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Experimental estimation and comparison of viscoelastic characteristics of rat aorta in vitro

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Abstract

The aim of the present work was to experimentally estimate and compare natural frequency, modulus of elasticity, and coefficient of viscosity of strip preparations of rat aorta. Materials and methods: The experimental setting included an improved apparatus, applying the forced oscillations method. Ten healthy Wistar rats, aged 4 (n = 7) and 14 months (n = 3), were sacrificed and the excised aortas were spirally cut into strip preparations. Forced oscillations were recorded for 4 suspended concentrated masses, equivalent to intraluminal pressure in the range 80-160 mmHg. Resonance curves were obtained and thereafter the natural frequency, 3dB width and octave length. Distension was measured at each equivalent pressure. Results: Natural frequency was estimated at 6.02-7.27 Hz (mature) versus 6.73-8.01 Hz (aged). Modulus of elasticity was higher for the aged aortas at 0.18-0.56 MPa versus 0.16-0.49 MPa for the mature. There was no statistically significant difference between the two age groups in terms of coefficient of viscosity. It was estimated at 0.008-0.023 N.s/m (mature) and 0.011-0.021 N.s/m (aged). Conclusion: The aged rat aorta is more threatened by high resonance oscillations than the mature aorta. The aged artery is less distensible than the mature one.

Keywords: viscoelasticity, rat, aorta

1. Introduction

As part of the mammalian circulatory system, arteries serve as conduits that provide oxygen- and nutrient-rich blood to tissues and organs. Except for the pulmonary arteries, which carry deoxygenated blood to the lungs, most other arteries deliver oxygenated blood to peripheral capillaries throughout the body. The largest artery in the mammalian body, the aorta, consists of the ascending section, an arch and a descending section. Beginning from the aortic arch, the descending aorta runs throughout the thoracic cavity, crosses the diaphragm via the aortic hiatus and extends into the abdominal cavity.

Larger arteries, and especially the aorta, are subjected to significant mechanical loads caused by the oscillating nature of heart beats. These pulsations lead to time-dependent variability of the resulting blood flow and thence the pressure. Further downstream along the arteries, the oscillating blood pressure is smoothed down to steady flow, which is necessary for maintaining of the physiological homeostasis at the capillary level. The aorta is both the largest artery and the one closest to the heart. It possesses significant distensibility, which helps it to store some of the blood volume pumped by the heart during the systolic phase of its cycle. In the diastolic phase, the stored elastic energy is released as recoil, contributing to the general equalization of blood pressure often referred to as the Windkessel effect. This physiological oscillation of blood flow is the main source of mechanical stress, acting on the arterial wall [1, 2].

The pulsatile blood flow generates two types of force: pressure and shear stress. As a consequence of these forces, the resulting strain sustained by the aorta is both circumferential and along its main axis [3]. There is evidence that the circumferential strain during cardiac systole, in healthy human thoracic descending aorta, is between 7.3% and 8.1% in young individuals, and decreases to between 2% and 4.2% in aged individuals. The longitudinal strain decreases from 2% to about 1% with age. The frame of reference for these measurements, in respect to the physiological state, is the diastolic circumference of the lumen

boundary or the diastolic length, respectively [4]. One study has shown that the aorta stretches up to 10% of its diastolic during cardiac systole [3].

The aortic wall consists of three concentric layers. The innermost layer, tunica intima, covers the internal wall surface, which is in immediate contact with the lumen. This layer includes the endothelium and the internal elastic lamina. The middle layer, tunica media, is on its own made of concentric layers called lamellar units [5]. Each unit consists of a layer of overlapping smooth muscle cells ordered in circumferential direction, which is covered on the two sides by elastic lamellae. The elastic lamellae are composed of elastin fibres. The lamellar units also include elastin interlamellar struts, elastin cell-lamellae connections, collagen fibres and collagen envelopes of smooth muscle cells. The collagen fibres are also ordered in circumferential direction. The third layer, tunica adventitia, consists of connective tissue such as collagen and elastin fibres, although the elastin network is less dense than in the media.

