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# Preserving and restoring bone with continuous insulin infusion therapy in a mouse model of type 1 diabetes

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ABSTRACT

Those with type 1 diabetes (T1D) are more likely to suffer a fracture than age- and sex-matched individuals without diabetes, despite daily insulin therapy. In rodent studies examining the effect of bone- or glucose-targeting therapies on preventing the T1D-related decrease in bone strength, insulin co-therapy is often not included, despite the known importance of insulin signaling to bone mass accrual. Therefore, working toward a relevant pre-clinical model of diabetic bone disease, we assessed the effect of continuous subcutaneous insulin infusion (CSII) therapy at escalating doses on preserving bone and the effect of delayed CSII on rescuing the T1D-related bone deterioration in an established murine model of T1D. Osmotic minipumps were implanted in male DBA/2 J mice 2 weeks (prevention study) and 6 weeks (rescue study) after the first injection of streptozotocin (STZ) to deliver insulin at 0, 0.0625, 0.125, or 0.25 IU/day (prevention study; n = 4–5 per dose) and 0 or 0.25 IU/day (rescue study; n = 10 per group). CSII lasted 4 weeks in both studies, which also included age-matched, non-diabetic DBA/2 J mice (n = 8–12 per study). As the insulin dose increased, blood glucose decreased, body weight increased, a serum maker of bone resorption decreased, and a serum marker of bone formation increased such that each end-point characteristic was linearly correlated with dose. There were insulin dose-dependent relationships (femur diaphysis) with cross-sectional area of cortical bone and cortical thickness (micro-computed tomography) as well as structural strength (peak force endured by the mid-shaft during three-point bending). Likewise, trabecular bone volume fraction (BV/TV), thickness, and number (distal femur metaphysis) increased as the insulin dose increased. Delayed CSII improved glycated hemoglobin (HbA1c), but blood glucose levels remained relatively high (well above non-diabetic levels). Interestingly, it returned the resorption and formation markers to similar levels as those seen in non-T1D control mice. This apparent return after 4 weeks of CSII translated to a partial rescue of the structural strength of the femur mid-shaft. Delayed CSII also increased Tb.Th to levels seen in non-T1D controls but did not fully restore BV/TV. The use of exogenous insulin should be considered in pre-clinical studies investigating the effect of T1D on bone as insulin therapy maintains bone structure without necessarily lowering glucose below diabetic levels.


## 1. Introduction

Individuals affected with type 1 diabetes (T1D) are 6 times more likely to suffer a fracture than the non-diabetic population (Janghorbani et al., 2007), and low areal bone mineral density (aBMD) is associated with poor glycemic control (Danielson et al., 2009). Even though T1D patients receive insulin via multiple daily injection therapy (MDI) or via continuous subcutaneous insulin infusion therapy (CSII) to achieve glycemic control, they have a greater fracture risk in adulthood compared to age- and sex-matched non-diabetic individuals (Weber and Schwartz, 2016). The reason for this elevated risk is not entirely explained by low aBMD (Vestergaard, 2007), the primary clinical measurement to predict fracture risk. Thus, there is a need for pre-clinical models that mimic the human condition in order to identify the deleterious changes to bone that explain how T1D increases fracture risk or decreases fracture resistance.

The administration of streptozotocin (STZ), which is cytotoxic to beta cells of the pancreas, is a well-established rodent model of T1D.

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The toxin causes a reduction in insulin production and subsequent hyperglycemia with the severity depending on the strain of the rodent (Rodrigues et al., 1997; Shimizu et al., 2012) and the dose of STZ (Ventura-Sobrevilla et al., 2011). Typically induced between 11-week to 14-week of age in either mice or rats, STZ-T1D impedes normal bone mass accrual resulting in lower structural strength of cortical bone and reduced trabecular bone within several weeks as compared to non-diabetic controls (Nyman et al., 2011; Silva et al., 2009). The resulting hypoinsulinemia/hyperglycemia in the STZ model also affects the material properties of cortical bone (fracture resistance independent of bone structure), but durations of T1D exceeding 8 weeks and 14 weeks are required before significant differences in material strength and toughness between diabetic and control rodents, respectively, are typically observed (Nyman, 2012).

Despite the known importance of insulin signaling in osteoblasts to bone mass accrual (Fulzele et al., 2010; Thrailkill et al., 2014), insulin co-therapy is not always included in pre-clinical studies investigating the effect of bone- or glucose-targeting therapies on preventing the T1D-related decrease in bone strength (Altan et al., 2007; Glorie et al., 2014; Maycas et al., 2016; Thrailkill et al., 2016; Zhang et al., 2014). In early rat studies, daily subcutaneous injections of insulin improved the structural strength of the femur mid-shaft (Dixit and Ekstrom, 1980) and femoral neck (Hou et al., 1993) when compared to untreated alloxan-induced T1D rats. Recent rat studies report daily subcutaneous injections of insulin nearly restored the STZ-related loss i) in peak force endured by the femur mid-shaft during three-point (3 pt) bending (Rao Sirasanagandla et al., 2014), ii) in total femur aBMD (Zhang et al., 2008), iii) in femoral neck and mid-shaft diameters (Abd El Aziz et al., 2015), iv) in femur mid-shaft aBMD (but not in tensile mechanical properties) (Erdal et al., 2012), and v) in peak bending stress (but not in peak force during 3 pt. bending) of the tibia mid-shaft (Bortolin et al., 2016). While the insulin dosing (~2 IU/day) tended to be similar among these studies, discrepancies in the protective effect on mechanical properties could be due to other experimental factors (e.g., age and strain of the rat, duration of T1D before therapy, dose of STZ, method of mechanical testing). There are at least two rat studies of T1D involving 4 weeks of CSII: one reporting an improvement in trabecular bone volume fraction with treatment compared to untreated rats (Hie et al., 2011) and one reporting no treatment effect on the osteointegration of miniscrews even when combined with intermittent parathyroid hormone (PTH) injections (Rybaczek et al., 2015).

