Low Dose Aspirin Therapy Decreases Blood Glucose Levels but Does not Prevent Type I Diabetes-induced Bone Loss

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Key Words

Bone • Diabetes • Aspirin • Glucose • Adipocyte

Abstract

Background: Diabetes is strongly associated with increased fracture risk. During T1-diabetes onset, levels of blood glucose and pro-inflammatory cytokines (including $\text{TNF}\alpha$) are increased. At the same time, levels of osteoblast markers are rapidly decreased and stay decreased 40 days later at which point bone loss is clearly evident. Inflammation is known to suppress bone formation and induce bone loss. Previous co-culture studies indicate that diabetic bone is inflamed and diabetic bone marrow is capable of enhancing osteoblast death *in vitro*. Here we investigate a commonly used non-steroidal anti-inflammatory drug, aspirin, to prevent T1-diabetic bone loss *in vivo*. **Methods:** We induced diabetes in 16-week-old male C57BL/6 mice and administered aspirin in the drinking water. **Results:** Our results demonstrate that aspirin therapy reduced diabetic mouse non-fasting blood glucose levels to less than 400 mg/dl, but did not prevent trabecular and cortical bone loss. In control mice, aspirin treatment

increased bone formation markers but did not affect markers of bone resorption or bone density/volume. In diabetic mice, bone formation markers and bone density/volume are decreased and unaltered by aspirin treatment. Bone resorption markers, however, are increased and 2-way ANOVA analysis demonstrates an interaction between aspirin treatment and diabetes ($p < 0.007$). Aspirin treatment did not prevent the previously reported diabetes-induced marrow adiposity. **Conclusion:** Taken together, our results suggest that low dose aspirin therapy does not negatively impact bone density in control and diabetic mice, but could potentially increase bone resorption in T1-diabetic mice.

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Introduction

Type I (T1) diabetes is a metabolic disorder that affects nearly 1 million Americans. Intensive treatments for diabetic patients to maintain euglycemia are critical

but remain difficult. Prolonged exposure to hyperglycemia results in increased incidence of secondary complications such as retinopathy, neuropathy, neuropathy, heart disease and osteoporosis [1-6]. Osteoporosis increases fracture risk. Both male and female T1-diabetic adults and adolescents are vulnerable to decreased bone density and increased fracture risk [7-18]. Animal models of T1-diabetes display comparable bone loss to patients thus allowing for extensive investigation of bone pathologies and mechanisms contributing to diabetes-induced bone loss [6, 19, 20]. To date, bone loss in T1-diabetic mice appears to occur at a number of skeletal sites and is independent of gender [6, 19, 20], similar to what is seen in human patients. While the exact mechanisms accounting for T1-diabetes-induced bone loss remain unknown, recent studies demonstrate a role for bone inflammation [6, 19-22].

T1-diabetes is strongly associated with inflammation. Hyperglycemia contributes to increased systemic inflammation, as well as local (bone) inflammation [23]. Recent reports demonstrate an elevation of several pro-inflammatory cytokines, including serum and bone TNF α , IFN γ , and IL-1, during diabetes onset [23, 24]. At the same time osteoblast death is increased and osteocalcin expression is reduced [22, 23]. IFN γ , TNF α , and IL-1 are known mediators of osteoblast death [25-33]. Furthermore, treatment with TNF α neutralizing antibodies reduces osteoblast death caused by diabetic marrow cells [22]. Correspondingly, two clinically used TNF α inhibitors, entanercept or pegsunercept, suppress diabetes-enhanced death of fibroblasts, bone-lining cells, and osteoblasts arising from periodontal disease *in vivo* [34-36]. Thus, decreasing bone inflammation could prevent diabetes-induced bone loss.

A commonly used anti-inflammatory drug, aspirin, is known to reduce diabetes related inflammation [37, 38] and decrease blood glucose levels in T1-diabetic rats [39]. Aspirin binds and inhibits cyclooxygenase (COX) enzyme and decreases prostaglandin (PG) production [40]. COX exists as two isoforms: COX-1 and COX-2. COX-1 is constitutively active and highly expressed in various tissues throughout the body and functions to maintain normal prostaglandin levels. COX-2 is an inducible enzyme correlated with inflammation and more highly expressed in osteoblasts compared to COX-1 [41, 42]. Some studies (although controversial) have advocated that aspirin treatment is advantageous to bone health by improving bone mineral density in trabecular and cortical bone parameters in aged populations [43, 44]. Similar results indicate that ovariectomized mice treated

with aspirin had higher bone density than non-aspirin treated mice. Furthermore, aspirin has been reported to decrease bone marrow stromal cell apoptosis [45].

