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Hyperglycemia caused reduction of cortical bone thickness in streptozotocin-induced diabetic rat



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Maria Cellina Wijaya,^{1*} Gadis Meinar Sari,² Damayanti Tinduh³

ABSTRACT

Background: Diabetes is a chronic metabolic disease characterized by hyperglycemia due to insulin deficiency (type 1 diabetes) or insulin insensitivity/resistance (type 2 diabetes). The significant metabolic changes that occur in diabetes also affect the skeleton and cause bone loss and/or altered bone matrix and strength, thereby increasing the risk of fracture. Hyperglycemia can alter cellular metabolic processes through the formation of advanced glycation end products (AGEs), which caused dysregulation of various cytokines as the underlying mechanism in the decrease of bone density. This study aimed to see the bone loss caused by hyperglycemia in rats, using cortical bone thickness as a parameter.

Methods: This study was an analytical experimental study, which was conducted in a laboratory. The sample of this study was Wistar

rat (*Rattus norvegicus*). Diabetes in the rats were induced using streptozotocin. The diabetic rats were then sacrificed 11 days later, and the left femur bone was obtained. The bone was decalcified for 1 week, then prepared for histological slides. Cortical bone thickness was measured microscopically using *Optilab Image Raster* software at 10 different axis points, and then averaged.

Results: The mean of cortical bone thickness were $32.43 \pm 2.65 \mu\text{m}$ in the control (non diabetic) rats, and $26.64 \pm 2.89 \mu\text{m}$ in streptozotocin-induced diabetic rats ($p < 0.001$). The diabetic rats had lower mean of cortical bone thickness than control by $5.79 \mu\text{m}$.

Conclusion: Cortical bone thickness in the diabetic rats were lower by 18% compared to control 11 days after induction of streptozotocin.

Keywords: hyperglycemia, diabetes, bone, cortical bone thickness, streptozotocin, rat

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¹Faculty of Medicine, Airlangga University, Surabaya-Indonesia.

²Department of Physiology, Faculty of Medicine Airlangga University, Surabaya-Indonesia.

³Department of Physical and Rehabilitation Medicine, RSUD Dr. Soetomo, Airlangga University, Surabaya-Indonesia

INTRODUCTION

Diabetes is a chronic metabolic disease manifested as hyperglycemia due to abnormality in the production and/or function of insulin. Diabetic condition has been known to induce metabolic changes which may cause complications such as cardiovascular disease, retinopathy, neuropathy, nephropathy, foot ulcer, and many more. Diabetes affects bone metabolism,¹ causing loss of bone density and changes in the matrix and bone strength.²

There are many theories for the mechanism behind the effect diabetes had on bone metabolism. One of the most popular one is that hyperglycemic condition in diabetic patients caused changes in cellular metabolism though the formation of advanced glycation end products (AGEs). AGEs triggered an increase in activation of RANKL,³ which ensued in increased osteoclastogenesis. Increased osteoclastogenesis and osteoclast activity resulted in extreme bone resorption, imbalance in bone remodelling cycle and the outcome is decreased bone mass.

Various studies regarding bone density performed by measured the cancellous or the trabecular part of the bone. According to Garn et al,⁴ decrease in bone density may be indicated by measurements of total tubular bone width, medullary cavity width, cortical thickness, cortical area,

and percent cortical area. Brandi⁵ mentioned that manifestation of decreased bone mass can be seen both in the trabecular and cortical bone.

An understanding about the effects of diabetes towards bone is imperative in formulating a holistic and targeted therapy to restore bone strength and increase life qualities of diabetic patients.

RESEARCH DESIGN AND METHODS

Animal model

This study has been approved by the Ethics Committee of Universitas Airlangga. Wistar rats were chosen as the sample because there is a homology between diabetes in humans and in Wistar rats.⁶ A total of 20 male Wistar rats were randomly divided into 2 groups, each consisting of 10 rats, which were the control (nondiabetic) group and the diabetic group. The diabetic group was given streptozotocin injection to induce diabetes in the rats.

