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ORIGINAL ARTICLE

Potential preventive effects of angiotensin-(1-7) on bone matrix quality in diabetic rats through modulation of the organic matrix

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ABSTRACT

Objectives: This experimental study aims to investigate the effects of angiotensin (1-7) (Ang-[1-7]) on the microstructural, biomechanical, and biophysicochemical properties of bone tissue in diabetic rats.

Materials and methods: Forty-eight male Wistar rats, aged three months and weighing between 280 and 330 g, were used in this study. Four groups, each containing 12 rats, were established: Control, diabetes mellitus (DM), DM-Ang-(1-7), and Ang-(1-7). The samples underwent analysis through micro-computed tomography (CT), Raman spectroscopy, and three-point bending biomechanical test.

Results: Diabetes significantly impaired bone quality, with reduced cortical thickness, maximum load, and flexural strength ($p<0.05$). The Ang-(1-7) treatment improved flexural strength ($p<0.05$), but did not fully restore mechanical function. Micro-CT showed decreased bone volume and trabecular thickness in both diabetic groups ($p<0.05$), with no significant recovery by Ang-(1-7). Raman spectroscopy revealed lower mineral-to-matrix ratio and disrupted collagen quality in diabetic bone ($p<0.05$), while Ang-(1-7) partially restored collagen-related parameters.

Conclusion: These findings highlight that Ang-(1-7) has minimal impact on bone minerals in DM rats. However, it may have a potential preventive effect on the triple-helix structural impairment within the bone organic matrix in this model.

Keywords: Angiotensin-(1-7), biomechanics, bone organic matrix, bone quality, diabetes mellitus, raman spectroscopy, renin-angiotensin system.

Diabetes mellitus (DM) is a devastating disease affecting millions of individuals worldwide. In 2015, it was estimated that 415 million individuals were affected by diabetes, five million individuals died due to diabetes and its complications, and health expenditures due to diabetes worldwide amounted to 673 billion US Dollars. This disease is characterized by hyperglycemia and a lack of insulin and its effect and is expected to affect more than 600 million individuals by 2040.[1] The most well-known chronic complications of diabetes, which affect many systems, are retinopathy, nephropathy, and neuropathy, but the disease affects many other organs, including bones.[2] Although the mechanisms have not been fully elucidated, it is known that

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type 1 DM (T1DM) and type 2 DM (T2DM) increase the risk of fracture in bone tissue.[3] While the risk of hip fracture in patients with T1DM is ~5 times higher than in the control group without diabetes, this risk is ~1.3 times higher in individuals with T2DM.[4,5] Individuals with T1DM cannot reach the peak bone mass expected in adulthood due to insulin deficiency during growth, which is another factor that increases the risk of fracture.[6] Several studies have shown that bone turnover is impaired and osteoblast activity is reduced in individuals with diabetes.[7] Verhaeghe et al.[8] demonstrated a decreased serum osteocalcin level in diabetic rats, while Suzuki et al.[9] showed that bone resorption markers increased in serum and urinary samples.

The renin-angiotensin system (RAS) which consists of proinflammatory and anti-inflammatory axes balanced in the normal physiological condition[10] and is an endocrine system which regulates blood pressure by controlling various factors, including plasma sodium concentration and extracellular volume. The RAS can be found in many tissues, including bone.[11-14] However, activation of the RAS can have negative effects on bone tissue, mainly due to the actions of angiotensin II (Ang II). Angiotensin II has been shown to affect calcium metabolism and promote calcium removal from bones, resulting in bone loss in healthy individuals.[15] The angiotensin-converting enzyme (ACE) converts the peptide angiotensin I (Ang I) into Ang II, one of the primary vasoactive peptides of the RAS. By attaching to the Ang II type 1 receptor (AT1R), it has proinflammatory effects.[16] Angiotensin-converting enzyme 2 (ACE2), on the other hand, causes the production of angiotensin-(1-7) (Ang-[1-7]), a protective RAS axis component. It has actions which are both anti-inflammatory and anti-pressor when it binds to the G-protein coupled MasR.[17] In some pathological situations, the ACE/Ang II/AT1R arm overactivation shifts the balance in favor of the inflammatory axis. To prevent the overactivation of the axis and restore the disturbed equilibrium, it is acceptable to endogenously raise the level of Ang-(1-7) and to strengthen the protective arm.[10] The Ang-(1-7) is a RAS hormone which has recently been extensively studied that is produced by the breakdown of either Ang II by the ACE2 or Ang I by endopeptidases such as neprilysin.[18,19] In animal models, Ang-(1-7) induces cardioprotective benefits such as vasodilation, endothelial nitric oxide (NO) release, improved baroreflex function, and decreases in sympathetic tone, cardiac hypertrophy, inflammation, oxidative stress, and fibrosis.[20,21]

The adverse effects of RAS on bone tissue are attributed to the ACE/Ang II/AT1R pathway of RAS, which is known for its vasoconstrictive, proliferative, and proinflammatory actions. Conversely, the ACE2/Ang-(1-7)/MasR cascade has vasodilatory and anti-inflammatory properties. The Ang-(1-7), a vasodilator heptapeptide found in the RAS, mainly affects the cardiovascular system, including the heart, blood vessels, and kidneys, and typically exhibits effects opposite to those of Ang II.[22,23] Queiroz-Junior et al.[24] demonstrated that Ang-(1-7) increases the expression of osteocalcin, a key marker of osteoblastic activity. Similarly, Sha et al.[23] reported that Ang-(1-7) administration enhanced bone mineral density (BMD) and ultimate force in both hypertensive and diabetic rats. In addition to its anabolic effects, Ang-(1-7) has been shown to reduce osteoclastogenesis in the bone marrow and attenuate metastatic prostate cancer progression in bone tissue.[25] Furthermore, ovariectomized (OVX) rats receiving Ang-(1-7) infusions exhibited significant recovery from bone loss after six weeks of treatment.[26]

Although the effects of Ang-(1-7) have been extensively studied in the context of the cardiovascular system, its impact on bone tissue remains under investigation. Therefore, a comprehensive study involving biomechanical, structural and biochemical aspects of the effects of Ang-(1-7) on diabetic bone tissue could provide important insight into this topic and could have also potential implications for the treatment of bone-related disorders associated with DM. Raman spectroscopy (RS) is a powerful technique for assessing bone matrix composition and is also gaining more attention in the bone field due to its non-destructive and non-invasive measurement capability.[27] Previously, RS has been used to investigate changes in DM-related bone matrix in animal models and humans.[28-30] Yet, to the best of our knowledge, it has not been used for investigating the preventive effects of Ang-(1-7) on bone matrix. In the present study, we aimed to investigate the effects of Ang-(1-7) on the microstructural, biomechanical, and physicochemical properties of bone tissue in diabetic rats.

MATERIALS AND METHODS

Animal preparations and experimental design

Forty-eight male Wistar rats, aged three months and weighing between 280 and 330 g, were used in

this experimental study. The animals were housed in a room with controlled room temperature (25±1°C) and humidity and maintained on a 12-h standard light/dark cycle with free access pellet feed and water through the experimental period.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (50 mg kg-1) (S0130, Sigma-Aldrich, USA) in citrate buffer (0.1 M, pH 4.5), while the control group received only citrate buffer. Blood glucose levels were daily measured using a standard automated glucometer. A week after the injection of STZ, blood glucose levels were measured, and only the rats with blood glucose levels of ≥300 mg dL-1 were selected for further experiments. Experimental animals with blood glucose levels exceeding 300 mg/dL at both the end of the first week and the fourth week, corresponding to a five-week period post-induction, were considered to have developed diabetic conditions, in accordance with established criteria in the literature (Figure 1).

