfaces (bottom row) as a function of tissue age revealed that long-term T1D postmenopausal patients had lower sub-micron pores tissue water content compared to age-matched controls. Moreover, they had higher mineral crystallinity (MMC) values compared to controls in the cancellous compartment. Moreover, at the cancellous compartment forming surfaces, T1D had significantly lower glycosaminoglycan (GAG) content. *p*-values shown are of the 2-way ANOVA, while asterisks denote significance based on the post-hoc tests (*** $p < 0.001$).

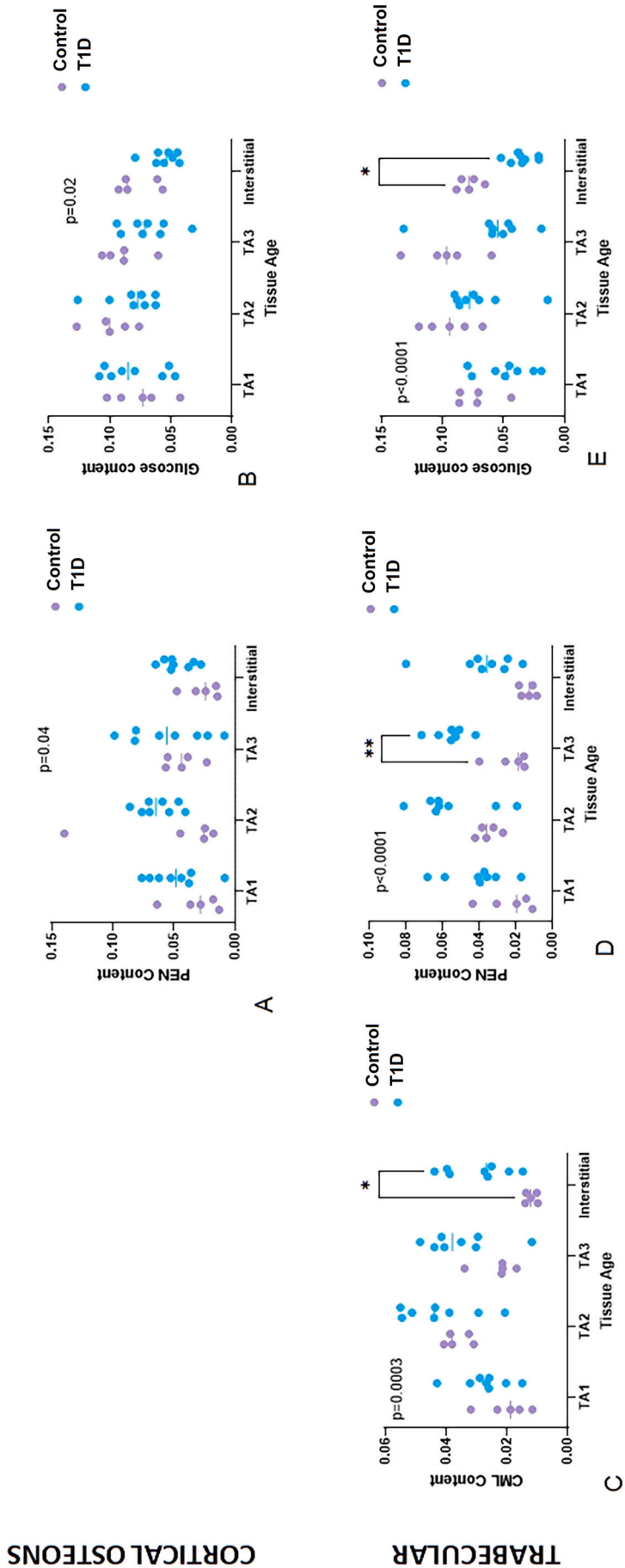


Fig. 4. Summary of 2-way ANOVA in the cortical (top row) and trabecular (cancellous- bottom row) compartments, of CML (ε-*N*-Carboxymethyl-ʟ-lysine) and PEN (Pentosidine) (non-enzymatic) collagen cross-links, and glucose content. Long-term T1D postmenopausal patients had significantly elevated PEN (in both compartments) and CML (in the cancellous compartment) content compared to age-matched controls. Moreover, T1D patients had lower glucose content in both anatomical compartments. p-values shown are of the 2-way ANOVA, while asterisks denote significance based on the post-hoc tests (* $p < 0.05$, ** $p < 0.01$).

