

Efficacy of Shear Wave Elasticity for Evaluating Myocardial Hypertrophy in Hypertensive Rats

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Abstract

Shear wave (SW) imaging is a novel ultrasound-based technique for assessing tissue characteristics. SW elasticity may be useful to assess the severity of hypertensive left ventricular (LV) hypertrophy. This study aimed to evaluate the efficacy of SW elasticity for assessing the degree of myocardial hypertrophy using hypertensive rats. Rats were divided into hypertension group and control group. SW elasticity was measured on the excised heart. Myocardial hypertrophy was assessed histologically. LV weight was greater in hypertension group. An increase in interventricular septum and LV free wall thicknesses was observed in hypertension group. SW elasticity was significantly higher in hypertension group than in control group (14.6 ± 4.3 kPa vs. 6.5 ± 1.1 kPa, $P < 0.01$). The cross-sectional area of cardiomyocytes was larger in hypertension group than in control group ($397 \pm 50 \mu\text{m}^2$ vs. $243 \pm 14 \mu\text{m}^2$, $P < 0.01$), and SW elasticity was positively correlated with the cross-sectional area of cardiomyocytes ($R = 0.96$, $P < 0.01$). This study showed that SW elasticity was higher in hypertensive rats and was closely correlated with the degree of myocardial hypertrophy, suggesting the efficacy of SW elasticity for estimating the severity of hypertensive LV hypertrophy.

Introduction

Shear wave (SW) imaging is a novel ultrasound technique for assessing characteristics of tissues¹⁻³. SW is generated by pushing pulse of ultrasound wave which deforms a part of the tissue. SW velocity within the tissue is detected by tracking pulse. SW imaging has been evaluated in several organs, including the liver⁴⁻⁹. SW elasticity, which is calculated by SW velocity, has been reported to be correlated with the degree of fibrosis in liver diseases^{4,5}. In the field of cardiac diseases, some studies reported that SW elasticity could be used to measure myocardial stiffness in animal models¹⁰⁻¹². In a clinical setting, one study reported that SW elasticity was higher in patients with hypertrophic cardiomyopathy¹³. However, hypertrophic cardiomyopathy has remarkable left ventricular (LV) hypertrophy. It remains unclear whether SW elasticity can assess the severity of hypertensive LV hypertrophy. Therefore, we hypothesized that SW elasticity histologically reflects the degree of myocardial hypertrophy. This study aimed to evaluate the efficacy of SW elasticity for assessing myocardial hypertrophy of left ventricle using hypertensive model rats.

Methods

Animal experiments.

Seven-week-old male Dahl salt-sensitive rats (Japan SLC, Shizuoka, Japan) were housed under conditions of constant temperature (22°C) and humidity (60%), exposed to a 12-hour light/dark cycle, and offered tap water to drink. Rats were divided into two groups: hypertension group (n = 6), which were fed a high-salt (8% NaCl) diet for 9 weeks to develop LV hypertrophy, and control group (n = 6), which were fed a normal diet. All surgery was performed under anesthesia, and all efforts were made to minimize

suffering. All protocols for animal experiments were approved in accordance with the recommendations of the Okayama University Animal Care and Use Committee, and all methods were performed in accordance with the relevant guidelines and regulations. This study was reported in accordance with the ARRIVE guidelines.

Echocardiography.

Transthoracic echocardiography was performed using Aplio ver. 6.0 with a 10-MHz sector probe (Canon Medical Systems, Otawara, Japan). The rats were anesthetized from inhalation of 2% isoflurane while lying in a left recumbent position. LV end-diastolic and end-systolic diameters were measured in the short-axis view, and fractional shortening was calculated. Interventricular septum and LV free wall thicknesses were measured.

Shear wave elasticity.

