Bone Reports 1 (2015) 16–19

Contents lists available at ScienceDirect

Bone Reports

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

Original Full Length Article

Effects of long-term doxycycline on bone quality and strength in diabetic male DBA/2J mice

John L. Fowlkes [a,b], Jeffry S. Nyman [c,d], R. Clay Bunn [a,b], Gael E. Cockrell [a,b], Elizabeth C. Wahl [a,b], Mallikarjuna R. Rettiganti [a,b], Charles K. Lumpkin Jr. [a,b], Kathryn M. Thrailkill [a,b,*]

[a] Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR 72202, USA
[b] Arkansas Children's Hospital Research Institute, Little Rock, AR 72202, USA
[c] VA Tennessee Valley Health Care System, Department of Orthopaedic Surgery, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[d] Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 15 September 2014
Accepted 15 October 2014
Available online 4 December 2014

Edited by Peter Ebeling

Keywords:
Diabetic bone disease
Type 1 diabetes
Tetracycline
Trabecular bone
Cortical bone
Microarchitecture

ABSTRACT

In type 1 diabetes, diabetic bone disease (DBD) is characterized by decreased bone mineral density, a state of low bone turnover and an increased risk of fracture. Animal models of DBD demonstrate that acquired alterations in trabecular and cortical bone microarchitecture contribute to decreased bone strength in diabetes. With anti-collagenolytic and anti-inflammatory properties, tetracycline derivatives may prevent diabetes-related decreases in bone strength. To determine if doxycycline, a tetracycline class antibiotic, can prevent the development of DBD in a model of long-term diabetes, male DBA/2J mice, with or without diabetes, were treated with doxycycline-containing chow for 10 weeks (dose range, 28–92 mg/kg/day). Long-term doxycycline exposure was not deleterious to the microarchitecture or biomechanical properties of healthy bones in male DBA/2J mice. Doxycycline treatment also did not prevent or alleviate the deleterious changes in trabecular microarchitecture, cortical structure, and biomechanical properties of bone induced by chronic diabetes.

1. Introduction

Type 1 diabetes (T1D) has detrimental effects on the skeleton, resulting in decreased bone formation and enhanced risk of fracture (Vestergaard et al., 2009; Thrailkill et al., 2005). The underlying mechanisms leading to skeletal fragility in T1D are likely multifactorial, but are associated with decreased endogenous insulin production, hyperglycemia, inflammation, and alterations in systemic hormones, such as insulin-like growth factor-1 (IGF-1) (Thrailkill et al., 2005; Fowlkes et al., 2011; Motyl et al., 2009). Members of the tetracycline family of antibiotics have been shown to reduce bone loss related to periodontal disease in animal models of T1D (Rifkin et al., 1993). While these antibiotics may be useful to prevent bacterial infections, they may also prevent bone breakdown due to their inhibition of collagenases (Rifkin et al., 1993). In a short-term streptozotocin (STZ)-induced diabetic rat model, minocycline, a tetracycline derivative, prevented cancellous bone loss and protected against a decrease in bone formation rates, as assessed for cancellous bone of the peripheral skeleton (Bain et al., 1997). While these studies have shown positive effects of tetracycline derivatives on boney tissues in models of diabetes, other studies suggest greater ambivalence regarding the ability of tetracycline derivatives, such as doxycycline, to prevent diabetes-related alveolar bone loss or osteo-integration in experimentally induced periodontitis (Alkan et al., 2002; Kopman et al., 2005). No definitive studies have been carried out to assess the long-term effects that a tetracycline derivative, like doxycycline, might have on the prevention of bone loss and fragility associated with T1D. Herein, we have used a well-described mouse model of T1D (STZ-induced diabetes in male DBA/2J mice) in which significant skeletal complications are observed, including diaphysial and metaphysial microarchitectural changes, as well as enhanced risk to fracture (Nyman et al., 2011). Diabetic mice were treated with long-term doxycycline (10 weeks) to determine the ability of doxycycline to prevent the development of diabetic bone disease (DBD) in this model.