To the best of our knowledge, there are no rodent studies reporting how variable insulin therapy, delivered as escalating doses of insulin via CSII, impacts bone early in the course of T1D (i.e., first 4 weeks of established diabetes) or whether delayed insulin therapy could also be beneficial in promoting bone strength in T1D. Therefore, we first studied the dose-dependent relationship between insulin therapy and bone strength in mice by assessing the effect of continuous insulin delivery on cortical structure and trabecular architecture in the early phase of diabetic bone disease. Next, we studied the effect of starting CSII after ~4 weeks of persistent STZ-induced T1D on mouse bone. The two hypotheses then were: i) CSII early in the course of T1D could prevent the diabetes-induced deterioration of cortical bone structure and strength as well as the deterioration of trabecular bone microarchitecture in a dose-dependent fashion (prevention study) and ii) CSII could rescue the diabetes-induced deterioration of cortical bone structure and trabecular bone microarchitecture (Study 2: rescue study). In both studies, CSII lasted for 4 weeks (see Fig. 1 for specifics of each study design).

## 2. Materials and methods

### 2.1. Study design

#### 2.1.1. Prevention study

Over 5 days, 11-week old, male, DBA/2 J mice (The Jackson Laboratories, Bar Harbor, ME) received intraperitoneal (ip) injections of STZ at 40 mg/kg/day in 100 mM citrate buffer. After confirming persistent hyperglycemia (non-fasting blood glucose remaining above 250 mg/dl), 13-week, STZ-diabetic mice were treated with saline vehicle (0.0 dose) or insulin (Humulin R Insulin, Eli Lilly and Co., Indianapolis, IN) at 1 of 3 doses (0.0625, 0.125, and 0.25 IU/day; n = 4–5 per group) using an osmotic minipump (Model 2004, ALZET®, Cupertino, CA) for 4 weeks and then euthanized. Following the instructions from the manufacturer, the Alzet pump was implanted subcutaneously in the left anterodorsal region. Matched for age, additional male DBA/2 J mice were also administered ip injections of STZ at 40 mg/kg/day (T1D: n = 9 but 8 individual femurs available for analysis) or only buffer (100 mM citrate buffer) over 5 days (non-T1D: n = 10) and euthanized at 17-weeks without

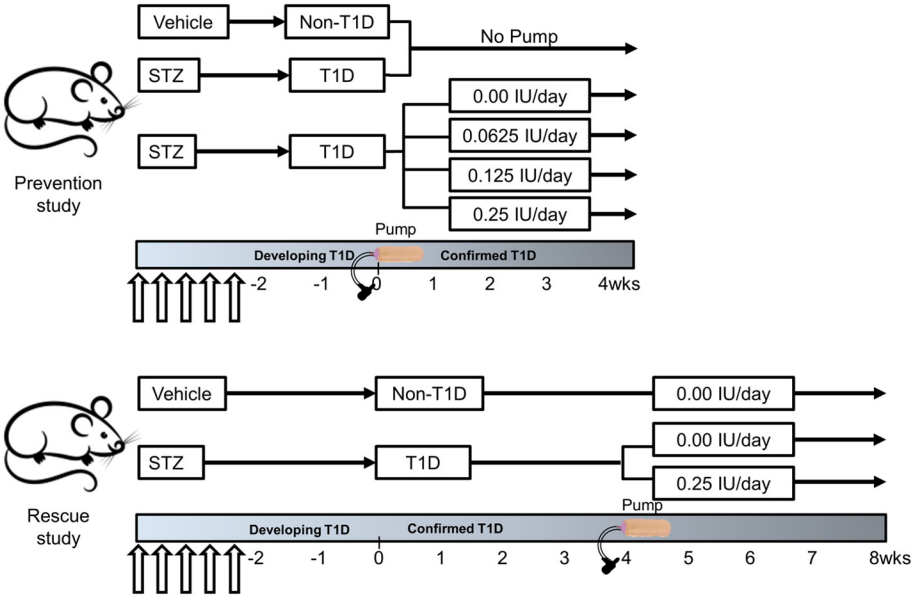


**Fig. 1.** Overview of the two study designs. In the prevention study, insulin was administered via CSII at escalating doses early in T1D duration. Non-diabetic and diabetic mice were age-matched to the mice receiving the insulin pumps in order to establish the effect of diabetes on bone. In the rescue study, insulin therapy did not commence until 6 weeks after the first STZ injection (arrows).