SW imaging was performed by ex vivo experiment. A retrograde perfusion system was used to maintain the rat's heart in a completely relaxed state¹⁴. After sacrifice under inhalation of 2% isoflurane anesthesia, the heart was quickly excised and submerged in the Tyrode solution (136 mmol/L NaCl, 5.4 mmol/L KCl, 1.8 mmol/L CaCl₂, 0.53 mmol/L MgCl₂, 5.5 mmol/L HEPES, and 1% Glucose, pH 7.4, 37°C) with 20 mmol/L butanedione monoxime, an inhibitor of actin-myosin interaction, and 10 μmol/L blebbistatin, a specific myosin II inhibitor. The ascending aorta was cannulated with an 18-gauge blunted needle connected to a retrograde perfusion system. The heart was perfused with the Tyrode solution with butanedione monoxime and blebbistatin to induce complete relaxation. The heart was set in a water tank of agar phantom.

SW elasticity was measured using Aplio i900 with an 18-MHz linear probe (Canon Medical Systems). B-mode image was obtained in the long-axis view. A rectangular region of interest was placed on LV free wall. SW was generated by pushing pulse, and SW velocity was obtained based on the tissue Doppler technique (Fig. 1). After confirming a proper SW propagation in "wave front" style display, a circular region of interest of 1-mm in diameter was placed on the image. SW elasticity was measured automatically using the equation: $3\rho c^2$ (c : SW velocity, ρ : tissue density). Each measurement was repeated five times, and the average value was calculated.

Histology.

The heart was sectioned transversely at the mid-papillary level, and then fixed with 10% formalin, embedded in paraffin, and cut into 5-μm-thick sections. Sections were stained with hematoxylin-eosin for assessing myocardial hypertrophy, and with picosirius red for assessing myocardial fibrosis. The cross-sectional area of cardiomyocytes was quantitatively measured at 50 locations using ImageJ software

(version 1.52v, National Institutes of Health, Bethesda, MD, USA), and the average value was calculated. The extent of myocardial fibrosis was also measured, and the percentage was calculated.

Statistical analysis.

Data are presented as mean \pm standard deviation for continuous variables. Variables were compared by unpaired t-test. Relationships of SW elasticity with myocardial hypertrophy and fibrosis were analyzed by Pearson's correlation coefficient. Statistical analysis was performed with JMP version 14.2 (SAS Institute Inc., Cary, NC, USA), and significance was defined as a value of $P < 0.05$.

Results

Baseline characteristics.

Baseline characteristics are shown in Table 1. Body weight was significantly lower in hypertension group than in control group. Heart weight and LV weight were greater in hypertension group than in control group. An increase in LV end-diastolic and end-systolic diameters with a decrease in fractional shortening was observed in hypertension group. Interventricular septum and LV free wall thicknesses were increased in hypertension group.

Table 1
Baseline Characteristics. Data are presented as mean \pm standard deviation. LV left ventricular.

Variables	Hypertension group (n = 6)	Control group (n = 6)	P
Body weight (g)	272 \pm 15	338 \pm 8	< 0.01
Heart weight (g)	1.6 \pm 0.1	1.4 \pm 0.1	< 0.01
LV weight (g)	1.2 \pm 0.1	0.9 \pm 0.1	< 0.01
Echocardiography			
LV end-diastolic diameter (mm)	8.4 \pm 0.4	6.8 \pm 0.3	< 0.01
LV end-systolic diameter (mm)	5.7 \pm 0.6	3.2 \pm 0.7	< 0.01
Fractional shortening (%)	32 \pm 7	54 \pm 9	< 0.01
Interventricular septum thickness (mm)	1.4 \pm 0.1	0.9 \pm 0.1	< 0.01
LV free wall thickness (mm)	1.5 \pm 0.1	1.1 \pm 0.2	< 0.01

Shear wave elasticity.

Comparison of SW elasticity of left ventricle between hypertension group and control group is shown in Fig. 2. SW elasticity was significantly higher in hypertension group than in control group (14.6 ± 4.3 kPa vs. 6.5 ± 1.1 kPa, $P < 0.01$).

Histological assessment.

Hematoxylin-eosin staining of left ventricle in hypertension group and control group is shown in Fig. 3A. The cross-sectional area of cardiomyocytes was significantly larger in hypertension group than in control group ($397 \pm 50 \mu\text{m}^2$ vs. $243 \pm 14 \mu\text{m}^2$, $P < 0.01$). Picrosirius red staining is shown in Fig. 3B. The amount of myocardial fibrosis was small, and the percentage of fibrosis was not different between hypertension group and control group ($2.1 \pm 1.1\%$ vs. $1.7 \pm 0.2\%$, $P = 0.40$).