Streptozotocin induction protocol

For the induction of diabetes in the diabetic group, we used single-dose streptozotocin in the amount of 50 mg/kg per rat, dissolved in 22.5 mg/ml citrate buffer. Streptozotocin (STZ) is a toxin that damages DNA structure, triggering apoptosis of the

*Correspondence to: Maria Cellina Wijaya, Faculty of Medicine, Airlangga University, Surabaya-Indonesia.
celline1130@gmail.com

pancreatic β cells, which induced diabetes in rats. After the induction, the rats were given sucrose or dextrose 10% during the first 24 hours to prevent sudden hypoglycemia post injection.⁷

Hyperglycemic condition in rats

The definition of hyperglycemia in rats is a blood glucose level above 200mg/dl, using blood sample from the rat's tail. According to Purwanto and Liben,⁷ significant hyperglycemia in the rats can be found 5-12 days after induction. Blood glucose levels were measured in the diabetic group one week post injection of STZ and right before termination. All the rats in the diabetic group have blood glucose measurement consistent with diabetes.

Histological examination

The rats were euthanized 11 days post induction. Bone tissue were obtained from the left femur of each rat, and were then decalcified for 1 week in Na citrate. After that the sample were sent to be made into histological slides with hematoxylin and eosin staining. The slides were viewed through a computer software, *Optilab Viewer*, which capture images from the microscope to be interpreted.

Measurement of cortical thickness

The cortical bone thickness was measured in 10 axis points. The axis points were chosen in random, with consideration of variability in cortical thickness by choosing points which were thought to represent the

thickest and thinnest parts of the overall cortical bone. Cortical thickness in each axis was measured from the distance of the periosteal to the endosteal surface.⁸ Measurement from the 10 random axis points were then averaged to achieve the cortical thickness for each sample. All measurements were done using *Optilab Image Raster* software.

Data analysis

All data were analyzed with SPSS. Mean \pm standard deviation (SD) and median (minimum-maximum) were used to present data with and without normal distribution, respectively. Shapiro-Wilk test was used to determine normality of data. Variance of data was analyzed with Levene's test. Independent t-test was performed to test the statistical significance in cortical bone thickness of control and diabetic groups. P value of < 0.05 was considered significant.

RESULTS

Histopathology examination was performed to determine the cortical bone thickness in both group by measured the 10 axis points ([figure 1](#) and [figure 2](#)).

The mean score of the control group is $32.43 \pm 2.65 \mu\text{m}$, and the diabetic group is $26.64 \pm 2.89 \mu\text{m}$ ([table 1](#)). The average difference between the control and diabetic group is $5.79 \mu\text{m}$. We found 18% decrease in cortical bone thickness in the diabetic group compared to control.

The result of Shapiro-Wilk test and Levene's test showed that our data were homogen and has normal distribution. Therefore, we chose independent t-test to test for statistical significance. There was significant decrease of cortical bone thickness in diabetic group compared to control group ($p < 0.001$). The result was presented in [table 2](#).

DISCUSSION

According to Follak et al¹ diabetes has an effect towards bone metabolism. Previous studies showed that in hyperglycemic condition, there is an increase in osteoclastogenesis and osteoclast activity.^{9,10} The mechanism behind this effect might be because in hyperglycemic condition, AGEs is formed. AGEs primarily affects tissues with slow regeneration rate, such as the cortical bone. AGEs increased activation of RANKL.³

Other cytokines which may play a part in increased osteoclastogenesis are TNF- α , M-CSF, and RANKL. In diabetic rats, there is increased secretion of TNF- α , M-CSF, and RANKL.¹¹ TNF- α works by increasing expression of RANKL. M-CSF causes activation of the RANKL receptor, RANK.¹² RANKL stimulates