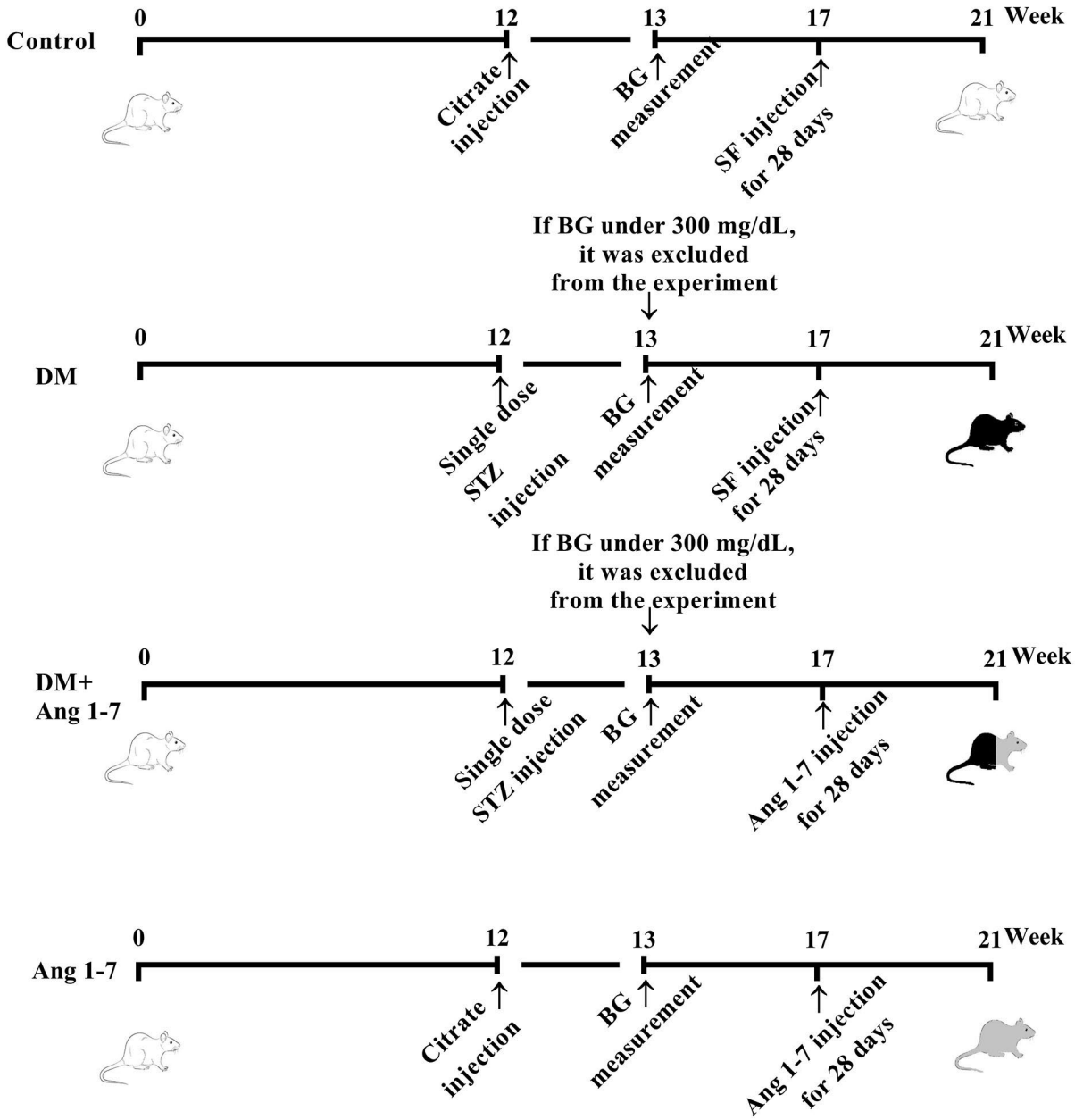
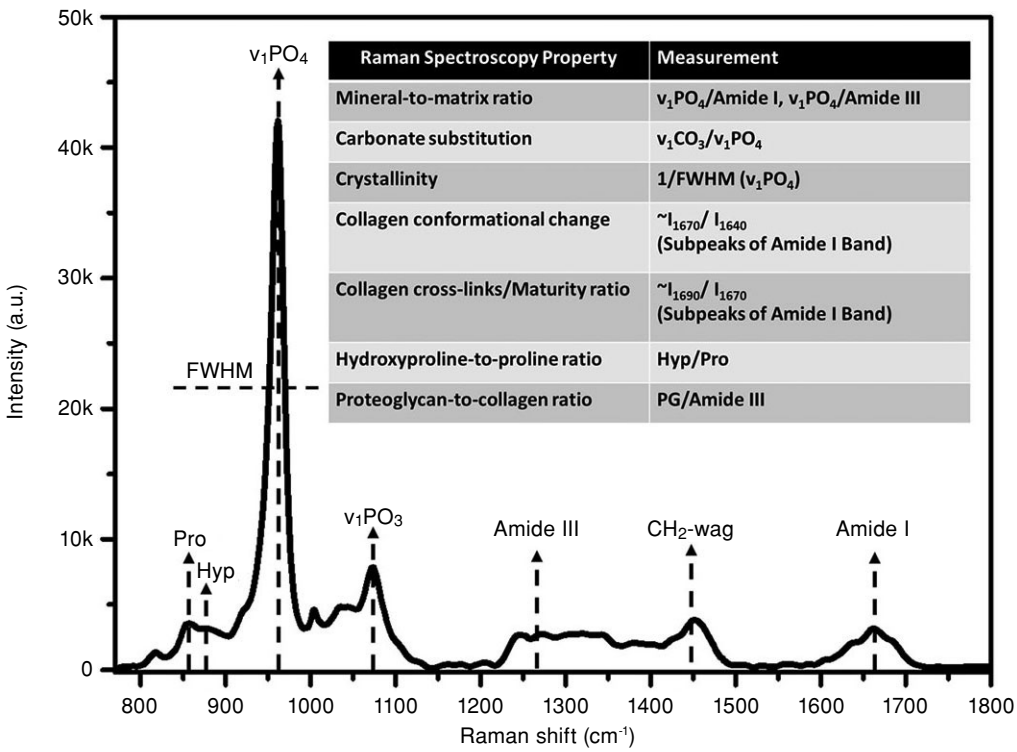


FIGURE 1. Schematic representation of the formation of experimental groups.
BG: Blood glucose; DM: Diabetes mellitus; STZ: Streptozotocin; Ang 1-7: Angiotensin 1-7.

At the end of Week 5, animals which developed diabetes were randomly divided into two groups. One group received no intervention and was monitored for an additional 28 days; this group constituted the STZ-induced diabetes (DM) group. The second group received Ang-(1-7) at a dose of 600 µg/kg/day via daily intraperitoneal bolus injections for 28 days and was designated as the DM + Ang-(1-7) group. Additionally, a separate group of non-diabetic animals, which did not receive STZ and were under standard care until the end of Week 5, was also administered Ang-(1-7) (600 µg/kg/day, intraperitoneally for 28 days). These animals formed the Ang-(1-7)-only group. In this context, four experimental groups were prepared: *(i)* control group (C) (n=12), *(ii)* STZ-induced diabetes (DM) (n=12), *(iii)* STZ-induced diabetes and administration of Ang-(1-7) (DM-Ang-[1-7]) (n=12), and *(iv)* administration of Ang-(1-7) (Ang-[1-7]) (n=12) (Figure 1). At the end of Week 9, bone tissues were collected from the experimental animals and the related measurements were performed. The study protocol was approved by the Akdeniz University Faculty of Medicine Ethics Committee (Date: 05.02.2025, No: 2025.02.B.009). The study was conducted in accordance with its ethics guidelines for the care and use of laboratory animals. After the completion of the applications, we euthanized the rats and removed all tibias and femurs. The bones were separated from the surrounding muscles and ligaments, cleaned with physiological saline solution, and stored at –20°C in phosphate-buffer saline (PBS) solution to prevent dehydration. Twelve right femurs were used for RS, followed by the three-point bending test. The left femurs were used for micro-computed tomography (CT) analysis.