Relationship of shear wave elasticity with myocardial hypertrophy.

SW elasticity was positively correlated with the cross-sectional area of cardiomyocytes ($R = 0.96$, $P < 0.01$) (Fig. 4). There was no correlation between SW elasticity and the percentage of myocardial fibrosis.

Discussion

The major findings of the present study were: 1) SW elasticity was higher in hypertensive rats; and 2) SW elasticity was positively correlated with myocardial hypertrophy. To the best of our knowledge, this is the first study to show the efficacy of SW elasticity for assessing the degree of myocardial hypertrophy of left ventricle.

The severity of LV hypertrophy is associated with adverse outcomes in the clinical setting¹⁵. LV hypertrophy is a common cause of heart failure with preserved LV ejection fraction, resulting in hospitalization. LV hypertrophy involves changes in myocardial tissue. Assessing the degree of myocardial hypertrophy can be useful for identifying the disease severity in the hypertensive population.

SW imaging is a novel technique for characterizing tissue structures by ultrasound beam. SW elasticity clarifies the contrast of anatomical structures consisting of tissues with different shear coefficients, thereby enabling tissue identification^{1,16}. Although SW elasticity has been recognized as a useful tool for characterization of liver diseases^{4,5,17-19}, the efficacy is limited in cardiac diseases. Regarding the heart, the utility of SW elasticity was investigated in animal models¹⁰⁻¹². Pernot et al. reported that SW elasticity could be used to measure myocardial stiffness in isolated rat hearts¹⁰. They also reported that SW elasticity differentiated between stiff, noncompliant infarcted myocardial walls and softer walls containing stunned myocardium in ischemic hearts of an ovine model, showing that SW elasticity was

higher in infarcted myocardium¹². In recent years, the feasibility of SW elasticity was investigated in human subjects^{13,20,21}. Villemain et al. reported that SW elasticity could assess myocardial stiffness quantitatively, showing that SW elasticity was higher in patients with hypertrophic cardiomyopathy than in healthy volunteers¹³. Pislaru et al. reported that higher SW elasticity was observed in patients with cardiac amyloidosis²⁰. Therefore, SW elasticity could be useful in detecting cardiomyopathy with severe hypertrophy of left ventricle. However, the utility of SW elasticity for assessing the severity of hypertensive LV hypertrophy remains unclear. The relationship of SW elasticity with myocardial histological tissue has not been well investigated.

To clarify these findings, we used hypertensive model rats and evaluated the efficacy of SW elasticity for histologically assessing myocardial hypertrophy. In the present study, SW elasticity was higher in hypertensive rats. Larger cross-sectional area of cardiomyocytes was observed in hypertensive rats, and SW elasticity was correlated with the cross-sectional area of cardiomyocytes. Our findings suggest that SW elasticity can evaluate the severity of hypertensive LV hypertrophy. Additionally, in the myocardium of hypertensive rats, myocardial fibrosis was slight, which was similar to that in control rats. SW elasticity has been reported to be correlated with fibrosis^{4,5}, whereas the present study found that SW elasticity was related to myocardial hypertrophy in the myocardium with less fibrosis.

Clinical application.

SW imaging is a non-invasive and quantitative method. The present study suggests that SW elasticity can provide valuable information for assessing the severity of LV hypertrophy. Additionally, SW elasticity may be useful as a clinical tool for assessing treatment effects for LV hypertrophy. SW elasticity may have the potential for identifying myocardial tissue characteristics, leading to the differentiation of cardiac diseases. Further studies are needed to determine the usefulness of SW elasticity. For clinical application of SW imaging, it is necessary to remove the influence of the beating of heart on SW velocity. A few recent studies have reported the feasibility of SW imaging under the beating hearts¹³. The application seems to be possible.

Limitations.

There are limitations in the present study. This study evaluated SW elasticity in non-beating hearts, because the heart rate of rats is too rapid to obtain SW imaging. This study evaluated SW elasticity under a retrograde perfusion system to make the heart fully relaxed for simulating the end-diastolic phase. Additionally, SW elasticity was measured in the long-axis view, whereas myocardial histology was assessed in the short-axis view.

