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Osteo-promoting effects of insulin-like growth factor I (IGF-I) in a mouse model of type 1 diabetes

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Abstract

Objective—Using a streptozotocin (STZ)-induced mouse model of type 1 diabetes (T1D), we have previously demonstrated that long-term diabetes inhibits regenerative bone formation during tibial distraction osteogenesis (DO) and perturbs skeletal integrity by decreasing cortical thickness, bone mineral density and bone's resistance to fracture. Because long-standing T1D is also associated with a deficiency of insulin-like growth factor I (IGF-I), we examined the effects of systemic IGF-I treatment on skeletal microarchitecture and strength, as well as on bone formation in diabetic mice.

Research Design and Methods—Streptozotocin-induced diabetic or control mice were treated with recombinant human IGF-I (rhIGF-I, 1.5 mg/kg/day as subcutaneous infusion) or vehicle throughout a 14 day DO procedure. Thereafter, trunk blood was assayed for glucose, insulin, rhIGF-I, mouse IGF-I and leptin. Bone formation in distracted tibiae was quantified. Effects on cortical bone strength and trabecular bone architecture were assessed by μ CT analysis and three-point bend testing of contralateral femurs.

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Results—New bone formation during DO was reduced in diabetic mice but significantly improved with rhIGF-I treatment. The contralateral femurs of diabetic mice demonstrated significant reductions in trabecular thickness, yield strength and peak force of cortical bone, which were improved with rhIGF-I treatment. rhIGF-I also reduced intracortical porosity in control mice. However, treatment with rhIGF-I did not normalize serum glucose, or correct concurrent deficiencies of insulin or leptin seen in diabetes.

Conclusions—These findings demonstrate that despite persistent hyperglycemia, rhIGF-I promoted new bone formation and improved biomechanical properties of bone in a model of T1D, suggesting that it may be useful as a fracture preventative in this disease.

Keywords

Distraction osteogenesis; IGF-I; diabetes; bone formation

1.1 INTRODUCTION

Type 1 diabetes (T1D) is associated with decreased bone density and portends a higher risk for hip and extremity fractures [1,2]. While the underlying mechanisms contributing to skeletal deficits in T1D are likely multifactorial, a consistent feature in T1D, both in humans and rodents, is a deficiency of insulin within the hepatic portal circulation. Lack of portal insulin, in turn, is associated with systemic deficiencies of insulin-like growth factor I (IGF-I) [3]. Because IGF-I mediates a number of osteo-promoting actions within skeletal tissues [4], deficiencies of IGF-I could contribute to skeletal abnormalities observed in T1D. IGF-I concentrations correlate positively with serum markers of bone formation in humans with T1D and IGF-I concentrations are lowest in osteopenic diabetic individuals compared to those without osteopenia [5–7]. Nevertheless, the role that IGF-I may play in the pathogenesis and/or prevention of T1D skeletal deficits has not been fully established. To address the concept that IGF-I might protect against development of skeletal deficits in T1D, we have studied the effects of exogenous IGF-I on skeletal microarchitecture, strength and regenerate bone in a mouse model of diabetic osteopathy [8].

1.2 MATERIALS AND METHODS

1.2.1 Animal Model

Forty 10-week-old, female, CD-1 mice from The Jackson Laboratory (Bar Harbor, Maine) were injected with streptozotocin (STZ: 40 mg/kg/d × 5 days, n=20) or vehicle (10 mM citrate buffer, n=20). Urine samples were monitored weekly for glucosuria to verify the development of diabetes. Twenty-one days after STZ administration, diabetic (STZ) or control (CON) mice were treated with rhIGF-I (1.5 mg/kg/day, n=10 in each group) or vehicle (PBS, n=10 in each group) as subcutaneous infusion via Alzet mini-osmotic pump, throughout a 14 day distraction osteogenesis procedure. The mini-osmotic pump was implanted on the same day that surgery (described below) was performed.

