Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy Dovepress
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HYPOTHESIS

A Potential Participant in Type 2 Diabetes Bone Fragility: TIMP-1 at Sites of Osteocyte Lacunar-Canalicular System

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Abstract: Type 2 diabetes mellitus (T2DM) is associated with an increased risk of bone fracture, but the bone mineral density (BMD) is typically normal or higher in such patients. Because the fracture risk is independent of reduced BMD, bone fragility in T2DM may be partially due to poor bone quality. The mechanisms triggering bone quality abnormalities in T2DM are complex, and include the accumulation of advanced glycation end-products, the increased inflammation, and low bone turnover. Matrix metalloproteinases (MMPs) in bone can hydrolyze the bone matrix. Tissue inhibitors of MMPs (TIMPs) can inhibit the activity of MMPs. Both MMPs and TIMPs participate in mediating bone quality. Among all types of TIMPs, TIMP-1 is mostly reportedly increased in the serum of T2DM patients. Because osteocytes can express TIMP-1, and osteocyte pericellular matrix influences bone quality partially regulated by perilacunar/canalicular remodeling, we hypothesized that TIMP-1 at sites of osteocyte lacunar-canalicular system is involved in T2DM bone fragility.

Keywords: bone fragility, lacunar-canalicular system, osteocyte, TIMP-1, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of fragility bone fractures, despite individuals with T2DM having normal or greater bone mineral density (BMD).[1–5] This increased fracture risk remains even after accounting for potential confounders such as falls, and it may be partially due to poor bone quality. Bone quality encompasses several bone properties reflecting bone geometry or microarchitecture, and the organization and/or composition of collagen and minerals in the bone matrix. Various mechanisms of bone quality deterioration in T2DM have been reported. Animal model studies indicated that impaired bone quality in T2DM is multifactorial, and includes changes in microarchitecture, abnormal collagen crosslinking, and matrix mineralization. Hunt et al[6] investigated several aspects of bone quality in a clinical population of men with and without T2DM, and detected high concentrations of advanced glycation end-products and altered mineral maturity in femoral neck cancellous bone from T2DM men. Karim et al[7] reported that cortical bone had worse indentation properties and higher advanced glycation end-products in the proximal femurs of adults with T2DM.

Apart from changes in bone extracellular matrix (ECM) at the tissue level in T2DM, Eckhardt et al[8] reported that accelerated osteocyte senescence may be

Received: 19 October 2021
Accepted: 12 December 2021
Published: 23 December 2021

involved in the skeletal fragility observed in the high-fat diet/streptozotocin mouse model of T2DM. Several prior studies indicate that the osteocyte network is altered in different diabetes models.[9–11] Osteocytes are the most abundant cells in bone, and have a large number of dendritic cell processes through which osteocytes communicate with the surrounding osteocytes and bone surface cells such as osteoblasts and osteoclasts.[12] Osteocytes and their cell processes reside in a mineralized matrix that forms the lacunar-canalicular system (LCS).[13–15] The primary function of LCS is to help osteocytes to obtain nutrients, sense mechanical stimuli, and communicate with other cells via molecular signaling.[10] The osteocyte LCS is believed to control both "outside-in" (mechanosensing) and "inside-out" (signaling molecule transport) processes.[10,16] Morphological alterations in LCS could impact the "outside-in" mechanosensing processes by modulating the levels of mechanical stimulus (fluid shear stresses[17] and drag forces[16]). The level of mechanical stimuli is related to osteocyte apoptosis which promotes osteoclastogenesis and is a mechanism by which osteocytes regulate bone adaptation.[18,19] It is presumable that the osteocyte mechanotransduction-mediated bone adaptation might be affected by altered osteocyte LCS network in T2DM.