Conclusions

SW elasticity was higher in hypertensive rats. SW elasticity was closely correlated with the degree of myocardial hypertrophy of left ventricle. Our findings suggest that SW elasticity has the potential for estimating the severity of hypertensive LV hypertrophy.

Declarations

Funding:

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Author contributions

Y.T. wrote the manuscript. Y.T., N.K., S.A., M.Y., T.M. and H.I. planned the experiment. R.N., O.H., N.A., M.K., K.A., Y.O., K.I. and Y.S. collected data. All authors reviewed the manuscript.

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Figures

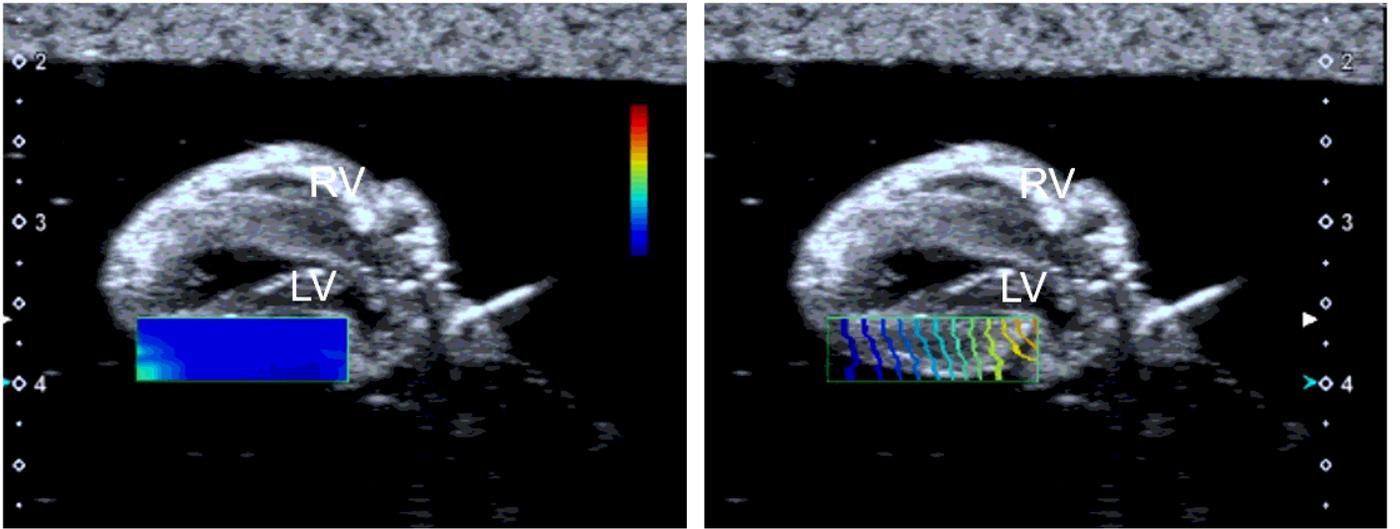


Figure 1

SW elasticity was automatically measured. LV left ventricle, RV right ventricle, SW shear wave.

