THE EFFECT OF GLUCOVANCE THERAPY ON BIOMECHANICAL DETERIORATION OF BONE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Aim. Is to evaluate the influence of glucovance therapy on biomechanical properties of bone in streptozotocin - induced diabetes mellitus (DM) in rats.

Materials and Methods. A total of 28 male Wistar-Albino rats (12-week-old; 210-300 g) were divided into 4 groups including control (C; no treatment; n=7), sham [Sh; distilled water (gavage, for 8 weeks); n=7], diabetes [DM; streptozotocin (45 mg/kg, single i.p injection); n=7] and diabetes+ Glucovance treatment [DM+G; streptozotocin (45 mg/kg, single i.p injection) + Glucovance (Glucovance, 500/5 mg/kg/day/rat, gavage, for 8 weeks); n=7] groups. Body weight, blood glucose levels (BGLs), bone mineral density (BMD) and geometric/mechanical properties of bone tissue were evaluated. BGLs in diabetic rats were significantly increased compared to non-diabetic rats, while the body weights were decreased (p<0.05).

Results. A significant difference was not detected between groups with regard to cross-sectional area of diaphyseal femur (p>0.05). Maximum load, energy absorption capacity, ultimate stress, ultimate strain, toughness and displacement were shown to decrease and stiffness was shown to increase in DM rats (p<0.05). Ultimate stress and maximum load were significantly increased in DM+G groups compared to DM groups (p<0.05).

Conclusion. Glucovance treatment seems to be effective in restoration of biomechanical deterioration of bone specific to STZ-induced DM.

Key words: Diabetes mellitus, glucovance, bone, geometric, biomechanical, bone mineral density.

INTRODUCTION

There is a close relationship between diabetes mellitus (DM) and fragility fracture. The increase in fracture risk was related with the decrease in bone mineral density (BMD) in Type 1 DM (T1DM), whereas BMD is usually normal or increases in Type 2

DM (T2DM) (1, 2). It was shown that the femur fracture risk increased two fold in T2DM (3). Additionally, it was set forth that the biomechanical quality of the bone changed in T2DM owing to the presence of no relationship between fracture risk and BMD. Studies indicated that the bone quality was impaired, which was related to the decreased bone strength in diabetic rats (1). Changes in the stiffness of diaphyseal femur, energy absorption capacity, and tensional strength were reported (4, 5). Nevertheless, the entire biomechanical quality and especially the changes in intrinsic properties of the bone are unclear (1).

Metformin is widely used as the first-line antidiabetic drug of choice in T2DM patients (6). There are conflicting results in the literature about the influence of metformin on fracture risk in T2DM. ADOPT trial showed no effect of metformin on fracture risk in T2DM (7). However, another study indicated that metformin reduced the fracture risk (8). Glyburide is an insulin secretagogue of sulphonylurea group. Glyburide treatment was shown not to have an effect on bone mass in ADOPT and Rochester trials (8, 9). However, another study showed that glyburide treatment reduced osteoblastic marker levels (10).

Combination treatments are widely used in T2DM. Glucovance is an oral antidiabetic agent which includes metformin and glyburide (11). Influence of Glucovance treatment on intrinsic and extrinsic biomechanical properties of diabetic bones has not been evaluated until today. The maximum fracture strength which reflects the best the structural integrity of the tissue and the maximum deformation amount, which is used for determining the structural properties of the tissue, are the most frequently used important biomechanical parameters. In this study, we investigated the effect of glucovance treatment on biomechanical impairment of the bone in rats with DM.

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MATERIALS AND METHODS

Animals

28 male Wistar-Albino rats (12-week-old) weighing 210-300 g were used in the present study. Favourable conditions for rats were provided. The rats were assigned randomly to 4 experimental groups including sham [Sh; distilled water (orally by gavage, for 8 weeks); n=7], DM (n=7), DM + Glucovance treatment [DM+G; (Glucovance, 500/5 mg/kg/day/rat, orally by gavage, for 8 weeks)] and control (C; no treatment; n=7) groups.

The ethical approval was taken by the Medical Faculty Experimentation Ethics Committee of Mersin University.

Induction of DM

Intraperitoneal injection of STZ (45 mg/kg) was done to occur rats with DM (Sigma-Aldrich, Turkey). Three days later, those with fasting blood glucose levels (BGLs) >250 mg/dL were accepted as diabetic (12).

Bone evaluations

The study was concluded after eight weeks. Later, the femur bone from each animal were harvested under ketamine anesthesia (Ketalar, Turkey) and stored at -20°C.