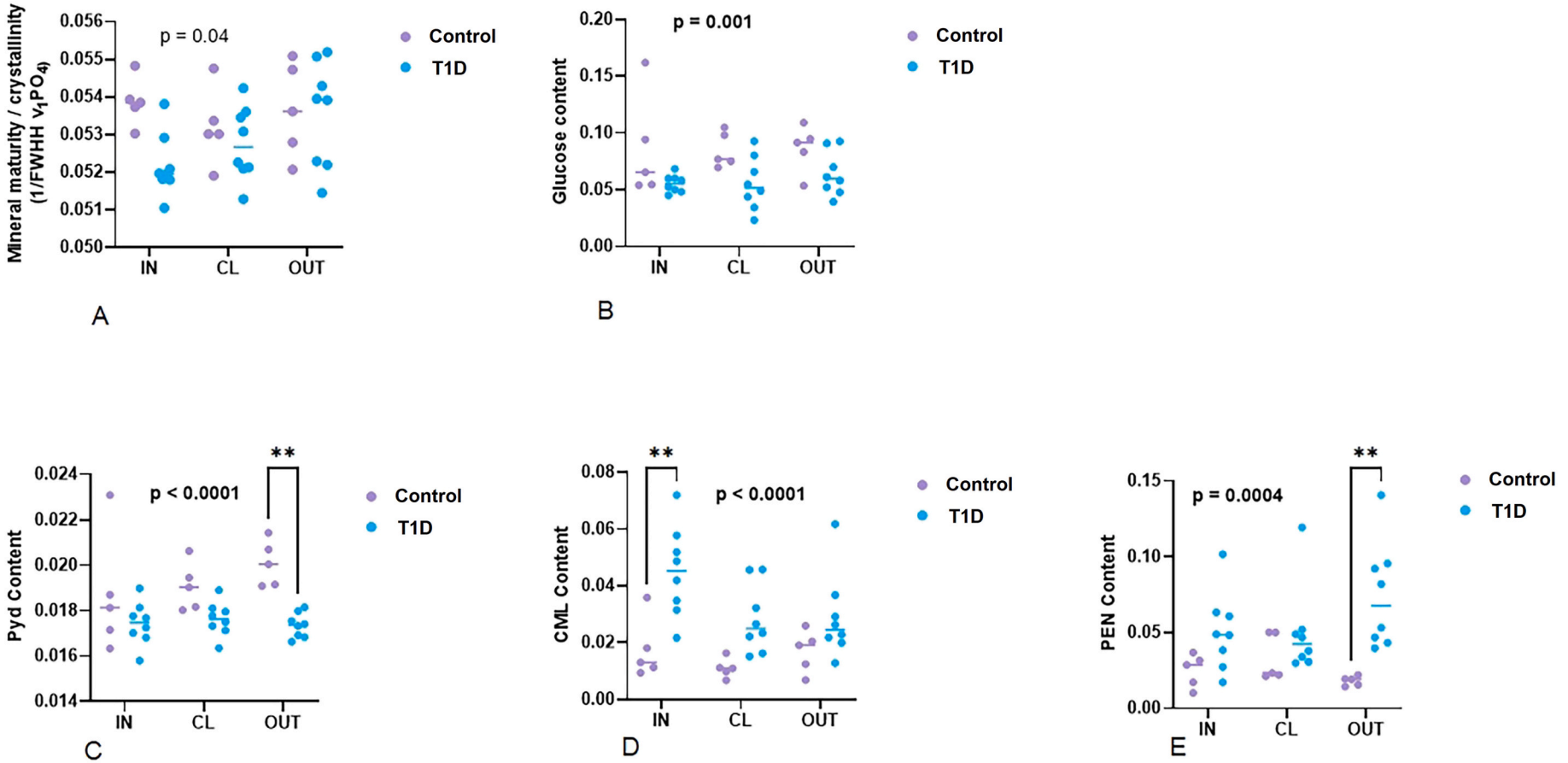


Fig. 5. Summary of significant differences between long term T1D postmenopausal patients and age-matched controls in the area of cement lines. T1D patients had altered mineral crystallinity (MMC) values compared to controls. Moreover, cement lines of T1D patients had elevated CML (ε-*N*-Carboxymethyl-L-lysine) and PEN (Pentosidine) content, and reduced pyridinoline (Pyd) and glucose content compared to age-matched controls.

p-values shown are of the 2-way ANOVA, while asterisks denote significance based on the post-hoc tests (** $p < 0.01$, Controls vs. T1D at equivalent anatomical locations per Tukey's post hoc test).

Table 2
Correlations between nanoindentation and Raman outcomes.

	Control hardness (GPa)	T1D hardness (GPa)
MMC	0.8794 (r) 0.0494 (p)	0.9614 (r) 0.0006 (p) −0.8683 (r)
TW	–	0.0112 (p) −0.8691 (r)
PEN	–	0.0111 (p)

between the two patient groups, indicating that although the number of BMUs (basic multicellular units or bone remodelling period) may be different between T1D and Controls, the rate of organic matrix deposition is similar in both groups.

