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AGEs and Bone Ageing in Diabetes Mellitus

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Abstract

Type 1 and type 2 Diabetes mellitus are associated with a decrease in bone quality that leads to an increase in low-stress fractures, a condition called diabetic osteopathy. A growing body of evidence strongly indicates that one of the main pathological mechanisms of diabetic osteopathy is an excess accumulation of advanced glycation end products (AGEs) on collagen of bone extracellular matrix. This accumulation increases exponentially during ageing, and is further increased in conditions of substrate carbonyl stress such as chronically uncompensated Diabetes mellitus. AGEs can form covalent crosslinks throughout collagen fibrils, progressively increasing bone fragility and decreasing bone post-yield strain and energy, fracture resistance and toughness. In addition, bone marrow mesenchymal cells, osteoblasts and osteoclasts express receptors such as RAGE that can bind AGEs with high affinity, altering normal cellular homeostasis. Binding of AGEs by RAGE diminishes the osteogenic potential of mesenchymal cells, inhibits osteoblastic bone-forming capacity and induces a long-term decrease in osteoclastic recruitment and bone-resorbing activity. Altogether, these cellular effects of AGEs depress bone turnover, and thus induce an even greater accumulation of AGEs. Recent *in vivo*, *ex vivo* and *in vitro* evidence indicates that anti-diabetic and anti-osteoporotic treatment may prevent the deleterious effects of AGEs on bone cells, providing alternative options for the pharmacological treatment of diabetic osteopathy.

Keywords: Diabetes mellitus; Advanced glycation end products; Osteoporosis; Receptor for AGEs; Metformin; Strontium ranelate; Alendronate

Introduction

Diabetes mellitus (DM) and osteoporosis are highly prevalent global diseases and represent an increasing burden for health care systems. There is a growing body of clinical and experimental evidence reporting the association of type 1 and type 2 DM with bone abnormalities, including osteopenia, osteoporosis and/or an increased incidence of low-stress fractures, in what has been termed diabetic osteopathy [1]. Many adult patients with type 1 DM show mild osteopenia. Although their decrease in bone mineral density (BMD) is frequently around 10% [2] and this would be expected to double hip fracture risk [3], in actual fact the incidence of low-stress fractures is 7-12 times that of age-matched non-diabetic individuals [4,5]. On the other hand, people with type 2 DM usually have normal or moderately elevated BMD that would be expected to be associated with a reduced incidence of osteoporotic fractures, however they actually show an approximately 2-fold increase in hip, extremity and vertebral fractures [3-7]. These clinical observations have been put forward as evidence for a significant decrease in the material properties of bone tissue (i.e., bone quality) associated with both types of DM [8].

Although not completely elucidated, several mechanisms have been implicated in diabetic osteopathy, such as disturbed glucose metabolism, systemic and local (bone) low-grade inflammation, alterations in levels of growth factors and/or cytokines, increased oxidative stress and excess accumulation of advanced glycation endproducts (AGEs) in bone. A chronic pro-inflammatory state develops during the early stages of DM, suggesting a loss of defence mechanisms. Thus, inflammation-associated cytokines such as TNFα are elevated and can directly affect the growth and apoptosis of bone cells [9]. Increased levels of reactive oxygen species (ROS) are also observed in DM, and they have been shown to induce cellular alterations in various tissues. Several mechanisms can contribute to this Diabetes-induced oxidative stress, such as AGEs accumulation, increased polyol pathways, activation of protein kinase C isoforms, glucose oxidation and/or superoxide overproduction [10].

Chronic hyperglycaemia in uncompensated diabetes leads to an excessive non-enzymatic glycosylation of proteins with accumulation of AGEs, especially on long-lived proteins such as collagen present in the extracellular matrix of connective tissues (e.g., cartilage, bone, tendon, and skin) [11-13]. AGEs accumulated on extracellular matrix (ECM) proteins, as well as circulating AGEs, can interact with cell-surface AGEs-specific receptors. Three classes of receptors for AGEs have been described: AGER1, which is involved in the clarification of AGEs and in the suppression of ROS and inflammation induced by AGEs; AGER2 or Galectin-3; and RAGE (receptor for AGEs) [14]. RAGE is a member of the immunoglobulin receptor superfamily and binds multiple ligands, including high mobility group box 1 protein (HMGB1), S100 proteins, certain variants of amyloid β protein, and AGEs-modified proteins and lipids. The binding of AGEs to RAGE generates intracellular ROS production and pro-inflammatory responses, up-regulation of RAGE and a cascade of signal transduction pathways, which in pancreatic beta cells leads to impaired insulin secretion and in peripheral insulin-dependent tissues can induce insulin resistance and micro-vascular complications. The binding of several ligands to RAGE on different cell types activates various signalling pathways including p38, JNK MAP kinases, Rho GTPases, PI3K, JAK/STAT, as well as NF-κB signalling which induces ROS generation and oxidative stress. Thus, activation of

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RAGE further increases ROS and inflammation in Diabetes and other chronic diseases [14].

In bone ECM, excess accumulation of AGEs occurs as a function of ageing and duration of Diabetes, and has been found to impair the mechanical properties of bone [15]. We and other researchers have demonstrated the expression of RAGE on bone cells, and found several deleterious effects of AGEs-RAGE interaction on the physiology of these cell types [16] (Figure 1). In addition, we have shown that anti-diabetic and anti-osteoporotic drugs can be useful to prevent *in vivo*, *ex vivo* and *in vitro* deleterious effects of AGEs on bone cells, and thus could provide useful options for the treatment of diabetic osteopathy [17-19].

Enzymatic and Non-enzymatic Crosslinks in Bone Collagen

The mechanical properties of bone are influenced by different factors, including the degree of mineralization of its individual basic structure units, micro-damage accumulation and formation of collagen cross-links [15]. In particular, collagen cross-links differ in their origin and localization, and have been classified in two categories: enzymatic and non-enzymatic.

Enzymatic cross-links are formed in a site-specific and closely controlled process by the combined action of the enzymes lysyl oxidase and lysyl hydroxylase. Initially these enzymes induce the formation of immature intra-fibrillar divalent keto-imines, which can then spontaneously form mature inter-fibrillar trivalent pyridinium cross-links [20]. Since a deficit in their enzymatic formation leads to a decrease in bone strength, these finely regulated divalent and trivalent cross-links are considered to have a beneficial effect on bone mechanical properties [21]. However, divalent cross-links are more prevalent in bone collagen and have thus been proposed to be more important in maintaining the mechanical properties of bone [15].