A custom-made biomaterial testing machine measured the tensile strength of the femur bones including the energy capacity, maximum load, stress, displacement, strain, stiffness, elastic modulus, and toughness (MAY BTS03, Turkey). There was measured bone mineral density (BMD) by using dual-energy X-ray absorptiometry (DEXA; Norland 45 XR, USA) and cross-sectional area of femur by using computerized tomography (Toshiba 64, USA) from mid-diaphysis femoral region (13, 14).

Statistical analysis

Statistical analysis was made using SPSS software (Version 11.5.1). Continuous variables for normality were analysed by using Shapiro-Wilk test.

Variables were analysed by using Tukey's and one-way analysis of variance (ANOVA) tests.

RESULTS

The initial body weight and BGLs were similar in test groups. At the end of 8 week, while BGLs increased significantly in DM and DM +G groups compared to C and Sh groups ($p<0.001$, for each), body weight declined significantly ($p<0.001$, for each). The reduction in body weight was greater in DM rats compared to DM+G group ($p<0.01$; Table 1).

Mechanic parameters of diaphyseal femur

Absorbed energy, displacement and toughness were lower while elastic modulus, stiffness and ultimate strain were higher in DM and DM+G rats (Table 3).

Maximum load was significantly lower in DM group compared to C, Sh and DM +G groups ($p<0.001$ for C, $p<0.01$ for Sh, $p<0.05$ for DM+G). Maximum load levels of DM+G, C and Sh groups were similar ($p>0.05$).

Ultimate stress was significantly lower in DM group compared to C, Sh and DM+G groups ($p<0.01$, $p<0.05$, $p<0.05$, respectively).

Geometric parameters and BMD

Geometric parameters and BMD of the diaphyseal femur are summarized in Table 2.

Diaphyseal femur length was significantly shorter in DM group (Table 2). While femur length was similar in DM+G group and C group ($p>0.05$), it was shorter than in Sh group ($p<0.001$).

A significant difference was not detected between groups in terms of the cross-sectional area of the diaphyseal femur ($p>0.05$, for each).

BMD was greater in DM group compared to the other groups (Table 2). BMD of DM+G group was similar with C and Sh groups ($p>0.05$).

DISCUSSION

In this study, many biomechanical parameters

Table 1. The mean values of blood glucose levels (BGLs) and body weights (BW) in groups of rats

Groups	C	Sh	T2DM	T2DM+G
BGLs (mg/dL)				
Initial	97.4±1.23	107.1±3.51	102.3±2.35	104.2±2.46
Final	100.1±3.10	104.3±5.74	357.4±34.81 ^{a†,b†}	245.9±28.54 ^{a†,b†}
BWs(g)				
Initial	276.2±2.49	291.5±6.50	218.9±8.38	236.7±8.83
Final	302.0±8.16	315.6±8.78	192.7±14.82 ^{a†,b†}	242.9±7.23 ^{a†,b†,c‡}

Each group consists of seven rats. C: Untreated control rats, Sh: Sham rats (treated with distilled water), DM: Diabetic rats, DM + G: Diabetic rats treated with Glucovance. a Compared to C rats, b Compared to Sh rats, c Compared to DM rats; † P <0.0001, ‡ P <0.01, * P <0.05.

Table 2. Geometric properties and bone mineral density (BMD) of diaphyseal femur in groups of rats

Groups	Length (mm)	Cross-sectional area (mm ²)	BMD (g/cm ³)
C	31.84±0.49	9.4±0.36	0.1449±0.004
Sh	32.02±0.41	11.17±0.21	0.1592±0.001
DM	29.46±0.32 ^{a†,b‡}	9.91±0.33	0.1664±0.007 ^{a†,b‡}
DM+G	31.07±0.47 ^{b‡}	9.16±0.22	0.1605±0.004 ^{c*}

Each group consists of seven rats. C: Untreated control rats, Sh: Sham rats (treated with distilled water), DM: Diabetic rats, DM + G: Diabetic rat treated with Glucovance. a Compared to C rats, b Compared to Sh rats, c Compared to DM rats; † P <0.0001, ‡ P <0.01, * P <0.05.