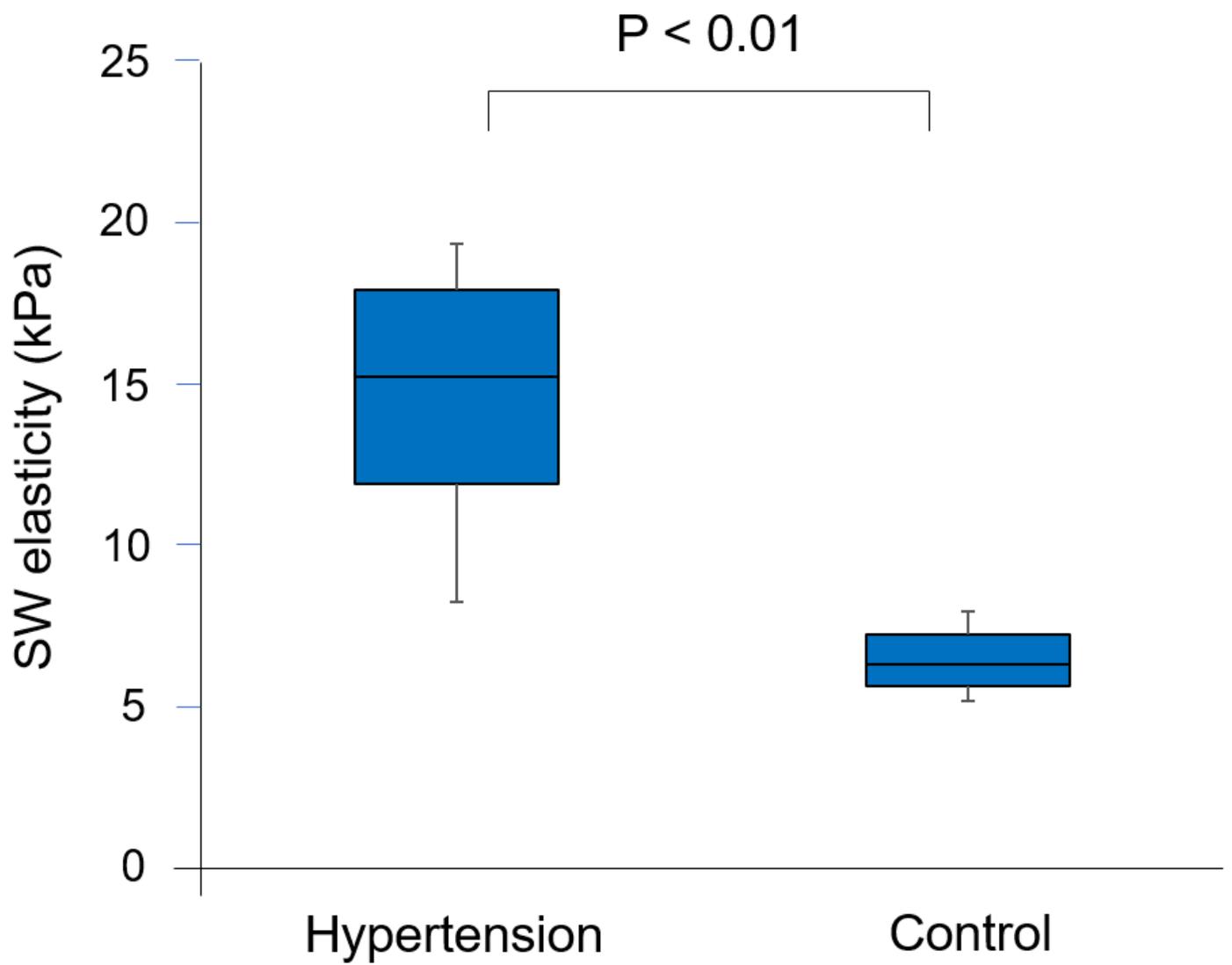
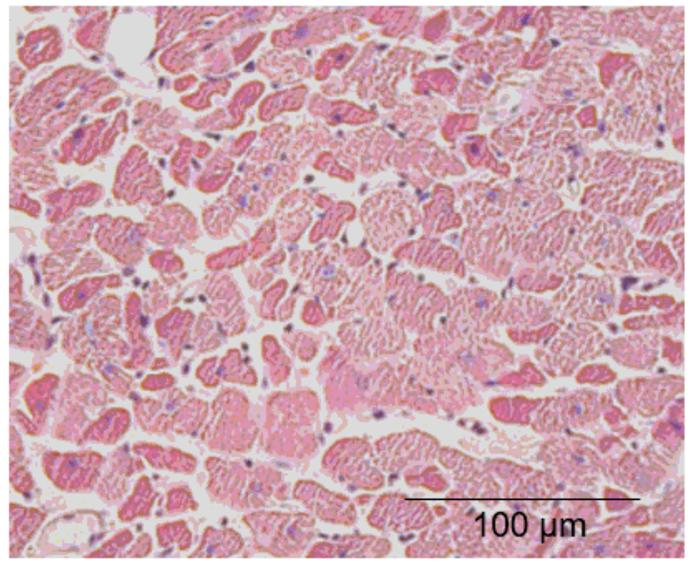
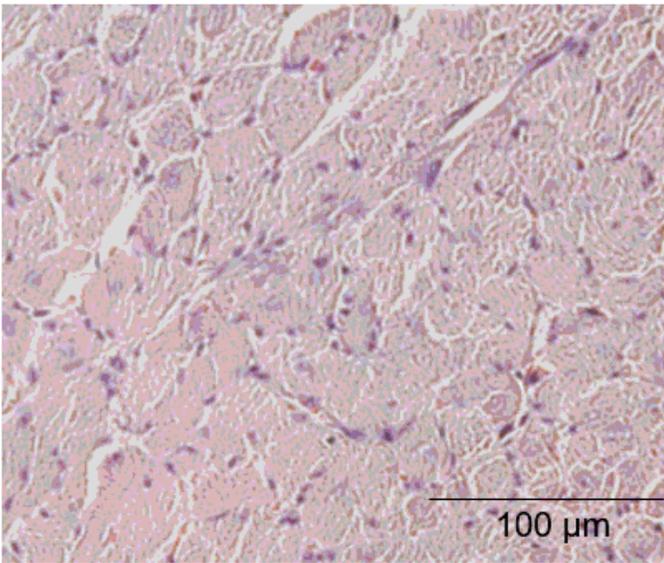