The GAG content was reduced in T1D in both osteoid (freshly deposited, unmineralized tissue) as well as trabecular mineralized tissue. The decrease in osteoid, may be due to a decrease in proteoglycan synthesis coupled with an increase in destruction due to upregulation of enzymes degrading GAGs or destruction by reactive oxygen species [21]. T1D had also lower GAG content in mineralized trabecular bone but not in cortical. Based on the experimental design of the present study, no definite explanation may be offered for the lack of differences in the cortical bone. One of the many roles proteoglycans play in bone homeostasis, is keeping osteocyte canaliculi free of mineral [16]. Thus, the lower GAG content in the mineralized bone tissue of the T1D may signify an altered canalicular network in these patients compared to controls, a hypothesis that would be in agreement with the findings in an animal model of T1D [22]. The difference between Controls and T1D may be further attributed to the fact that in an ovariectomized animal model, it was shown that estrogen depletion resulted in the enlargement of canalicular size [23]; in the present study, the Control group consists of postmenopausal women. Finally, it has been shown that there is a direct correlation between tissue GAG content and bone toughness [24]. Thus, the decrease in GAGs in T1D is expected to decrease toughness, causing an increase in modulus and hardness (as reported here) as these metrics are inversely related to toughness.

T1D had reduced TW content in the trabecular osteoid, in agreement with the lower osteoid GAG content, as most of the tissue water is adsorbed onto the GAGs of the proteoglycans [27]. The lack of any differences in this metric in the cortical (osteons) osteoid may be due to differences in osteoblastic output due to different surface curvature [28–33]. TW was also significantly reduced in T1D compared to Controls in both the cortical and trabecular bone's compartments. Bone tissue water is an important modulator of bone's mechanical attributes [34–39], while it has been suggested that water-generated tensile forces may play a pivotal role in the mechanical properties of collagen-based materials such as bone [40,41]. Thus, it is plausible that the reduced content in T1D compared to Controls contributes to the increase in fracture risk inherent with these patients. Unfortunately, the analysed bone tissue was embedded rather than fresh, it was impossible to determine other types of tissue water [42–45].

Among the various non-enzymatic collagen cross-links (AGEs), carboxymethyl-lysine (CML) and pentosidine (PEN) are the most extensively studied to date. In mineralized tissues, AGEs formation and accumulation associates with more brittle bone, and is believed to be a major culprit in the increased fracture incidence evident in diabetes [46,47]. In an animal model of T1D, they have been shown to correlate inversely with macroscopic bone toughness [48]. In the present study, PEN was significantly elevated in T1D in both cortical and trabecular bone tissue, while CML content was elevated in trabecular (cancellous) bone compared to Controls, in agreement with what has been reported for diabetes. The elevated AGEs content in T1D patients would be consistent with the observed increased modulus and hardness since both are inversely related to toughness. On the other hand, we are uncertain how this translates to fragility fracture occurrence as a recent review article pointed out, that we cannot be certain whether AGEs are causing or are just associated with fragility fractures [49], while another concluded that increases in AGE content (induced through ribose incubation) may not be sufficient to affect the fracture toughness of human cortical bone [50].

Glucose (Glu) content within mineralized bone tissue decreased in T1D compared to control. The important role of vascular supply for bone formation, remodelling, and fracture repair is well documented [51,52]. A common complication of Type 1 diabetic patients is microvascular disease resulting in reduced blood flow [5]. Thus, bone tissue's lower glucose levels observed in the present study may be attributable to reduced blood flow in T1D. The decreased glucose content may seem counterintuitive in view of the increased AGEs (CML and PEN), unless one considers that AGEs do not form on collagen exclusively, but rather on oxidized proteins and lipids [17,53]. One plausible scenario consistent with these two observations would be that although the glucose content is decreased, the available substrata for AGEs formation are increased.

Using our recruitment criteria, no fracturing TD1 or Controls were selected. There were no differences in Pyd enzymatic collagen cross-links content between the two groups in either the osteoid or the mineralized tissue. This organic matrix quality index has previously been shown to correlate with fragility fracture occurrence, independent of clinical indicators of fracture risk such as BMD by DXA [54–60]. Thus, the lack of differences in Pyd between the two groups analysed in the present study is encouraging. The results of the present study suggest that enzymatic collagen cross-links may be the real culprit in diabetes fragility fractures, a hypothesis we plan to test in future experiments.

Fragility fractures are mostly attributed to compromised toughness [25] which depends on interfaces within the bone material [26]. Thus, in the present work we focused on the transition (inside the osteon, through the cement line, out to interstitial bone) at cement lines, a major bone interface. Right on the cement lines, T1D patients were found to have greater modulus and hardness values by nanoindentation, coupled with higher MMC, CML, and PEN, and lower Pyd and Glu values compared to controls. Raman imaging experiments also confirmed the presence of elevated CML and PEN in the vicinity of cement lines in T1D patients compared to Controls (Appendix I). The elevated MMC, CML, and PEN values would be consistent with compromised mechanical properties. It is plausible then to hypothesize that the elevated values contribute to the increased modulus and hardness values measured in these patients, while the lower Pyd content may contribute to the lack of fragility fractures in the analysed patients, as elevated Pyd content has been reported to result in collagen fibers with more brittle-like behaviour [61]. Interestingly, there were no correlations between any of the nanoindentation and Raman outcome values obtained right on the cement lines. On the other hand, when the slopes of the lines defined by the three Raman measurements through the cement lines were considered, significant positive correlations were evident between both Controls' and T1D's MMC and hardness, and negative ones between TW and PEN, and T1D patients hardness values. These data highlight the potentially pivotal role that the rate of change in compositional properties across cement lines may play in local mechanical properties. Of interest is also the observation that although PEN content significantly and inversely correlated with T1D hardness, CML did not. This result may be due to the fact that unlike PEN, the CML is a non-cross-linking AGE. Interestingly, it has been reported that although PEN significantly associates with prevalent vertebral fractures, CML does not.