On the other hand, non-enzymatic intra- and inter-fibrillar cross-links are a sub-class of AGEs that include fluorescent structures such as pentosidine, and non-fluorescent moieties such as the more abundant glucosepane. These AGEs form intra- or inter-molecular covalent bonds in long-lived proteins such as collagen. Non-enzymatic cross-links can potentially be formed on any site in which there is an amino acid with appropriate side-chains (such as lysine or arginine), and their abundance relative to collagen depends on bone turnover (i.e. collagen half-life) and on the concentration over time of carbohydrate or lipid substrates that give rise to reactive carbonyl compounds (i.e., "carbonyl stress"). Thus, in clinical conditions with carbonyl stress such as DM, AGEs cross-links tend to accumulate at a far greater rate than in non-diabetic individuals [22]. As this accumulation is not controlled by cellular processes and is non-site-specific, it is generally believed to deteriorate the mechanical properties and biological functions of bone [23].

Excess Formation of AGEs in Bone is a Hallmark of Diabetes and Ageing

There is a growing body of evidence confirming the accumulation of

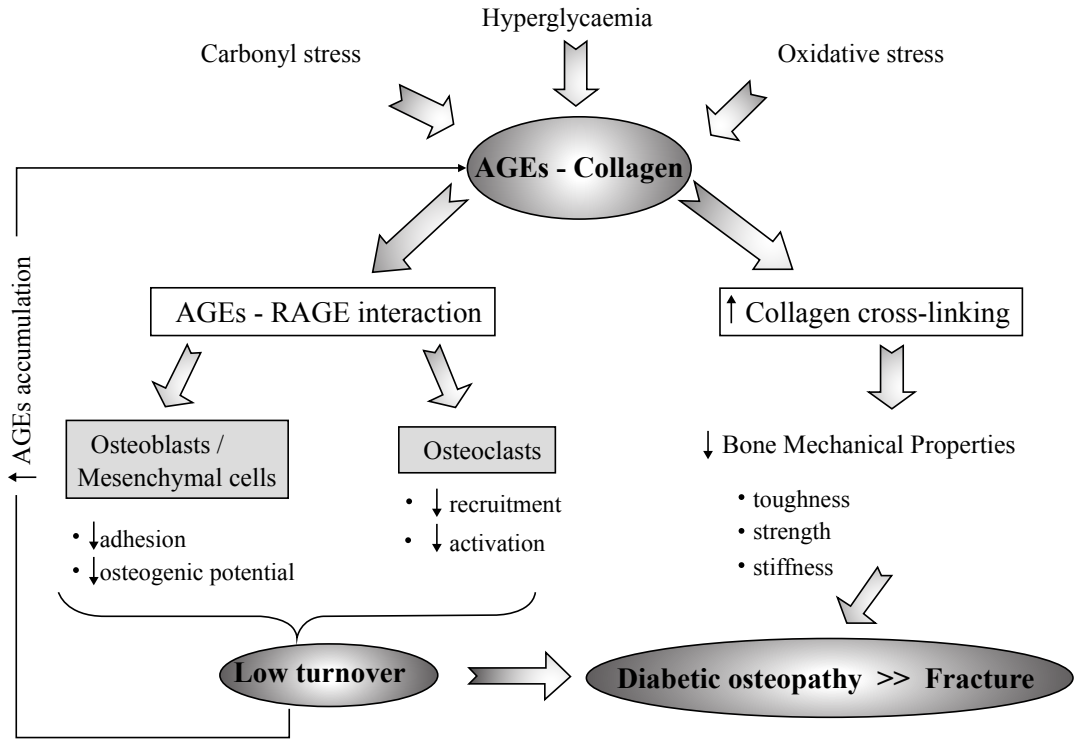

Figure 1: AGEs accumulation on bone collagen is a central mechanism for Diabetic osteopathy. Uncompensated Diabetes mellitus elevates circulating reactive oxygen species, glucose and/or carbonyl stress, which can induce excess AGEs formation on bone extracellular matrix. Accumulation of collagen-AGEs reduces bone quality, strength and post-yield properties. Additionally, collagen-AGEs interact with the receptor for AGEs expressed by bone cells, inhibiting their functionality and decreasing bone turnover. This induces an even greater accumulation of AGEs in bone with development of diabetic osteopathy, thus increasing fracture risk. AGEs: Advanced Glycation End Products. RAGE: Receptor for AGEs.

AGEs in bone ECM and in the articular cartilage of ageing individuals. Almost 20 years ago, collagen non-enzymatic glycosylation and AGEs-associated fluorescence was shown to increase in the cortical bone of ageing and/or diabetic rats [24-26]. Several authors have also described a progressive accumulation of AGEs in human cortical and trabecular bone as a function of ageing. The levels of AGEs in aged human bone have been estimated both by measurement of collagen-associated fluorescence [27,28] and by determining levels of pentosidine in bone ECM as a marker of cross-linking AGEs [29-31]. Given that glucosepane has been shown to be the most prevalent AGEs structure in skin ECM and glomerular basement membrane collagen [32], this non-enzymatic cross-link would also be expected to play an important role in the ageing of mineralized tissues. However, to date its accumulation in bone ECM has not yet been evaluated.

In the study reported by Odetti and co-workers, pentosidine was measured in femoral and knee cortical bone samples obtained from 104 non-diabetic individuals. The concentration of this AGEs collagen cross-link was found to increase exponentially with patient age, with a doubling time of around 20 years [29]. Interestingly, these authors also found a significant positive correlation between pentosidine levels in serum and in cortical bone.

In articular cartilage the turnover of ECM is very low. Articular cartilage is thus a candidate tissue for progressive accumulation of AGEs with ageing. This has been demonstrated for human individuals by different authors [33,34] and has been proposed to be one of the causal factors of ageing-associated osteoarthritis [35]. However, recent studies have found that *in vivo* AGEs formation on cartilage by intra-articular injection of ribose as a glycating agent was not able to enhance disease progression in an animal model of spontaneous knee osteoarthritis [36].

In theory, the effect of Diabetes on the accumulation of AGEs in bone should be even greater than that of physiological ageing. Surprisingly, this effect of Diabetes has been studied in animal models but not yet in humans. A significant increase in non-enzymatic glycosylation of bone collagen was found in alloxan- and streptozotocin-induced models of type 1 Diabetes, in both young and ageing rats [8,24-26]. The accumulation of AGEs in bone was found to correlate positively with the duration of Diabetes and with the level of hyperglycaemia. Recently, bone AGEs content has also been evaluated in partially insulin-deficient spontaneously diabetic rats [37]. These authors found that as Diabetes evolved over time there was a progressive increase in pentosidine (AGEs) cross-links of bone collagen, together with a significant decrease in divalent immature enzymatic cross-links, which coincided with impaired bone mechanical properties.