Figure 2

SW elasticity was higher in hypertension group than in control group. SW shear wave.

(A)



(B)

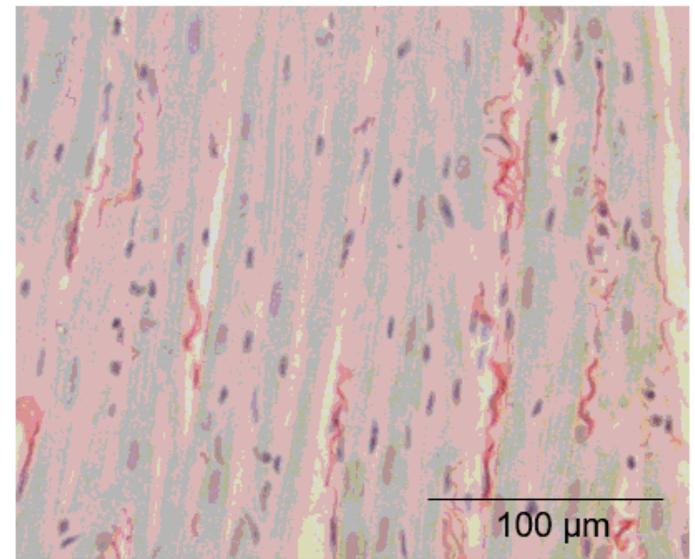
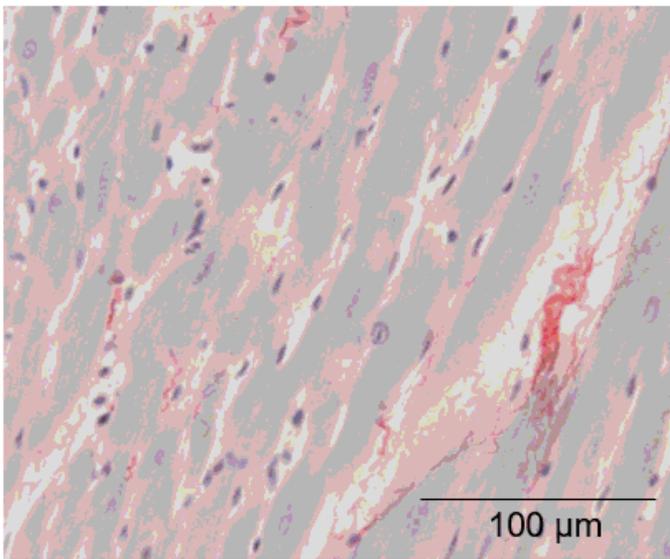


Figure 3

(A) Histological findings stained with hematoxylin-eosin in hypertensive group (left) and control group (right). The cross-sectional area of cardiomyocytes was measured. (B) Histological findings stained with picrosirius red (shown as red) in hypertensive group (left) and control group (right). Scale bar indicated 100 μm.