A previous publication has examined whether bone matrix from fracturing and nonfracturing T1DM contains elevated AGEs than bone from healthy patients (CTL) and compared the degree of mineralization of bone and hardness between fracturing and nonfracturing T1DM versus CTL [3]. There are differences between the published and the present study; while in the present study we compared postmenopausal women against postmenopausal women diagnosed with long term T1D, the already published one included both male and female subjects, while

the female ones were premenopausal. As menopause induces changes in material / compositional properties of bone [62], comparing the two studies inappropriate. Additionally, material/compositional properties are greatly dependent on tissue age [8,63], and in the present study we utilized double fluorescence labels to adjust for it. Moreover, in the present study nanoindentation was performed in interstitial bone at random, as well as specific anatomical locations (cement lines).

Another recent study using multimodal analysis of cadaveric bone from male donors that were diagnosed with either Type 1 Diabetes or Type 2 Diabetes, and appropriate controls, reported similar mineral and matrix maturity as well as collagen enzymatic collagen cross-links between the diabetic groups and controls, coupled with elevated non-enzymatic cross-links in the diabetic group [64]. Again, the results of the published study are not directly comparable with the present ones, as they did not normalize for tissue age.

Limitations of this study include the low number of patients in each group, which has a potential of type-II error. Although the numbers are comparable to recently published studies [3,65–67] and we do plan to analyse more bone biopsies as they become available. On the other hand, the clinical characteristics of the two patient groups are similar, further supporting the notion that the observed differences are due to diabetes. Another limitation is that there is no age- and BMD-matched fracture-sustaining T1D group. Furthermore, the bone tissue in the biopsy site (iliac bone) may not be reflective of a fracture- and loading-site compared to femoral neck and tibia. Nonetheless, the bone biopsy tissue provides a window into the intrinsic properties and composition of skeletal health with regards to T1D and Controls.

5. Conclusion

The results of the present study comparing bone intrinsic material and compositional properties between postmenopausal women and postmenopausal women diagnosed with long-duration T1D, confirm the widely reported elevation of AGE content in the latter, and highlight the differences in MMC and GAG content between the two patient groups, thus potentially offering two targets for the management of the disease. Moreover, the differences at the interface of cement lines, especially regarding CML, PEN, and MMC may result in compromised toughness, thus increase of the fracture risk inherent in T1D that is not captured by BMD outcomes. Additionally, the correlation between nanoindentation and rate of change in compositional outcomes rather than absolute values at the cement lines, highlight the importance of considering kinetics rather than absolute values given the compositional heterogeneity of bone as a function of tissue age. Finally, because the analysis was performed at equivalent tissue ages and/or anatomical micro-locations, the observed differences may not be attributed to the decreased bone turnover rates widely reported in T1D patients. Nevertheless, the suggestions formulated based on the presented results should be considered in light of the small number of biopsies available for analysis. Moreover, the fact that these patients did not suffer from fragility fractures, indicates that they may represent a negative control group in the effort to understand what results in the fragility fractures in T1D patients. These data suggest that the enzymatic collagen cross-links maybe the real culprit in diabetes fragility fractures, a hypothesis we plan to test in future experiments to understand the cause of stiffness increase and toughness decline in T1D bone tissue.

CRediT authorship contribution statement

Wen Qian: Data curation. **Sonja Gamsjaeger:** Data curation. **Eleftherios P. Paschalis:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Laura A. Graeff-Armas:** Investigation, Formal analysis. **Sue P. Bare:** Data curation. **Joseph A. Turner:** Supervision, Investigation, Data curation. **Joan M. Lappe:** Investigation. **Robert R. Recker:** Funding acquisition, Conceptualization. **Mohammed P. Akhter:** Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

There is NO financial or any other conflict of interest.

1) This study was funded by National Institute of Health (*NIH-5R01DK122558, Dr. Recker, PI, Dr. Akhter Co-PI*)
2) The study design was approved by Internal Review Board (IRB) Creighton University, Omaha, NE, USA (*study title: Skeletal Effects of Type 1 Diabetes; approval date-5/7/2019, IRB#142564*).

Data availability

Data will be made available on request.

Acknowledgments

The present study is supported by NIH grant # 5R01DK122558-01, RFA NIDDK), (Drs. Recker-PI, Akhter-CoPI), the AUVA (Research funds of the Austrian workers compensation board), and OEGK. We thank Dr. Claudia Gragnoli, MD, PhD, for her help in the process of recruitment of subjects. Hysitron nanoindentation analysis on bones tissues were performed at the NanoEngineering Research Core Facility (NERCF), which is partially funded by the Nebraska Research Initiative.

