3P5-15

Evaluation of Longitudinal Wave Velocity in Diabetic Rat Tibia using Micro-Brillouin Scattering Technique

顕微Brillouin光散乱法を用いた糖尿病rat脛骨中の縦波音速評価

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1. Introduction

U.S. National Institutes of Health (NIH) has reported the importance of the evaluation of both BMD (bone mineral density) and bone quality [1]. Bone quality depends on several factors, such as collagen, mineralization, micro-crack, bone turnover, etc. Inada et al. have reported that diabetic patients have higher bone fracture risks despite of the normal BMD [2], which implies the importance of bone quality. Diabetes also causes Chronic kidney disease (CKD), which is known to result in the weak bone.

In case of diabetes, the amount of insulin decreases and Advanced Glycation End Products (AGEs) cross-links tend to be generated. Imoto et al. have artificially induced AGEs cross-links in bovine cortical bone and measured the longitudinal wave velocity in a small area by a micro-Brillouin scattering technique [3]. As a result, the wave velocity decreased as the glycation advanced.

In this study, by using the micro-Brillouin scattering technique, we measured the wave velocity in bone (cortical bone and single trabeculae) of SDT (Spontaneously Diabetic Torii), SDT (Spontaneously Diabetic Torii) fatty and healthy SD (Sprague Dawley) rats, to check the effect of diabetes on the bone.

2. Material and Methods

2.1. Comparison of SD and SDT rats

Specimens were 20-week-old male rats (four Slc:SD Rats, four SDT/Jcl Rats CLEA Japan, Inc.). The blood glucose levels of SDT rats (approximately 519 mg/dL) were more hyperglycemic than those of SD rats (approximately 155 mg/dL). Four cortical bone specimens were obtained from the proximal posterior part of tibia. We have totally fabricated eight bone specimens (bone axis and tangential plate) with the thickness around 70 μm.

Cancellous bone specimens were also obtained from the proximal part of tibia. We have fabricated eight bone specimens (in the plate of bone axis and medial-lateral direction) with the thickness around 100μm. As Tsubota et al. have pointed, the elastic properties of trabeculae depend on the trabecular length [4]. Then, we selected trabeculae with similar length (400 ± 20 μm) for velocity measurements. The propagation direction of the measured wave was along trabecular axis.

2.2. Comparison of SD and SDT fatty rats

Specimens were 17-week-old male rats (three Slc: SD Rats, three SDT fatty/Jcl Rats, CLEA Japan, Inc.). The blood glucose levels of SDT fatty rats (two kidneys: approximately 574 mg/dL, one kidney: approximately 430 mg/dL) were more hyperglycemic than those of SD rats (approximately 110 mg/dL). These cortical bone specimens were obtained from the proximal posterior part of tibia. We have fabricated three bone Specimen (anterior-posterior and medial-lateral plate) with the thickness around 80 μm.

2.3. Brillouin scattering technique [5]

The micro-Brillouin scattering technique is a non-destructive method to observe wave velocity in the GHz range. Brillouin scattering measurements were carried out with a six-pass tandem TFPI (Fabry-Pérot interferometer) using a solid state the

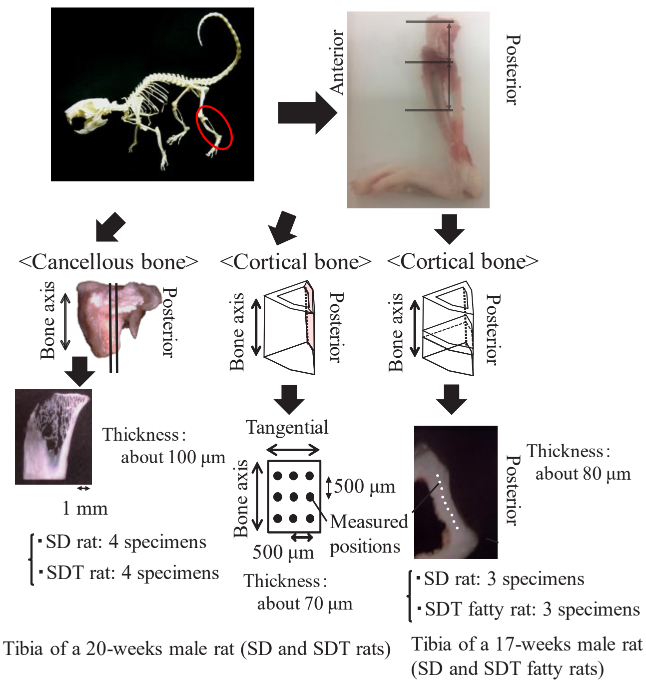

Fig. 1 Preparation of specimens.s

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laser (Spectra-Physics) with wavelength of 532 nm. the actual measured area in the specimen was approximately 10 µm. The velocity in the specimen was defined as the average velocity obtained from the data at different 9 positions.