Table 3. Mechanical parameters of diaphyseal femur in groups of rats

	C	Sh	DM	DM+G
Displacement (mm)	2.22±0.11	2.61±0.25	0.86±0.32 ^{a†,b‡}	0.86±0.21 ^{a†, b*}
Maximum Load (N)	460.64±29.70	457.88±21.27	263.96±19.56 ^{a†, b‡}	386.12±34.38 ^{c*}
Stiffness (N/mm)	188.66±8.83	156.69±19.87	811.06±268.95 ^{a*, b*}	638.84±138.29 ^{a*, b*}
Absorbed Energy (mJ)	571.35±43.93	638.79±52.43	106.70±44.84 ^{a†, b‡}	171.92±46.668 ^{a†, b‡}
Ultimate Stress (MPa)	47.10±4.41	43.50±3.58	26.81±2.26 ^{a‡,b*}	42.45±4.24 ^{c*}
Ultimate Strain (mm/mm)	0.07±0.01	0.08±0.01	0.02±0.01 ^{a‡,b‡}	0.02±0.01 ^{a‡, b‡}
Elastic Modulus (GPa)	0.63±0.04	0.57±0.04	2.46±0.87 ^{a†,b‡}	2.19 ±0.49 ^{a†, b‡}
Toughness (MPa)	1.78±0.23	1.70±0.15	0.37±0.14 ^{a†,b‡}	0.60±0.16 ^{a†, b‡}

Each group consists of seven rats. C: Untreated control rats, Sh: Sham rats (treated with distilled water), DM: Diabetic rats, DM + G: Diabetic rats treated with Glucovance. a Compared to C rats, b Compared to Sh rats, c Compared to DM rats; †P<0.0001, ‡P<0.01, *P<0.05.

of the bone were found different in diabetic rats. This condition supports the view that bone quality is significantly impaired in DM. The significant improvements in ultimate stress and maximum load that were obtained through the Glucovance treatment put forward that this treatment positively affects both bone integrity and bone strength.

BMD measured with DEXA is a strong determining factor of fracture risk. However, it is not a marker of bone quality and bone strength. BMD level may not have always shown bone fragility risk. While T1DM is characterized by a reduction in BMD at both vertebrae and femur level, BMD is normal or increased compared to general population in T2DM (3, 15). In this study, BMD was higher in diabetic rats without glucovance treatment. However, there was not a significant difference between glucovance treatment - receiving diabetic group and C and Sh groups ($p>0.05$ for each). These results suggest that glucovance treatment may play a role in BMD restoration.

The exact mechanisms of the effect of T2DM on bone fragility are not known. Recently, the relationship between the duration of diabetes and fracture was evaluated in T2DM and bone strength was seen to be better in the early stages of the disease (16, 17). Type 1 collagen is the most frequently found collagen in the bone. Collagen matrix plays an important role in the mechanical power of the bone through forming cross-links between molecules. There are two types of collagen cross-links in the bone as enzymatic cross-link and glycation or oxidation-induced advanced glycation end

products (AGEs). Bone strength decreases independently of BMD in diabetic bones due to AGEs formation in case of hyperglycemia and oxidative stress (18, 19). In recent studies, enzymatic and AGEs cross links were shown to affect toughness, stiffness and elastic modulus of the bone (18, 20-22).

Biomechanical properties such as toughness, elastic modulus, ultimate strain, and ultimate stress are the main parameters used for evaluating the fragility of the femur. These features show the biomechanical integrity of the bone. It is accepted that crystallinity increase, mineralization defects and collagen deformation increase indicate the combined changes in these parameters (1, 14, 23, 24). In this study, all these parameters were detected to decrease in DM rats. That ultimate stress increased and reached the levels of control group in glucovance treatment-receiving diabetic rats compared to the ones which do not receive suggests that this treatment has a partially beneficial effect on biomechanical integrity of the bone.

Mechanical features such as absorbed energy, displacement, stiffness, and maximum load are the structural parameters of the bone. These parameters are closely related with the quality and strength of the bone (1). Bone strength and displacement were shown to decrease and stiffness was shown to increase in T2DM rats (1, 25). In our study, maximum load, energy absorption capacity and displacement were shown to decrease and stiffness was shown to increase in DM rats and this result indicates that bone quality is impaired and femur strength is decreased in DM. Maximum load

is the power which is applied until the bone is broken (14). Presence of a significant increase in maximum load in glucovance-receiving diabetic rats compared to DM ($p < 0.05$) and its reaching to similar levels with C group ($p > 0.05$) suggest that this treatment has a healing effect on the bone quality and femur strength.

Geometric properties are important factors for evaluating the quality and strength of the bone (13, 26-28). In this study, femur length was lower in diabetic rats. There was not a significant difference between Glucovance-receiving diabetic rats and C group with regard to femur length.

In conclusion, an improvement in ultimate stress and maximum load, the impaired biomechanical parameters of the bone, following Glucovance treatment suggests that this treatment may have a partial improvement in increase of the quality of the diabetic bone and in decrease of fragility in STZ-induced diabetic rat model.

Conflict of interest

The authors declare that they have no conflict of interest.

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