1.2.2 Distraction Osteogenesis (DO)

Mice were anesthetized with Nembutal (71 mg/kg). Four 27-gauge, 1.25 inch needles were drilled through the left tibia and an external fixator was secured to the pins. The skin was incised distal to the tibia crest, and a single hole was manually drilled through both cortices of the middiaphysis. Surgical scissors were used to fracture the cortex on either side of the hole. The fibula was fractured by direct lateral pressure. Distraction began 3 days after surgery at a rate of 0.075 mm twice a day (0.15mm/day) and continued for 14 days, resulting in an average final distraction gap length of 1.9 mm, or ~10% of the total tibia length. All mice were sacrificed on postoperative day 17 and limbs were harvested. After 48 hours of

fixation in 10% neutral buffered formalin, the left tibiae (distracted tibiae) were removed from the fixators for high-resolution single beam radiography, as we have previously described [8]. For quantification of mineralized bone by histology, H & E stained sections were selected to represent a central or near central gap location. A quadrilateral region of interest was outlined and recorded. New bone was defined as all organized osteoid/sinusoid columns. Both the proximal and distal endosteal new bone matrix was outlined and recorded. The % of new bone area within the distraction gap was calculated by dividing the new bone matrix area by the total distraction gap area [9]. The contralateral femurs (non-distracted leg) were analyzed by μ CT and biomechanical testing.

1.2.3 Micro-computed tomography (μ CT) analysis

To assess the effects of diabetes and rhIGF-I treatment on the trabecular architecture and cortical bone structure, femurs were immersed in PBS, and the long axis of the bone was aligned with the scanning axis of the μ CT40 (Scanco Medical, Brüttisellen, Switzerland). Cross-sectional images of regions of interest [cortex of each femur at the midpoint (2 mm in length) and the trabeculae within the distal metaphysis of the femur (0.24–1.2 mm below the growth plate)] were acquired with an isotropic voxel size of 12 μ m, at energy settings of 55 kVp and 145 mA, with 1000 projections per rotation, and an integration time of 300 ms, as we have previously described [10]. To select the trabecular compartment, the Scanco auto-contouring script was used to identify the endocortical surface [11]. Visual inspections were routinely performed to confirm fidelity of the contours within the endosteum. Bone tissue was segmented from air or soft tissue such that each region of interest had unique global threshold (trabecular: 205 per mille or 406.7 mgHA/cm³ and cortical: 400 per mille or 979.6 mgHA/cm³) and image filtration parameters (Gaussian support of 2 and sigma of 0.8 for cortical and 0.2 for trabecular) that were held constant across the experimental groups. The density calibration was checked by weekly scans of a hydroxyapatite phantom and did not vary during the course of the study. A beam hardening correction developed by the manufacturer specifically for bone applications was used in all scans, and in calculating the tissue mineral density (TMD), partial volume effects were suppressed by peeling surface voxels following segmentation of the bone [12]. Scanco evaluation software was used to quantify structural parameters of the mid-shaft, including mean cortical thickness (Ct.Th), average cross-sectional area (Ct.Ar), porosity ($Po = 1 - BV/TV$ in which TV is defined by outer and inner contour of the cortex), and the average moment of inertia with respect to the orientation of three-point bending test (I_{MIN}). Standard architectural characteristics of trabecular bone within the metaphysis included bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), bone surface per bone volume (BS/BV) and connectivity density (Conn.D). The average volumetric tissue mineral density of the mineralized tissue in mgHA/cm³ was also measured for the trabecular (Tb.TMD) and cortical (Ct.TMD) compartments.

1.2.4 Biomechanical Testing

To determine the differences in biomechanical properties between the experimental groups, a pre-load (1 N) first held each hydrated femur in place on the lower support points of a three-point bending fixture with the anterior side down (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, determined by three-point bending, included the yield force, peak force, yield strength and peak strength. Yielding occurred when the secant stiffness was 15% less than the initial stiffness, and the post-yield deflection was the displacement at fracture minus the displacement at the yield point. Material properties of modulus and strength of the cortex

were also estimated using standard beam theory [13]. The μ CT scans provided the moment of inertia and the distance between the neutral axis of bending and the outermost point in the anterior-posterior direction ($C_{\min}$).

1.2.5 Biochemical Assays

Serum was obtained on post-operative day 17 for measurement of glucose, rhIGF-I (ELISA AC-27F1, Immunodiagnostic Systems, Inc., Fountain Hills, AZ), mouse IGF-I (ELISA AC-42F1, Immunodiagnostic Systems Inc., Fountain Hills, AZ.; < 0.04% cross-reactivity with human IGF-I), leptin (Mouse Serum Adipokine Milliplex MAP kit MADPK-71K, Millipore Corp., Billerica, MA) and insulin (Mouse Bone panel 1A Milliplex MAP kit MBN1A-41K, Millipore Corp., Billerica, MA).