Based on the above studies, we hypothesized that bone inflammation, illustrated by increased pro-inflammatory cytokine levels, contributes to the diabetic bone pathology. Here, we demonstrate that regular, low dose aspirin treatment decreases blood glucose levels in diabetic mice, but does not prevent diabetes-induced bone loss. Additionally, aspirin treatment increased diabetic marrow adiposity and bone loss beyond the normal diabetic phenotype.

Materials and Methods

Diabetic Mouse Models

Diabetes was induced in adult (15-16 week old) C57BL/6 male mice (Harlan Laboratories, Indianapolis, Indiana) by 5 daily intraperitoneal (IP) injections of streptozotocin (50 mg/kg body weight in 0.1 M citrate buffer, pH 4.5). Controls were given citrate buffer alone. Aspirin was delivered in the water of control and diabetic mice on 1-day post first injection (dpi) at a concentration of 200 μ g/kg (comparable to a human dose of 70mg/kg) for the entire experiment (40 dpi). The water was changed every third day to maintain dosage. Mice were maintained on a 12-hour light, 12-hour dark cycle at 23°C, and given standard lab chow and water *ad libitum* (if not treated with aspirin). Body weight and food and water intake were monitored during diabetes induction and throughout the experiment. Diabetes was confirmed 12 days after initial STZ injection using an Accu-Check compact glucometer (Roche Diagnostics Corporation, Indianapolis, IN) with a drop of blood from the saphenous vein. Total body, tibialis anterior, and subcutaneous femoral fat pad mass were recorded. Animal procedures have been completed with the approval of Michigan State University Institutional Animal Care and Use Committee.

RNA Analysis

Immediately after euthanasia, one tibia and femur were cleared of soft tissue and snap frozen in liquid nitrogen and stored at -80°C. Frozen tibias were crushed under liquid nitrogen conditions, homogenized, and placed in Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH). RNA integrity was determined through formaldehyde-agarose gel electrophoresis. cDNA was synthesized through a reverse transcriptase reaction utilizing Superscript II Reverse Transcriptase Kit and oligo dT (12-18) primers (Invitrogen, Carlsbad, CA), amplified by quantitative real time PCR with iQ SYBR Green Supermix (Bio-Rad, Hercules, CA), and gene-specific primers. Forward and reverse primers used as previously stated: aP2, HPRT, OPG, RANKL, and TRAP5 [21, 22, 46]. Expression of HPRT does not alter in diabetes and therefore used as a housekeeping gene. Real Time PCR was carried out for 40 cycles, each cycle consisting of 95°C for 15 seconds, 60°C for 30 seconds (except osteocalcin which has an

annealing temperature of 65°C) and 72°C for 30 seconds. RNA-free samples were used as a negative control and did not produce amplicons. PCR products were separated on 1.5% agarose gel electrophoresis and sequenced to verify the desired gene is being amplified.

Micro-computed Tomography (μ CT) analysis

Femurs and vertebrae were fixed in 70% EtOH and scanned using the GE Explore μ CT system at a voxel resolution of 20 μ m from 720 views with a beam strength of 80kvp and 450uA. Scans included bones from each condition and a phantom bone to standardize the grayscale values and maintain consistency between runs. Using the systems auto-threshold (800) and an isosurface analysis, trabecular bone densities: bone volume fraction (BV/TV), bone mineral density (BMD) and content (BMC), and trabecular thickness (Tb. th), number (Tb. n) and spacing (Tb. sp) are measured in the trabecular region defined at 0.17mm under the growth plate of the femur extending 2mm toward to diaphysis, and excluding the outer cortical shell. Cortical bone measurements are determined with a 2-mm³ region of interest (ROI) in the mid-diaphysis. Cortical BMC, BMD, moment of inertia, cortical thickness, perimeter and area and trabecular parameters were computed by the GE Microview Software for visualization and analysis of volumetric image data.