Accumulation of AGEs on bone ECM impairs its mechanical properties

Bone is a two-phase composite material with an organic phase of cross-linked type 1 collagen molecules, and an inorganic phase of hydroxyapatite nanocrystals. The mechanical properties of bone (toughness, strength and stiffness) are derived from its structural properties at multiple length scales in its microarchitecture, ranging from nanometric covalent and non-covalent molecular interactions to near-millimetric osteonal organization [28]. The mineral phase provides stiffness whereas collagen fibres provide tensile strength, ductility and toughness. Thus, changes in enzymatic and non-enzymatic collagen cross-links can affect bone mechanical properties.

In physiological conditions, formation of divalent (immature) enzymatic cross-links and their maturation to trivalent moieties is closely regulated by bone collagen turnover, by the expression of lysyl hydroxylases and lysyl oxidases, and possibly also by interaction with proteoglycans and collagen-binding proteins such as periostin [38], in order to optimize the mechanical properties of collagen fibres within a narrow beneficial range. Indeed, the ratio of divalent to trivalent enzymatic crosslinks in bone is usually very close to 2:1. In pathological situations this balance can be altered, affecting post-yield properties of bone. For example, in partially insulin-deficient rats, before their spontaneous development of Diabetes (i.e., in their pre-diabetic phase) a decrease in bone strength was observed which was attributed to a 25% reduction in divalent cross-links, while trivalent cross-links and AGEs remained unchanged [37].

On the other hand, formation of AGEs cross-links on bone collagen fibres is a non-regulated and accumulative process, believed to be an important determinant of the age- and Diabetes-related deterioration of bone post-yield properties such as ductility and toughness. This has been demonstrated by the *in vitro* induction of AGEs in bone samples, and determination of changes in their mechanical properties as a consequence of glycation. Several studies have found that the *in vitro* formation of AGEs cross-links on bone collagen generates an increase in bone fragility and a decrease in bone post-yield strain, energy, fracture resistance and toughness, in this last case due to multiple changes in bone tissue micro-damage mechanisms that lead to an overall reduction in its ability to dissipate energy [27,39,40]. AGEs have been proposed to stiffen the collagen matrix so that its fibrils dissipate less energy, and this would allow for an increase in the formation and propagation of micro-damage throughout bone ECM [41]. In a recent study, *in vitro* AGEs formation on bovine bone samples on one hand induced inferior cortical post-yield strain and flexural toughness, but on the other hand correlated positively with measures of strain accommodation and energy absorption before failure [42]. These results suggest that the increase in AGEs cross-linking cannot completely explain the embrittlement of bone associated with ageing and Diabetes.

In vivo, many AGEs can be formed between lysine and arginine side-chains within the helical (non-telopeptide) domain of collagen. They would be expected to increase the resistance of collagen to enzymatic breakdown and thus its half-life, further promoting the accumulation of AGEs. This has been demonstrated in alloxan-induced type 1 Diabetes in rats [25]. In addition, Diabetes-induced AGEs accumulation in bone ECM has been associated with a decrease in femoral trabecular bone volume and cortical width [8,25], and with a decrease in long-bone stiffness, energy absorption, elastic modulus, maximum load and fatigue life [8,37].

Excess levels of AGEs in bone ECM have also been associated with incident and prevalent osteoporotic fractures in ageing non-diabetic human individuals [43,44]. In particular, Saito and co-workers have found an increase in pentosidine cross-links and a decrease in enzymatic cross-links in bone ECM from ageing patients with intra-capsular hip fracture versus gender- and age-matched post-mortem controls [44]. These results are similar to those found by the same authors in a rat model of spontaneous insulin-deficient Diabetes [37]. In combination, both studies suggest that absolute and/or relative changes in the levels of enzymatic and non-enzymatic collagen cross-links could be important determinants of bone quality in osteoporosis and diabetic osteopathy. Interestingly, in a recent study the accumulation of AGEs in skin was evaluated by non-invasive trans-cutaneous auto-fluorescence, and found to correlate negatively with calcaneal ultrasound osteo-sono

assessment index suggesting an inverse correlation between AGEs accumulation and bone strength [45]. Although both determinations used in this study were surrogate markers (for bone AGEs and mechanical properties respectively) the results suggest that this method could be useful for long-term prospective studies.

Turnover of AGEs-modified collagen generates low molecular weight AGEs-peptides, which localize to plasma prior to their renal excretion in urine [46]. Based on these considerations, different authors have evaluated the correlation between plasma or urine levels of AGEs moieties such as pentosidine and bone alterations such as osteoporosis, diabetic osteopathy and/or incident and prevalent fractures. As mentioned above, plasma pentosidine has been shown to have a significant linear correlation with cortical bone pentosidine [29]. In an interesting study by Hein and colleagues, serum pentosidine concentrations were found to be significantly higher in patients with osteoporosis than in age- and gender-matched controls [47]. However, the authors were only able to find a significantly positive correlation between serum pentosidine levels and participant age for the group of healthy controls, suggesting that osteoporosis could involve a more intensified generation of AGEs. Yamamoto and co-workers recently showed that in postmenopausal women with type 2 Diabetes, increased serum pentosidine was independently associated with prevalent vertebral fractures [48]. In a 5-year observational study with elderly non-diabetic women, prevalent and incident vertebral fractures were positively correlated with elevated levels of urinary pentosidine [49]. However, in another observational cohort study with elderly men and women with or without type 2 Diabetes, urine pentosidine levels were found to be independent predictors of clinical incident fractures and prevalent vertebral fractures in patients with type 2 Diabetes, but not in non-diabetic individuals [50].

As discussed above, the excess accumulation of AGEs on bone ECM that can be observed in ageing and Diabetes alters its mechanical properties and can thus predispose to increased fracture incidence. However, formation of AGEs such as pentosidine or glucosepane irreversibly alters the side-chains of arginine residues, and could thus also interfere with integrin-mediated interactions between RGD sequences of the ECM and bone cells. In order to prove this hypothesis, we demonstrated that the *in vitro* modification of type 1 collagen by AGEs decreased its free arginine residues, and thus significantly inhibited the attachment of osteoblasts *via* integrin receptors [51]. These effects, together with other specific actions of AGEs on bone-derived cells that will be described below, can further affect bone metabolism and contribute to osteoporosis, diabetic osteopathy and their clinical consequences including low-stress fractures.

