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Bone's responses to mechanical loading are impaired in type 1 diabetes

Ashutosh Parajuli [a,1], Chao Liu [c,1], Wen Li [b,1], Xiaoyu Gu [b], Xiaohan Lai [b], Shaopeng Pei [b], Christopher Price [a], Lidan You [c], X. Lucas Lu [a,b,*], Liyun Wang [a,b,*]

[a] Department of Biomedical Engineering, University of Delaware, Newark, DE 19716, USA
[b] Department of Mechanical Engineering, University of Delaware, Newark, DE 19716, USA
[c] Department of Mechanical and Industrial Engineering, Institute of Biomaterials and Biomedical Engineering, University of Toronto, Ontario, Canada

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ABSTRACT

Diabetes adversely impacts many organ systems including the skeleton. Clinical trials have revealed a startling elevation in fracture risk in diabetic patients. Bone fractures can be life threatening: nearly 1 in 6 hip fracture patients die within one year. Because physical exercise is proven to improve bone properties and reduce fracture risk in non-diabetic subjects, we tested its efficacy in type 1 diabetes. We hypothesized that diabetic bone's response to anabolic mechanical loading would be attenuated, partially due to impaired mechanosensing of osteocytes under hyperglycemia. Heterozygous C57BL/6-Ins2^{Akita}/J (Akita) male and female diabetic mice and their age- and gender-matched wild-type (WT) C57BL/6J controls (7-month-old, N = 5–7 mice/group) were subjected to unilateral axial ulnar loading with a peak strain of 3500 με at 2 Hz and 3 min/day for 5 days. The Akita female mice, which exhibited a relatively normal body weight and a mild 40% elevation of blood glucose level, responded with increased bone formation (+6.5% in Ct.B.Ar, and 4 to 36-fold increase in Ec.BFR/BS and Ps.BFR/BS), and the loading effects, in terms of changes of static and dynamic indices, did not differ between Akita and WT females ($p \geq 0.1$). However, loading-induced anabolic effects were greatly diminished in Akita males, which exhibited reduced body weight, severe hyperglycemia (+230%), diminished bone formation (ΔCt.B.Ar: 0.003 vs. 0.030 mm^2, $p = 0.005$), and suppressed periosteal bone appositions (ΔPs.BFR/BS, $p = 0.02$). Hyperglycemia (25 mM glucose) was further found to impair the flow-induced intracellular calcium signaling in MLO-Y4 osteocytes, and significantly inhibited the flow-induced downstream responses including reduction in apoptosis and sRANKL secretion and PGE_2 release. These results, along with previous findings showing adverse effects of hyperglycemia on osteoblasts and mesenchymal stem cells, suggest that failure to maintain normal glucose levels may impair bone's responses to mechanical loading in diabetics.

1. Introduction

Obesity and diabetes are epidemic health problems associated with sedentary life-style and high-fat diets. According to the Center for Disease Control and Prevention's 2005–2008 National Health and Nutrition Examination Survey, 14% of adults between ages 45–64 and 27% of adults older than 65 are diabetic. Diabetes causes serious health complications including heart disease, neuropathy, blindness, kidney failure, and lower-extremity amputations. It is the seventh leading cause of death in the United States (www.cdc.gov). However, the negative impacts of diabetes on bone health have not been well recognized until recently. In 2007, two meta-analyses of 13–16 large clinical trials showed a startling elevation in bone fracture risk for diabetic patients compared with normal population, which was a 7-fold increase in type 1 diabetes (T1D, formerly called juvenile-onset diabetes) and a 1.4-fold increase in type 2 diabetes (T2D, formerly called adult-onset diabetes) [1,2]. Since nearly 1 in 6 patients with hip fracture died within one year [3], bone fractures can be life threatening. The risk is especially high for diabetic patients, whose impaired vasculature and wound healing capability contribute to increased mortality [4].

