Longitudinal wave velocity in bones of streptozotocin induced diabetic rat

顕微 Brillouin 光散乱法を用いたストレプトゾトシン誘発糖尿病ラット骨中の縦波音速

Keita Yano[1‡], Yoshihiko Maekawa, Yuhi Haneda, Koki Shirai, Masaya Ikegawa, Mami Matsukawa (Doshisha Univ.)
矢能 啓太‡, 前川 慶彦, 羽田 雄飛, 白井 浩貴, 池川 雅哉, 松川 真美, (同志社大)

1. Introduction

Hyperglycemia in diabetes causes serious damage to the eye, kidneys, nerves, and peripheral vascular system. Diabetes also affects bone and increases the bone fracture risk. The two main types of diabetes are type 1 (insulin dependent) and type 2 (insulin independent). Insulin has been suggested to exert both systemic and local anabolic effects in bone tissues [1]. Recently, higher bone fracture risks were found not only in the type 1 patients but also in type 2 patients which often have normal bone mineral density (BMD). One reason seems to result from the deterioration of collagen which occupies 50 % of the total bone volume [2]. In case of diabetes, Advanced Glycation End Products (AGEs) cross-links tend to be generated due to the decreases of insulin amounts or deficient action. The abnormal collagen crosslinks in bone are also expected to affect bone elasticity. However, the bone elasticity cannot be evaluated by BMD. In contrast, ultrasonic techniques, which are non-invasive, inexpensive, and safe, can provide information of not only BMD but also elasticity [3].

In this study, using a micro-Brillouin scattering (BR) technique, we studied longitudinal wave velocity in bones of streptozotocin induced diabetic rats.

2. Material and methods

2.1. Sample preparation

Samples were fabricated from 4 male Sprague Dawley (SD) rats. Two samples were from diabetic model rats and 2 samples were from normal rats. For the diabetic samples, the 10-week-old SD rats were injected with streptozotocin (STZ) solution (STZ dissolved in physiological saline solution) to induce diabetes. For normal samples, SD rats were injected with the equivalent volume of physiological saline solution. We fabricated samples 4 and 9 weeks after the injection. As shown in Fig.1, the small cortical plate samples (thickness 70μm) were fabricated from tibia (plate of the bone axis and tangential directions) and skull (plate of the anterior-posterior and medical-lateral directions) partitioned into 4 parts along by the suture line. The average velocity in each sample was obtained from the BR measurements at 9 different positions.

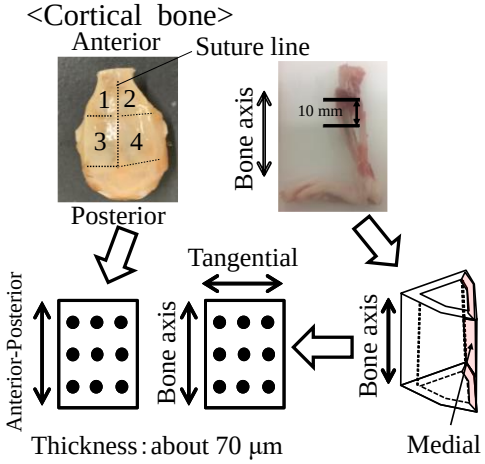

Fig. 1 Sample preparation and BR measurement positions.

2.2. Brillouin scattering technique

Brillouin scattering measurements were carried out with a six-pass tandem Fabry-Pérot interferometer using a solid state laser with wave length of 532 nm. The system included an optical microscope. The actual spot diameter of the focused laser beam on the specimen was approximately 10 μm. For Brillouin scattering measurements, the Reflection Induced θ Angle scattering geometry was used [4]. This geometry enables to observe ultrasound waves propagating in 2 directions ($q^{\theta A}$ and q^{180}). Here, we focused on the in-plane $q^{\theta A}$ direction. From the following equation, we can obtain wave velocity.

$$v^{\theta A} = f^{\theta A} \frac{\lambda_0}{2\sin(\frac{\theta}{2})} \qquad (1)$$

Here, $v^{\theta A}$ is the wave velocity, $f^{\theta A}$ is the measured shift frequency and λ_0 is the incident light wave length and θ is the incident angle of light.

3. Results and discussion

Figure 2 shows the X-ray CT (computed tomography) cross section images of tibias. The

cortical thickness of STZ samples were smaller than those of control samples.

