Diabetic mellitus aggravates cortical bone status in aging mice

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Introduction. Bone loss characterized by a decrease in bone mass and structure occurs with aging. Diabetes mellitus (DM), a prevalent condition in aged subjects, causes bone deterioration and an increased fracture risk[1]. DM hampers osteoblast maturation[2] and angiogenesis[3], associated with changes in bone microarchitecture[3] and increased oxidative stress[5]. However, the underlying mechanisms by which aging and DM produce bone deterioration are yet ill-defined. Here, we induced DM in old mice using a well characterized protocol (multiple streptozotozin s.c. injections) aiming at exploring the consequences of combined aging and DM on cortical bone microarchitecture and vascularity in the mouse long bones.

References

1- Hofbauer, L.C., et al., Osteoporosis in patients with diabetes mellitus. J Bone Miner Res, 2007; 22: 317-28.
2- Blakytny, R., M. Spraul, and E.B. Jude, Review: The diabetic bone: a cellular and molecular perspective. Int J Low Extrem Wounds, 2011; 10:16-32.
3- Lozano D, de Castro LF, Dapia S, Andrade-Zapata I, Manzarbeitia F, Alvarez-Arroyo MV, Gomez-Barrena E, Esbrit P Role of parathyroid hormone-related protein in the decreased osteoblast function in diabetes-related osteopenia. Endocrinology 2009; 150:202735.
4- Saito, M. and K. Marumo, Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int, 2010; 21:195-214.
5- Christensen, J.O. and O.L. Svendsen, Bone mineral in pre- and postmenopausal women with insulin-dependent and non-insulin-dependent diabetes mellitus. Osteoporos Int, 1999; 10:307-11.

Methods: DM was induced in male CD1 mice (18 months-old) at 3 weeks after streptozotocin injections (45 mg/g BW, x5) (Old-DM). Mice of the same age (Old) or aged 3 months (Young) were included as controls (n=6 each group). Microcomputerized tomography (µCT): Mouse femora were scanned using a GE eXplore Locus µCT scanner (GE Healthcare, London, Canada), using 80 kvolts and 450 µA as X-ray tube settings. Raw acquired µCT images resulting from 400 projections during a 20-min full rotation of the gantry were reconstructed with a filtered back-projection algorithm to a final image of 93-µm resolution in all three spatial dimensions. Cortical bone analysis was performed in 35 slices of 46-mm length at the diaphysis starting at 25% of the femur height from the growth plate. The reconstructed images were viewed and analyzed using MicroView software, version 2.2 with Advanced Bone Analysis Plus (GE Healthcare). The following cortical parameters were estimated: cortical thickness (Ct.Th), inner perimeter, outer perimeter, and the marrow area (Ma.Ar). Microindentation: It was performed on mouse tibia using a BioDent Reference Point Indenter (ActiveLife Scientific, Santa Barbara, CA). Each tibia was held in place using an ex vivo small bone stage and hydrated by submerging each simple in Hank's balanced salt solution. Three measurements were made within a 1 mm2 area at the tibiofibular junction on the anterior side of each sample using a published 2N, 10 cycle indentation protocol. A single BP3 probe assembly was used for all measurements (N=70). Prior to and after measurements, 5 indentations were performed on PMMA to verify no damage to the BP3 probe during the study. Three point bending test: Prior to the test, tibiae were scanned by µCT (as indicated above). The procedure was carried out as previously described in detail (Westbroek, JCB, 2007). Briefly, femora were placed in a 3-point bending device, with the loading posts 10 mm apart. Mechanical testing was performed using a Single Column Lloyd LRX System (Lloyd Instruments, Fareham, UK). Displacement (mm) and force (N) were registered and stiffness (N/mm) and work-to-failure (N.mm) calculated. The polar moment of inertia (MOI) and centroid value were determined from the µCT scans at the site of fracture. Maximum force (N), stress (N/mm2), strain and elastic modulus (GPa) were determined from the resulting displacement to force graphs as well as the MOI and centroid values (Turner, Bone, 1993). Blood vessels counting: Tibiae were de-calcified with osteosoft and then paraffin embedded. Four-µm sections were then obtained and stained with lectin from *Bandeiraea simplicifolia.* Blood vessels were identified by lectin staining and the presence of erythrocytes in their lumen. They were counted in the mid-diaphysis (5 fields per sample) with 400x magnification by three independent observers in a blinded fashion, and expressed as the number of vessels/mm2 for each group. Results are expressed as mean±SD. Statistical comparisons between experimental groups were done by U Mann-Whitney test using the GraphPad Prism© V 6.01 software (GraphPad software, Inc, La Jolla, CA); p< 0.05 was considered significant.