Bone Histology and Histomorphometry

Bones are fixed in 10% buffered formalin and transferred to 70% EtOH after 24 hours where they underwent dehydration, clearing and infiltration of fixed femurs on a routine overnight processing schedule. Samples are then paraffin embedded on a Sakura Tissue Tek II embedding center and sectioned at 5 μ m on a Reichert Jung 2030 rotary microtome. Osteoclasts were identified, by staining for tartrate resistant acid phosphatase (TRAP) (Sigma). Osteoclasts and adipocytes were identified in the trabecular region of the femur as defined at 0.17mm under the growth plate of the femur extending 2mm toward to diaphysis. Osteoclast surface was measured in three trabecular regions for each mouse and expressed as a percent of total trabecular surface. Visible adipocytes, greater than 30 μ m, were counted in the same trabecular region.

Mineral apposition rate

Mice were injected intraperitoneally with 200 μ l of 10mg/ml calcein (Sigma, St. Louis, MO, USA) dissolved in saline 7 and 2 days prior to harvest. L3-L4 vertebrae were fixed in formalin at time of harvest then transferred to 70% ethanol 24 hours later. Vertebrae were then embedded and sectioned at 5 μ m on a Reichert Jung 2030 rotary microtome. Sections were photographed (5 images per bone) under UV light and the distance between the calcein lines were measured.

Serum Measurements

Blood was collected at the time of harvest, allowed to clot at room temperature for 5 minutes, then centrifuged at 4,000 rpm for 10 minutes. Serum was removed and stored at -80°C. Serum went through no more than two freeze/thaw cycles.

Serum TRAP5b and Osteocalcin were measured using a Mouse TRAP and OC assay kits (SB-TR103, Immunodiagnostic Systems Inc., Fountain Hills, AZ, USA and BT-470, Biomedical Technologies Inc., Stoughton, MA, USA, respectively) according to the manufacturer's protocol.

Statistical Analysis

All data is presented as mean $\pm$ standard error. Statistical significance (p-value < 0.05) of main effects (aspirin treatment or diabetes) as well as aspirin treatment x diabetes interaction (which determines if diabetes alters the treatment effect or vice versa) was determined using factorial analysis of variance (ANOVA) with SPSS statistical software (Chicago, IL). Student's t-test was used where needed.

Results

Previous reports indicate that T1-diabetes causes bone inflammation. To test if a general anti-inflammatory therapy could be used to treat T1-diabetic bone loss, we induced diabetes in mice and began treatment with or without aspirin. After 40 days, mice were examined. Consistent with previous reports, blood glucose levels were decreased in treated compared to non-treated diabetic mice (383mg/dl and 494mg/dl, respectively). Analysis by 2-way ANOVA indicates that aspirin treatment, diabetes and treatment X diabetes all significantly affected blood glucose levels (p<0.010, p<0.000, and p<0.004, respectively). In contrast, aspirin treatment did not significantly modify any of the other general physiologic responses to T1-diabetes. Specifically, diabetes induction with or without aspirin treatment decreased total body mass (14% and 14%, respectively), tibialis anterior mass (33% and 27%, respectively) and femoral fat pad mass (61% and 58%, respectively) relative to corresponding condition controls (Fig. 1). Interestingly, aspirin therapy alone decreased the body weight of control mice by 7% (p<0.038).

To determine if aspirin treatment prevented T1-diabetes-induced bone loss, we imaged femurs by micro-computed tomography. Representative 3-dimensional isosurface images are shown in Fig. 2. Consistent with previous studies, diabetic mice lost an average of 30% bone volume fraction (BV/TV) and displayed decreased bone mineral content, bone mineral density, and trabecular number. Aspirin treatment did not alter control or diabetic mouse bone parameters. Specifically, a 2-way ANOVA analysis did not detect a treatment X disease association for this parameter. Cortical bone parameters decreased in diabetic mice

Fig. 1. Aspirin decreases blood glucose levels in diabetic mice compared to non-treated diabetic mice, but did not prevent other diabetes-induced physiological changes. Body mass, non-fasting blood glucose levels, peripheral fat mass and muscle (tibialis) mass were collected at time of harvest for control (white bars) and diabetic (black bars) mice treated with or without aspirin. Bars represent the average value $\pm$ SE (n=7 per group). Statistical analyses by factorial analysis of variance (ANOVA) was used to determine the effects of aspirin treatment, diabetes and treatment x diabetes on body parameters. * indicates $p < 0.05$.

