Changes in the Structure and Mechanical Properties of Bone Tissues Obtained from Experimental Animal Models of Lifestyle-Related Diseases

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ABSTRACT

Lifestyle-related diseases such as diabetes and hypertension are a major public health problem in the world. It is well known that these diseases directly affect the characteristics of cardiovascular tissues. However, there has been little information on the effects of diabetes or hypertension on the biomechanical properties of musculoskeletal tissues. We hypothesized that diabetes or hypertension is related to impaired material and structural properties of bones. To examine this hypothesis, mechanical loading tests were conducted to determine the stress-strain relations of cortical bone specimens obtained from experimental animal models of diabetes or hypertension. Zucker diabetic fatty rats (Diabetic group), stroke-prone spontaneously hypertensive rats (Hypertensive group), and normal Wistar rats (Control group) were used for the experiments. Cortical bone specimens were obtained from the femora of the rats, and compressive forces were applied to the specimens until failure using a conventional material tester. In addition, the trabecular structure of the femoral neck was observed using an X-ray micro-computed tomography. Bone compressive strength in the diabetic group was significantly lower than that of the control group. Trabecular bone in the hypertensive group has a higher porosity than that in

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the control group. These findings support the concept that bone metabolism may be impaired in diabetic or hypertensive subjects.

Keywords: *Biomechanics, Bone, Diabetes, Hypertension, Mechanical Properties.*

Introduction

The number of patients with lifestyle-related diseases such as diabetes mellitus and hypertension is annually increasing in Asia as well as in the United States and Europe. Diabetes and hypertension, which are major types of lifestyle-related diseases, induce vascular morbidity and nephropathy associated with changes in calcium metabolism [1-4]. Therefore, it is possible that bone abnormalities occur diabetic or hypertensive patients. For example, a study in the past has identified the association between diabetes and delayed fracture healing in bone [5]. Moreover, a previous study has demonstrated that postmenopausal women who have diabetes or in whom diabetes develops are at higher risk for hip fracture than nondiabetic postmenopausal women [6]. It has been previously reported that high blood pressure or diabetes mellitus is linked to an increased loss rate for bone mineral density (BMD) [7-10]. Recent studies have shown that the biochemical bone turnover markers are lower in diabetes patients compared with healthy subjects [11, 12].

Skeletal health is important to the maintenance of functionality in the aging population. Previous biological and observational studies reveal the degeneration of bone quantity and quality due to diabetes and hypertension, but the mechanisms responsible for them are unclear. Recent clinical data and epidemiological investigations suggested that decreased bone strength in the diseases may contribute to bone fracture risk. However, few studies have been done on the effects of lifestyle-related diseases on the mechanical properties of bone tissues. Bone fractures are ultimately a kind of mechanical event [13-15]. Consequently, a quantitative assessment for bone biomechanical performance is essential both biologically and clinically. A better understanding of the factors that determine bone strength in diabetes and hypertension is needed to inform fracture prevention efforts in order adults. However, less attention has been paid to the association of lifestyle-related diseases and bone strength. In the present study, we hypothesized that diabetes or hypertension is related to impaired material and structural properties of bone tissues. To examine this hypothesis, the stress-strain relations and microstructure of the tissues were determined by means of mechanical loading tests and X-ray micro-computed tomography (μCT) analyses.

Materials and Methods

Specimen preparation

Animal experimentation in the present study was carried out under the guideline of the Animal Care and Use Committee of Faculty of Biology-Oriented Science and Technology, Kindai University. Zucker diabetic fatty rats (Diabetic group) of 8 and 14 weeks of age, Stroke-prone spontaneously hypertensive rats (Hypertensive group) of 8 or 12 weeks of age, and the age-matched Wistar Kyoto rats (Control group) were used for the experiments. Soft tissues around the femur were removed, and the bone samples were stored at -20˚C until required. Ring-like specimens were sliced off from the midshaft of the femur using a rotating diamond saw blade. Bone tissues were kept submerged in physiological saline water during machining to minimize load- and heat-induced damage. The final length of each specimen was measured with a micrometer. The nominal length of the specimen was approximately 3 mm. The cross-sectional area of the specimen was measured using an image analyzer.

Mechanical tests and structural observation

A conventional material tester (AGS-H, Shimadzu, Kyoto, Japan) having polished stainless steel platens was used for compressive loading tests (Fig. 1). Platen surfaces were lubricated with mineral oil prior to each loading. To minimize the effects of the machine compliance of the material tester, the specimen deformation was measured by a laser micrometer (LT-8110, Keyence, Osaka, Japan) having a resolution of 2 μm. Bone specimens were immersed in a physiological saline solution of 37˚C during the mechanical testing. Compressive forces were applied to the specimens until failure at the rate of 1 mm/min. The proximal portion of the femur was used for X-ray micro-CT analyses (Inspexio SMX-90CT, Shimadzu, Kyoto, Japan). Cross-sectional images of the trabecular bone were obtained at the femoral neck portion.