1.2.6 Statistical Analyses

A one-way analysis of variance (ANOVA) was used to determine differences in phenotypic and metabolic parameters across the four experimental groups (Table 1). Data are presented as mean $\pm$ standard error of the mean (SEM), and statistical significance was defined as $p < 0.05$. For analyses of bone strength and architecture, a two-way ANOVA was used to determine whether differences in each parameter existed between Control and STZ groups (diabetes effect) or between vehicle- and rhIGF-I-treated groups (IGF-I effect), followed by post-hoc comparisons (vehicle vs. rhIGF-I within Control or within STZ), when sufficient differences ($p < 0.05$) existed for the IGF-I main effect ($p < 0.1$). In the event that normality and/or variance assumptions of the ANOVA were violated, the p -value was determined using a non-parametric analysis (Mann-Whitney test or Kruskal-Wallis for more than two groups) for each comparison. Data in Table 3 are presented as mean $\pm$ SEM, and statistical significance of the two IGF-I comparisons was defined as $p < 0.0253$ (Sidak correction for two hypotheses).

1.3 RESULTS

1.3.1 Physiological parameters

Between group differences in animal weights and biochemical parameters are shown in Table 1. In diabetic mice, at the time of surgery and prior to IGF-I treatment, body weights and serum insulin concentrations were statistically lower and serum glucose concentrations were statistically higher compared with non-diabetic controls (i.e., CON vs. STZ and CON + IGF-I vs. STZ + IGF-I). Concurrent treatment with rhIGF-I during DO prevented the post-operative weight loss observed in control mice (CON vs. CON + IGF-I), while restoring and normalizing body weight in diabetic mice (CON + IGF-I vs. STZ + IGF-I). However, rhIGF-I treatment only partially (~50%) rectified the extreme hyperglycemia experienced by untreated diabetic mice, and did not correct the concurrent deficiencies of insulin or the adipokine, leptin, in diabetic mice. But, coincident with its anabolic effects on body weight, rhIGF-I-treatment of *control* mice was associated with a significant lowering of leptin concentrations (CON vs. CON + IGF-I), possibly suggesting an increase in lean body mass in these animals. The model studied was a shorter-term model of STZ-induced diabetes; in this study, endogenous mouse IGF-I was not significantly different between control and diabetic mice (Table 1). Furthermore, treatment with rhIGF-I did not significantly impact endogenous mouse IGF-I concentrations in either control or diabetic animals.

Final lengths of the contralateral (non-distracted) tibiae were also measured. There were no significant differences in final tibia length between the 4 groups ($p = 0.299$) indicating that systemic bone growth rates across treatment groups were comparable.

1.3.2 Bone formation in DO of the tibia

As shown in Figure 1, vehicle and IGF-I treated control animals both generated robust new bone in the DO gap, as assessed radiographically (A) and histologically (B). New bone formation in the DO gap did not improve in control animals over a 14-day distraction period when given exogenous IGF-I. In contrast, diabetic animals showed poor bone formation in the DO gap compared to control animals; however, rhIGF-I therapy improved bone formation in diabetic animals, reaching near-normal values. Representative depictions of each condition are shown in Figure 2 A (radiography), B (histology; new bone formation is outlined), and C (μ CT), demonstrating qualitatively that more abundant bone formation was evident in the distracted tibiae of diabetic mice when IGF-I was administered.

1.3.3 Analysis of the femur

As determined by the ANOVA, short-term diabetes (5 weeks duration) and rhIGF-I treatment (during the final 2 weeks) each affected a few properties of trabecular bone (Table 2). From the subsequent post-hoc analysis of the metaphyseal measures, trabecular thickness was enhanced with IGF-I treatment in the diabetic (STZ) animals (Table 3), while there was a trend toward lower BS/BV in both groups with IGF-I therapy. There were no differences in trabecular bone volume fraction (BV/TV) or in the other architectural parameters (e.g., Tb.N, Conn.D) among the groups (Table 2 and Table 3). However, diabetes and IGF-I treatment affected several structural and biomechanical properties of cortical bone (Table 2). In particular, IGF-I reduced cortical porosity with a trend toward thicker cortices in the non-diabetic group (Table 3). Moreover, IGF-I significantly improved the strength parameters of femurs from the diabetic animals (Table 3). There were no differences in stiffness, modulus, and post-yield deflection between Control and STZ groups, and between vehicle- and rhIGF-I-treated groups.