Fig. 2. Aspirin treatment does not prevent diabetes-induced trabecular bone loss. Representative μ CT isosurface images of trabecular bone volume fraction (BV/TV) in distal femurs were obtained from control and diabetic mice with or without aspirin treatment. Graphical representation of BV/TV is displayed from control (white bars) and diabetic (black bars) in the presence or absence of aspirin. Bars represent the average value $\pm$ SE (n=7 per group). Statistical analyses by factorial analysis of variance (ANOVA) was used to determine the effects of treatment, diabetes and treatment x diabetes on bone volume fraction. * indicates $p < 0.05$.

treated with (-13%) or without (-8%) aspirin compared to respective controls, resulting in decreased cortical area and decreased percent cortical area per total area (Fig. 3, Table I).

T1-diabetes is a known suppressor of bone formation; therefore, we investigated the role of aspirin therapy on several markers of bone formation. Consistent with previous studies, diabetes decreased serum osteocalcin levels (-29%) and tibia osteocalcin mRNA levels (-40%) compared to control mice. Interestingly, aspirin treatment alone elevated serum and tibia

osteocalcin levels (35% and 60%, respectively) in control mice. Aspirin therapy did not, however, prevent diabetic suppression of bone formation markers (Fig. 4). Analysis by 2-way ANOVA indicated that aspirin treatment as well as diabetes induction affected serum and tibia osteocalcin levels ($p < 0.002$ and $p < 0.002$, respectively, and $p < 0.009$ and $p < 0.006$, respectively). However there was no interaction between treatment and disease.

We also examined markers of bone resorption: serum TRAP5b, osteoclast surface per total trabecular surface, and Trap5 and RANKL/OPG mRNA ratio.

Trabecular	Control (n=7)	Diabetic (n=7)	+Aspirin	
			Control (n=7)	Diabetic (n=7)
BMC (mg)	0.48 ± 0.02	0.34 ± 0.02*	0.43 ± 0.04	0.33 ± 0.03*
BMD (mg/cc)	154 ± 5	113 ± 9*	143 ± 13	108 ± 10*
BV/TV (%)	19.4 ± 1.2	13.4 ± 1.6*	19.1 ± 3.2	9.2 ± 1.5*
Tb. Th (μm)	40.0 ± 1.2	36.4 ± 2.0	40.0 ± 3.9	33.2 ± 2.8
Tb. N (1/mm)	4.79 ± 0.26	3.56 ± 0.27*	4.56 ± 0.42	3.27 ± 0.53*
Tb. Sp. (mm)	0.17 ± 0.01	0.26 ± 0.03*	0.19 ± 0.02	0.32 ± 0.05*
Cortical				
Ct. Th (mm)	224.2 ± 2.8	206.4 ± 4.7*	218.4 ± 9.0	189.5 ± 5.0*
I _{max} (mm ⁴)	0.2 ± 0.03	0.16 ± 0.01	0.18 ± 0.01	0.12 ± 0.01*
Ec. Pm (mm)	3.68 ± 0.07	3.79 ± 0.07	3.76 ± 0.06	3.97 ± 0.11
Ps. Pm (mm)	5.09 ± 0.08	5.01 ± 0.07	5.07 ± 0.06	5.07 ± 0.09
Ma. Ar (mm ²)	0.93 ± 0.03	0.96 ± 0.04	0.96 ± 0.03	1.05 ± 0.04
Ct. Ar (mm ²)	0.92 ± 0.02	0.78 ± 0.03*	0.88 ± 0.05	0.70 ± 0.03*
Tt.Ar (mm ²)	1.85 ± 0.06	1.76 ± 0.05	1.83 ± 0.05	1.75 ± 0.05
Ct. Ar/ Tt. Ar (%)	49.9 ± 0.4	44.6 ± 1.6*	47.8 ± 1.7	40.1 ± 1.5*

Table 1. μ CT femur parameters in control and diabetic mice. Significance: * $p < 0.05$ compared to non-treated control; ^ $p < 0.05$ compared to non-treated diabetic. Abbreviations: BMC, bone mineral content, BMD, bone mineral density, BV/TV, bone volume fraction, Tb. Th, trabecular thickness, Tb. Sp, trabecular spacing, Tb. N, trabecular number, I_{max}, maximum moment of inertia, Ec. Pm, Endocortical perimeter, Ps. Pm, Periosteal Perimeter, outer perimeter, Ma. Ar, Marrow area, Ct. Ar, Cortical area, Tt. Area, total area.