AGEs Modulate Bone Cell Metabolism *via* Specific Receptors

In many cell types, AGEs exert their deleterious actions *via* binding to specific receptors such as AGE-receptor 1, RAGE and Galectin-3 [14]. The presence of receptors for AGEs has also been demonstrated in bone cells. Osteoblasts express RAGE [52,53] and Galectin-3 [54], with receptor levels that depend on the stage of osteoblastic differentiation [55]. RAGE expression has also been shown in bone marrow mesenchymal/progenitor cells [56,57] and in osteoclasts [58].

After its discovery 20 years ago as a receptor for AGEs, RAGE has since been found to recognize certain proteins with high affinity, and so AGEs are currently considered to be accidental ligands for this member of the immunoglobulin superfamily of receptors. A physiological ligand for RAGE is the chromatin protein high mobility group box 1 (HMGB1), which is considered an "alarmin", i.e. an endogenous molecule released by dead and dying cells that acts as a signal for tissue damage indicating the need for repair. HMGB1 has been shown to be released by apoptotic osteoblasts and osteocytes *in vitro*, and thus could act as an osteocyte alarmin to mediate normal bone remodelling and/or pathological bone loss [59]. In addition, HMGB1 can trigger the differentiation of human bone marrow mesenchymal cells into an osteoblastic phenotype [60]. Another family of physiological ligands that have been described for RAGE are the S100 proteins. Through *in vitro* studies these proteins have been found to inhibit osteoblast mineralization and increase osteoclast recruitment and differentiation, *via* binding to RAGE [61]. A pathological ligand for RAGE appears to be the Swedish mutation of amyloid precursor protein (APPswe, associated with early-onset Alzheimer's disease). APPswe has been found to promote osteoclast activation *in vivo* and *in vitro via* RAGE, providing a potential mechanism underlying the increased bone fracture rate in patients with Alzheimer's disease [62]. Interestingly, in RAGE-knockout mice [10,63] an increase in bone mineral density and a decrease in osteoclast development and function have been observed.

The effect of AGEs on bone cells was first observed 20 years ago [64]. In this study de-mineralized bone particles were modified *in vitro* with AGEs prior to their sub-cutaneous implantation in rats, as a model for endochondral bone formation. The presence of AGEs induced a 90% decrease in osteoblastic differentiation determined by alkaline phosphatase activity (ALP), *in vivo* calcium uptake and histological analysis. These results encouraged further studies to determine the direct effect of AGEs on individual bone-derived cells such as bone marrow mesenchymal cells, osteoblasts and osteoclasts.

Kume and co-workers found that AGEs increased the apoptosis and thus decreased the viability of human mesenchymal cells *in vitro*. AGEs, *via* interaction with RAGE and up-regulation of this receptor, also decreased the adipogenic, chondrogenic and osteogenic potential of mesenchymal cells [56]. However, most studies with bone cells have focused on the actions of AGEs on osteoblasts in culture.

In our laboratory we have found that AGEs (soluble or attached to a collagen matrix) decrease the attachment, proliferation, differentiation and mineralization of osteoblastic cells in culture [51,65,66] *via* an increase in intracellular reactive oxygen species (ROS), in the expression of nitric oxide synthase [66] and in the activation of extracellular-regulated kinases (ERK) [52]. In addition, we have shown that exposure to AGEs (a) modifies the secretory pattern of IGF-1 and its binding proteins inducing a decrease in the levels of free IGF-1 [67], (b) increases osteoblast apoptosis and (c) up-regulates the expression of RAGE and Galectin-3 to exacerbate the receptor-mediated effects of AGEs in these cells [68]. We have recently found that the AGEs-mediated increase in osteoblast apoptosis is secondary to a disruption of its actin cytoskeleton with formation of geodesic domes [69]. Similarly, other authors have also found that AGEs stimulate osteoblast apoptosis *via* MAP kinase pathways [70] and up-regulate osteoblastic expression of RAGE [53,71-73].

Simultaneously with our first studies, other researchers reported coincident anti-osteogenic effects of AGEs on primary osteoblasts of rat [24] and human origin [74], and additionally found that AGEs can stimulate osteoblastic interleukin-6 secretion suggesting their involvement in the modulation of bone remodelling. All in all, these and our results are in agreement with the immunohistochemical findings of Hein and co-workers in trabecular bone biopsies from patients with osteoporosis. These authors described a positive association of bone

AGEs levels with patient age, and an inverse association with relative osteoblast-covered bone surface [47].

Recently, additional signal-transduction mechanisms have been described for RAGE-dependent anti-osteogenic effects of AGEs on osteoblasts. RAGE activation was found to consecutively suppress Wnt, PI3K and ERK signalling, thus inhibiting osteoblastic proliferation [75]. In another study, AGEs increased the apoptosis and inhibited the proliferation and differentiation of osteoblasts by decreasing the expression of Runx2 and Osterix, two transcription factors that are essential for osteoblastic progression [76].

In osteoclasts the effects of AGEs have been poorly defined, and published reports are controversial [58,77]. Using unfractionated mouse bone marrow cultures that included osteoclasts among other cell types, Miyata and co-workers found an increase in resorption pits (but not in osteoclast number) when the cultures were incubated for 4 days on AGEs-modified dentin slices [77]. In the other published report on this issue, Valcourt et al. found that both human and rabbit osteoclasts expressed significant levels of RAGE, and that when cultured on ivory slices modified with the AGEs cross-link pentosidine, osteoclasts transiently increased their resorptive action after 3 days but were greatly inhibited if cultures were extended to 8 days [58]. Thus, it appears that although the presence of AGEs may initially increase the activity of osteoclasts, exposure to AGEs for longer (and more clinically relevant) periods of time greatly diminishes their recruitment and activity. While further research is necessary to confirm these results, they are in agreement with the vision of diabetic osteopathy as an adynamic bone disease.

Bone healing also appears to be affected by the presence of AGEs. In an interesting study by Santana and co-workers, craniotomy defects healed significantly less (and expressed greater levels of RAGE) in streptozotocin-induced diabetic mice than in non-diabetic animals. A similar decrease in bone healing was observed when AGEs were applied locally to defects in non-diabetic mice [78]. Thus, AGEs-RAGE interaction appears to modulate the process of bone repair, although this must be confirmed in studies with weight-bearing bones of endochondral formation. Recently, a unifying mechanism has been proposed suggesting that inflammatory signalling secondary to excess AGEs and ROS increases osteoblast and chondrocyte apoptosis, as well as initial osteoclast survival, thus inducing impaired bone regeneration in Diabetes [9].