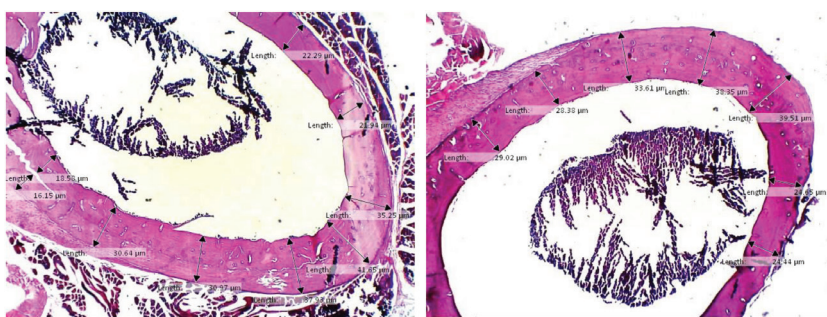


Figure 1 Histological image of samples from the control group, with measurements (40x enlargement)

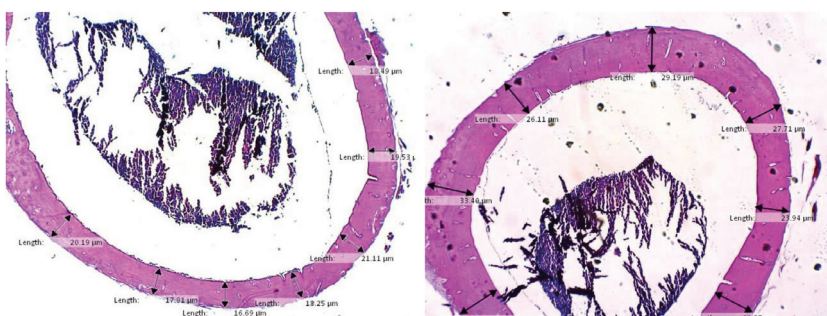


Figure 2 Histological image of samples from the diabetic group, with measurements (40x enlargement)

Table 1 Measurement of cortical bone thickness in control group and diabetic group

Sample Number	Cortical Bone Thickness (μm)	
	Control	Diabetic
1	30.71	27.39
2	32.39	26.79
3	30.88	25.48
4	34.33	29.50
5	32.84	28.57
6	37.57	24.39
7	33.61	26.61
8	27.29	30.15
9	32.17	20.08
10	32.50	27.47
Mean \pm SD	32.43 \pm 2.65	26.64 \pm 2.89

Table 2 Analysis of cortical bone thickness in control and diabetic group

	Control Group (n=10)	Diabetic group (n=10)	p-value
Cortical bone thickness, mean \pm SD (μm)	32.43 \pm 2.65	26.64 \pm 2.89	<0.001

Independent t-test

osteoclastogenesis by attaching to its receptor, RANK, which is found on the surface of osteoclast precursors and mature osteoclast cells, subsequently leading osteoclast precursors (macrophage/monocyte) towards the osteoclastogenesis pathway.¹²

This study chose cortical bone thickness as a marker for decreased bone density. To the author's knowledge, measuring cortical bone thickness as a marker for decreased bone density has been rarely done before. Decreased bone density can be measured from total tubular bone width, medullary cavity width, cortical thickness, cortical area, and percent cortical area.⁴ Manifestation of decreased bone density can be seen both in trabecular and cortical bone.⁵ Cortical bone thickness is measured by the distance of the periosteal to the endosteal surface.⁸

Results of independent t-test showed $p < 0.001$. The decrease in cortical bone thickness in the diabetic group compared to control group is 18%, which is achieved in 11 days after induction using streptozotocin. This result presented a possibility of a greater decrease in cortical bone thickness if the time period after induction is elongated.

The results of this study is in line with the researcher's hypothesis. Furthermore, the results of

this study also supported previous studies, in which it was said that diabetes cause a decrease in bone density.^{2,10,13,14,15}

CONCLUSION

This research concludes that there was a significant decrease of cortical bone thickness in streptozotocin-induced diabetic rats compared to non diabetic rats ($p < 0.001$). There was 18% decrease of cortical bone thickness in the diabetic rats compared to control 11 days after induction of streptozotocin.

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