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Results

	Young	Old	Old+DM
Ct. Th (µm)	398±8	360±12*	350±13*
Inner Pm (mm)	3.7±0.1	4.5±0.4*	4.2±0.2**
Outer Pm (mm)	6±0.1	7.7±0.6*	7.1±0.2**
Ma. Ar. (mm^2)	0.8±0.1	1.3±0.1**	1.4±0.1**

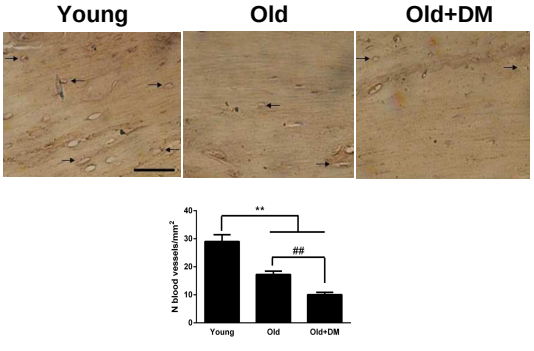
Micro CT analysis of mouse femoral diaphysis. Ct. Th: Cortical thickness; Pm: perimeter; Ma. Ar: Medullary area. Data are mean±SEM (n=6). *p<0.05; **p<0.01 vs young control mice;

	Young	Old	Old+DM
Max.Load (N)	21±1.8	18±2.5	16±2.1
Stiffness (N/mm)	49±0.6	58±7.2	61±8.6
MOI (mm^4)	0.36±0.07	0.41±0.04	0.36±0.04
Work-to-failure (Nmm)	4.5±0.5	3.4±0.7	1.9±0.2*
Stress Top (N/mm^2)*10^3	380±89	241±47	174±15
Stress bottom (N/mm^2)*10^3	299±40	183±34*	163±56
Strain top (N/mm^2)	113.5±18.2	90.1±10.6*	40.0±4.1*,#
Strain bottom (N/mm^2)	142±11	114±8	87±6
Young´s modulus (N/mm^2)	3,716±564	2,995±314	4,125±124

Three point bending analysis of tibia from different group of mice. Max. Load: Maximun load, MOI: Momenf of Inertia. Data represent mean±SEM (n=6). *p<0.05 vs Young; #p<0.05 vs Old. DM: Diabetic

	Young	Old	Old+DM
ID 1st (µm)	32±0.6	30±0.1*	30±0.8
US 1st (N/µm)	0.27±0.01	0.27±0.01	0.29±0.01
CID 1st (µm)	3.2±0.1	3.0±0.1	2.8±0.1
TID (µm)	35±0.6	33±0.1*	33±0.8*
IDI (µm)	4.9±0.16	4.7±0.15	4.5±0.28
Avg. CID (µm)	1.2±0.08	1.1±0.05	0.97±0.02**,#
Avg. ED (µJ)	3.9±0.16	3.6±0.18	3.4±0.10
Avg. US (N/µm)	0.27±0.01	0.27±0.01	0.30±0.01
Avg. LS (N/µm)	0.19±0.003	0.19±0.01	0.18±0.01

Microindentation analysis of tibiae from different groups of mice. ID 1st: Indentation distance first cycle; US 1st: Unloading slope first cycle; CID 1st: Creep indentation distance 1st cycle; TID: Total indentation distance; IDI: Indentation distance increase; Avg. CID: Average creep indentation distance; Avg. ED: Average energy dissipated; Avg. US: Average unloading slope; Avg. LS: Average loading slope. Data are mean±SEM (n=6) *p<0.05 **p<0.01 vs Young; #p<0.05 vs Old+DM. DM: Diabetic.



Representative light microscopy images of cortical vasculature of mouse femur mid-diaphysis stained with lectin (magnification, 400x). Arrows indicate a positive vessel with erythrocytes in the lumen. Bar denotes 100 µm. Blood vessels quantification in the cortical diaphysis of femur from the different groups of mice studied is shown below. Data are mean±SEM (n=6 per group). **p<0.01 vs Young; ##p<0.01 vs Old.

Conclusions

Our results indicate that DM aggravates the compromised cortical bone quality in Old mice, which might increase fracture risk in age-related osteopenia.