2. Materials and methods

2.1. Animal model

Male DBA/2J mice, 10–11 weeks of age, (The Jackson Laboratories, Bar Harbor, Maine) were treated either with streptozotocin (STZ) to induce diabetes (40 mg/kg/day × 5 days) or with vehicle (100 mM citrate,

* Corresponding author at: Arkansas Children's Hospital, 1 Children's Way, Slot 512-6, Little Rock, AR 72202, USA. Fax: +1 501 364 6299.
E-mail address: thrailkillkathrynm@uams.edu (K.M. Thrailkill).

http://dx.doi.org/10.1016/j.bonr.2014.10.001
2352-1872/

pH 4.5). At ~12–13 weeks of age, diabetic mice, as confirmed by glucosuria, were then randomly assigned to treatment with doxycycline (DOX) rodent chow (STZ + DOX: Bio-Serv product #S3888, doxycycline concentration of 200 mg/kg, Frenchtown, NJ; n = 20) or with a control (CON) chow for doxycycline diets (STZ + CON: Bio-Serv product #S4207; n = 20). Vehicle-treated (non-diabetic) mice were similarly fed, receiving either doxycycline chow (VEH + DOX; n = 10) or control chow (VEH + CON; n = 10). Mice were provided ad libitum access to water and to their assigned food. Food consumption and body weight (Table 1) were measured weekly for the next 10 weeks. Blood glucose was measured on trunk blood at sacrifice via glucometer (OneTouch® Ultra®2 Blood Glucose Monitoring System, LifeScan, Inc., Milpitas, CA; average intra-assay coefficient of variation of 1.7% across a range of 40–300 mg/dl target glucose concentrations). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

2.2. Skeletal assessment

After euthanasia, the left femur was harvested and femur length was measured using calipers. Bones were flash frozen with liquid nitrogen and stored at −80 °C until analysis. With each femur immersed in phosphate buffered saline (PBS) at room temperature, the long axis of the bone was aligned with the scanning axis of the μCT40 (Scanco Medical, Brüttisellen, Switzerland). All scans were acquired at an isotropic voxel size of 12 μm using the same energy settings (70 kVp/0.114 mA), acquisition parameters (integration time of 300 ms with 1000 projections per full rotation), and calibration to a hydroxyapatite (HA) phantom with a beam hardening correction from Scanco Medical, as we have previously described (Nyman et al., 2011). The regions of interest (ROI) included the trabecular bone of the metaphysis (0.24–1.4 mm above the growth plate) and the cortical bone of the mid-shaft (1.19 mm in length spanning the mid-point between the condyles and femoral neck). Each ROI had a unique threshold (323 mgHA/cm^3 and 809 mgHA/cm^3 for trabecular and cortical bone, respectively) and Gaussian noise filter (sigma of 0.6 with support of 1 for trabecular bone and sigma of 0.8 with support of 2 for cortical bone) that was used for all scans. Standard evaluation scripts from the manufacturer were used to determine the architectural and structural properties of trabecular and cortical bone, respectively. Moreover, in calculation of tissue mineral density (TMD), partial volume effects were suppressed by peeling the first voxel from all surfaces following segmentation of the bone.

2.3. Biomechanical testing

To determine the differences in bone strength between groups, a pre-load (1 N) held each hydrated femur in place on the lower support points of a three-point bending fixture with the anterior side down

Table 1
Phenotypic parameters (top); and selected properties of bone (metaphysis and diaphysis), as determined by μCT and three-point bending.