Raman spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique that provides molecular-level information on both the organic and inorganic components of bone tissue.[27] It has become a widely used method in musculoskeletal research due to its ability to non-destructively assess matrix composition, including mineral content, collagen integrity, and crystallinity.[27] Before the Raman analysis, we thawed the samples completely and washed them with PBS solution. We, then, gently wiped them with a surgical sponge to remove excess water. We collected Raman spectra from 50 bone



Raman Spectroscopy Property	Measurement
Mineral-to-matrix ratio	v_1PO_4/Amide I, v_1PO_4/Amide III
Carbonate substitution	v_1CO_3/v_1PO_4
Crystallinity	1/FWHM (v_1PO_4)
Collagen conformational change	~I_{1670}/ I_{1640} (Subpeaks of Amide I Band)
Collagen cross-links/Maturity ratio	~I_{1690}/ I_{1670} (Subpeaks of Amide I Band)
Hydroxyproline-to-proline ratio	Hyp/Pro
Proteoglycan-to-collagen ratio	PG/Amide III

FIGURE 2. A typical processed Raman spectrum of bone with major peaks and calculated Raman spectroscopy properties in this study.
FWHM: Full width at half maximum.

samples using a Renishaw inVia™ confocal Raman microscope (Wotton-under-Edge, Gloucestershire, England) with a 785 nm laser and a 20x objective lens. We followed the previous protocol and spectrum collection suggestions for bone.[27,31] Briefly, for each sample, three spectra were collected from randomly selected locations, with a 5-sec acquisition time per spectrum, each representing the average of 10 scans from the same point. We processed the raw spectra with LabSpec 5 software (Horiba Jobin Yvon, Edison, NJ, USA) and a custom MATLAB script. First, we averaged the spectra from three sites per sample to create one representative spectrum accounting for heterogeneity of bone matrix composition. Then, we removed background fluorescence by subtracting a 5th-order polynomial function. Finally, we applied a proprietary de-noising (D-n) algorithm provided by the LabSpec 5 software (HORIBA, Grenoble, France) to reduce noise. From the processed spectra, we identified several characteristic peaks of bone, such as proline ($C_5H_9NO_2$, 855 cm^{-1} and 920 cm^{-1}), hydroxyproline ($C_5H_9NO_3$, 876 cm^{-1}), Amide I (1600-1720 cm^{-1}), Amide III (1243-1320 cm^{-1}), methylene (CH2-wag, 1450 cm^{-1}), proteoglycan (PG: ~1375 cm^{-1}) for the organic matrix, and phosphate ($_{v1}PO_4$, 960 cm^{-1}) and carbonate ($_{v1}CO_3$, 1070 cm^{-1}) for the mineral content. We also calculated 1/FWHM (bandwidth of $_{v1}PO_4$ peak at the full width at half maximum) for crystallinity analysis (Figure 2).[31]

We used the peak intensity of each peak determined above to calculate the following RS properties of bone tissue: mineral-to-matrix ratio ($_{v1}PO_4$/Amide I, $_{v1}PO_4$/Amide III), mineral quality properties of crystallinity ($_{v1}PO_4$; 1/FWHM) and carbonate substitution ($CO_4/_{v1}PO_4$), organic matrix properties of hydroxyproline/proline (Hyp/Pro), collagen cross-links/matrix maturity ratio (~I_{1670}/I_{1690}) and ~I_{1670}/I_{1640} which is associated with collagen conformational changes,[32] and PG/Amide III (proteoglycan/collagen ratio) (Figure 2). After RS analysis, we stored the bone samples at –20°C, until we performed the three-point bending test.

Three-point bending test

We followed our previous protocol[33] for the experiment. First, we thawed the femurs from –20°C to room temperature. Then, using the Universal Testing Machine Inspekt Duo (Hegewald & Peschke Meß- und Prüftechnik GmbH, Nossen, Germany), we performed a three-point bending test. The femurs were positioned with a 15-mm separation between the two supports, and a compression force with a velocity of 2 mm/min was steadily applied until the bones broke. To analyze the results, we calculated the load, displacement, and maximum bending moment at the point of maximum load using the formula $M_{max} = F_{max} \times L/4$, where Mmax is the maximum bending moment, Fmax is the maximum load, and L is the distance between the two supports. Finally, we used the beam theory formula to determine the flexural strength (σ_{max}).

Micro-CT scanning and measurements

To assess microarchitecture properties, 12 left femurs from each group (a total of 48 femurs) were scanned using micro-CT (SkyScan, Kontich, Belgium). The scanning parameters were 80 kVp energy, 125 μA density, 360-degree shooting, 10 μm voxel size, 0.2 rotation per projection, 49 ms exposure time, and with the 0.1 mm Al filter. Radiological artifacts were removed using the NRecon reconstruction software (SkyScan, Antwerp, Belgium). The images were first reconstructed on multiple axes using the Dataviewer software (SkyScan, Antwerp, Belgium), then BV/TV (the ratio of bone volume in % to the total volume in the examined area), Tb.Th (average thickness of trabeculae assessed using direct three-dimensional [3D] measurement methods in mm), Tb.Sp (mean distance between trabeculae evaluated using direct 3D measurement in mm) values were calculated using the CTAn software (SkyScan, Antwerp, Belgium).[33]

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 software (GraphPad Software, Boston, MA, USA) for data obtained from micro-CT, biomechanical test, and RS. Descriptive data were expressed in mean ± standard error of mean (SEM) for each group. One-way analysis of variance (ANOVA) test and Tukey test, as a post-hoc test, were used. *P* values of $p<0.0001$, $p<0.001$, $p<0.01$, and $p<0.05$ were considered statistically significant.

RESULTS

Body weight and blood glucose level

As a result of the STZ application, an increase was observed in the blood glucose levels of the DM and DM-Ang-(1-7) groups as planned to develop diabetic conditions. The DM and DM-Ang-(1-7) groups exhibited a four-fold rise in final blood glucose levels compared to the control group (Table I). Although the blood glucose level in the DM-Ang-(1-7) group was in a relative

TABLE I
Blood glucose level and animal weight

	C	DM	DM-ANG 1-7	ANG 1-7
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
FBW (g)	295.6±7.07	309.7±6.42	316.8±11.54	307.8±6.72
LBW (g)	328.4±6.66	249.3±8.76*	271.7±10.81*	347.4±6.72
FBG (mg/dL)	103.1±3.41	113.5±3.40	118.4±4.26	108.7±4.85
LBG (mg/dL)	131.0±5.17	524.10±22.10*	491.40±31.84*	130.5±6.30

C: Control group; DM: Diabetes mellitus; ANG-(1-7): Angiotensin-(1-7); SE: Standard error; FBW: first body weight; LBW: last body weight; FBG: first blood glucose level; LBG: last blood glucose level; * Indicates statistical significance compared to the control group ($p<0.05$).