1.4 DISCUSSION

We and others have previously demonstrated that in long-term mouse models of T1D, skeletal microarchitecture and assessments of bone quality are significantly compromised, and regenerative bone formation is impaired [8,10]. In the current studies, we have shown that these deficits can, in large part, be restored when IGF-I is delivered systemically early in the course of diabetes. Even in the early stages of diabetes, regenerate bone formation is impaired; however, administration of rhIGF-I markedly improved bone formation in diabetic mice, but not in uncompromised control mice. Despite the fact that endogenous IGF-I deficiency was not present, IGF-I administration to diabetic mice also improved femur strength.

Similarly to IGF-I, insulin also has been shown to consistently improve microarchitecture, biomechanical properties and regenerate bone [8,14–16]. The ability of insulin action to improve skeletal homeostasis has been attributed to both direct effects on bone cells, as well as indirect effects through the lowering of glucose levels [1]. Exogenous IGF-I modestly lowered systemic glucose levels in diabetic mice; however, IGF-I did not lower glucose levels to normal ranges, suggesting that the effect of IGF-I on osteo-promotion may be principally through glucose-independent effects on bone cells.

Insulin and IGF-I can signal through overlapping downstream pathways in bone cells, suggesting they may be capable of performing similar roles to prevent diabetic bone disease [17]. Furthermore, IGF-I has been shown to increase beta cell replication in STZ-induced diabetic mice [18]. Thus, it is possible that IGF-I might improve endogenous insulin production in diabetic mice and thereby improve skeletal parameters indirectly via insulin.

However, this possibility seems unlikely in this short-term treatment model, since IGF-I treatment did not improve endogenous insulin production in diabetic animals (Table 1).

Several adipokines have been shown to possibly regulate bone mineral density [19]. We examined the adipokine leptin to see if it might be implicated in skeletal deficits observed in the diabetic mouse, and if IGF-I affected systemic concentrations of leptin. Currently, studies show that leptin may enhance or diminish bone architecture, and its effects may be mediated both through a central mechanism as well as a peripheral pathway [20]. Leptin concentrations were significantly lower in diabetic mice compared to controls, consistent with previous studies in STZ-induced diabetic rodents [21]. However, IGF-I therapy failed to alter leptin levels in diabetic mice, suggesting that while leptin deficiency might contribute to skeletal deficits observed in diabetic mice, improvements in bone microarchitecture, bone formation and strength achieved with IGF-I therapy cannot be attributed to changes in leptin.

This study was conducted using mice of an adolescent to young adult age equivalent, from start to completion of the study. As such, these results are particularly pertinent to a model of T1D, where childhood onset of disease is expected. Our results may not, however, be translatable to the model of diabetes and aging, in which further functional declines in skeletal integrity would be anticipated in later years. Never-the-less, we have previously demonstrated that both locally and systemically delivered IGF-I can significantly increase new bone formation in an aged mouse model (18-month-old C57BL/6 male mice) in which new bone formation is normally markedly impaired. It is possible, therefore, that even in older aged diabetic mice, IGF-I might improve bone quality [9].

Taken together, these findings demonstrate that despite persistent hyperglycemia, insulinopenia and leptin deficiency, rhIGF-I promotes new bone formation and improves biomechanical properties of bone in a short-term mouse model of T1D. While insulin and IGF-I are highly homologous peptides and can cross-signal through insulin receptors and IGF-I receptors, it is possible that they may exert independent effects on skeletal wellbeing. These findings in mice suggest that there may be a role for IGF-I in regulating bone integrity and skeletal regeneration in humans with T1D. Because T1D in humans is a condition of IGF-I deficiency, IGF-I, as a preventative therapy to improve upon morbidity incurred through diabetic bone disease and poor fracture healing, warrants further investigation.

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HIGHLIGHTS

- Systemic IGF-I treatment nearly normalizes diabetes-induced deficits in new bone formation during distraction osteogenesis in a mouse model of T1D.
- Despite persistent hyperglycemia and insulinopenia, 14 days of IGF-I treatment improves parameters of femur strength in diabetic mice.
- IGF-I treatment also increases trabecular thickness in diabetic mice.
- IGF-I administration, as preventative therapy, might lessen the morbidity incurred through diabetic bone disease and poor fracture healing.