						P values			
		STZ + DOX (A)	STZ + CON (B)	VEH + DOX (C)	VEH + CON (D)	Doxycycline		Diabetes	
		n = 20	n = 19[c]	n = 10	n = 10	A vs. B	C vs. D	A vs. C	B vs. D
Parameter									
Starting body weight	Grams	23.53 (1.79)	23.82 (1.73)	23.05 (1.27)	24.89 (2.01)	0.96	0.09	0.96	0.36
Ending body weight[c]	Grams	22.71 (2.28)	23.07 (1.81)	31.84 (3.37)	31.98 (2.77)	1.00	1.00	**<.0001**	**<.0001**
Week 5 food intake	gm/mouse/day	10.81 (1.39)	9.64 (2.85)	4.81 (0.36)	5.11 (0.42)	0.16	0.69	**<.0001**	**<.0001**
Week 10 food intake	gm/mouse/day	7.59 (0.67)	7.26 (1.18)	4.53 (0.43)	4.69 (0.46)	0.45	0.67	**<.0001**	**<.0001**
Blood glucose[c]	mg/dL	570.63 (70)	594 (12.6)	129.6 (21.82)	123.7 (22.75)	0.20	0.76	**<.0001**	**<.0001**
Serum bone biomarkers									
P1NP[c]	ng/mL	6.20 (1.80)	4.46 (1.27)	15.62 (3.72)	13.33 (2.72)	**0.04**	**0.04**	**<.0001**	**<.0001**
RatLAPS[c]	ng/mL	31.10 (22.02)	22.42 (9.21)	8.03 (1.28)	9.21 (3.05)	0.12	0.85	**0.0003**	0.054
Metaphysis (trabecular bone)									
VOX-BV/TV	mm^3	0.06 (0.01)	0.06 (0.01)	0.09 (0.02)	0.1 (0.02)	0.13	0.10	**<.0001**	**<.0001**
Conn.D	mm^{-3}	53.94 (19.31)	66.92 (20.73)	59.91 (24.37)	73.98 (16.22)	0.20	0.38	0.75	0.75
Tb.N	mm^{-1}	3.46 (0.26)	3.59 (0.25)	3.7 (0.4)	3.91 (0.26)	0.21	0.21	0.11	**0.02**
Tb.Th	mm	0.04 (0.003)	0.04 (0.002)	0.05 (0.001)	0.05 (0.003)	0.61	0.82	**<.0001**	**<.0001**
Tb.Sp	mm	0.29 (0.02)	0.28 (0.02)	0.27 (0.03)	0.25 (0.02)	0.25	0.25	**0.02**	**0.01**
SMI[a]	–	2.82 (0.21)	2.78 (0.21)	2.78 (0.22)	2.61 (0.16)	1.00	0.21	1.00	0.15
Tb.TMD	mgHA/cm^3	863.52 (25.3)	860.15 (25.99)	934.46 (6.99)	927.36 (19.73)	0.97	0.97	**<.0001**	**<.0001**
Diaphysis (cortical bone)									
Length	mm	13.76 (0.3)	13.84 (0.2)	13.76 (0.23)	13.8 (0.34)	1.00	1.00	1.00	1.00
Ma.V	mm^3	0.56 (0.05)	0.54 (0.04)	0.53 (0.04)	0.53 (0.04)	0.51	1.00	0.25	1.00
Ct.Ar	mm^2	0.65 (0.06)	0.69 (0.07)	0.81 (0.06)	0.85 (0.08)	0.07	0.18	**<.0001**	**<.0001**
Tt.Ar	mm^2	1.12 (0.08)	1.15 (0.07)	1.25 (0.07)	1.29 (0.10)	0.45	0.45	**0.0002**	**<.0001**
I_{min}	mm^4	0.05 (0.01)	0.06 (0.01)	0.07 (0.01)	0.07 (0.01)	0.30	0.30	**<.0001**	**<.0001**
Ct.Th	mm	0.21 (0.01)	0.22 (0.02)	0.25 (0.02)	0.26 (0.02)	**0.03**	0.25	**<.0001**	**<.0001**
Ct.TMD	mgHA/cm^3	1346.34 (11.67)	1357.64 (18.04)	1372.08 (16.51)	1368.74 (20.74)	0.11	0.65	**0.0006**	0.18
Ct.Po	%	3.07 (0.21)	2.92 (0.27)	2.56 (0.15)	2.58 (0.16)	0.06	0.77	**<.0001**	**0.0006**
Slenderness[b]	mm/mm^2	12.35 (0.79)	12.1 (0.56)	11.05 (0.6)	10.74 (0.88)	0.57	0.57	**<.0001**	**<.0001**
Stiffness	N/mm	81.94 (18.54)	88.5 (19.04)	108.74 (10.18)	116.43 (13.19)	0.46	0.46	**0.0004**	**0.0003**
Peak force	N	15.6 (2.52)	16.58 (2.7)	19.42 (1.96)	21.03 (2.02)	0.29	0.29	**0.0005**	**<.0001**
Work-to-fracture	N mm	3.56 (1.09)	3.52 (1.5)	3.8 (1.24)	4.49 (0.46)	1.00	0.62	1.00	0.17
Modulus	GPa	16.16 (3.17)	16.36 (2.92)	16.68 (2.51)	16.96 (2.82)	1.00	1.00	1.00	1.00
Bending strength	MPa	274.96 (34.01)	275.07 (33.01)	282.9 (33.75)	290.58 (24.31)	1.00	1.00	1.00	0.89
Toughness	N/mm	5.46 (1.53)	5.07 (2.09)	4.72 (1.39)	5.37 (0.84)	1.00	1.00	0.98	1.00