Appendices. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2023.116832.

References

[1] N. Napoli, M. Chandran, D.D. Pierroz, B. Abrahamsen, A.V. Schwartz, S.L. Ferrari, I.O.F. Bone, G., Diabetes working, mechanisms of diabetes mellitus-induced bone fragility, Nat. Rev. Endocrinol. 13 (4) (2017) 208–219.
[2] D.M. Maahs, N.A. West, J.M. Lawrence, E.J. Mayer-Davis, Epidemiology of type 1 diabetes, Endocrinol. Metab. Clin. N. Am. 39 (3) (2010) 481–497.
[3] D. Farlay, L.A. Armas, E. Gineyts, M.P. Akhter, R.R. Recker, G. Boivin, Nonenzymatic glycation and degree of mineralization are higher in bone from fractured patients with type 1 diabetes mellitus, J. Bone Miner. Res. 31 (1) (2016) 190–195.
[4] P. Fratzl, H. Gupta, E. Paschalis, P. Roschger, Structure and mechanical quality of the collagen–mineral nano-composite in bone, J. Mater. Chem. 14 (2004) 2115–2123.
[5] V.N. Shah, R.D. Carpenter, V.L. Ferguson, A.V. Schwartz, Bone health in type 1 diabetes, Curr. Opin. Endocrinol. Diabetes Obes. 25 (4) (2018) 231–236.
[6] P. Vestergaard, Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis, Osteoporos. Int. 18(4) (2007) 427–44.
[7] V.N. Shah, C.S. Shah, J.K. Snell-Bergeon, Type 1 diabetes and risk of fracture: meta-analysis and review of the literature, Diabet. Med. 32 (9) (2015) 1134–1142.
[8] E.P. Paschalis, P. Fratzl, S. Gamsjaeger, N. Hassler, W. Brozek, E.F. Eriksen, F. Rauch, F.H. Glorieux, E. Shane, D. Dempster, A. Cohen, R. Recker, K. Klaushofer, Aging versus postmenopausal osteoporosis: bone composition and maturation kinetics at actively-forming trabecular surfaces of female subjects aged 1 to 84 years, J. Bone Miner. Res. 31 (2) (2016) 347–357.
[9] A. Diez, J. Puig, M.T. Martinez, J.L. Diez, J. Aubia, J. Vivancos, Epidemiology of fractures of the proximal femur associated with osteoporosis in Barcelona, Spain, Calcif. Tissue Int. 44 (6) (1989) 382–386.
[10] C. Meier, T.V. Nguyen, J.R. Center, M.J. Seibel, J.A. Eisman, Bone resorption and osteoporotic fractures in elderly men: the dubbo osteoporosis epidemiology study, J. Bone Miner. Res. 20 (4) (2005) 579–587.
[11] D.W. Dempster, J.E. Compston, M.K. Drezner, F.H. Glorieux, J.A. Kanis, H. Malluche, P.J. Meunier, S.M. Ott, R.R. Recker, A.M. Parfitt, Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee, J. Bone Miner. Res. 28 (1) (2013) 2–17.
[12] S. Vennin, A. Desyatova, J.A. Turner, P.A. Watson, J.M. Lappe, R.R. Recker, M. P. Akhter, Intrinsic material property differences in bone tissue from patients suffering low-trauma osteoporotic fractures, compared to matched non-fracturing women, Bone 97 (2017) 233–242.