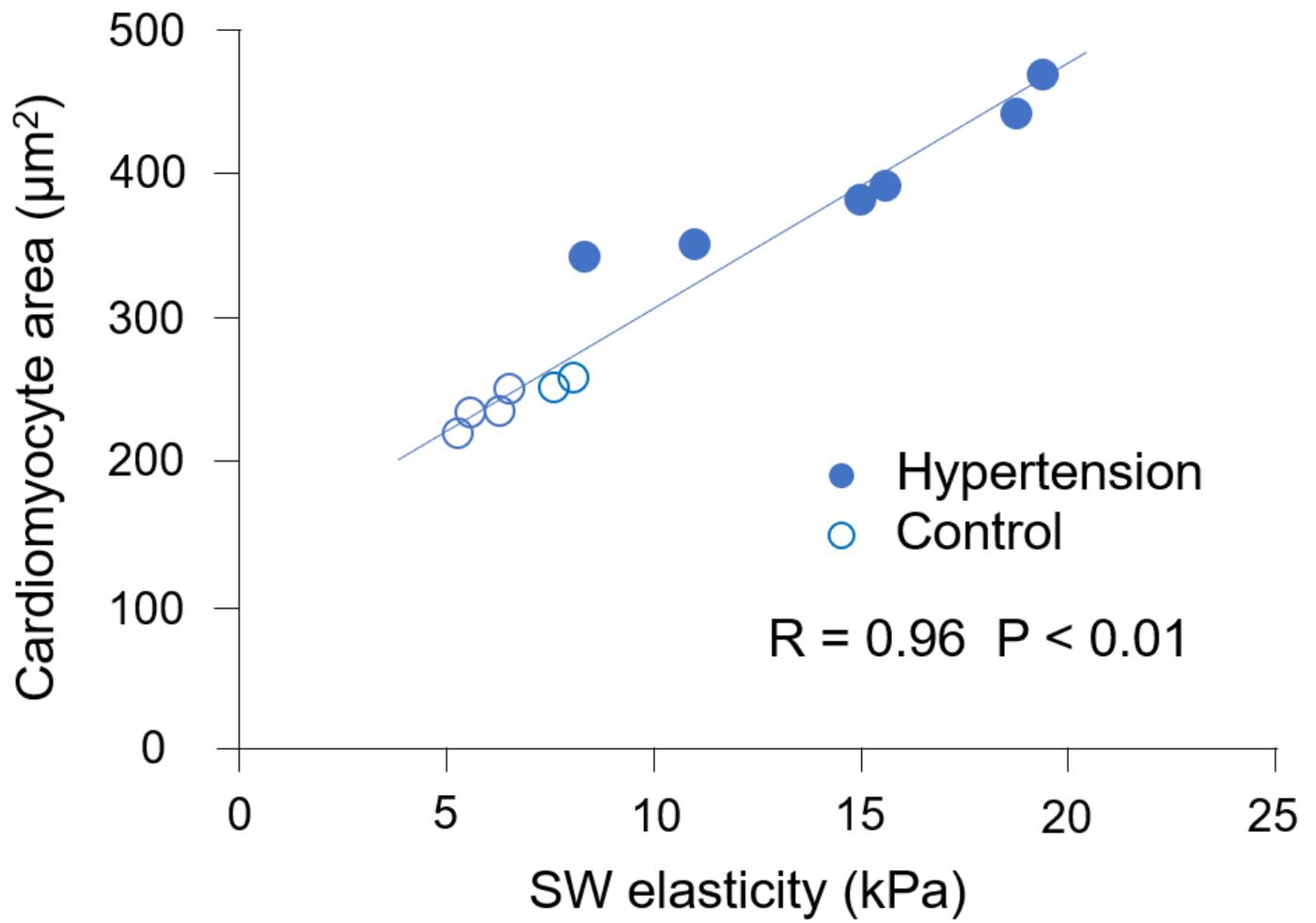


Figure 4

SW elasticity was correlated with the cross-sectional area of cardiomyocytes. SW shear wave.