The role of the elastin network is to provide strength and elasticity at low and medium strain levels. In contrast, the collagen fibres are tightly packed at low strain levels, providing only support. At higher blood pressure the collagen fibres unpack and lengthen so that excess strain could be restricted. Modulus of elasticity of collagen is about 100 MPa while for elastin it is between 0.4 and 0.6 MPa. Due to the non-homogenous distribution of elastin and collagen fibres, the medial structure is regarded as anisotropic [2,3]. The anisotropic nature of the aortic wall exhibits mechanical properties, which differ between perpendicular directions of the aorta – circumferential and longitudinal. On the other hand, some authors claimed that the aorta could be regarded as isotropic in human [6] and rat [7], as well as dog carotid artery [8]. The cited authors made their conclusions in experiments with small deformations in both directions – longitudinal and circumferential. According to the applied deformations in our experiments, the rat aorta was accepted here as isotropic.

A lot of studies have been performed to describe differences in biomechanical behaviour of arteries with regard to various influencing factors [9,10,11,12,13,14,15]. One of these factors is arterial wall ageing, a process developing at different rate either in physiological or pathological condition. It leads to progressively higher accumulation of collagen fibres in the arterial wall with lower contribution of elastin. The aorta becomes stiffer, while its diastolic diameter increases [3,4]. The natural process, generally known as arteriosclerosis, is often additionally worsened with time due to concomitant pathologies that have their influence on the circulatory system. For instance, diabetes, hypertension or genetic disorders may significantly increase wall stiffness, decrease its compliance or cause adaptive changes in the underlying tissue, leading to adjustments in its viscoelasticity and subsequent development of aortic aneurisms.

Arterial viscoelasticity has been adopted as a major determinant for diagnostics, prognosis and treatment of vascular disease. The most of the studies were carried out in *in vivo* experiments and are not considered in the present work because of the presence of the organism regulation. In other studies were obtained static and quasi-static elastic characteristics, and thus they can be only used as reference for the trend of changes because are still far from the real behaviour of the arterial wall in the organism. Viscoelasticity of the arterial wall might be investigated more precisely when the humoral and nervous regulation of the organism is eliminated - i.e., in *in vitro* experiments. In such a paradigm, the active tone of the smooth muscle cells and the passive tone of the cell and extracellular constituents of the arterial wall might be investigated. Such experiments were implemented previously mostly on post-mortem human and animal's preparations and were directed to estimation of the complex elastic modulus (consisting of dynamic modulus of elasticity and modulus of viscous loss) [13, 16, 17, 18, 19].

The viscoelasticity of rat aorta was studied particularly by our team, using an engineering method – the method of forced oscillations [20, 21]. Some of the experiments were carried out on strip preparations from Wistar and SHR mature rats (4 months) [22, 23], while others – on cylindrical segments from Wistar rats (4, 12, and 18 months in age) [24]. Viscoelastic characteristics such as natural frequency, dynamic modulus of elasticity, and coefficient of viscosity were estimated – dependent on the equivalent intraluminal pressure. That approach allowed the arterial wall to be regarded as a material possessing nonlinear elasticity. The method of forced oscillations enables to assess the proper dynamical behaviour of the arterial wall when it is not affected by the organism regulations.

The aim of the present work was to experimentally estimate the relationship between natural frequency, modulus of elasticity, coefficient of viscosity and the equivalent intraluminal pressure, using the forced oscillations' method. *In vitro* experiments were carried out with strip preparations of rat aorta at quasi-physiological conditions, simulating tissue behaviour in vivo. Another important objective of this study was

to compare the three characteristics between two subject groups, each at different age, in this way also investigating the effect of ageing on arterial viscoelasticity.

2. Materials and methods

2.1. *Subjects*

Two groups of healthy Wistar male rats at different age were studied: the first one at 4 months (mature) and the second one at 14 months (aged). The Ethics Committee of the Institute of Neurobiology has approved the experiments.

The rats were sacrificed by decapitation and exsanguinated. Then, the aorta was excised within 10 minutes after animal death and immersed in a chilled modified Tyrode solution, called nutrient medium. The solution has been used in other experiments containing (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 2.0, $MgCl_2$ 0.6, $NaHCO_3$ 11.9, NaH_2PO_4 0.5, glucose 11.5, pH 7.2–7.4 (oxygenated – 5% CO_2 in O_2, 37°C). The aorta was cleaned from the connective tissue and spirally cut at about 60° angle in a 3 mm wide strip, which was then cut into 2 separate preparations, each about 25 mm long. Four of the prepared strips were insufficiently long, therefore used for only one preparation. One of the experiments was destroyed by electrical disturbances, so one of preparations was excluded from the investigation.