Fig. 3. Cortical bone thickness is decreased in diabetic mice. Representative μ CT isosurface images of cortical bone thickness from the mid-diaphysis of femurs were obtained from control and diabetic mice with or without aspirin treatment. Graphical representation of cortical bone thickness is displayed from control (white bars) and diabetic (black bars) in the presence or absence of aspirin. Bars represent the average value $\pm$ SE (n=7 per group). Statistical analyses by factorial analysis of variance (ANOVA) was used to determine the effects of treatment, diabetes and treatment x diabetes on cortical thickness. * indicates $p < 0.05$.

Serum TRAP5b and the percent osteoclast surface/total trabecular surface were elevated in untreated diabetic mice by 30% and 47%; the former reached statistical significance. In aspirin treated diabetic mice, both serum Trap5b and osteoclast surface were significantly increased by 42% and 107%, respectively (Fig. 5). Two-way ANOVA analysis indicated that aspirin therapy, diabetes treatment, and aspirin X diabetes all account for the increase in serum TRAP5b levels ($p < 0.006$, $p < 0.000$, and $p < 0.007$, respectively). Furthermore, we measured the mRNA ratio of RANKL/OPG expression and observed

Fig. 4. Aspirin treatment increases bone formation markers. Serum and tibia mRNA osteocalcin expression was analyzed in control and diabetic mice and treated with or without aspirin water. Bars represent the average value $\pm$ SE (n=7 per group) in control (white bars) and diabetic (black bars) in the presence or absence of aspirin. RNA levels are calculated relative to the housekeeping gene HPRT. Statistical analyses by factorial analysis of variance (ANOVA) was used to determine the effects of aspirin, diabetes and aspirin x diabetes on bone formation markers. * indicates $p < 0.05$.

elevated ratios in aspirin treated diabetic mice compared to respective control, while non-treated diabetic mice displayed a trend toward an elevated RANK/OPG ratio (Fig. 5). Two-way ANOVA analysis determined that diabetes alone is responsible for these changes ($p < 0.032$).

Previous reports indicate a significant increase in bone marrow adiposity in response to diabetes; therefore, we measured tibia aP2 (marker for mature adipocytes) mRNA expression and total adipocyte number per total

Fig. 5. Increased bone resorption results from diabetes induction. Serum TRAP5 expression, percent osteoclast surface per total trabecular surface, and RANKL/OPG ratio and Trap5 mRNA were analyzed in control (white bars) and diabetic (black bars) mice in the presence or absence of aspirin treatment. Bars represent the average value $\pm$ SE (n=7 per group). RNA levels were calculated relative to the housekeeping gene HPRT. Statistical analyses by factorial analysis of variance (ANOVA) was used to determine the effects of aspirin treatment, diabetes and aspirin treatment x diabetes on bone resorption and remodeling markers. * indicates $p < 0.05$ compared to respective control. ^ indicates 0.05 compared to non-treated diabetic mice.

marrow area in response to aspirin therapy. As expected, diabetes induction resulted in a 70% increase in aP2 expression and a 92% increase in adipocyte number/marrow area compared to control mice (Fig. 6). Aspirin therapy did not prevent the diabetes-induced marrow adiposity. Specifically, aP2 expression increased 50% in aspirin treated diabetic mice compared to controls while adipocyte number/marrow area increased 171% (Fig. 6). Statistical analysis by 2-way ANOVA indicated that

Fig. 6. Diabetes increased marrow adiposity in aspirin treated and non-treated mice. Representative adipocyte images were obtained from femurs of control and diabetic mice given aspirin water or regular water. Graphical representation of adipocyte numbers in control (white bars) and diabetic (black bars) mice with or without aspirin treatment. Expression late adipocyte mRNA marker, aP2 was also examined and calculated relative to the housekeeping gene HPRT. Bars represent the average value $\pm$ SE (n=7 per group). Statistical analyses by factorial analysis of variance (ANOVA) was used to determine the effects of aspirin, diabetes and aspirin x diabetes on adipocyte parameters. * indicates $p < 0.05$ compared to respective control.

diabetes affected adipocyte number and aP2 expression ($p < 0.001$ and $p < 0.023$, respectively), while aspirin treatment alone did not alter marrow adiposity (Fig. 6).

Conclusion

T1-diabetes and its associated hyperglycemia contribute to increased systemic inflammation as well as

local bone inflammation [23]. For example, studies demonstrate elevated levels of pro-inflammatory cytokines, such as TNF α , in diabetic serum as well as in the bone during diabetes onset [22-24]. Inflammation in the bone microenvironment is known to contribute to bone loss. Therefore, decreasing bone marrow inflammation could serve as a potential therapeutic strategy for inhibiting bone loss. Here, we investigated the influence of aspirin therapy on control and diabetic mouse bone parameters. Despite aspirin treatment reducing diabetic blood glucose levels, it did not prevent the diabetes-induced bone pathology.