Osteocytes remodel its surrounding bone matrix by secreting several proteases, which include matrix metalloproteinase (MMP) 2, MMP13, and cathepsin K.[20] MMPs and tissue inhibitors of MMPs (TIMPs) regulate bone development and remodeling. As a family of proteolytic enzymes, MMPs may hydrolyze ECM.[21,22] Most MMP members have been detected in bone and mediate bone homeostasis.[23,24] Both low fracture toughness and low post-yield deflection have been reported in MMP9$^{-/-}$ mouse femurs.[25] MMP2 is mainly secreted by mature osteoblasts and may be a key enzyme involved in bone quality.[26,27] The precise coordination of TIMPs and MMPs maintains ECM homeostasis, and a TIMP/MMP imbalance adversely affects ECM, leading to bone diseases such as primary osteoporosis, osteoarthritis, and rheumatoid arthritis.[25,28,29] TIMPs are the natural inhibitor of many MMPs, are widely expressed in bone, and play an essential role in bone adaptation.[30] TIMP-1 is a soluble glycoprotein of 28kDa and a prominent member of the TIMP family. It can inhibit most MMPs, except membrane type-MMPs and MMP24.[31,32] Haeusler et al[33] observed that TIMP-1 was present in all zones of the growth plate, osteoblasts, and osteoclasts, some of osteocytes. It also has been reported that TIMP-1 can directly stimulate the bone resorbing activity independent of their inhibition of MMPs at certain physiological concentrations.[29] Prior studies have investigated the role of TIMP-1 in osteoblasts and/or osteoclasts,[29,34,35] but little is known about associations between TIMP-1 and the osteocyte network, and its influences on bone quality in T2DM. It is not known whether TIMP-1 located in the LCS is involved in bone fragility in T2DM patients.

Hypothesis

MMPs are a large family of zinc-dependent ECM-degrading enzymes and play a crucial role in bone matrix remodeling, thus dramatically affecting bone mechanical and material properties.[24,36,37] TIMPs inhibit the activity of MMPs.[34,38] Bone quality is dependent on a balance between TIMPs and MMPs. Among four types of TIMPs found in bone, only significant changes in TIMP-1 have reported in the serum of individuals with T2DM.[39–41] Several studies indicate that osteocytes can express TIMP-1.[26,34,42,43] The osteocyte LCS plays a vital role in mechanical sensing, strain experience, bone composition, and bone quality.[13] MMP13 influences perilacunar/canalicular remodeling (PLR),[36] so interaction between TIMP-1 and MMP13 or other MMPs may improve PLR in pathological conditions. We hypothesized that TIMP-1 located in the osteocyte LCS is involved in T2DM-associated bone fragility (Figure 1).

Evaluation of the hypothesis

Altered TIMP-1 Level in the Plasma/Serum of T2DM Patients

To our knowledge, the underlying mechanisms that lead to the changes of TIMP-1 in plasma/serum of T2DM patients remain scarce; but the positive association between TIMP-1 and T2DM in patients concur with the results from several case control and cross-sectional studies (Table 1). In case-control study nested within a prospective cohort among Chinese living in Singapore, Wang et al[40] demonstrated that serum TIMP-1 levels were significantly higher in T2DM patients (n=254) than in age and gender-matched non-diabetic patients (n=254), and elevated TIMP-1 levels were positively associated with T2DM risk. Lee et al[39] reported that plasma levels of TIMP-1 and MMP2 were elevated in Korea T2DM patients (without uremia) whereas there was no significant alteration in MMP9 (cases n=80, controls n=80), and the increase in TIMP-1 and MMP2 were independent of age, duration of diabetes,

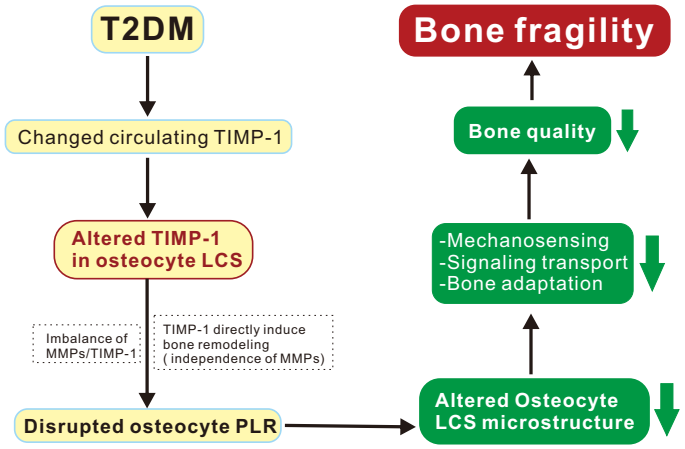

Figure 1 A conceptual framework of this hypothesis: TIMP-1 at sites of osteocyte lacunar-canalicular system (LCS) is partially involved in type 2 diabetes bone fragility. The two potential mechanisms by which TIMP-1 in LCS alters PLR are shown in the dotted boxes, and future studies are warranted to elucidate the precise mechanism for this hypothesis.