Pharmacological Treatment can Modulate the Deleterious Effects of AGEs on Bone

In recent years our research group has tested the hypothesis that pharmacological treatment may prevent the deleterious effects of AGEs on osteoblasts by mechanisms related to a decrease in the generation of oxidative stress, and by modulation of survival signals and of RAGE expression. In particular we have evaluated whether an anti-diabetic or anti-resorptive treatment can prevent or reverse the deleterious actions of AGEs on osteoblastic cells. This novel aspect of pharmacological treatment had not been previously investigated by other research groups and introduces an interesting perspective regarding possible beneficial side effects of widely used treatment options for Diabetes and osteoporosis.

We first demonstrated *in vitro* direct pro-osteogenic actions of metformin on osteoblastic proliferation, differentiation and mineralization [79]. We later found that metformin treatment could also prevent the *in vitro* AGEs-induced decrease in osteoblastic differentiation and induction of apoptosis, in this last case by decreasing caspase-3 activity and intracellular oxidative stress [80]. Interestingly, in recent *in vivo* and *ex vivo* studies with rats we found that orally administered metformin improves bone regeneration and femoral microarchitecture, and increases the osteogenic potential of bone marrow progenitor cells *via* an increase in the expression of Runx2 and in the phosphorylation/activation of AMPK, a well-known sensor of energetic balance. Metformin can additionally prevent the *in vivo* and *ex vivo* anti-osteogenic effects of the insulin-sensitizer rosiglitazone in rats [19,81].

Following our line of research, other investigators have also found *in vitro* and *in vivo* osteogenic effects of metformin in most [82-84] but not all studies [85]. It has recently been demonstrated that AMPK activation increases intracellular antioxidant defences and decreases the mitochondrial production of reactive oxygen and nitrogen species [86]. Activation of AMPK in other cell systems has been shown to reduce TNF-alpha-induced activation of caspase-3, and consequently inhibit cell apoptosis [87].

As an additional mechanism for metformin action, we also demonstrated that it curbs the up-regulation of RAGE induced by AGEs [80]. Although AGEs are not the only ligands for RAGE, AGEs-RAGE interaction has been shown to generate ROS, induce osteoblast apoptosis and activate inflammatory signalling cascades such as TNF-alpha, IL-1beta and IL-6 that affect bone homeostasis [17,53,67,88,89].

More recently we have demonstrated that the development of partially insulin-deficient Diabetes in rats induces deleterious effects on long-bone micro-architecture that are associated with an inhibition of bone marrow progenitor cell (BMPC) osteogenic potential. This in turn is mediated by a decrease in the Runx-2/PPAR-gamma ratio and up-regulation of RAGE in BMPC. All of these Diabetes-induced alterations can be totally or partially prevented by oral administration of metformin [57]. Although we did not directly measure AGEs levels in diabetic bone ECM, they would be expected to be elevated. Thus we speculate that metformin could be exerting its preventive effect on diabetic osteopathy in our rat model, in part by inhibiting the deleterious effects of bone AGEs. Interestingly in a nation-wide observational case-control study in Denmark, use of metformin in patients with type 2 Diabetes was associated with a significantly decreased fracture risk [90].

A presently unresolved issue is the precise effect in Diabetes of currently used anti-osteoporotic treatments. At present their prescription in patients with diabetic osteopathy has not been firmly recommended, since results of clinical studies tend to be contradictory [91-93]. In an attempt to shed light on this issue, our group demonstrated for the first time the preventive effects of anti-osteoporotic drugs such as strontium ranelate and the N-containing bisphosphonate alendronate, on the deleterious actions of AGEs on osteoblasts [17,18,69]. Other authors previously demonstrated that the bisphosphonates incadronate and minodronate were able to supress anti-angiogenic effects of AGEs *in vitro* [94,95].

For both strontium ranelate and alendronate, the initial mechanism of action against AGEs-induced cytotoxicity is activation of L-type calcium channels. However, the downstream cascades appear to be different. On one hand, low doses of alendronate prevent intracellular oxidative stress and in consequence revert morphological changes and apoptosis induced by AGEs, but do not affect expression of ERK [18,69]. On the other hand, strontium ranelate prevents the osteoblastic secretion of IL-1beta and TNF-alpha induced by AGEs, an effect that is downstream to the activation of ERK. In addition, strontium ranelate

also prevents the AGEs-induced decrease of beta-catenin activation, a key regulator of osteoblastic function [17].

In vivo studies are currently under way in our laboratory to evaluate the possible preventive effects of orally administered alendronate or strontium ranelate, on bone alterations induced in models of type 1 and type 2 Diabetes.

Conclusions and Perspectives

Chronically elevated ROS, glucose levels and/or carbonyl stress associated with uncompensated Diabetes mellitus induce an excess accumulation of AGEs on long-lived proteins such as bone collagen. Excess AGEs in bone ECM reduce bone quality, strength and post-yield properties. In addition, collagen AGEs can interact with RAGE expressed by bone cells, impairing the homeostasis and activity of mesenchymal cells, osteoblasts and osteoclasts. This in turn decreases bone turnover inducing an even greater accumulation of AGEs with development of diabetic osteopathy, thus increasing fracture risk (Figure 1). Pharmacological treatment with anti-diabetic and anti-osteoporotic drugs may prevent the deleterious effects of AGEs on bone cells.

The progressive accumulation of AGEs in diabetic bone would be expected to correlate with the deterioration in bone quality. This has been shown in animal models, but surprisingly has not been demonstrated yet in patients with Diabetes. Studies to prove this hypothesis are necessary in order to design specific strategies to prevent diabetic osteopathy and its associated bone fractures.