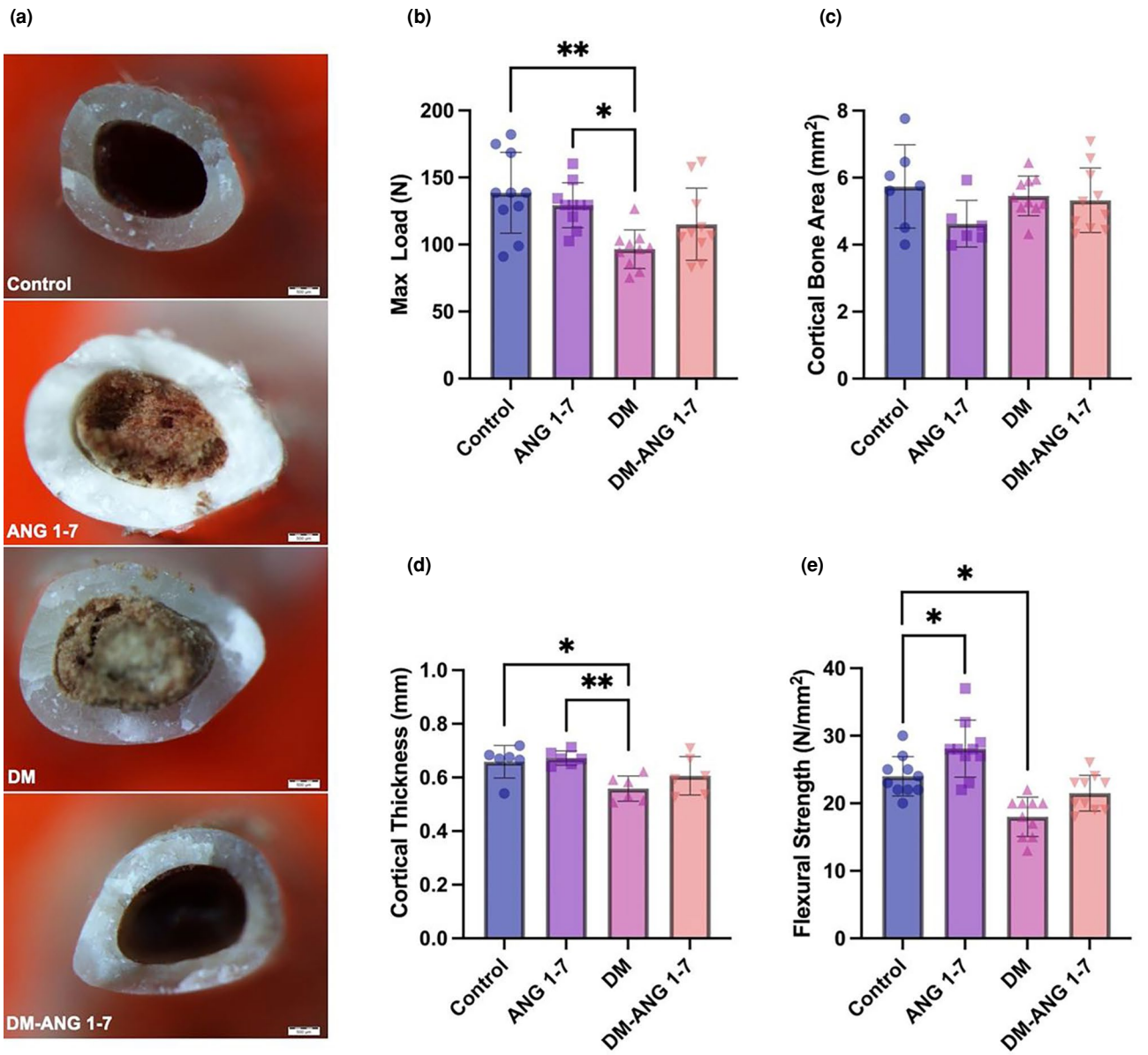


FIGURE 3. Ang-(1-7) did not affect biomechanical properties of bone.
DM: Diabetes mellitus; Ang-(1-7): Angiotensin-(1-7).

decrease compared to the DM group, it was not statistically significant, indicating that Ang-(1-7) administration had no significant effect on improving blood glucose or diabetic conditions. We further measured the body weight of the rats for the morphometric evaluation of diabetic conditions. After STZ application, we observed a decrease in body weight in the groups of DM and DM-Ang-(1-7).

Biomechanical properties of bone

Figure 3 illustrates the average values of total cortical bone area (A), cortical bone thickness (B), maximum load (C), and flexural strength (D) of the bone samples. There was no statistically significant difference in total cortical bone area among the groups ($p>0.05$). However, the DM group exhibited a significant reduction in cortical bone thickness and maximum load, compared to both the control group and the Ang-(1-7) group ($p<0.05$). Regarding flexural strength, the DM group showed a significant decrease compared to the control group, whereas the Ang-(1-7) group displayed a significant increase compared to the control group ($p<0.05$). No significant differences were observed between the DM-Ang-(1-7) group and the other groups in any of the parameters ($p>0.05$) (Figure 3). Biomechanical findings suggest that Ang-(1-7) treatment tended to enhance bone tissue material properties, although this did not reach statistical significance.

Microarchitecture of diabetic bone

While the average bone volume, total volume, and structural thickness of both the DM and DM-Ang-(1-7) groups were significantly lower than those of the control group ($p<0.05$), there were no significant differences between the DM and DM-Ang-(1-7) groups ($p>0.05$). Furthermore, there were no significant differences in structure separation and percent volume among any of the groups (Figure 4) ($p>0.05$).

Biophysiochemical properties of bone tissue by Raman spectroscopy

The mineral-to-matrix ratios were significantly higher in the control group compared to the DM group ($p<0.05$), indicating greater mineral content relative to the organic matrix in healthy bone tissue (Figure 5). However, no significant differences were observed between the other experimental groups ($p>0.05$). Additionally, mineral quality parameters, including crystallinity and carbonate substitution, did not differ significantly among any of the groups ($p>0.05$). However, the organic matrix parameters of Hyp/Pro, PG/Amide III, and ~I_{1670}/I_{1690} showed significant differences between the control and DM groups, as well as between the DM and DM-Ang-(1-7) groups ($p<0.05$). Specifically, the Hyp/Pro ratio was higher in the DM group, while both PG/Amide III and ~I_{1670}/I_{1690} ratios were lower compared to the