[a] Structural model index characterizes the shape of trabecular bone (1: plate-like; 3: rod-like).
[b] Slenderness is the ratio of the length to the total cross-sectional area of the femur mid-shaft. Abbreviations: P1NP, procollagen type 1 N-terminal propeptide; RatLAPS, C-terminal telopeptides of type I collagen; BV/TV, bone volume/tissue volume; Conn.D, connectivity density; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; SMI, structural model index; Tb.TMD, trabecular tissue mineral density; Ma.V, marrow volume; Ct.Ar, cortical area; Tt.Ar, total cross-sectional area; I_{min}, moment of inertia; Ct.Th, cortical thickness; Ct.TMD, cortical tissue mineral density; Ct.Po, cortical porosity. Significant differences ($P < 0.05$) are highlighted in bold font.
[c] n = 19 for STZ + CON, for the four marked variables, due to animal demise on study end date.

(i.e., bending about the medial–lateral plane). The span between the lower supports was 8 mm, and the load rate was 3.0 mm/min. Then, forces from a 100 N load cell (Honeywell, Morristown, NJ) and displacements from the LVDT (Dynamight 8841, Instron, Canton, OH) were recorded at 50 Hz during a monotonic load-to-failure test. Measured differences in biomechanical properties included stiffness and the peak force endured by the bone. Material properties of modulus and strength of the cortex were also estimated using standard beam theory (Nyman et al., 2011). The μCT scans provided the moment of inertia (I_{min}) and the distance between the neutral axis of bending and the outermost point in the anterior–posterior direction (C_{min}).

2.4. Bone biomarker analyses

Procollagen type 1 N-terminal propeptide (P1NP), a marker of bone formation, was measured in serum at sacrifice, using the Rat/Mouse P1NP Enzyme immunoassay (Immunodiagnostics Systems, Inc., Fountain Hills, AZ; #AC-33F1). Serum C-terminal telopeptides of type I collagen (RatLAPS), a marker of bone resorption, was measured using the RatLAPs ELISA (Immunodiagnostics Systems, Inc., Fountain Hills, AZ; #AC-06F1).

2.5. Statistical analyses

A one-way analysis of variance (ANOVA) was performed to test whether the means of bone biomarkers and bone parameters were equal between the four groups: STZ + DOX, STZ + CON, VEH + DOX, and VEH + CON. Linear contrasts for the group variable were used to test the following four pairwise comparisons: STZ + DOX vs. STZ + CON, VEH + DOX vs. VEH + CON, STZ + DOX vs. VEH + DOX, and STZ + CON vs. VEH + CON. For each parameter, a step-down Bonferroni method was used to keep the overall family-wise error rate under 0.05 to adjust for multiple comparisons. An analysis of covariance (ANCOVA) model was used to compare the mean cortical thickness (Ct.Th) between STZ + DOX and STZ + CON groups, after adjusting for RatLAPS. All tests were two-sided assuming a significance level of 5%. All statistical analyses were generated using SAS/STAT software, Version 9.4 of the SAS System for Windows 7, Copyright © 2002–2012 SAS Institute Inc.

3. Results and discussion

Changes in body weight, food consumption and blood glucose across the ten-week treatment period are shown in Table 1. No statistically significant differences in average starting body weight existed between mice in each of the four groups (overall ANOVA, $P = 0.11$). Both non-diabetic groups (VEH + DOX, VEH + CON) consumed comparable amounts of food, by weight, over time ($P = 0.69$ for week 5; $P = 0.67$ for week 10). Moreover, by week ten, final body weights ($P = 1$) and final blood glucose levels ($P = 0.76$) were not significantly different between these two non-diabetic groups. However, as expected, both groups of diabetic DBA/2J mice (STZ + DOX, STZ + CON), when assessed after 10 weeks of diabetes, demonstrated polyphagia (approximately double the food consumption relative to control groups; $P < 0.0001$ for STZ + DOX vs. VEH + DOX and STZ + CON vs. VEH + CON), severe hyperglycemia ($P < 0.0001$ for both comparisons) and weight loss ($P < 0.0001$ for both comparisons; Table 1). Hence, based on the changes in average weekly food consumption and in body weight over time, the doxycycline dose during the 10 week treatment period for mice in the STZ + DOX group ranged from ~92 mg/kg/day (at week 5) to 67 mg/kg/day (week 10) and in the VEH + DOX group ranged from 42 mg/kg/day (week 5) to 28 mg/kg/day (week 10).