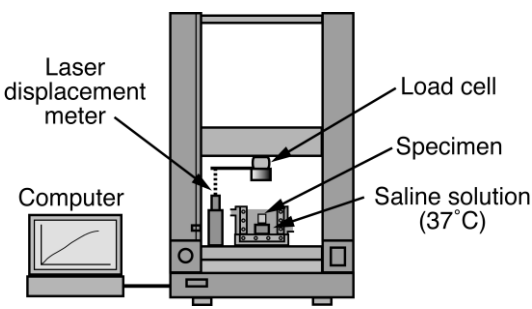

Figure 1: Schematic diagram of a material tester.

Results and Discussion

Body weight of the animals

Irrespective of age and gender of animals, the body weight in the Diabetic group was significantly higher than that in the Control group (Table 1). Increase in the body weight from 8 to 14 weeks old was more remarkable in the Diabetic group than in the Control group. For example, the percentage ratio of the weight at 14 weeks old to that at 8 weeks old in male rats was 132% and 112% in the Diabetic and Control groups, respectively. In both male and female animals, the body weight in the Hypertensive group was significantly lower than that in the Control group (Table 2).

Table 1: Body weight of experimental animals in the Diabetic and Control groups.

Body weight (g)				
Group	Male		Female	
	8 weeks	14 weeks	8 weeks	14 weeks
Control	262.7 ± 24.1 (n = 5)	294.0 ± 9.6 (n = 5)	164.9 ± 34.0 (n = 5)	183.4 ± 6.7 (n = 5)
Diabetic	350.1 ± 9.3* (n = 5)	463.8 ± 15.2* (n = 5)	267.3 ± 14.1* (n = 5)	376.6 ± 11.0* (n = 5)

* $p < 0.05$, vs. Control
(Mean ± S.D.)

Table 2: Body weight of experimental animals in the Hypertensive and Control groups.

Body weight (g)		
Group	Male	Female
	12 weeks	12 weeks
Control	337.8 ± 13.5 (n = 8)	206.5 ± 4.9 (n = 8)
Hypertensive	278.8 ± 6.6* (n = 8)	183.3 ± 9.3* (n = 8)

* $p < 0.05$, vs. Control
(Mean ± S.D.)

Mechanical properties in the Diabetic group

The stress-strain relations of cortical bones in the Diabetic and Control groups were shown in Figs. 2 (8 weeks old) and 3 (14 weeks old). There were no significant differences in the compressive strength and failure strain between the Diabetic and Control groups at 8 weeks of age in both male and female animals. At 14 weeks of age in both genders, the compressive strength in the Diabetic group was significantly lower than that in the Control

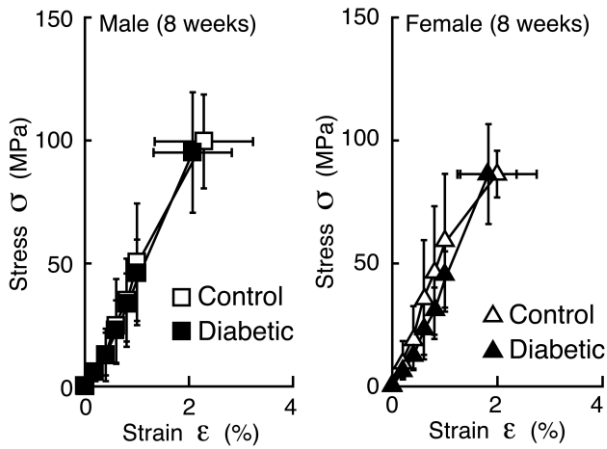

Figure 2: Stress-strain curves in the Diabetic and Control groups (8 weeks of age).

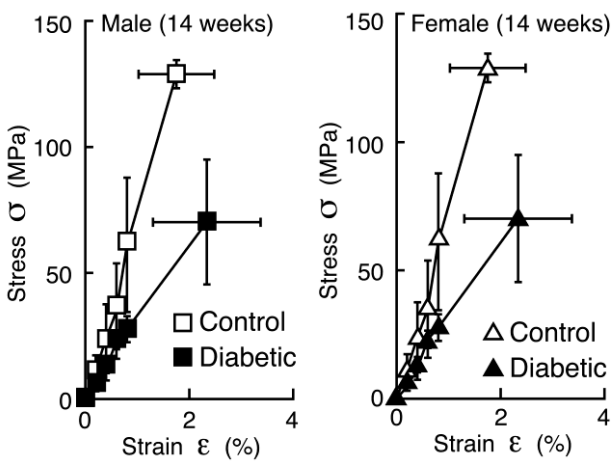

Figure 3: Stress-strain curves in the Diabetic and Control groups (14 weeks of age).