[13] R. Mcreery, Signal-to-noise in Raman Spectroscopy, in: J. Winefordner, R. McCreery (Eds.), Raman Spectroscopy for Chemical Analysis2000, pp. 49–71.
[14] E.P. Paschalis, S. Gamsjaeger, K. Klaushofer, Vibrational spectroscopic techniques to assess bone quality, Osteoporos. Int. 28 (8) (2017) 2275–2291.
[15] S. Gamsjaeger, A. Masic, P. Roschger, M. Kazanci, J.W. Dunlop, K. Klaushofer, E. P. Paschalis, P. Fratzl, Cortical bone composition and orientation as a function of animal and tissue age in mice by Raman spectroscopy, Bone 47 (2010) 392–399.
[16] W.R. Thompson, S. Modla, B.J. Grindel, K.J. Czymmek, C.B. Kirn-Safran, L. Wang, R.L. Duncan, M.C. Farach-Carson, Perlecan/Hspg2 deficiency alters the pericellular space of the lacunocanalicular system surrounding osteocytic processes in cortical bone, J. Bone Miner. Res. 26 (3) (2011) 618–629.
[17] E.B. Frye, T.P. Degenhardt, S.R. Thorpe, J.W. Baynes, Role of the Maillard reaction in aging of tissue proteins, Advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins, J. Biol. Chem. 273 (30) (1998) 18714–18719.
[18] E. Wiercigroch, E. Szafraniec, K. Czamara, M.Z. Pacia, K. Majzner, K. Kochan, A. Kaczor, M. Baranska, K. Malek, Raman and infrared spectroscopy of carbohydrates: a review, Spectrochim. Acta A Mol. Biomol. Spectrosc. 185 (2017) 317–335.
[19] X. Sun, Glucose detection through surface-enhanced Raman spectroscopy: a review, Anal. Chim. Acta 1206 (2022), 339226.
[20] E.P. Paschalis, S. Gamsjaeger, K. Condon, K. Klaushofer, D. Burr, Estrogen depletion alters mineralization regulation mechanisms in an ovariectomized monkey animal model, Bone 120 (2019) 279–284.
[21] L.M. Hiebert, Proteoglycans and diabetes, Curr. Pharm. Des. 23 (10) (2017) 1500–1509.
[22] X. Lai, C. Price, S. Modla, W.R. Thompson, J. Caplan, C.B. Kirn-Safran, L. Wang, The dependences of osteocyte network on bone compartment, age, and disease, Bone Res. 3 (1) (2015) 15009.
[23] D. Sharma, C. Ciani, P.A. Marin, J.D. Levy, S.B. Doty, S.P. Fritton, Alterations in the osteocyte lacunar-canalicular microenvironment due to estrogen deficiency, Bone 51 (3) (2012) 488–497.
[24] X. Wang, R. Hua, A. Ahsan, Q. Ni, Y. Huang, S. Gu, J.X. Jiang, Age-related deterioration of bone toughness is related to diminishing amount of matrix glycosaminoglycans (GAGS), JBMR Plus 2 (3) (2018) 164–173.
[25] C.J. Hernandez, M.C. van der Meulen, Understanding bone strength is not enough, J. Bone Miner. Res. 32 (6) (2017) 1157–1162.
[26] W. Wagermaier, K. Klaushofer, P. Fratzl, Fragility of bone material controlled by internal interfaces, Calcif. Tissue Int. 97 (3) (2015) 201–212.
[27] Q. Wang, Y.Y. Yang, H.J. Niu, W.J. Zhang, Q.J. Feng, W.F. Chen, An ultrasound study of altered hydration behaviour of proteoglycan-degraded articular cartilage, BMC Musculoskelet. Disord. 14 (2013) 289.
[28] C.M. Bidan, K.P. Kommareddy, M. Rumpler, P. Kollmannsberger, Y.J. Brechet, P. Fratzl, J.W. Dunlop, How linear tension converts to curvature: geometric control of bone tissue growth, PLoS One 7 (5) (2012), e36336.
[29] C.M. Bidan, K.P. Kommareddy, M. Rumpler, P. Kollmannsberger, P. Fratzl, J. W. Dunlop, Geometry as a factor for tissue growth: towards shape optimization of tissue engineering scaffolds, Adv. Healthc. Mater. 2 (1) (2013) 186–194.
[30] K.P. Kommareddy, C. Lange, M. Rumpler, J.W. Dunlop, I. Manjubala, J. Cui, K. Kratz, A. Lendlein, P. Fratzl, Two stages in three-dimensional in vitro growth of tissue generated by osteoblastlike cells, Biointerphases 5 (2) (2010) 45–52.
[31] I. Manjubala, A. Woesz, C. Pilz, M. Rumpler, N. Fratzl-Zelman, P. Roschger, J. Stampfl, P. Fratzl, Biomimetic mineral-organic composite scaffolds with controlled internal architecture, J. Mater. Sci. Mater. Med. 16 (12) (2005) 1111–1119.
[32] M. Rumpler, A. Woesz, J.W. Dunlop, J.T. van Dongen, P. Fratzl, The effect of geometry on three-dimensional tissue growth, J. R. Soc. Interface 5 (27) (2008) 1173–1180.
[33] M. Rumpler, A. Woesz, F. Varga, I. Manjubala, K. Klaushofer, P. Fratzl, Three-dimensional growth behavior of osteoblasts on biomimetic hydroxylapatite scaffolds, J. Biomed. Mater. Res. A 81 (1) (2007) 40–50.