Selected bone parameters, as determined by serum bone biomarker assays, μCT, and biomechanical testing are also shown in Table 1. Serum P1NP values were significantly lower in diabetic mice (STZ + DOX vs. VEH + DOX and STZ + CON vs. VEH + CON, $P < 0.0001$ for both), indicating that long-term diabetes was characterized by a significant reduction in bone formation. Serum RatLAPS values were significantly higher in doxycycline-treated diabetic mice, compared with non-diabetic mice (STZ + DOX vs. VEH + DOX, $P < 0.0001$) and demonstrated a similar, nearly significant, trend in control-fed mice (STZ + CON vs. VEH + CON, $P = 0.054$), reflecting a disease-associated increase in bone resorption. Interestingly, doxycycline treatment itself, both in the diabetic mice and in the control mice, increased P1NP values (STZ + DOX vs STZ + CON and VEH + DOX and VEH + CON, $P = 0.04$ for both), suggesting a modest positive drug treatment effect on osteoblast function. RatLAPS values, however, were not further affected by doxycycline treatment.

When examining bone quality, consistent with our previous findings (Nyman et al., 2011) long-term diabetes was associated with deficits in trabecular bone microarchitecture, including decreases in trabecular bone volume fraction (BV/TV; $P < 0.0001$), trabecular thickness ($P < 0.0001$), and trabecular TMD ($P < 0.0001$). In cortical bone, deficits in cortical bone area ($P < 0.0001$), cortical thickness ($P < 0.0001$), cortical TMD ($P = 0.0006$ for STZ + DOX vs. VEH + DOX), I_{min} ($P < 0.0001$), and bone stiffness ($P = 0.0004$ for STZ + DOX vs. VEH + DOX; $P = 0.0003$ for STZ + CON vs. VEH + CON) were apparent in diabetic animals, in addition to an increase in cortical porosity ($P < 0.0001$ for STZ + DOX vs. VEH + DOX; $P = 0.0006$ for STZ +CON vs. VEH + CON). Femur slenderness, the ratio of the length to the total cross-sectional area of the femur mid-shaft, was also increased in diabetic animals ($P < 0.0001$ for STZ + DOX vs. VEH + DOX and STZ + CON vs. VEH + CON). Consequently, diabetes caused a decrease in the structural strength of the femur in bending (peak force; $P = 0.0005$ for STZ + DOX vs. VEH + DOX; $P < 0.0001$ for STZ + CON vs. VEH + CON). However, no significant differences were identified in any of the measured microarchitectural or biomechanical bone parameters between doxycycline-treated and control-fed *non-diabetic* mice (VEH + DOX, VEH + CON, respectively). Moreover, when comparing doxycycline-treated and control-fed *diabetic* mice (STZ + DOX, STZ + CON, respectively), a modest further decrement in cortical thickness was observed in the STZ + DOX group ($P = 0.03$). This difference was not explained by differences in body weight; however, mean ratLAPs values were higher in the STX + DOX group and Ct.Th was negatively associated with ratLAPS among the diabetic mice ($r = -0.33$, $p = 0.045$), inferring slightly greater bone resorption in the STZ + DOX group. After adjusting for ratLAPS, Ct.Th was not significantly different between the STZ + DOX and STZ + CON groups ($P = 0.052$). All other microarchitectural and biomechanical parameters were unaffected by doxycycline exposure. Hence, doxycycline treatment, in this model of male mice, did not prevent or alleviate the development of diabetic bone disease, as determined by microarchitectural analysis and biomechanical testing, even when given at twice the exposure dose relative to control animals.

Similar to our findings in animal models (Nyman et al., 2011), deterioration in trabecular bone microarchitecture, in particular, has been inferred from studies of T1D subjects with a history of fracture. Our expectation that tetracycline derivatives might improve bone deficits in diabetes comes from experiments demonstrating that, in other in vivo models of diabetes, tetracyclines can: 1) increase osteoblast-specific collagen synthesis (Sasaki et al., 1992); 2) maintain normal bone formation rates and prevent trabecular bone loss (Bain et al., 1997); and 3) inhibit proinflammatory mediators (Golub et al., 1998). Additionally, in women with post-menopausal systemic osteoporosis, the use of sub-antimicrobial dose doxycycline (SDD) has also been shown to significantly reduce serum biomarkers of bone resorption and of inflammation (Payne and Golub, 2011; Payne et al., 2011). In the present study, however, we found no evidence for improvement in diabetic bone deficits by doxycycline.