3. Results and Discussions

Comparison of SD and SDT rats

Figure 2 shows a Brillouin spectrum of a trabecular bone in a SD rat. The wave velocity estimated from frequency shift was 4.94 $\times 10^3$ m/s.

Measured wave velocities in the cortical and trabeculae bones of SD rats were faster than those of the reported velocities of bovine femur [3, 4]. **Figure 3** shows the average wave velocity of four specimens from the bones of SD and SDT rats. The wave velocites in bones of SDT rats were lower than those of SD rats (cortical bone: 2.0 %, trabeculae bone: 3.8%) ($p<0.05$). Fujii et al. have reported that the BMD values of both SD and SDT rats were similar [6]. Because elasticity depends on both density and velocity, elastic moduli of SDT rats then seemed lower (cortical bone: 4.0 %, trabeculae bone: 7.5 %). Compared to the cortical bone, the cancellous bone has a large surface area in the bone marrow, then the effects of diabetes were possibly stronger.

Comparison of SD and SDT fatty rats

Figure 4 shows the average wave velocity of three specimens from cortical bones of SD and SDT fatty (two kidneys or one kidney) rats. These velocities were in the tangential direction of bone, then, the values were smaller than the values in the axial directions. The wave velocites of SDT fatty rats were lower than those of SD rats. The decrease seemed to depend on the number of kidneys, however, the effect of the number of kidney seems small. The blood glucose level of SDT fatty rats (two kidneys) were constantly over 550 mg/dL, whereas those of SDT fatty rats (one kidney) were around 300 mg/dL when they were alive. As the hyperglycemic condition continues, the kidneys tend to be suffered from CKD, which gradually weaken the kidney functions. In this study, we found these metabolic diseases seemed to affect bone elasticity.

4. Summary

The wave velocities in tibia of healthy SD, SDT and SDT fatty (two kidneys or one kidney) rats were measured by a micro-Brillouin scattering technique. In the tibia, the average wave velocities of diabetic rats were much lower than those of SD rats. The tendency was clear in the cancellous bone. Compared to the cortical bone, the effect of diabetes seemed stronger in the cancellous bone.

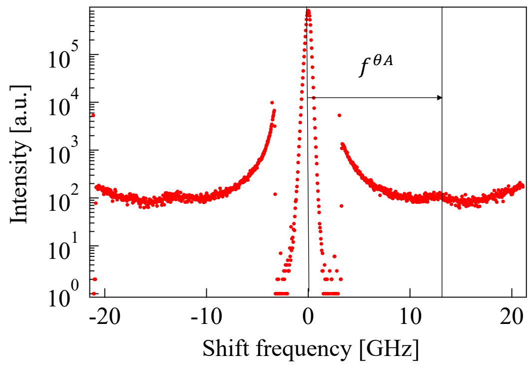

Fig. 2 An observed spectrum of Brillouin scattering from cortical bone of SD rat.

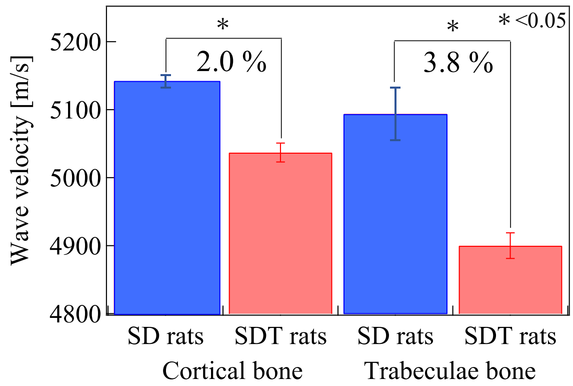

Fig 3 Comparison of wave velocity in bone (cortical and trabeculae bones) of SD and SDT rats

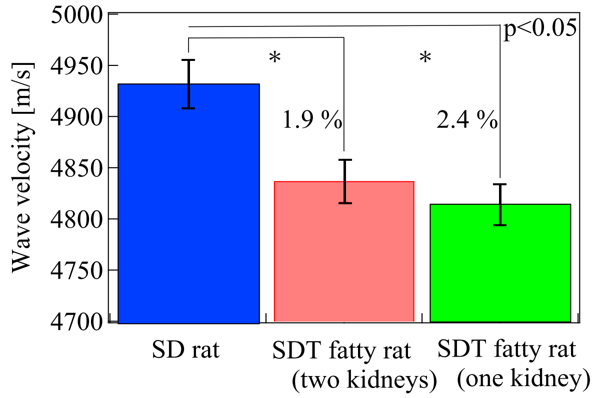

Fig. 4 Comparison of wave velocities in cortical bone of SD and SDT fatty (two kidneys and one kidney) rats.

References

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