[34] M.R. Allen, C.L. Newman, N. Chen, M. Granke, J.S. Nyman, S.M. Moe, Changes in skeletal collagen cross-links and matrix hydration in high- and low-turnover chronic kidney disease, Osteoporos. Int. 26 (3) (2015) 977–985.
[35] A. Creecy, S. Uppuganti, A.R. Merkel, D. O'Neal, A.J. Makowski, M. Granke, P. Voziyan, J.S. Nyman, Changes in the fracture resistance of bone with the progression of type 2 diabetes in the ZDSD rat, Calcif. Tissue Int. 99 (3) (2016) 289–301.
[36] M. Granke, A.J. Makowski, S. Uppuganti, M.D. Does, J.S. Nyman, Identifying novel clinical surrogates to assess human bone fracture toughness, J. Bone Miner. Res. 30 (7) (2015) 1290–1300.
[37] J.S. Nyman, M. Granke, R.C. Singleton, G.M. Pharr, Tissue-level mechanical properties of Bone contributing to fracture risk, Curr. Osteoporos. Rep. 14 (4) (2016) 138–150.
[38] J.S. Nyman, A. Roy, X. Shen, R.L. Acuna, J.H. Tyler, X. Wang, The influence of water removal on the strength and toughness of cortical bone, J. Biomech. 39 (5) (2006) 931–938.
[39] J.S. Nyman, S. Uppuganti, M. Unal, C.J. Leverant, S. Adabala, M. Granke, P. Voziyan, M.D. Does, Manipulating the amount and structure of the organic matrix affects the water compartments of human cortical bone, JBMR Plus 3 (6) (2019), e10135.
[40] A. Masic, L. Bertinetti, R. Schuetz, S.W. Chang, T.H. Metzger, M.J. Buehler, P. Fratzl, Osmotic pressure induced tensile forces in tendon collagen, Nat. Commun. 6 (2015) 5942.
[41] L. Bertinetti, A. Masic, R. Schuetz, A. Barbetta, B. Seidt, W. Wagermaier, P. Fratzl, Osmotically driven tensile stress in collagen-based mineralized tissues, J. Mech. Behav. Biomed. Mater. 52 (2015) 14–21.
[42] M. Unal, O. Akkus, Shortwave-infrared Raman spectroscopic classification of water fractions in articular cartilage ex vivo, J. Biomed. Opt. 23 (1) (2018) 1–11.
[43] M. Unal, O. Akkus, J. Sun, L. Cai, U.L. Erol, L. Sabri, C.P. Neu, Raman spectroscopy-based water content is a negative predictor of articular human cartilage mechanical function, Osteoarthr. Cartil. 27 (2) (2019) 304–313.
[44] M. Unal, R.L. Wilson, C.P. Neu, O. Akkus, Raman spectroscopy-based water measurements identify the origin of MRI T2 signal in human articular cartilage zones and predict histopathologic score, J. Biophotonics 15 (1) (2022), e202100212.
[45] M. Unal, S. Yang, O. Akkus, Molecular spectroscopic identification of the water compartments in bone, Bone 67 (2014) 228–236.
[46] S.Y. Tang, M.R. Allen, R. Phipps, D.B. Burr, D. Vashishth, Changes in non-enzymatic glycation and its association with altered mechanical properties following 1-year treatment with risedronate or alendronate, Osteoporos. Int. 20 (6) (2009) 887–894.
[47] S.Y. Tang, U. Zeenath, D. Vashishth, Effects of non-enzymatic glycation on cancellous bone fragility, Bone 40 (4) (2007) 1144–1151.
[48] M.R. Rubin, E.P. Paschalis, A. Poundarik, G.E. Sroga, D.J. McMahon, S. Gamsjaeger, K. Klaushofer, D. Vashishth, Advanced glycation endproducts and bone material properties in type 1 diabetic mice, PLoS One 11 (5) (2016), e0154700.
[49] T.L. Willett, P. Voziyan, J.S. Nyman, Causative or associative: a critical review of the role of advanced glycation end-products in bone fragility, Bone 163 (2022), 116485.
[50] M. Unal, S. Uppuganti, D.Y. Dapaah, R. Ahmed, J.S. Pennings, T.L. Willett, P. Voziyan, J.S. Nyman, Effect of ribose incubation on physical, chemical, and mechanical properties of human cortical bone, J. Mech. Behav. Biomed. Mater. 140 (2023), 105731.
[51] B. Thompson, D.A. Towler, Arterial calcification and bone physiology: role of the bone-vascular axis, Nat. Rev. Endocrinol. 8 (9) (2012) 529–543.
[52] J.F. Griffith, D.K. Yeung, P.H. Tsang, K.C. Choi, T.C. Kwok, A.T. Ahuja, K.S. Leung, P.C. Leung, Compromised bone marrow perfusion in osteoporosis, J. Bone Miner. Res. 23 (7) (2008) 1068–1075.
[53] R. Ramasamy, S.J. Vannucci, S.S. Yan, K. Herold, S.F. Yan, A.M. Schmidt, Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation, Glycobiology 15 (7) (2005) 16R–28R.