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References

1. Wongdee K, Charoenphandhu N (2011) Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms. World J Diabetes 2: 41-48.
2. Bouillon R (1991) Diabetic bone disease. Calcif Tissue Int 49: 155-160.
3. Hofbauer LC, Brueck CC, Singh SK, Dobnig H (2007) Osteoporosis in patients with diabetes mellitus. J Bone Miner Res 22: 1317-1328.
4. Forsén L, Meyer HE, Midthjell K, Edna TH (1999) Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trøndelag Health Survey. Diabetologia 42: 920-925.
5. Nicodemus KK, Folsom AR; Iowa Women's Health Study (2001) Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women. Diabetes Care 24: 1192-1197.
6. Melton LJ 3rd, Leibson CL, Achenbach SJ, Therneau TM, Khosla S (2008) Fracture risk in type 2 diabetes: update of a population-based study. J Bone Miner Res 23: 1334-1342.
7. Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, et al. (2001) Older women with diabetes have an increased risk of fracture: a prospective study. J Clin Endocrinol Metab 86: 32-38.
8. Silva MJ, Brodt MD, Lynch MA, McKenzie JA, Tanouye KM, et al. (2009) Type 1 diabetes in young rats leads to progressive trabecular bone loss, cessation of cortical bone growth, and diminished whole bone strength and fatigue life. J Bone Miner Res 24: 1618-1627.
9. Roszer T (2011) Inflammation as death or life signal in diabetic fracture healing. Inflamm Res 60: 3-10.
10. Hamada Y, Fujii H, Fukagawa M (2009) Role of oxidative stress in diabetic bone disorder. Bone 45 Suppl 1: S35-38.
11. Verzijl N, DeGroot J, Oldehinkel E, Bank RA, Thorpe SR, et al. (2000) Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochem J 350 Pt 2: 381-387.
12. Viguet-Carrin S, Roux JP, Arlot ME, Merabet Z, Leeming DJ, et al. (2006) Contribution of the advanced glycation end product pentosidine and of maturation of type I collagen to compressive biomechanical properties of human lumbar vertebrae. Bone 39: 1073-1079.
13. Reiser KM (1991) Nonenzymatic glycation of collagen in aging and diabetes. Proc Soc Exp Biol Med 196: 17-29.
14. Vlassara H, Striker GE (2011) AGE restriction in diabetes mellitus: a paradigm shift. Nat Rev Endocrinol 7: 526-539.
15. Saito M, Marumo K (2010) Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int 21: 195-214.
16. Zhou Z, Xiong WC (2011) RAGE and its ligands in bone metabolism. Front Biosci (Schol Ed) 3: 768-776.
17. Fernández JM, Molinuevo MS, Sedlinsky C, Schurman L, Cortizo AM, et al. (2013) Strontium ranelate prevents the deleterious action of advanced glycation endproducts on osteoblastic cells via calcium channel activation. Eur J Pharmacol 706: 41-47.
18. Gangoiti MV, Cortizo AM, Arnol V, Felice JI, McCarthy AD (2008) Opposing effects of bisphosphonates and advanced glycation end-products on osteoblastic cells. Eur J Pharmacol 600: 140-147.
19. Molinuevo MS, Schurman L, McCarthy AD, Cortizo AM, Tolosa MJ, et al. (2010) Effect of metformin on bone marrow progenitor cell differentiation: *in vivo* and *in vitro* studies. J Bone Miner Res 25: 211-221.
20. Eyre DR, Dickson IR, Van Ness K (1988) Collagen cross-linking in human bone and articular cartilage. Age-related changes in the content of mature hydroxypyridinium residues. Biochem J 252: 495-500.
21. Oxlund H, Barckman M, Ortoft G, Andreassen TT (1995) Reduced concentrations of collagen cross-links are associated with reduced strength of bone. Bone 17: 365S-371S.
22. McCarthy AD (2000) Glycation, glycoxidation and carbonyl stress: role in the vascular complications of Diabetes mellitus. Rev Arg Endocrinol Metab 37: 141-163.
23. Vashishth D (2007) The role of the collagen matrix in skeletal fragility. Curr Osteoporos Rep 5: 62-66.
24. Katayama Y, Akatsu T, Yamamoto M, Kugai N, Nagata N (1996) Role of nonenzymatic glycosylation of type I collagen in diabetic osteopenia. J Bone Miner Res 11: 931-937.
25. Locatto ME, Abranzon H, Caferra D, Fernandez MC, Alloatti R, et al. (1993) Growth and development of bone mass in untreated alloxan diabetic rats. Effects of collagen glycosylation and parathyroid activity on bone turnover. Bone Miner 23: 129-144.
26. Tomasek JJ, Meyers SW, Basinger JB, Green DT, Shew RL (1994) Diabetic and age-related enhancement of collagen-linked fluorescence in cortical bones of rats. Life Sci 55: 855-861.
27. Tang SY, Zeenath U, Vashishth D (2007) Effects of non-enzymatic glycation on cancellous bone fragility. Bone 40: 1144-1151.
28. Zimmermann EA, Schaible E, Bale H, Barth HD, Tang SY, et al. (2011) Age-related changes in the plasticity and toughness of human cortical bone at multiple length scales. Proc Natl Acad Sci U S A 108: 14416-14421.
29. Odetti P, Rossi S, Monacelli F, Poggi A, Cirnigliaro M, et al. (2005) Advanced glycation end products and bone loss during aging. Ann N Y Acad Sci 1043: 710-717.
30. Saito M, Marumo K, Fujii K, Ishioka N (1997) Single-column high-performance liquid chromatographic-fluorescence detection of immature, mature, and senescent cross-links of collagen. Anal Biochem 253: 26-32.
31. Wang X, Li X, Shen X, Agrawal CM (2003) Age-related changes of noncalcified collagen in human cortical bone. Ann Biomed Eng 31: 1365-1371.
32. Sell DR, Biemel KM, Reihl O, Lederer MO, Strauch CM, et al. (2005) Glucosepane is a major protein cross-link of the senescent human extracellular matrix. Relationship with diabetes. J Biol Chem 280: 12310-12315.
33. Bank RA, Bayliss MT, Lafeber FP, Maroudas A, Tekoppele JM (1998) Ageing

and zonal variation in post-translational modification of collagen in normal human articular cartilage. The age-related increase in non-enzymatic glycation affects biomechanical properties of cartilage. Biochem J 330: 345-351.

34. Verzijl N, DeGroot J, Thorpe SR, Bank RA, Shaw JN, et al. (2000) Effect of collagen turnover on the accumulation of advanced glycation end products. J Biol Chem 275: 39027-39031.

35. Lotz M, Loeser RF (2012) Effects of aging on articular cartilage homeostasis. Bone 51: 241-248.

36. Willett TL, Kandel R, De Croos JN, Avery NC, Grynpas MD (2012) Enhanced levels of non-enzymatic glycation and pentosidine crosslinking in spontaneous osteoarthritis progression. Osteoarthritis Cartilage 20: 736-744.

37. Saito M, Fujii K, Mori Y, Marumo K (2006) Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. Osteoporos Int 17: 1514-1523.