Figure 3 shows the blood glucose level (BGL) and body weight (BW) changes in the rats. STZ rats died just before 19 weeks. Threshold for the diagnostic criteria of hyperglycemia diabetes is BGL of 300 mg/dl [5]. The STZ rats showed hyperglycemia 4 days after injection, and BW gradually decreased. The control rats kept normal BGL, and BW gradually increased. The estimated ages of the rats in 10-week-old correspond to those of young human (9 years) [6]. The rats are still in the growth stages, and the BW increased until 19-week-old (almost 16 years old in human). The body weight difference may affect bone elasticity of tibia (weight bearing bone). Therefore, we also measured the velocities in skull bone (non-weight bearing bone).

Figure 4 shows averaged wave velocities in tibia and skull samples. The wave velocities in skulls were lower than those of tibias. The velocity difference due to the site may come from the different structures [7]. In case of 14-week-old rats, the velocities in the STZ samples were 4.6 % (tibia) and 3.7 % (skull) lower than those in the control samples (*p<0.01). However, in the 19-week-old samples, the velocities showed no significant difference between STZ and control samples.

Insulin has effects on bone formation to enhance the bone forming process from matrix formation to mineralization [1]. The velocity decreases in the 14-week-old may be caused by production of AGEs in the bones or constricted bone growth such as mineralization because of impaired insulin secretion. Yasui, et al. reported velocity decrease even in the young rats in the early stages of hyperglycemia diabetes [8]. The bone probably has excessive AGEs due to long term hyperglycemia in the 19-week samples because the rodents have almost no bone metabolism. Crosslinks often increase the elasticity. However, these results showed that in the early stages of hyperglycemia, the velocities decrease in bones, but the velocities may increase in long-term hyperglycemia.

4. Summary

Longitudinal wave velocities in the tibia and skull of streptozotocin induced diabetic rats were investigated by a micro-Brillouin scattering technique. The velocities in 4 weeks later after inducing diabetes were lower than those of the control samples. However, the velocities in the 9 weeks later showed no significant difference compared with the control samples. The mechanism of bone growth under hyperglycemia should be investigated in detail.

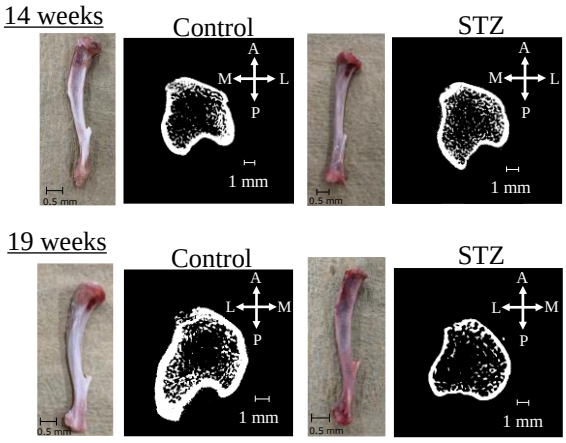

Fig. 2 Control and STZ rat tibias measured by X-ray CT.

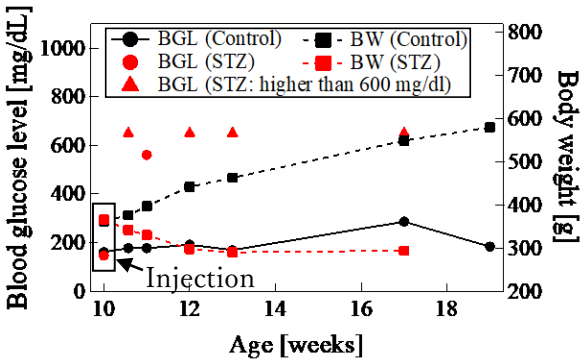

Fig. 3 Changes of body weight (BW) and Blood glucose level (BGL).

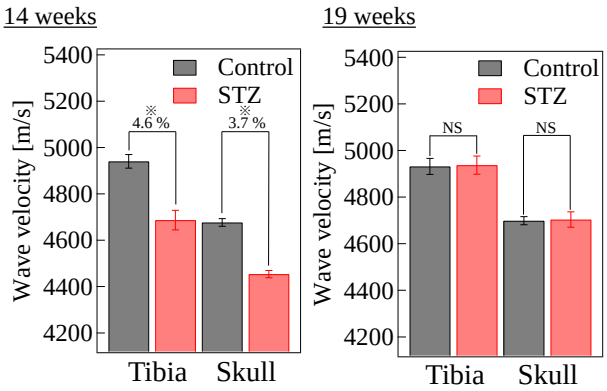

Fig. 4 Averaged wave velocities in tibia and skull samples.

References

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