These negative findings do, nevertheless, merit attention. Recent studies demonstrating that doxycycline can inhibit M2-type (alternatively activated) macrophage polarization and pathologic neovascularization suggest a potential therapeutic role for doxycycline in conditions of pathological angiogenesis (He and Marneros, 2014), including

diabetic complications such as retinopathy, as confirmed in a recent proof-of-concept clinical trial (Scott et al., 2014). Hence, the lack of a significant *detrimental* effect of long-term exposure on diabetic skeletal tissue is important to note, should chronic doxycycline therapy become a reality. Equally, the absence of any detrimental effect of long-term doxycycline exposure on healthy skeletal tissues is a pertinent finding for experimental models employing tissue-specific doxycycline repressible Cre-recombinase gene expression methodologies, especially those that target skeletal tissues (e.g., Osterix-Cre) (Elefteriou and Yang, 2011). Doxycycline delivery via rodent chow, at a concentration of 200 mg/kg as was used in this experiment, is the recommended delivery route for these doxycycline regulated gene expression systems (Cawthorne et al., 2007).

4. Conclusions

While the tetracycline class of antibiotics has been shown to have beneficial effects on boney tissues in models of diabetes, in male DBA/2J mice subjected to 10 weeks of diabetes, diabetic bone disease was not improved by doxycycline treatment. Moreover, long-term doxycycline exposure was not deleterious to the microarchitecture or biomechanical properties of healthy bone in male DBA/2J mice.

Author contributions

J.L.F. and K.M.T. researched the data, contributed to the discussion, wrote the manuscript and reviewed/edited the manuscript. J.S.N., R.C.B., G.E.C., and E.C.W. researched the data, contributed to the discussion and reviewed/edited the manuscript. M.R.R. provided the statistical analyses and reviewed/edited the manuscript. C.K.L. contributed to the discussion and reviewed/edited the manuscript.

Funding source

This work was supported by grants from the Children's University Medical Group fund of the Arkansas Children's Hospital Research Institute (to K.M.T.), the Arkansas Biosciences Institute (to J.L.F.), and in part by National Institutes of Health Grants R01DK055653 (to J.L.F.), and C06RR16517 (to Arkansas Children's Hospital Research Institute), as well as the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development 1I01BX001018 (to J.S.N.).

Acknowledgments

This work was supported by grants from the Children's University Medical Group fund of the Arkansas Children's Hospital Research Institute (to K.M.T.), the Arkansas Biosciences Institute (to J.L.F.), and in part by National Institutes of Health Grants R01DK055653 (to J.L.F.), and C06RR16517 (to ACHRI), as well as the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development 1I01BX001018 (to J.S.N.).

References

Alkan, A., Erdem, E., Gunhan, O., Karasu, C., 2002. Histomorphometric evaluation of the effect of doxycycline on the healing of bone defects in experimental diabetes mellitus: a pilot study. J. Oral Maxillofac. Surg. 60 (8), 898–904 (PubMed PMID: 12149735).

Bain, S., Ramamurthy, N.S., Impeduglia, T., Scolman, S., Golub, L.M., Rubin, C., 1997. Tetracycline prevents cancellous bone loss and maintains near-normal rates of bone formation in streptozotocin diabetic rats. Bone 21 (2), 147–153 (PubMed PMID: 9267690).

Cawthorne, C., Swindell, R., Stratford, I.J., Dive, C., Welman, A., 2007. Comparison of doxycycline delivery methods for Tet-inducible gene expression in a subcutaneous xenograft model. J. Biomol. Tech. 18 (2), 120–123 (PubMed PMID: 17496224; PubMed Central PMCID: PMC2062538).

Elefteriou, F., Yang, X., 2011. Genetic mouse models for bone studies — strengths and limitations. Bone 49 (6), 1242–1254. http://dx.doi.org/10.1016/j.bone.2011.08.021 (PubMed PMID: 21907838; PubMed Central PMCID: PMC3331798).

Fowlkes, J.L., Bunn, R.C., Thrailkill, K.M., 2011. Contributions of the insulin/insulin-like growth factor-1 axis to diabetic osteopathy. J. Diabetes Metab. 1 (3). http://dx.doi.org/10.4172/2155-6156.S1-003 (PubMed PMID: 23484069; PubMed Central PMCID: PMC3593087).