38. Shimazaki M, Nakamura K, Kii I, Kashima T, Amizuka N, et al. (2008) Periostin is essential for cardiac healing after acute myocardial infarction. J Exp Med 205: 295-303.

39. Tang SY, Vashishth D (2010) Non-enzymatic glycation alters microdamage formation in human cancellous bone. Bone 46: 148-154.

40. Jungmann R, Szabo ME, Schitter G, Tang RY, Vashishth D, et al. (2011) Local strain and damage mapping in single trabeculae during three-point bending tests. J Mech Behav Biomed Mater 4: 523-534.

41. Nyman JS, Makowski AJ (2012) The contribution of the extracellular matrix to the fracture resistance of bone. Curr Osteoporos Rep 10: 169-177.

42. Willett TL, Sutty S, Gaspar A, Avery N, Grynpas M (2013) *In vitro* non-enzymatic ribation reduces post-yield strain accommodation in cortical bone. Bone 52: 611-622.

43. Ager JW 3rd, Nalla RK, Balooch G, Kim G, Pugach M, et al. (2006) On the increasing fragility of human teeth with age: a deep-UV resonance Raman study. J Bone Miner Res 21: 1879-1887.

44. Saito M, Fujii K, Soshi S, Tanaka T (2006) Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation-induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. Osteoporos Int 17: 986-995.

45. Momma H, Niu K, Kobayashi Y, Guan L, Sato M, et al. (2012) Skin advanced glycation end-product accumulation is negatively associated with calcaneal osteo-sono assessment index among non-diabetic adult Japanese men. Osteoporos Int 23: 1673-1681.

46. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, et al. (1991) Advanced glycosylation end products in patients with diabetic nephropathy. N Engl J Med 325: 836-842.

47. Hein G, Wiegand R, Lehmann G, Stein G, Franke S (2003) Advanced glycation end-products pentosidine and N epsilon-carboxymethyllysine are elevated in serum of patients with osteoporosis. Rheumatology (Oxford) 42: 1242-1246.

48. Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T (2008) Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 93: 1013-1019.

49. Shiraki M, Kuroda T, Tanaka S, Saito M, Fukunaga M, et al. (2008) Nonenzymatic collagen cross-links induced by glycoxidation (pentosidine) predicts vertebral fractures. J Bone Miner Metab 26: 93-100.

50. Schwartz AV, Garnero P, Hillier TA, Sellmeyer DE, Strotmeyer ES, et al. (2009) Pentosidine and increased fracture risk in older adults with type 2 diabetes. J Clin Endocrinol Metab 94: 2380-2386.

51. McCarthy AD, Uemura T, Etcheverry SB, Cortizo AM (2004) Advanced glycation endproducts interefere with integrin-mediated osteoblastic attachment to a type-I collagen matrix. Int J Biochem Cell Biol 36: 840-848.

52. Cortizo AM, Lettieri MG, Barrio DA, Mercer N, Etcheverry SB, et al. (2003) Advanced glycation end-products (AGEs) induce concerted changes in the osteoblastic expression of their receptor RAGE and in the activation of extracellular signal-regulated kinases (ERK). Mol Cell Biochem 250: 1-10.

53. Franke S, Rüster C, Pester J, Hofmann G, Oelzner P, et al. (2011) Advanced glycation end products affect growth and function of osteoblasts. Clin Exp Rheumatol 29: 650-660.

54. Mercer N, Ahmed H, McCarthy AD, Etcheverry SB, Vasta GR, et al. (2004) AGE-R3/galectin-3 expression in osteoblast-like cells: regulation by AGEs. Mol Cell Biochem 266: 17-24.

55. McCarthy AD, Etcheverry SB, Cortizo AM (1999) Advanced glycation endproduct-specific receptors in rat and mouse osteoblast-like cells: regulation with stages of differentiation. Acta Diabetol 36: 45-52.

56. Kume S, Kato S, Yamagishi S, Inagaki Y, Ueda S, et al. (2005) Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. J Bone Miner Res 20: 1647-1658.

57. Tolosa MJ, Sedlinsky C, Schurman L, McCarthy AD, Molinuevo MS, et al. (2013) Insulin-deficient Diabetes-induced bone microarchitecture alterations are associated with a decrease in the osteogenic potential of bone marrow progenitor cells. Preventive effects of metformin. Diab Res Clin Pract. [doi: 10.1016/j.diabres.2013.05.016. [Epub ahead of print].

58. Valcourt U, Merle B, Gineyts E, Viguet-Carrin S, Delmas PD, et al. (2007) Non-enzymatic glycation of bone collagen modifies osteoclastic activity and differentiation. J Biol Chem 282: 5691-5703.

59. Bidwell JP, Yang J, Robling AG (2008) Is HMGB1 an osteocyte alarmin? J Cell Biochem 103: 1671-1680.

60. Meng E, Guo Z, Wang H, Jin J, Wang J, et al. (2008) High mobility group box 1 protein inhibits the proliferation of human mesenchymal stem cells and promotes their migration and differentiation along osteoblastic pathway. Stem Cells Dev 17: 805-813.

61. Yoshida T, Flegler A, Kozlov A, Stern PH (2009) Direct inhibitory and indirect stimulatory effects of RAGE ligand S100 on sRANKL-induced osteoclastogenesis. J Cell Biochem 107: 917-925.

62. Cui S, Xiong F, Hong Y, Jung JU, Li XS, et al. (2011) APPswe/Aî² regulation of osteoclast activation and RAGE expression in an age-dependent manner. J Bone Miner Res 26: 1084-1098.

63. Ding Q, Xia W, Liu JC, Yang JY, Lee DF, et al. (2005) Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. Mol Cell 19: 159-170.

64. Fong Y, Edelstein D, Wang EA, Brownlee M (1993) Inhibition of matrix-induced bone differentiation by advanced glycation end-products in rats. Diabetologia 36: 802-807.

65. McCarthy AD, Etcheverry SB, Bruzzone L, Cortizo AM (1997) Effects of advanced glycation end-products on the proliferation and differentiation of osteoblast-like cells. Mol Cell Biochem 170: 43-51.

66. McCarthy AD, Etcheverry SB, Bruzzone L, Lettieri G, Barrio DA, et al. (2001) Non-enzymatic glycosylation of a type I collagen matrix: effects on osteoblastic development and oxidative stress. BMC Cell Biol 2: 16.

67. McCarthy AD, Etcheverry SB, Cortizo AM (2001) Effect of advanced glycation endproducts on the secretion of insulin-like growth factor-I and its binding proteins: role in osteoblast development. Acta Diabetol 38: 113-122.