The mechanisms underlying the observed elevation in fracture risk among diabetics are not fully understood, although human and animal studies have documented bone defects such as retarded bone accrual and reduced bone size [5–7] (for T1D) and/or altered bone matrix [8,9] and increased cortical porosities [10–12] (for T2D) [11,12]. The fragile bone phenotypes have been recapitulated in various diabetic animal models [13–16]. On the cellular and molecular levels, hyperglycemia and hormonal disturbances, present in both T1D and T2D [17,18], were found to i) inhibit the proliferation and differentiation of bone marrow mesenchymal stem cells into bone-forming osteoblasts [19], ii) suppress osteoblast's functions [20,21], and iii) increase

* Corresponding authors at: Center for Biomedical Engineering Research, Department of Mechanical Engineering, 130 Academy Street, Newark, DE 19716, USA.
E-mail addresses: parajuli.ashutosh@gmail.com (A. Parajuli), wenli@udel.edu (W. Li), davegu@udel.edu (X. Gu), laixhan@udel.edu (X. Lai), shaopeng@udel.edu (S. Pei), cprice@udel.edu (C. Price), youlidan@mie.utoronto.ca (L. You), xlu@udel.edu (X.L. Lu), lywang@udel.edu (L. Wang).
[1] These three authors contributed equally to this work.

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nonenzymatic glycation of collagen (the major organic constitute of bone matrix) [8,9,15]. Paradoxically, long-term use of some diabetes treatments such as thiazolidinediones, a class of insulin-sensitizing drug including rosiglitazone and pioglitazone, was recently found to stimulate adipogenesis and inhibit osteogenesis, leading to even more bone deterioration [11,22]. Thus, there is a great need to improve bone health in diabetics using non-pharmaceutical interventions.

Mechanical stimulation associated with exercise and physical activities is long recognized as a potent anabolic factor in promoting bone health [23–25]. The beneficial effects of mechanical stimulation can be best demonstrated in the stronger bone seen in the accrual of bone mass in the dominant arms of professional tennis players, in contract with the rapid bone loss seen in astronauts and bed-rest patients as their skeletons are deprived from mechanical stimulation [26,27]. During these bone adaptation processes, osteocytes, the most abundant cells in bone, play a central role. Dispersed in bone matrix and being well-connected with each other as well as the cells lining the bone surfaces, osteocytes serve not only as the primary sensors that detect external mechanical stimuli, but also as a paracrine regulator of osteoblasts and osteoclasts via signaling molecules such as PGE_2, RANKL, OPG and sclerostin/SOST [28–31]. However, the efficacy of applying mechanical stimulation in rescuing diabetic bone diseases is not known and whether diabetic hyperglycemia impairs osteocyte's mechanosensing has yet to be determined.

The objective of the present study was to test the hypothesis that bone's response to anabolic mechanical loading is attenuated in diabetes due to, at least partially, impaired mechanosensing in osteocytes. We first investigated bone's acute responses to exogenously applied ulnar loading in T1D female and male mice as well as their age- and gender-matched normal controls. We then further studied how hyperglycemia associated with severe diabetes affected the responses of osteocytic MLO-Y4 cells to fluid flow stimulation. Our results demonstrated that in vivo bone formation was impaired in severe diabetic T1D mouse and hyperglycemia inhibited osteocyte's sensitivity or responses to mechanical stimulation in vitro. This study suggests the use of proper glycemic control to restore bone's response to mechanical signals and to improve bone health in diabetic patients.

2. Methods

2.1. In vivo response to ulnar loading

2.1.1. Animals

To test whether hyperglycemia negatively affect in vivo bone responses to loading, we used heterozygous C57BL/6-Ins2^{Akita}/J (Akita) male and female mice and their age matched wild-type (WT) C57BL/6J controls (7-month-old, N = 5–7 mice/group, Jackson Laboratory, Bar Harbor, Maine). Due to the spontaneous Akita mutation that impairs the normal folding and secretion of insulin, Akita male mice develop T1D diabetes at the age of 5 weeks, manifesting severe hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, while Akita females demonstrated milder diabetic symptoms with less severe impairment in β-cell functions [13,32]. Both Akita females and males were used due to their different stages of disease severity. Fasting blood glucose level was determined using retro-orbital bleeding and a glucometer (OneTouch®, LifeScan, Inc., Milpitas, CA) prior to acute ulnar loading. Body weight was measured at both the beginning and the end of experiments. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Delaware.