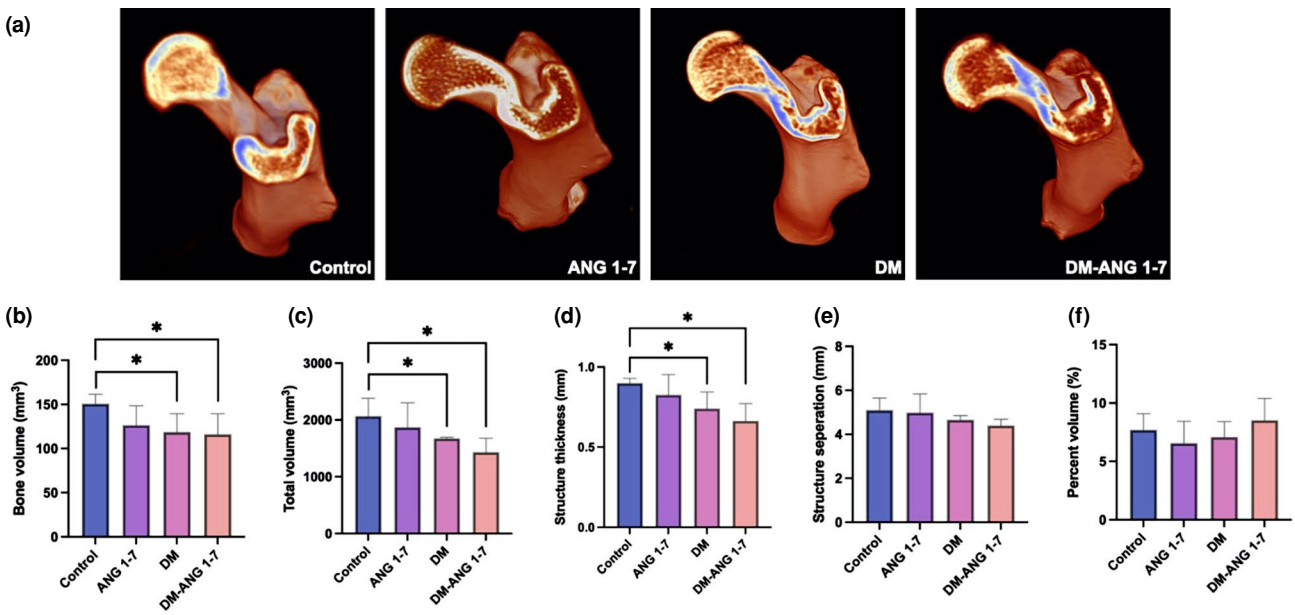


FIGURE 4. Effects of Ang-(1-7) on diabetic bone properties based on micro-CT results.
DM: Diabetes mellitus; Ang-(1-7): Angiotensin-(1-7); CT: Computed tomography.

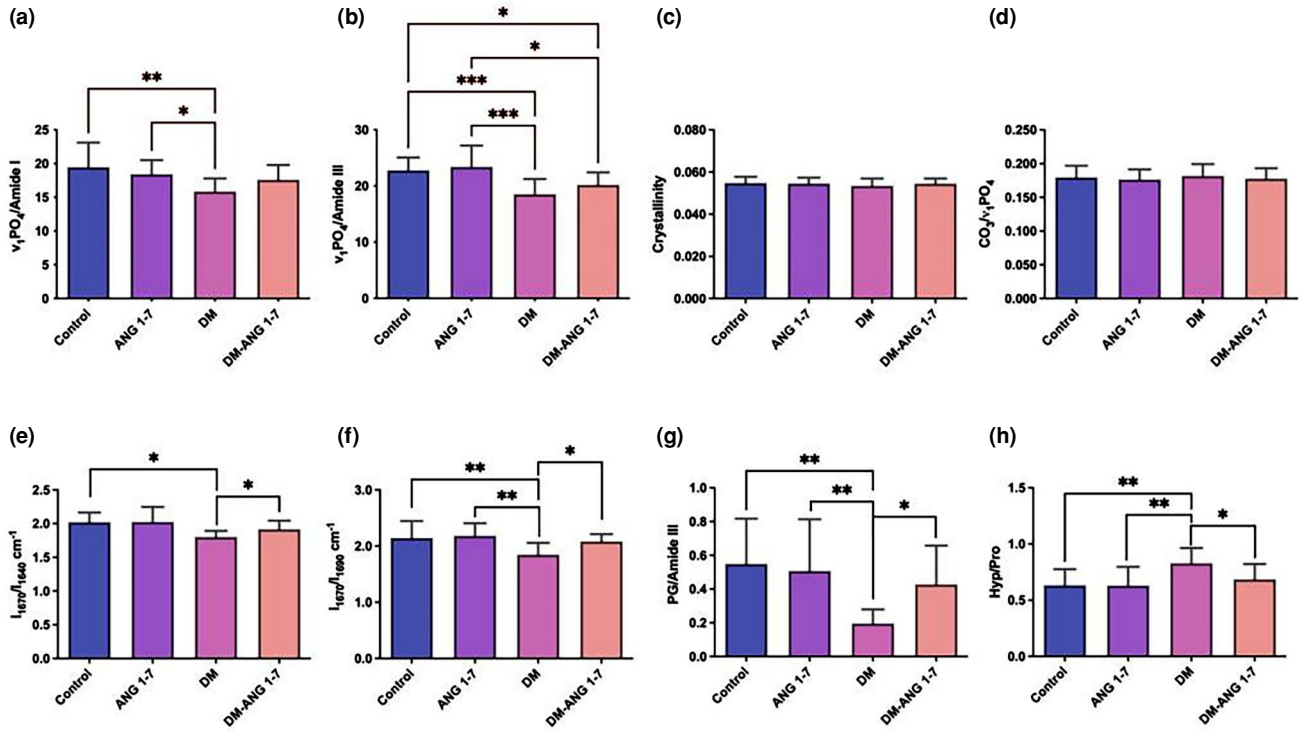


FIGURE 5. Effects of Ang-(1-7) on biophysicochemical properties of bone tissue obtained by Raman spectroscopy.
DM: Diabetes mellitus; Ang-(1-7): Angiotensin-(1-7).

control group, indicating impaired proteoglycan content and collagen cross-linking in diabetic bone. The ~I_{1670}/I_{1640} ratio was also significantly higher in the control group than in the DM group ($p<0.05$) and showed a trend toward recovery in the DM-Ang-(1-7) group, approaching control levels.

DISCUSSION

In addition to fracture toughness and fatigue resistance, strength is one of the essential factors determining bone fracture resistance.[34,35] Therefore, a successful therapeutic agent for bone fragility caused by diseases would also improve bone strength. However, the increased fracture risk and changes in bone biomechanical properties are frequently linked to degradation in BMD and bone microarchitecture in the conditions of osteoporosis and diabetic osteopenia.[2,15,33] Additionally, the role of bone composition is crucial in understanding the deterioration of bone quality in both T1DM and T2DM conditions. This includes alterations in the organic matrix of bone, such as the accumulation of advanced glycation end products (AGEs), and structural changes in collagen molecules and minerals along with their amount.[2,36-38] Many studies have been conducted so far to gain insight into the changes in bone quality caused by DM.[39,40] and to explore ways to prevent or treat such negative effects, as they ultimately impact bone fracture resistance. There are many proposed strategies, including drug candidates to prevent or treat such negative effects of diabetic conditions.[41,42] The Ang-(1-7) could be one of these potential strategies given the limited evidence that Ang-(1-7) may have the potential to increase the expression of osteocalcin.[24] and improve the mechanical and microarchitectural properties of bone.[23] In the present study, we investigated the potential preventive effect of Ang-(1-7) on deterioration in bone quality, particularly its potential preventive ability to the biophysicochemical properties of bone composition at the material/tissue level. This is important, as the origin of bone strength also lies at the material/tissue level, encompassing various determinants of bone strength that result from the individual contribution of bone components as well as their hierarchical organization.[43]

For this purpose, using multiple RS-based bone quality properties, we evaluated biophysicochemical properties of bone tissues comprehensively.