blood pressure, and blood lipids. Tayebjee et al[44] showed that circulating MMP-9, TIMP-1 are raised in treated hypertensive T2DM patients from the UK (n=86) compared with normotensive control subjects (n=63). Sundström et al[45] presented their interesting data from the USA that plasma TIMP-1 was higher in men compared with women, and increased with age, and positively related to diabetes. However, Papazafiropoulou et al[41] reported that serum TIMP-1 was lower in patients with T2DM, and that they exhibited no differences in MMP2 or MMP9. In another study, serum MMP9 was increased in patients with diabetic retinopathy, but there was no

Table 1 Summary of Association Between TIMP-1 Level in Blood and T2DM in Patients

Reference	Subjects			Outcome of TIMP-1 (ng/mL)	Association of TIMP-1 and T2DM
	Numbers	Age (Years)	Country		
Wang Y. et al, 2020[40]	Patients: 254 Control: 254	59±5.83 59.3±6.03	Singapore[a]	227.9±39* 213.5±35.6 (Serum Level)	Positive
Lee SW. et al, 2005[39]	Patients: 80 Control: 80	49.9±9.9 47.4±7.9	Korea	362.1±187.7* 172.5±184.1 (Plasma level)	Positive
Tayebjee M.H. et al, 2004[44]	Patients: 86 Control: 63	68±6 66±10	UK	397 (300–496) * 280 (225–305) (Plasma level)	Positive
Sundstrom J. et al, 2004[45]	Whole samples: 1069, Diabetes: 9%	56.3±9.9	USA	1.03[b], (p=0.04) * (Plasma level)	Positive
Papazafiropoulou A. et al, 2010[41]	Patients: 60 Control: 60	60.3±7.4 59.2±10.1	Greece	197.5±76.5* 233.6±61.4 (Plasma level)	Negative
Jayashree K. et al, 2018[46]	Patients: 41 Control: 41	35–65	India	454.40 (359–529) 432.8 (295.3–539.35) (Plasma level, p=0.337)	No significant difference

Note: [a]The Chinese population in Singapore; [b]Ratio of plasma level TIMP-1 between diabetes and control subjects; *P<0.05 was taken as statistically significant.

significant difference in TIMP-1 between diabetic retinopathy patients and controls.[46] The heterogeneous results could be explained by the different characteristics of patients. Compared with the other studies, patients in Papazafiropoulou's study were at more advanced stage of T2DM, and may have received more intensive treatment.[41] The diabetes therapy have shown to decrease TIMP-1 levels significantly.[44] Collectively these studies indicate that serum TIMP-1 levels are altered in T2DM patients.

Altered Osteocyte Network in T2DM

Osteocytes play an essential role in regulating bone homeostasis. The osteocyte LCS plays a critical role in mechanical sensing and transduction, thus affecting the osteocyte function.[12,16] Mabilleau et al[11] reported that profound alterations of osteocyte network was present in a mouse model of high fat-induced type 2 diabetes, and perilacunar mineralization heterogeneity was reduced by 48%, but the mean perilacunar mineralization remained unchanged. Villarino et al[47] reported reduced lacunar density of alveolar bone in acute streptozotocin-induced diabetic rats. de Mello-Sampayo et al[48] described both lacunar density and tibial cortical bone size in 5-month-old streptozotocin-induced diabetic rats, but did not provide quantitative confirmation. Ay et al[49] reported that lacunar density increased in diabetic rats, and osteocyte territorial matrix decreased. Notably, however, Kerckhofs et al[50] reported that there were no significant differences in lacunar density or porosity between mice with obesity-driven T2DM and control mice. The discrepancy presented in the effect of T2DM on osteocyte network can be explained by the diversity of the diabetes models used as well as by the differences between the analysis techniques. Apart from dendritic network connectivity and lacunar density in T2DM bone, the pericellular matrix should be considered because the surface area of the osteocyte LCS within bone is several orders of magnitude greater than the area of the bone surface.[12] Altered osteocyte connectivity and LCS network could be the critical factors of T2DM bone fragility.