2.1.2. Ulnar loading experiments

Similar to previous studies [33,34], we subjected the right forearms of the anesthetized mice to cyclical compression that induced a peak of ~3500 με near the lateral mid-shaft surface (Fig. 1). Due to the different body weights and bone sizes of each group, the applied load magnitude was determined by strain gauging performed in a separate set of animals (n ≥ 8 ulnae/group). A single-element gauge (EA-06-015DJ-120; Measurements Group, Inc., Raleigh, NC) was fixed on a relatively flat surface (1–2 mm proximal of the mid-shaft) of the intact ulna attached to the body. The ulna was axially compressed with a gradually increasing load (0.4 N/s to 4.2 N) using a Bose LM1 TestBench® loading system (Bose Corporation, Framingham, MA), while the strain gauge's output voltage was recorded by LM1's data acquisition unit and converted to strain using a calibrated conversion coefficient [35]. From the strain vs. load curves for the compression tests, the mean compressive rigidity of the ulnae per group was thus calculated. To achieve a consistent 3500 με strain on the ulnar surface, the loading magnitudes for the four groups were calculated to be 2.7 ± 0.5 N, 3 ± 0.4 N, 2.2 ± 0.4 N, and 3 ± 0.5 N for Akita females, WT females, Akita males and WT males, respectively. Thus, the group averages were chosen to be the applied loading magnitudes, i.e., 2.7 N for Akita females (n = 7), 3 N for WT females (n = 5), 2.2 N for Akita males (n = 5), and 3 N for WT males (n = 7). Daily mechanical stimulation was applied at 2 Hz, 3 min/day, for 5 consecutive days as published [33]. The mice received dynamic bone labels (calcein 10 mg/kg) on Day 4 and Day 15 and were sacrificed on Day 18.

2.1.3. Sample processing and data collection

Both loaded and contralateral non-loaded ulnae were harvested, chemically fixed, embedded in methylmethacrylate, sectioned and polished, and analyzed using an OsteoMeasure® software package and an upright epifluorescent microscope, following the protocols published previously [35,36]. Static and dynamic histomorphometric measurements were performed on two mid-shaft sections and the average values were used for each animal. For each gender, two types of comparisons were performed. First, the loading effects (loaded ulnae vs. non-loaded ulnae) were tested within the Akita and WT groups with either Student's t tests (for normally distributed data such as bone morphology) or Mann–Whitney U tests (for data without normal distributions). The second type of comparison focused on whether the responses of diabetic Akita mice differed from those of controls. We performed Whitney–Mann U tests on the relative changes, Δ(loaded − nonloaded) data, between the Akita and WT mice. All analyses were performed using Origin (OriginLab, Northampton, MA) with significance set at $p < 0.05$.

2.2. In vitro responses to hyperglycemia

Although in vivo bone formation involves osteoblasts, osteocytes, and mesenchymal progenitor cells, we focused our studies on osteocytes, because the inhibitory effects of hyperglycemia on osteoblast proliferation and mineralization have been well established in literature [21,37,38]. To test the effects of hyperglycemia on osteocyte mechanosensitivity, we utilized an in vitro model where cultured osteocytes were exposed to fluid flow stimulation that mimicked the interstitial fluid flow occurring in vivo [39]. Several outcome measures were assessed, such as intracellular Ca^{2+} ($[Ca^{2+}]_i$) peaks, one of the earliest responses of osteocytes to physical stimulation [40–42], and important downstream responses including the secretion of anabolic cytokine (PGE_2), catabolic cytokine (RANKL) and osteocyte apoptosis, which play a role in initiation of bone remodeling [23,43,44].

2.2.1. Cells and culture media

MLO-Y4 cells, a generous gift from Dr. Lynda Bonewald (University of Missouri-Kansas City, Kansas City, MO) and a well-characterized model for osteocytes [45], were cultured on type I collagen (BD Biosciences, San Jose, CA, USA) coated Petri-dishes. Three culture media were used: i) the regular medium consisted of alpha MEM with 5.5 mM D-glucose (Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum and 5% calf serum (Hyclone Laboratories Inc., Logan, UT, USA); ii) the osmotic control medium consisted of the regular medium described above with the addition of 20 mM L-glucose