Figure 1. Assessment of bone formation occurring during tibial distraction osteogenesis
Percent bone formation was assessed by radiology (A) and histology (B) as described in *Materials and Methods* and presented as means $\pm$ SEM. NS = non-significant. * $p < 0.05$ CON vs. STZ.

Figure 2. Bone formation during tibial distraction osteogenesis

Representative depictions of each condition (CON, CON + IGF-I, STZ, and STZ + IGF-I) are shown in panels A (radiography), B (histology; new bone formation is outlined), and C (μ CT).

TABLE 1

Selected phenotypic and metabolic properties of mice (mean $\pm$ SEM; $p < 0.05$).

PARAMETER	CONTROL	CON + IGF-I	STZ	STZ + IGF-I	p-value*			
					A	B	C	D
N	9	10	7	9				
Body weight at Surgery (gm)	31.4 $\pm$ 0.7	30.3 $\pm$ 0.4	28.6 $\pm$ 0.9	27.9 $\pm$ 0.9	0.261	0.538	0.022	0.014
Body weight at Sacrifice (gm)	29.7 $\pm$ 0.6	30.5 $\pm$ 0.7	27.1 $\pm$ 0.5	30.9 $\pm$ 0.7	0.439	0.001	0.609	0.014
Glucose at Surgery (mg/dL)	125.6 $\pm$ 11.7	136.6 $\pm$ 8.7	455.4 $\pm$ 32.5	416.8 $\pm$ 12.9	0.624	0.124	<0.001	<0.001
Glucose at Sacrifice (mg/dL)	99.9 $\pm$ 5.5	104.9 $\pm$ 7.1	495.4 $\pm$ 39.3	267.7 $\pm$ 36.0	0.882	<0.001	<0.001	<0.001
Insulin (pg/mL)	158.2 $\pm$ 23.1	117.8 $\pm$ 26.3	47.3 $\pm$ 7.6	50.8 $\pm$ 9.8	0.151	0.908	0.020	0.001
rhIGF-I (μ g/L)**	ND	81.5 $\pm$ 10.1	ND	82.9 $\pm$ 7.4	NA	NA	0.886	NA
mIGF-I (ng/mL)***	290.5 $\pm$ 47.1	203.5 $\pm$ 30.1	306.8 $\pm$ 63.5	186.5 $\pm$ 38.9	0.165	0.125	0.744	0.711
Leptin (pg/ml)	796.1 $\pm$ 188.3	470.0 $\pm$ 124.6	154.8 $\pm$ 65.8	203.5 $\pm$ 12.5	0.061	0.794	0.123	0.002

* A = CON vs. CON + IGF-I; B = STZ vs. STZ + IGF-I; C = CON + IGF-I vs. STZ + IGF-I; D = CON vs. STZ.

** Measures recombinant human IGF-I, reflecting therapeutic delivery via minipump.

*** Measures mouse IGF-I.

ND = not detected. NA = not applicable.

TABLE 2

Two-way ANOVA p-values for selected properties of trabecular and cortical bone ($p < 0.05$ and $p < 0.1$).

PARAMETER	Diabetes	IGF-I	Interaction
Trabecular bone volume fraction	0.910	0.185	0.926
Trabecular number	0.162	0.938	0.608
Trabecular thickness	0.038	0.002	0.456
Connectivity density	0.348	0.815	0.926
Bone surface per bone volume	<i>0.087</i>	0.004	0.464
Trabecular tissue mineral density	<i>0.057</i>	<i>0.056</i>	0.576
Cortical porosity	0.003	0.033	0.459
Cortical thickness	0.014	<i>0.065</i>	0.372
Bone cross-sectional area	0.010	0.041	0.495
Moment of inertia	0.214	0.989	0.803
Cortical tissue mineral density	0.011	0.260	0.614
Stiffness	<i>0.087</i>	<i>0.086</i>	0.328
Modulus	0.537	<i>0.080</i>	0.211
Yield force	0.009	<i>0.054</i>	0.519
Yield strength	0.027	0.043	0.542
Peak force	0.036	<i>0.062</i>	0.273
Bending strength	0.172	0.038	0.221
Post-yield deflection	0.435	0.159	0.599

A two-way analysis of variance was completed, examining the effects of diabetes or IGF-I treatment on structural and biomechanical properties of bone. Significant effects of each condition are highlighted in bold ($p < 0.05$) or italic ($p < 0.1$) font. No significant interactions between the two conditions were identified.