[54] R.D. Blank, T.H. Baldini, M. Kaufman, S. Bailey, R. Gupta, Y. Yershov, A.L. Boskey, S.N. Coppersmith, P. Demant, E.P. Paschalis, Spectroscopically determined collagen Pyr/deH-DHLNL cross-link ratio and crystallinity indices differ markedly in recombinant congenic mice with divergent calculated bone tissue strength, Connect. Tissue Res. 44 (3–4) (2003) 134–142.
[55] B.M. Misof, S. Gamsjaeger, A. Cohen, B. Hofstetter, P. Roschger, E. Stein, T. L. Nickolas, H.F. Rogers, D. Dempster, H. Zhou, R. Recker, J. Lappe, D. McMahon, E.P. Paschalis, P. Fratzl, E. Shane, K. Klaushofer, Bone material properties in premenopausal women with idiopathic osteoporosis, J. Bone Miner. Res. 27 (12) (2012) 2551–2561.
[56] E.P. Paschalis, E. Shane, G. Lyritis, G. Skarantavos, R. Mendelsohn, A.L. Boskey, Bone fragility and collagen cross-links, J. Bone Miner. Res. 19 (12) (2004) 2000–2004.
[57] E.P. Paschalis, E.V. Glass, D.W. Donley, E.F. Eriksen, Bone mineral and collagen quality in iliac crest biopsies of patients given teriparatide: new results from the fracture prevention trial, J. Clin. Endocrinol. Metab. 90 (8) (2005) 4644–4649.
[58] H.H. Malluche, D.S. Porter, H. Mawad, M.C. Monier-Faugere, D. Pienkowski, Low-energy fractures without low T-scores characteristic of osteoporosis: a possible bone matrix disorder, J. Bone Joint Surg. Am. 95 (19) (2013) e1391–e1396.
[59] S. Gourion-Arsiquaud, D. Faibish, E. Myers, L. Spevak, J. Compston, A. Hodsman, E. Shane, R.R. Recker, E.R. Boskey, A.L. Boskey, Use of FTIR spectroscopic imaging to identify parameters associated with fragility fracture, J. Bone Miner. Res. 24 (9) (2009) 1565–1571.
[60] S. Rokidi, E.P. Paschalis, K. Klaushofer, S. Vennin, A. Desyatova, J.A. Turner, P. Watson, J. Lappe, M.P. Akhter, R.R. Recker, Organic matrix quality discriminates between age- and BMD-matched fracturing versus non-fracturing post-menopausal women: a pilot study, Bone 127 (2019) 207–214.
[61] B. Depalle, Z. Qin, S.J. Shefelbine, M.J. Buehler, Influence of cross-link structure, density and mechanical properties in the mesoscale deformation mechanisms of collagen fibrils, J. Mech. Behav. Biomed. Mater. 52 (2015) 1–13.
[62] S. Gamsjaeger, W. Brozek, R. Recker, K. Klaushofer, E.P. Paschalis, Transmenopausal changes in trabecular bone quality, J. Bone Miner. Res. 29 (3) (2014) 608–617.
[63] E. Donnelly, A.L. Boskey, S.P. Baker, M.C. van der Meulen, Effects of tissue age on bone tissue material composition and nanomechanical properties in the rat cortex, J. Biomed. Mater. Res. A 92 (3) (2010) 1048–1056.
[64] E.M. Wolfel, F.N. Schmidt, A. Vom Scheidt, A.K. Siebels, B. Wulff, H. Mushumba, B. Ondruschka, K. Puschel, J. Scheijen, C.G. Schalkwijk, E. Vettorazzi, K. Jahn-Rickert, B. Gludovatz, E. Schaible, M. Amling, M. Rauner, L.C. Hofbauer, E. A. Zimmermann, B. Busse, Dimorphic mechanisms of fragility in diabetes mellitus - the role of reduced collagen fibril deformation, J. Bone Miner. Res. 37 (11) (2022) 2259–2276.
[65] T. Rodic, E.M. Wolfel, P. Milovanovic, I.A.K. Fiedler, D. Cvetkovic, K. Jahn, M. Amling, J. Sopta, S. Nikolic, V. Zivkovic, B. Busse, M. Djuric, Bone quality analysis of jaw bones in individuals with type 2 diabetes mellitus-post mortem anatomical and microstructural evaluation, Clin. Oral Investig. 25 (7) (2021) 4377–4400.

[66] E.M. Wolfel, F.N. Schmidt, A. Vom Scheidt, A.K. Siebels, B. Wulff, H. Mushumba, B. Ondruschka, K. Puschel, J. Scheijen, C.G. Schalkwijk, E. Vettorazzi, K. Jahn-Rickert, B. Gludovatz, E. Schaible, M. Amling, M. Rauner, L.C. Hofbauer, E. A. Zimmermann, B. Busse, Dimorphic mechanisms of fragility in diabetes mellitus: the role of reduced collagen fibril deformation, J. Bone Miner. Res. 37 (11) (2022) 2259–2276.

[67] S.D. Kolibova, E.M. Wolfel, H. Hemmatian, P. Milovanovic, H. Mushumba, B. Wulff, M. Neidhardt, K. Puschel, A.V. Failla, A. Vlug, A. Schlaefer, B. Ondruschka, M. Amling, L.C. Hofbauer, M. Rauner, B. Busse, K. Jahn-Rickert, Osteocyte apoptosis and cellular micropetrosis signify skeletal aging in type 1 diabetes, Acta Biomater 162 (2023) 254–265.

[68] J.D. Currey J. Biomech., Volume 2, Issue 4, October 1969, Pages 477–480.