Research investigating aspirin's effects on serum blood glucose levels dates back to 1877 when researchers found that high doses of sodium salicylate (aspirin) reduced glucosuria in diabetic patients, however conflicting results have been reported [38, 47-53]. More recent clinical studies report that T2-diabetic patients showed a 40 mg/dl decrease in fasting plasma glucose levels and a 21% reduction in blood glucose levels during a mixed-meal tolerance test after only 2 weeks of aspirin treatment [38]. Additionally, diabetic rodents treated with aspirin for a short term (7 days) or long-term (45 days to 5 months) regimes display significantly decreased blood glucose and HbA1c levels, similar to our findings [39, 49, 50]. In contrast, a small clinical study showed that neither aspirin treatment nor ibuprofen reduced fasting blood glucose levels in T2-diabetic patients, however this study had a small number of subjects compared to other studies, making their results difficult to draw conclusions from [54]. How does aspirin treatment reduce hyperglycemia? It is hypothesized that aspirin contributes to decreased basal rates of hepatic glucose production, enhanced tissue insulin sensitivity, decreased insulin clearance, and decreases oxidative stress in diabetic patients [38, 50].

Interestingly, although aspirin treatment decreased blood glucose levels, it did not prevent T1-diabetes-induced bone loss. This is likely because aspirin-treated mice still displayed average blood glucose levels well above 300mg/dl, the level we use to diagnose T1-diabetes in mice. Past studies in our laboratory that examined leptin treatment as a therapy for diabetic bone pathology also demonstrated a significant reduction in blood glucose levels; however, similar to the current study, the reduction in blood glucose levels did not prevent bone loss [55]. This may indicate that hyperglycemia is not the sole mediator of diabetic bone loss and/or, more likely, that even modest hyperglycemia which is on the order of what is seen in T1-diabetic human patients is capable of contributing to the T1-diabetic bone pathology.

The direct effects of aspirin treatment on bone remain controversial. Some studies indicate that aspirin therapy significantly benefits both trabecular and cortical bone density parameters. One clinical report indicates that aspirin users had elevated whole body BMD (+4.2%) and hip BMD (+4.6%) compared to non-users [43, 56]. Additionally, aspirin treatment improved bone density and remodeling in OVX mice as well as in aged human population by inhibiting bone resorption [43-45]. Similar to the increased osteogenesis seen in aspirin treated OVX mice, our studies revealed that aspirin increased serum OC levels in control mice, although BV/TV did not change. The former study did not include aspirin treated controls, so we cannot make a direct comparison for this condition. In our study aspirin treatment also caused a decrease in body weight that could contribute to the lack of a notable change in BV/TV.

In contrast, several studies indicate that aspirin/NSAIDs can suppress overall bone remodeling and density, especially at high doses. This is thought to result from a reduction in prostaglandins specifically PGE₂ [56, 57], because increasing PGE₂ levels can increase bone formation [58] and induce bone remodeling [57] in rodent models. Interestingly, NSAID suppression of inflammation has been proposed to be detrimental to certain stages of fracture repair, since some cytokines may promote osteogenesis and angiogenesis which are required for healing [59]. We previously demonstrated that expression of pro-inflammatory cytokines, such as IL-1, TNF α , and IL-6, is elevated in T1-diabetic bone [23]. These same cytokines also mediate osteoblast

PGE₂ production through enhanced expression of COX-2 [60]. Too much inflammation, can suppress fracture healing as indicated in a previous study in T1-diabetic mice where treatment with a targeted anti-inflammatory approach, a TNF α , improved fracture healing [36].

In summary, low dose aspirin treatment, which is often prescribed for diabetics at risk for cardiovascular complications, reduced blood glucose levels but did not prevent T1-diabetes-induced bone loss or bone marrow adiposity. While aspirin treatment did raise bone resorption markers in T1-diabetic mice, it did not cause further bone loss. It is possible that more specific inhibitors of pro-inflammatory cytokines could provide more targeted anti-inflammatory effects to reduce diabetes-induced inflammation and osteoblast apoptosis. Further research is needed to accurately dissect which pro-inflammatory cytokines are key to mediating diabetes-induced inflammation and bone loss.

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