Our findings suggest that DM causes a severe decrease in local amount of mineral per organic matrix at the sampling volume as measured by the mineral-to-matrix ratio. More intriguingly, Ang-(1-7) treatment partially protected the bone from this negative effect, but this did not reach statistical significance. The mineral quality parameter of carbonate/phosphate ratio did not show any significant difference between the groups which is, indeed, consistent with the findings of previous studies that reported DM mainly affects organic matrix rather than mineral part.[37,44] While the decreased mineral-to-matrix ratio in the DM group could be interpreted as an increase in organic matrix content, this interpretation does not align with the literature,[37,44] which consistently reports impaired collagen quality, not increased quantity in diabetic.[45-47] Instead, our results likely reflect a deterioration in the structural integrity of the organic matrix, particularly in collagen's secondary and tertiary structures. Thus, the lower mineral-to-matrix ratio in the DM group likely results from both impaired mineralization and a less mature, structurally compromised collagen matrix rather than an overproduction of matrix components. This interpretation aligns with prior reports highlighting the compositional and structural degradation of bone matrix in diabetic models, ultimately contributing to compromised mechanical strength.[45-47] However, such studies also found that individuals with DM had a higher risk of bone fractures, suggesting that the organic matrix plays a more important role in alteration of bone strength in DM.[39] Disruption of enzymatic cross-linking, increased non-enzymatic glycation, and compromised proteoglycan integrity are well-documented in diabetic conditions[32,38,48] and may explain the spectral signatures observed. Diabetes mellitus caused an alteration in the RS-based organic matrix properties reflecting the secondary structure in collagen molecules and the enzymatic cross-links of collagen.[32,38,48] Taken together, the treatment with Ang-(1-7) provides protective effect against negative alteration in collagen secondary structures as reflected by the ratios of I1640/I1670 and Hyp/Pro in which their levels are comparable to the levels of the control group. This result supports the hypothesis that Ang-(1-7) may have a beneficial effect on the organic matrix in DM. In addition, the ratio of I1670/I1690 reflects the enzymatic cross-links in collagen.[27,31] In the DM group, this ratio was significantly lower than in the control group, suggesting that there may be significant changes in triple helix structure of collagen, since enzymatic cross-links have a significant role of maintaining stability of collagen fibrils within bone matrix.[36] However, in the group treated with Ang-(1-7), this ratio was significantly higher, indicating that Ang-(1-7) prevents the impairment of enzymatic cross-links; therefore, the impairment of triple helix structure of collagen caused by DM conditions. Moreover, the ratio of PG/Amide III refers the proteoglycans composition of the bone, which are non-collagenous organic matrix components with high water-binding properties.[49] This ratio was significantly lower in the DM group than in the control group, but the PG/Amide III value was comparable to the DM-Ang-(1-7) group. Two implications can be concluded from these findings as reflected by the comparable level of this parameter with control group upon treatment with Ang-(1-7): (a) DM can reduce the amount of PGs in the bone, but Ang-(1-7) helps to maintain its level in DM condition; (b) DM may also cause a change in hydration status of bone, since PGs have a strong tendency to bind water molecules;[50] and any change in PGs composition may result in alteration of the hydration status of bone. Our cumulative results indicate that Ang-(1-7) has a limited effect on bone minerals in rats with DM. On the other hand, Ang-(1-7) has a positive preventive effect on aspects such as impairment of the triple helix structure in the bone organic matrix in the rats with DM.

In the current study, the flexural strength of the bone samples in DM group had lower than the control group, and the Ang-(1-7) group had higher flexural strength than that of the control group ($p<0.05$). There was a trend towards the level of the control group in the treatment group, in which diabetic rats received Ang-(1-7) (DM-Ang-(1-7) group), indicating that Ang-(1-7) may have a positive effect on overall bone strength. This is also the case for overall the microarchitecture of bone tissue in DM-Ang-(1-7) group. Although, the micro-CT results showed no significant differences between the treatment group and the other groups, DM-Ang-(1-7) group had a higher value than the DM group, but the trend did not reach statistically significant level. A recent study by Sha et al.[23] reported that an increase in maximum load in a three-point bending test on rat tibias. They compared a diabetic and hypertensive rats group treated with Ang-(1-7) with other groups, including one that received valsartan, an anti-hypertension drug that blocks Ang II receptors. They found that Ang-(1-7) was more effective than valsartan in improving the mechanical property of bone at a macro-level, specifically the maximum

load. This improvement depended mostly on the structural properties of bone, going beyond its material properties and biophysicochemical properties. This study sheds light on the effects of Ang-(1-7) treatment on bone biophysicochemical properties and overall matrix quality, providing the first evidence of Ang-(1-7) effects on bone tissue matrix. Thus, RS has the potential to provide new insights into bone matrix changes in DM and the effects of Ang-(1-7) on it by examining changes in the bone matrix at the tissue level.

Abuohashish et al.[26] demonstrated that treating OVX rats with Ang-(1-7) (200 ng/kg/min for a total of six weeks) resulted in increased several microarchitectural properties such as bone volume, BMD, bone volume to total volume ratio, trabecular thickness, and trabecular number. In our study, we observed limited protective effects of Ang-(1-7) on bone microarchitectural properties. A possible explanation for this discrepancy is that Ang-(1-7) was administered to rats at different doses or for different durations. On the other hand, in T1DM, the insulin-secreting pancreatic β-cells are largely destroyed by STZ, thereby leading to hyperglycemia and devastating complications of the disease. Due to administration of STZ, there were several noticeable alterations to the mitochondrial organization, coarsening, and separation of endoplasmic reticulum, and no presence of mature granules in the Golgi area of pancreatic beta islets.[51-53] Furthermore, this type of cell is subject to necrosis and eventual disappearance after a certain period of time.[51,54] This results in increased blood glucose levels and significant decreases in plasma insulin and pancreatic insulin levels compared to normal levels.[55] Therefore, treatment with Ang-(1-7) cannot fully prevent the occurrence of diabetic-related conditions in bone microarchitectural and biomechanical properties, since there are no longer any pancreatic beta cells on which Ang-(1-7) may act, nor any insulin available to increase its activity. The partial preventive effects of Ang-(1-7) on bone matrix, thus, may emerge from direct interaction between Ang-(1-7) and the bone matrix in a beta cell- and insulin- independent manner, similar to the observed interaction between raloxifene, an estrogen receptor modulator which acts as an estrogen agonist in the skeleton, and the bone matrix.[56] However, this prospect needs to be investigated further. Our findings also align with prior study showing tissue-specific regulatory effects of Ang-(1-7). Castelo-Branco et al.[57] demonstrated a biphasic, dose-dependent effect of Ang-(1-7) on renal bicarbonate transport via Mas and AT1 receptors in hypertensive rats, highlighting its modulatory role beyond a single tissue type. In the context of skeletal health, Abuohashish et al.[58] demonstrated that ovariectomy-induced reductions in bone mineralization and microstructural integrity were ameliorated by ACE-1 inhibition, which activated the local ACE-2/Ang-(1–7)/Mas axis and suppressed osteoclastogenesis. This underscores Ang-(1-7)'s potential to counteract osteoporotic bone loss by modulating bone remodeling. Unlike prior studies, our work is the first to examine the effects of Ang-(1-7) on bone matrix composition under diabetic conditions using RS, revealing specific alterations in collagen quality and mineral-to-matrix ratio. Collectively, these findings highlight Ang-(1-7) as a promising therapeutic candidate for preserving skeletal integrity in diabetes through Mas receptor-mediated pathways.

Nonetheless, this study has certain limitations. Specifically, the current design restricts our ability to fully assess the therapeutic effectiveness of angiotensin 1-7 and to provide comprehensive molecular insights. To address these limitations in future research, we plan to implement a more detailed methodological approach involving varied dosing regimens and treatment durations of angiotensin 1-7. In addition, we will evaluate bone formation markers using RT-PCR to better elucidate its underlying molecular mechanisms.