TIMP-1 Expressed by Osteocytes

TIMP-1 can be expressed in osteoblasts, osteoclasts, or lining cells of the various bone tissues, including rib bone, ectopic bone, and osteophytes, and is expressed differently in different physiological and pathological conditions.[43] Several studies have indicated that osteocytes secrete TIMP-1. In an in situ hybridization study Hatori et al[26] reported that TIMP-1 can be expressed in osteoblasts and osteocytes. Prideaux et al[42] reported that MLO-A5 cells (an osteoid preosteocyte-like cell) secrete TIMP-1 and MMPs, and that TIMP-1 expression was decreased during the mineralization of MLO-A5, along with MMP2, MMP23, and MMP28. These prior observations suggest that TIMP-1 may be secreted by osteocytes and may be involved in the mineralization of bone matrix. In addition, the molecular weight of TIMP-1 (~28 kDa) is smaller than the sieving cutoff for the LCS (~70 kDa),[51,52] indicating that TIMP-1 produced by osteocytes is capable of reaching target cells such as osteoblasts and osteoclasts. Future studies are warranted to elucidate the mechanisms by which type 2 diabetes could alter the expression of TIMPs in osteocytes or other bone cells.

TIMP-1/MMPs Participate in Osteocytic PLR

As terminally differentiated cell, osteocytes reside in the bone matrix, and have functions similar to osteoclasts with respect to secreting specific molecules that affect local bone matrix remodeling, called osteocyte PLR. In this process osteocytes can directly resorb and replace the local bone ECM. Some members of the MMP family play an important role in PLR, such as MMP2, MMP13 and MMP14. An absence of MMP2 reported had negative effects on bone structure, including destruction of the bone canalicular network, reduced BMD, and increased cortical porosity in long bones.[25,53] TIMP-1 is a natural inhibitor of MMPs. It has been reported that mesenchymal stem cells can inhibit MMP2 by secreting TIMP-1, and TIMP-1 can inhibit the activation of MMP2 under pathological conditions and protect blood vessels from protease damage.[54] These studies provoke a question as to whether the inhibitory effects of TIMP-1 on MMP2 under physiological or pathological conditions can affect the osteocyte lacunar-canalicular network. MMP13 is essential for remodeling the lacunar-canalicular pericellular matrix. MMP13 deficiency may impair the osteocyte network, collagen, and mineral tissue.[36,55] MMP2 and MMP9 can be activated by MMP13; and MMP13 deficiency may alter MMP2 and MMP9 activity, interrupting bone remodeling.[22] TIMP-1 can bind strongly to MMP13, downregulating its activity.[56] Inhibiting MMP13 activity can affect pericellular matrix remodeling. Given the importance of TIMP-1 in the remodeling of bone matrix and the lacunar

canalicular network, further research should be done to elucidate the effects of TIMP-1 on bone tissue and the function of TIMP-1 on bone quality in T2DM. The reported parameter of osteocyte lacunae density in diabetes bone is inconsistent, and the altered osteocyte dendritic network connectivity has been confirmed. Geoffroy et al[57] reported that overexpression of TIMP-1 in osteoblast-lineage cells did not affect osteocyte lacunae (number density at tissue level in bone). Therefore, altered TIMP-1 level may affect osteocyte pericellular matrix, not the lacunae density at the whole bone tissue level. Impaired osteocyte pericellular matrix by TIMP-1 may be involved in bone fragility of T2DM in which changes of TIMP-1 has been reported.

Conclusions

Skeletal fracture is a devastating event with dismal health consequences. Individuals with T2DM are associated with an increased risk of bone fracture. By several literature evidence and our view point, it is possible that TIMP-1 may have a specific role in the mechanism of bone fragility associated with T2DM. Prior research indicates that TIMP-1 may regulate the LCS pericellular matrix by inhibiting MMPs, and serum TIMP-1 levels are changed in T2DM patients. It is not clear what role does TIMP-1 play in the development of bone fragility in T2DM. Confirming the specific location and local roles of TIMP-1 in the osteocyte LCS may better explain the mechanism of bone fragility associated with T2DM, and provide a target for preventing bone fragility in T2DM patients.

Funding

This work was supported by the National Natural Science Foundation of China (11602046, 82070899), the Science and Technology Research Program of Chongqing Municipal Education Commission (KJQN201900418, KJQN202000421), the Project of Science and Technology of Chongqing Yuzhong District (20190114).

Disclosure

The authors declare no conflicts of interest.

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