68. Mercer N, Ahmed H, Etcheverry SB, Vasta GR, Cortizo AM (2007) Regulation of advanced glycation end product (AGE) receptors and apoptosis by AGEs in osteoblast-like cells. Mol Cell Biochem 306: 87-94.

69. Gangoiti MV, Anbinder PS, Cortizo AM, McCarthy AD (2013) Morphological changes induced by advanced glycation endproducts in osteoblastic cells: Effects of co-incubation with alendronate. Acta Histochem .

70. Alikhani M, Alikhani Z, Boyd C, MacLellan CM, Raptis M, et al. (2007) Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. Bone 40: 345-353.

71. Franke S, Siggelkow H, Wolf G, Hein G (2007) Advanced glycation endproducts influence the mRNA expression of RAGE, RANKL and various osteoblastic genes in human osteoblasts. Arch Physiol Biochem 113: 154-161.

72. Ogawa N, Yamaguchi T, Yano S, Yamauchi M, Yamamoto M, et al. (2007) The combination of high glucose and advanced glycation end-products (AGEs) inhibits the mineralization of osteoblastic MC3T3-E1 cells through glucose-induced increase in the receptor for AGEs. Horm Metab Res 39: 871-875.

73. Sanguineti R, Storace D, Monacelli F, Federici A, Odetti P (2008) Pentosidine effects on human osteoblasts *in vitro*. Ann N Y Acad Sci 1126: 166-172.

74. Takagi M, Kasayama S, Yamamoto T, Motomura T, Hashimoto K, et al. (1997) Advanced glycation endproducts stimulate interleukin-6 production by human bone-derived cells. J Bone Miner Res 12: 439-446.

75. Li G, Xu J, Li Z (2012) Receptor for advanced glycation end products inhibits proliferation in osteoblast through suppression of Wnt, PI3K and ERK signaling. Biochem Biophys Res Commun 423: 684-689.

76. Okazaki K, Yamaguchi T, Tanaka K, Notsu M, Ogawa N, et al. (2012) Advanced glycation end products (AGEs), but not high glucose, inhibit the osteoblastic differentiation of mouse stromal ST2 cells through the suppression of osterix expression, and inhibit cell growth and increasing cell apoptosis. Calcif Tissue Int 91: 286-296.

77. Miyata T, Notoya K, Yoshida K, Horie K, Maeda K, et al. (1997) Advanced glycation end products enhance osteoclast-induced bone resorption in cultured mouse unfractionated bone cells and in rats implanted subcutaneously with devitalized bone particles. J Am Soc Nephrol 8: 260-270.

78. Santana RB, Xu L, Chase HB, Amar S, Graves DT, et al. (2003) A role for advanced glycation end products in diminished bone healing in type 1 diabetes. Diabetes 52: 1502-1510.

79. Cortizo AM, Sedlinsky C, McCarthy AD, Blanco A, Schurman L (2006) Osteogenic actions of the anti-diabetic drug metformin on osteoblasts in culture. Eur J Pharmacol 536: 38-46.

80. Schurman L, McCarthy AD, Sedlinsky C, Gangoiti MV, Arnol V, et al. (2008) Metformin reverts deleterious effects of advanced glycation end-products (AGEs) on osteoblastic cells. Exp Clin Endocrinol Diabetes 116: 333-340.

81. Sedlinsky C, Molinuevo MS, Cortizo AM, Tolosa MJ, Felice JI, et al. (2011) Metformin prevents anti-osteogenic *in vivo* and ex vivo effects of rosiglitazone in rats. Eur J Pharmacol 668: 477-485.

82. Jang WG, Kim EJ, Bae IH, Lee KN, Kim YD, et al. (2011) Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. Bone 48: 885-893.

83. Kanazawa I, Yamaguchi T, Yano S, Yamauchi M, Sugimoto T (2008) Metformin enhances the differentiation and mineralization of osteoblastic MC3T3-E1 cells via AMP kinase activation as well as eNOS and BMP-2 expression. Biochem Biophys Res Commun 375: 414-419.

84. Shah M, Kola B, Bataveljic A, Arnett TR, Viollet B, et al. (2010) AMP-activated protein kinase (AMPK) activation regulates *in vitro* bone formation and bone mass. Bone 47: 309-319.

85. Jeyabalan J, Viollet B, Smitham P, Ellis SA, Zaman G, et al. (2013) The anti-diabetic drug metformin does not affect bone mass *in vivo* or fracture healing. Osteoporos Int .

86. Wang S, Song P, Zou MH (2012) AMP-activated protein kinase, stress responses and cardiovascular diseases. Clin Sci (Lond) 122: 555-573.

87. Kewalramani G, Puthanveetil P, Wang F, Kim MS, Deppe S, et al. (2009) AMP-activated protein kinase confers protection against TNF-{alpha}-induced cardiac cell death. Cardiovasc Res 84: 42-53.

88. Bierhaus A, Humpert PM, Stern DM, Arnold B, Nawroth PP (2005) Advanced glycation end product receptor-mediated cellular dysfunction. Ann N Y Acad Sci 1043: 676-680.

89. Mosquera JA (2010) [Role of the receptor for advanced glycation end products (RAGE) in inflammation]. Invest Clin 51: 257-268.

90. Vestergaard P, Rejnmark L, Mosekilde L (2005) Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. Diabetologia 48: 1292-1299.

91. Dagdelen S, Sener D, Bayraktar M (2007) Influence of type 2 diabetes mellitus on bone mineral density response to bisphosphonates in late postmenopausal osteoporosis. Adv Ther 24: 1314-1320.

92. Vestergaard (2011) Diabetes and Bone. J Diabetes Metab S:1.

93. Yamauchi M; World Health Organization (2007) [Absolute risk for fracture and WHO guideline. Treatment of patients with secondary osteoporosis]. Clin Calcium 17: 1106-1113.

94. Okamoto T, Yamagishi S, Inagaki Y, Amano S, Takeuchi M, et al. (2002) Incadronate disodium inhibits advanced glycation end products-induced angiogenesis *in vitro*. Biochem Biophys Res Commun 297: 419-424.

95. Yamagishi S, Matsui T, Nakamura K, Takeuchi M (2005) Minodronate, a nitrogen-containing bisphosphonate, inhibits advanced glycation end product-induced vascular cell adhesion molecule-1 expression in endothelial cells by suppressing reactive oxygen species generation. Int J Tissue React 27: 189-195.

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