group (Fig. 4). The compressive strength in the Diabetic group at 14 weeks old in male and female animals was 70.2 ± 24.7 MPa and 83.8 ± 20.0 MPa, respectively. These values were approximately 60% of the compressive strength in the Control group. Figure 5 shows the relations between the compressive strength and body weight in the Diabetic and Control groups. Irrespective of the animal gender, there were positive linear correlations between the strength and weight in the Control group. In contrast, no positive correlations were observed between the strength and weight in the Diabetic group. In particular, the compressive strength in male animals decreased with the increase in the body weight. The body weight of animals in the Diabetic group was significantly higher than that in the Control group. In contrast, no significant increases were observed in the compressive strength between the Diabetic and Control groups. The body weight of animals is one of the most important factors that influence mechanical stresses applied to such musculoskeletal tissues as bone. It is possible that bone tissues in the diabetic animals are incapable of remodeling in response to in vivo applied load which is related to the body weight.

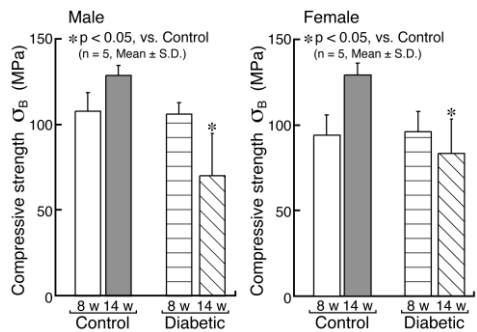

Figure 4: Compressive strength in the Diabetic and Control groups.

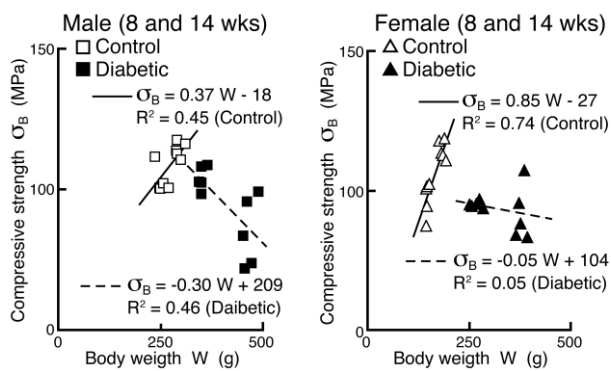

Figure 5: Relations between the compressive strength and body weight in the Diabetic and Control groups.

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Figure 6 shows the cross-sectional area, compressive strength, and failure load in the Hypertensive and Control groups. The cross-sectional area in the Hypertensive group was significantly lower than that in the Control group. However, no remarkable difference in the compressive strength was observed between the Hypertensive and Control groups. These results indicate that hypertension is more closely related to the structure than to the material properties of cortical bone. In the present study, we investigated the bone properties only at 12 weeks of age. To understand the effects of the duration of hypertension, it is necessary to conduct the mechanical test for cortical bone obtained from elderly animals. Figure 7 shows the trabecular structure of femoral neck in the Hypertensive and Control groups (8 weeks old). Trabecular bone in the Hypertensive group has a higher porosity than that in the Control group. These findings obtained from mechanistic viewpoint support the concept that bone metabolism may be impaired in hypertensive subjects. Epidemiologically, it is reported that bone remodeling markers are significantly elevated in the hypertensive subjects [14]. Molecular and cellular biological studies should be conducted to know the mechanisms of the effects of hypertension on the bone strength. Further understanding of how metabolism of lifestyle-related diseases affect bone quality would improve fracture prevention efforts in older adults.

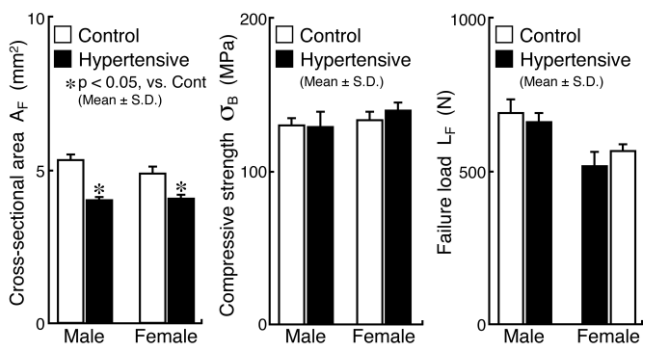

Figure 6: Cross-sectional area, compressive strength, and failure load in the Hypertensive and Control groups.

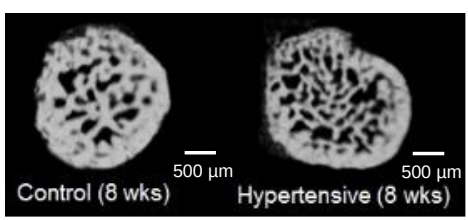

Figure 7: Trabecular bone structure in the Hypertensive and Control groups (8 weeks old).

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