In conclusion, our study results suggest that Ang-(1-7) can have a potential preventive effect on bone matrix quality, but the preventive effects against DM-induced changes in bone tissue at all hierarchical levels are not fully achievable due to the limited improvement in architectural properties. It seems that the treatment with Ang-(1-7) at the current dose enhances organic matrix quality of diabetic bone at the material-level, but this improvement does not fully extend to its extrinsic structural properties and strength. This can be attributed to an inadequate dose, short treatment period, or other factors. Therefore, future studies are still needed to establish the optimal dosage and duration of Ang-(1-7) treatment for bone tissue impaired by DM. Another speculative hypothesis posits that the influence of Ang-(1-7) on the collagen matrix could potentially be driven by an interaction between Ang-(1-7) and the collagen matrix, which operates independently of cellular and insulin pathways. However, this potential scenario requires further in-depth investigation. Nonetheless, the findings presented in this study

lend support to the concept that Ang-(1-7) holds the potential to be utilized as a therapeutic approach for enhancing the quality of the bone's organic matrix in individuals with DM.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017;128:40-50. doi: 10.1016/j.diabres.2017.03.024.
2. Donmez BO, Unal M, Ozdemir S, Ozturk N, Oguz N, Akkus O. Effects of losartan treatment on the physicochemical properties of diabetic rat bone. J Bone Miner Metab 2017;35:161-70. doi: 10.1007/s00774-016-0748-9.
3. Hygum K, Starup-Linde J, Langdahl BL. Diabetes and bone. Osteoporos Sarcopenia 2019;5:29-37. doi: 10.1016/j.afos.2019.05.001.
4. Wang H, Ba Y, Xing Q, Du JL. Diabetes mellitus and the risk of fractures at specific sites: A meta-analysis. BMJ Open 2019;9:e024067. doi: 10.1136/bmjopen-2018-024067.
5. Fan Y, Wei F, Lang Y, Liu Y. Diabetes mellitus and risk of hip fractures: A meta-analysis. Osteoporos Int 2016;27:219-28. doi: 10.1007/s00198-015-3279-7.
6. Hough FS, Pierroz DD, Cooper C, Ferrari SL; IOF CSA Bone and Diabetes Working Group. MECHANISMS IN ENDOCRINOLOGY: Mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. Eur J Endocrinol 2016;174:R127-38. doi: 10.1530/EJE-15-0820.
7. Rico H, Hernandez ER, Cabranes JA, Gomez-Castresana F. Suggestion of a deficient osteoblastic function in diabetes mellitus: The possible cause of osteopenia in diabetics. Calcif Tissue Int 1989;45:71-3. doi: 10.1007/BF02561404.
8. Verhaeghe J, Visser WJ, Einhorn TA, Bouillon R. Osteoporosis and diabetes: Lessons from the diabetic BB rat. Horm Res 1990;34:245-8. doi: 10.1159/000181834.
9. Suzuki K, Kurose T, Takizawa M, Maruyama M, Ushikawa K, Kikuyama M, et al. Osteoclastic function is accelerated in male patients with type 2 diabetes mellitus: The preventive role of osteoclastogenesis inhibitory factor/osteoprotegerin (OCIF/OPG) on the decrease of bone mineral density. Diabetes Res Clin Pract 2005;68:117-25. doi: 10.1016/j.diabres.2004.08.006.
10. Khajeh Pour S, Ranjit A, Summerill EL, Aghazadeh-Habashi A. Anti-inflammatory effects of ang-(1-7) bone-targeting conjugate in an adjuvant-induced arthritis rat model. Pharmaceuticals (Basel) 2022;15:1157. doi: 10.3390/ph15091157.
11. De Mello WC, Danser AH. Angiotensin II and the heart: On the intracrine renin-angiotensin system. Hypertension 2000;35:1183-8. doi: 10.1161/01.hyp.35.6.1183.
12. Paul M, Wagner J, Dzau VJ. Gene expression of the renin-angiotensin system in human tissues. Quantitative analysis by the polymerase chain reaction. J Clin Invest 1993;91:2058-64. doi: 10.1172/JCI116428.
13. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. Physiol Rev 2006;86:747-803. doi: 10.1152/physrev.00036.2005.
14. Ribeiro-Oliveira A Jr, Nogueira AI, Pereira RM, Boas WW, Dos Santos RA, Simões e Silva AC. The renin-angiotensin system and diabetes: An update. Vasc Health Risk Manag 2008;4:787-803.
15. Donmez BO, Ozdemir S, Sarikanat M, Yaras N, Koc P, Demir N, et al. Effect of angiotensin II type 1 receptor blocker on osteoporotic rat femurs. Pharmacol Rep 2012;64:878-88. doi: 10.1016/s1734-1140(12)70882-4.
16. Wei CC, Tian B, Perry G, Meng QC, Chen YF, Oparil S, et al. Differential ANG II generation in plasma and tissue of mice with decreased expression of the ACE gene. Am J Physiol Heart Circ Physiol 2002;282:H2254-8. doi: 10.1152/ajpheart.00191.2001.
17. da Silveira KD, Coelho FM, Vieira AT, Sachs D, Barroso LC, Costa VV, et al. Anti-inflammatory effects of the activation of the angiotensin-(1-7) receptor, MAS, in experimental models of arthritis. J Immunol 2010;185:5569-76. doi: 10.4049/jimmunol.1000314.
18. Miller AJ, Arnold AC. The renin-angiotensin system in cardiovascular autonomic control: Recent developments and clinical implications. Clin Auton Res 2019;29:231-43. doi: 10.1007/s10286-018-0572-5.
19. Kamo T, Akazawa H, Suzuki JI, Komuro I. Roles of renin-angiotensin system and Wnt pathway in aging-related phenotypes. Inflamm Regen 2016;36:12. doi: 10.1186/s41232-016-0018-1.
20. Santos RA. Angiotensin-(1-7). Hypertension 2014;63:1138-47. doi: 10.1161/HYPERTENSIONAHA.113.01274.
21. Miller AJ, Arnold AC. The renin-angiotensin system and cardiovascular autonomic control in aging. Peptides 2022;150:170733. doi: 10.1016/j.peptides.2021.170733.
22. Mo C, Ke J, Zhao D, Zhang B. Role of the renin-angiotensin-aldosterone system in bone metabolism. J Bone Miner Metab 2020;38:772-9. doi: 10.1007/s00774-020-01132-y.
23. Sha NN, Zhang JL, Poon CC, Li WX, Li Y, Wang YF, et al. Differential responses of bone to angiotensin II and angiotensin(1-7): Beneficial effects of ANG(1-7) on bone with exposure to high glucose. Am J Physiol Endocrinol Metab 2021;320:E55-70. doi: 10.1152/ajpendo.00158.2020.
24. Queiroz-Junior CM, Santos ACPM, Galvão I, Souto GR, Mesquita RA, Sá MA, et al. The angiotensin converting enzyme 2/angiotensin-(1-7)/Mas Receptor axis as a key player in alveolar bone remodeling. Bone 2019;128:115041. doi: 10.1016/j.bone.2019.115041.
25. Krishnan B, Smith TL, Dubey P, Zapadka ME, Torti FM, Willingham MC, et al. Angiotensin-(1-7) attenuates metastatic prostate cancer and reduces osteoclastogenesis. Prostate 2013;73:71-82. doi: 10.1002/pros.22542.
26. Abuohashish HM, Ahmed MM, Sabry D, Khattab MM, Al-Rejaie SS. Angiotensin (1-7) ameliorates the structural and biochemical alterations of ovariectomy-induced osteoporosis