TABLE 3

Selected properties (mean $\pm$ SEM) for the femur metaphysis and mid-shaft.

PARAMETER	CONTROL	CON + IGF-I	STZ	STZ + IGF-I	p-value	
					A	B
N	9	10	7	9		
Metaphysis – trabecular bone						
BV/TV (%)	8.8 $\pm$ 1.3	10.7 $\pm$ 1.5	8.7 $\pm$ 1.8	11.0 $\pm$ 1.4	NS	-
Tb.N (1/mm)	3.58 $\pm$ 0.18	3.44 $\pm$ 0.24	3.77 $\pm$ 0.28	3.87 $\pm$ 0.19	NS	-
Tb.Th (mm)	0.045 $\pm$ 0.001	0.052 $\pm$ 0.003	0.040 $\pm$ 0.001	0.047 $\pm$ 0.001	0.062	0.001*
Conn.D (1/mm ³)	76.4 $\pm$ 13.1	77.3 $\pm$ 13.3	87.7 $\pm$ 19.4	93.1 $\pm$ 13.5	NS	-
BS/BV (mm ² /mm ³)	58.9 $\pm$ 2.4	51.3 $\pm$ 2.7	64.9 $\pm$ 3.5	55.4 $\pm$ 2.1	0.052	0.059
Tb.TMD (mgHA/cm ³)	971 $\pm$ 13	993 $\pm$ 10	953 $\pm$ 5	972 $\pm$ 7	0.203	0.027
Diaphysis – cortical bone						
Cortical Porosity (%)	9.8 $\pm$ 0.2	9.1 $\pm$ 0.2	10.7 $\pm$ 0.5	10.1 $\pm$ 0.2	0.024*	0.255
Cortical thickness (mm)	0.190 $\pm$ 0.005	0.205 $\pm$ 0.004	0.179 $\pm$ 0.010	0.186 $\pm$ 0.005	0.034	0.474
Cl.Ar (mm ²)	0.775 $\pm$ 0.031	0.846 $\pm$ 0.031	0.697 $\pm$ 0.025	0.757 $\pm$ 0.027	0.126	0.133
Moment of inertia (mm ⁴)	0.123 $\pm$ 0.008	0.125 $\pm$ 0.008	0.114 $\pm$ 0.011	0.112 $\pm$ 0.007	NS	-
Cl.TMD (mgHA/cm ³)	1328 $\pm$ 4	1335 $\pm$ 5	1316 $\pm$ 6	1321 $\pm$ 4	0.305	0.494
Stiffness (N/mm)	118 $\pm$ 10	128 $\pm$ 10	93 $\pm$ 13	116 $\pm$ 10	NS	-
Modulus (GPa)	10.4 $\pm$ 0.9	11.1 $\pm$ 0.7	8.8 $\pm$ 1.2	11.3 $\pm$ 1.2	0.551	0.082
Yield force (N)	18.4 $\pm$ 1.0	21.3 $\pm$ 1.8	14.2 $\pm$ 1.4	17.3 $\pm$ 1.2	0.185	0.115
Yield strength (MPa)	196 $\pm$ 9	224 $\pm$ 17	163 $\pm$ 15	194 $\pm$ 10	0.171	0.100
Peak force (N)	21.7 $\pm$ 1.2	23.3 $\pm$ 1.8	17.0 $\pm$ 1.2	21.3 $\pm$ 1.1	0.472	0.020*
Bending strength (MPa)	231 $\pm$ 7	246 $\pm$ 17	195 $\pm$ 14	240 $\pm$ 12	0.428	0.023*
Post-yield deflection (mm)	0.263 $\pm$ 0.036	0.190 $\pm$ 0.037	0.276 $\pm$ 0.046	0.238 $\pm$ 0.037	NS	-

* Significant at p<0.0253 for CON vs. CON + IGF-I (A) and STZ vs. STZ + IGF-I (B)

NS = IGF-I effect was not significant as determined by ANOVA.