Golub, L.M., Lee, H.M., Ryan, M.E., Giannobile, W.V., Payne, J., Sorsa, T., 1998. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. Adv. Dent. Res. 12 (2), 12–26 (PubMed PMID: 9972117).

He, L., Marneros, A.G., 2014. Doxycycline inhibits polarization of macrophages to the proangiogenic M2-type and subsequent neovascularization. J. Biol. Chem. 289 (12), 8019–8028. http://dx.doi.org/10.1074/jbc.M113.535765 (PubMed PMID: 24505138; PubMed Central PMCID: PMC3961635).

Kopman, J.A., Kim, D.M., Rahman, S.S., Arandia, J.A., Karimbux, N.Y., Fiorellini, J.P., 2005. Modulating the effects of diabetes on osseointegration with aminoguanidine and doxycycline. J. Periodontol. 76 (4), 614–620. http://dx.doi.org/10.1902/jop.2005.76.4.614 (PubMed PMID: 15857103).

Motyl, K.J., Botolin, S., Irwin, R., Appledorn, D.M., Kadakia, T., Amalfitano, A., et al., 2009. Bone inflammation and altered gene expression with type I diabetes early onset. J. Cell. Physiol. 218 (3), 575–583. http://dx.doi.org/10.1002/jcp.21626 (PubMed PMID: 19006181).

Nyman, J.S., Even, J.L., Jo, C.H., Herbert, E.G., Murry, M.R., Cockrell, G.E., et al., 2011. Increasing duration of type 1 diabetes perturbs the strength-structure relationship and increases brittleness of bone. Bone 48 (4), 733–740. http://dx.doi.org/10.1016/j.bone.2010.12.016 (PubMed PMID: 21185416; PubMed Central PMCID: PMC3062641).

Payne, J.B., Golub, L.M., 2011. Using tetracyclines to treat osteoporotic/osteopenic bone loss: from the basic science laboratory to the clinic. Pharmacol. Res. 63 (2), 121–129. http://dx.doi.org/10.1016/j.phrs.2010.10.006 (PubMed PMID: 20937388; PubMed Central PMCID: PMC3031719).

Payne, J.B., Golub, L.M., Stoner, J.A., Lee, H.M., Reinhardt, R.A., Sorsa, T., et al., 2011. The effect of subantimicrobial-dose-doxycycline periodontal therapy on serum biomarkers of systemic inflammation: a randomized, double-masked, placebo-controlled clinical trial. J. Am. Dent. Assoc. 142 (3), 262–273 (PubMed PMID: 21357860; PubMed Central PMCID: PMC3077029).

Rifkin, B.R., Vernillo, A.T., Golub, L.M., 1993. Blocking periodontal disease progression by inhibiting tissue-destructive enzymes: a potential therapeutic role for tetracyclines and their chemically-modified analogs. J. Periodontol. 64 (8 Suppl.), 819–827. http://dx.doi.org/10.1902/jop.1993.64.8s.819 (PubMed PMID: 8410621).

Sasaki, T., Ramamurthy, N.S., Golub, L.M., 1992. Tetracycline administration increases collagen synthesis in osteoblasts of streptozotocin-induced diabetic rats: a quantitative autoradiographic study. Calcif. Tissue Int. 50 (5), 411–419 (PubMed PMID: 1534508).

Scott, I.U., Jackson, G.R., Quillen, D.A., Larsen, M., Klein, R., Liao, J., et al., 2014. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in patients with severe nonproliferative or non-high-risk proliferative diabetic retinopathy: a randomized clinical trial. JAMA Ophthalmol. 132 (5), 535–543. http://dx.doi.org/10.1001/jamaophthalmol.2014.93 (PubMed PMID: 24604308).

Thrailkill, K.M., Lumpkin Jr., C.K., Bunn, R.C., Kemp, S.F., Fowlkes, J.L., 2005. Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. Am. J. Physiol. Endocrinol. Metab. 289 (5), E735–E745. http://dx.doi.org/10.1152/ajpendo.00159.2005 (PubMed PMID: 16215165; PubMed Central PMCID: PMC2387001).

Vestergaard, P., Rejnmark, L., Mosekilde, L., 2009. Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes. Calcif. Tissue Int. 84 (1), 45–55. http://dx.doi.org/10.1007/s00223-008-9195-5 (PubMed PMID: 19067021).