in rats via activation of ACE-2/Mas receptor axis. Sci Rep 2017;7:2293. doi: 10.1038/s41598-017-02570-x.
27. Unal M, Ahmed R, Mahadevan-Jansen A, Nyman JS. Compositional assessment of bone by Raman spectroscopy. Analyst 2021;146:7464-90. doi: 10.1039/d1an01560e.
28. Hammond MA, Gallant MA, Burr DB, Wallace JM. Nanoscale changes in collagen are reflected in physical and mechanical properties of bone at the microscale in diabetic rats. Bone 2014;60:26-32. doi: 10.1016/j.bone.2013.11.015.
29. Hunt HB, Torres AM, Palomino PM, Marty E, Saiyed R, Cohn M, et al. Altered tissue composition, microarchitecture, and mechanical performance in cancellous bone from men with type 2 diabetes mellitus. J Bone Miner Res 2019;34:1191-206. doi: 10.1002/jbmr.3711.
30. Rubin MR, Paschalis EP, Poundarik A, Sroga GE, McMahon DJ, Gamsjaeger S, et al. Advanced glycation endproducts and bone material properties in type 1 diabetic mice. PLoS One 2016;11:e0154700. doi: 10.1371/journal.pone.0154700.
31. Unal M, Uppuganti S, Timur S, Mahadevan-Jansen A, Akkus O, Nyman JS. Assessing matrix quality by Raman spectroscopy helps predict fracture toughness of human cortical bone. Sci Rep 2019;9:7195. doi: 10.1038/s41598-019-43542-7.
32. Unal M, Jung H, Akkus O. Novel Raman spectroscopic biomarkers indicate that postyield damage denatures bone's collagen. J Bone Miner Res 2016;31:1015-25. doi: 10.1002/jbmr.2768.
33. Ozturk S, Cuneyit I, Altuntas F, Karagur ER, Donmez AC, Ocak M, et al. Resveratrol prevents ovariectomy-induced bone quality deterioration by improving the microarchitectural and biophysicochemical properties of bone. J Bone Miner Metab 2023;41:443-56. doi: 10.1007/s00774-023-01416-z.
34. Sun Y, Li X, Ma S, Chong H, Cai TC, Li KM, et al. Comparison of the efficacy and safety of unilateral and bilateral approach kyphoplasty in the treatment of osteoporotic vertebral compression fractures: A meta-analysis. Jt Dis Relat Surg 2024;35:491-503. doi: 10.52312/jdrs.2024.1701.
35. Hernandez CJ, van der Meulen MC. Understanding bone strength is not enough. J Bone Miner Res 2017;32:1157-62. doi: 10.1002/jbmr.3078.
36. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: A possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int 2010;21:195-214. doi: 10.1007/s00198-009-1066-z.
37. Karim L, Bouxsein ML. Effect of type 2 diabetes-related non-enzymatic glycation on bone biomechanical properties. Bone 2016;82:21-7. doi: 10.1016/j.bone.2015.07.028.
38. Unal M, Uppuganti S, Leverant CJ, Creecy A, Granke M, Voziyan P, et al. Assessing glycation-mediated changes in human cortical bone with Raman spectroscopy. J Biophotonics 2018;11:e201700352. doi: 10.1002/jbio.201700352.
39. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL, et al. Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol 2017;13:208-19. doi: 10.1038/nrendo.2016.153.
40. Murray CE, Coleman CM. Impact of diabetes mellitus on bone health. Int J Mol Sci 2019;20:4873. doi: 10.3390/ijms20194873.
41. Sarmah S, Roy AS. A review on prevention of glycation of proteins: Potential therapeutic substances to mitigate the severity of diabetes complications. Int J Biol Macromol 2022;195:565-88. doi: 10.1016/j.ijbiomac.2021.12.041.
42. Shah M, Appuswamy AV, Rao SD, Dhaliwal R. Treatment of bone fragility in patients with diabetes: Antiresorptive versus anabolic? Curr Opin Endocrinol Diabetes Obes 2021;28:377-82. doi: 10.1097/MED.0000000000000645.
43. Unal M, Creecy A, Nyman JS. The role of matrix composition in the mechanical behavior of bone. Curr Osteoporos Rep 2018;16:205-15. doi: 10.1007/s11914-018-0433-0.
44. Willett TL, Voziyan P, Nyman JS. Causative or associative: A critical review of the role of advanced glycation end-products in bone fragility. Bone 2022;163:116485. doi: 10.1016/j.bone.2022.116485.
45. Emerzian SR, Chow J, Behzad R, Unal M, Brooks DJ, Wu IH, et al. Long-duration type 1 diabetes is associated with deficient cortical bone mechanical behavior and altered matrix composition in human femoral bone. J Bone Miner Res 2024;40:87-99. doi: 10.1093/jbmr/zjae184.
46. Dixit M, Liu Z, Poudel SB, Yildirim G, Zhang YZ, Mehta S, et al. Skeletal response to insulin in the naturally occurring type 1 diabetes mellitus mouse model. JBMR Plus 2021;5:e10483. doi: 10.1002/jbm4.10483.
47. Bolger MW, Tekkey T, Kohn DH. The contribution of perilacunar composition and mechanical properties to whole-bone mechanical outcomes in streptozotocin-induced diabetes. Calcif Tissue Int 2023;113:229-45. doi: 10.1007/s00223-023-01098-9.
48. Unal M. Letter to the Editor. Connect Tissue Res 2020;61:420-2. doi: 10.1080/03008207.2019.1666113.
49. Gamsjaeger S, Klaushofer K, Paschalis EP. Raman analysis of proteoglycans simultaneously in bone and cartilage. J Raman Spectrosc 2014;45:794-800.
50. Wang X, Xu H, Huang Y, Gu S, Jiang JX. Coupling effect of water and proteoglycans on the ın situ toughness of bone. J Bone Miner Res 2016;31:1026-9. doi: 10.1002/jbmr.2774.
51. Arison RN, Ciaccio EI, Glitzer MS, Cassaro JA, Pruss MP. Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. Diabetes 1967;16:51-6. doi: 10.2337/diab.16.1.51.
52. Ganda OP, Rossini AA, Like AA. Studies on streptozotocin diabetes. Diabetes 1976;25:595-603. doi: 10.2337/diab.25.7.595.
53. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J Diabetes Metab Disord 2013;12:60. doi: 10.1186/2251-6581-12-60.
54. Hayashi K, Kojima R, Ito M. Strain differences in the diabetogenic activity of streptozotocin in mice. Biol Pharm Bull 2006;29:1110-9. doi: 10.1248/bpb.29.1110.
55. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J Clin Invest 1969;48:2129-39. doi: 10.1172/JCI106180.
56. Bivi N, Hu H, Chavali B, Chalmers MJ, Reutter CT, Durst GL, et al. Structural features underlying raloxifene's biophysical interaction with bone matrix. Bioorg Med Chem 2016;24:759-67. doi: 10.1016/j.bmc.2015.12.045.
57. Castelo-Branco RC, Leite-Dellova DCA, Fernandes FB, Malnic G, de Mello-Aires M. The effects of angiotensin-(1-7) on the exchanger NHE3 and on [Ca2+]i in the proximal tubules of spontaneously hypertensive rats. Am J Physiol Renal Physiol 2017;313:F450-60. doi: 10.1152/ajprenal.00557.2016.
58. Abuohashish HM, Ahmed MM, Sabry D, Khattab MM, Al-Rejaie SS. ACE-2/Ang1-7/Mas cascade mediates ACE inhibitor, captopril, protective effects in estrogen-deficient osteoporotic rats. Biomed Pharmacother 2017;92:58-68. doi: 10.1016/j.biopha.2017.05.062.