INTRODUCTION

In diabetes mellitus bone mass decreases and leads to increased skeletal fragility and impaired fracture healing. The underlying mechanisms are still unclear and efficient therapies are limited. Zucker Diabetic Fatty (ZDF) rats are a well-established animal model for type 2 diabetes mellitus. They are characterized by an impaired beta cell function, low bone mass, and delayed bone defect healing due to reduced osteoblast function. Currently, the underlying mechanisms are still unclear and efficient therapies are limited. Zucker Diabetic Fatty (ZDF) rats are a well-established animal model for type 2 diabetes mellitus. They are characterized by an impaired beta cell function, low bone mass, and delayed bone defect healing. The underlying mechanisms are still unclear and efficient therapies are limited.

MATERIALS AND METHODS

A subcritical femoral defect of 3 mm was created in 11 weeks old diabetic ZDF (fa/fa) and non-diabetic ZDF (+/+ ) rats. PTH (75 µg/kg) or vehicle was administered subcutaneously five days per week over 12 weeks. The non-operated femora, the lumbar vertebrae, and the filling of the femur gaps were analyzed by µCT at the distal metaphysis and mid-diaphysis. Three-point bending test was performed at the mid-diaphysis of the non-operated femora and lumbar vertebrae. To analyse dynamic histomorphometry all rats received two intraperitoneal injections of calcein (20 mg/kg) 10 and 3 days before sacrifice. Bone formation rate/bone surface (BFR/BS) was determined on unstained sections of lumbar vertebra using fluorescence microscopy and Osteomeasure® software. Third lumbar vertebrae (L3) were fixed in formalin, and afterwards stained with von Kossa and toluidine blue. Intraoperative glucose tolerance test (IpGTT) was performed at treatment weeks 0, 3, and 6. Blood glucose levels were measured by using a glucometer and recorded at time point 0 and then every 30 minutes up to 3 hours post glucose injection (2g/kg). Paraffin embedded pancreata were stained with hematoxylin and eosin (HE). For immunohistochemistry, primary antibodies like mouse anti-human insulin and rabbit anti-human glucagon were used. Signal was amplified using the VENTANA amplification kit and visualized using avidin-biotin labelling and 3,3′-diaminobenzidine. To analyze the effects of diabetes and PTH treatment 2-way factorial analysis of variance (ANOVA) was performed using Graphpad Prism® software.

RESULTS

1) µCT data of femur and lumbar vertebra
Significant increase in bone volume after intermittent treatment with PTH in diabetic and non-diabetic rats.

2) Histology and histomorphometry of lumbar vertebra
Significant elevation of bone volume and bone formation rate after intermittent PTH therapy in diabetic and non-diabetic rats.

3) Biomechanical testing of femur and lumbar vertebra
Intermittent PTH treatment improved bone strength significantly in lumbar vertebra of diabetic and non-diabetic rats, but not in femur.

4) Healing of femoral defect
Intermittent application of PTH increased filling of created bone gap.

5) Intraoperative glucose tolerance test (IpGTT)
No effect of intermittent PTH treatment on beta cell function in diabetic and non-diabetic rats.

CONCLUSION

Intermittent PTH therapy is capable of reversing the adverse effects of type 2 diabetes mellitus on bone mass and delayed bone defect regeneration in ZDF rats. PTH or vehicle treatment had no influence on diabetes onset or disease progression.