In general, there were investigated 7 mature and 3 aged rat aortas, divided in 10 preparations from mature aorta and 5 preparations from aged aorta. In one experimental day only one laboratory rat could be investigated. This limitation was enjoined by the time for getting up the preparation (about 1 hour) and the time for following investigation of two preparations – each about 2 hours. It was previously evidenced [23] that the experimental protocol ensures at least 4 hours of preservation the vitality of the preparation and allowed biomechanical investigation in quasi-physiological conditions. The vitality of preparations was evidenced by their resonance curves [23] that were of hardening type for vital preparation and of softening type for devitalised preparation.

2.2. Method and experimental protocol

Each strip preparation was suspended in an organ bath chamber (see Fig.1) and continuously superfused with the nutrient medium. The latter was constantly oxygenated – 5% CO_2 in O_2, and heated up to 37°C. While experimenting with the first preparation, the second one was kept immersed in the nutrient medium and refrigerated to 4°C. Once suspended, the experimental preparation was allowed to adapt to the experimental conditions for 30 min. After the adaptation time had elapsed, the suspended preparation was subjected to the method of forced oscillations [21]. Following this method, low frequency sinusoidal oscillations with constant amplitude were applied at the upper end of the preparation – named excitation oscillations. At the lower end of the preparation were sequentially suspended several concentrated masses, this way imitating an extension, equivalent to that made by the aortic intraluminal pressure. Each mass corresponds to a fixed intraluminal mean-pressure level.

The excitation oscillations frequency was swept across the range of 3 to 30 Hz in an increment-decrement mode. The used frequency range is in the physiologically normal to rat's heart rate, which is from 2 to 10 Hz. In the same time the response displacement oscillations at the lower end were being recorded, which were with the same frequency but with different oscillation amplitudes. The goal was to pass through the displacement resonance by sweeping the range of physiological frequencies, normal to rats. The experimental procedure was repeated at several mean-pressure levels, corresponding to selected applied concentrated masses. On Fig.2 are shown typical resonance curves at increasing and decreasing excitation frequencies for a fixed mean-pressure level. On the vertical axis are represented the amplitudes of the response oscillations in dB, and on the horizontal axis - the excitation frequency in Hz in logarithmic scale. The natural frequency was measured as the projection of the inclined bone of the resonance curve on the frequency axis.

Fig. 1. Organ batch chamber and experimental setting

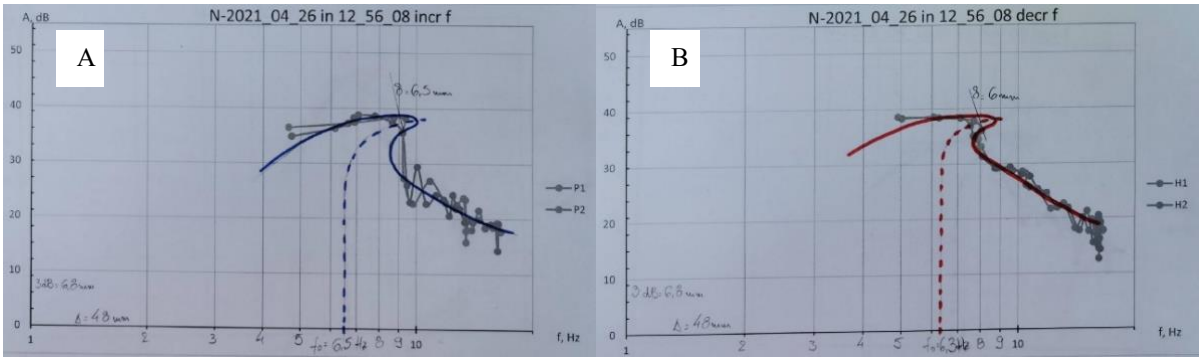


Fig. 2. Resonance curves for a fixed mean-pressure level at increasing (left, A) and decreasing (right, B) excitation frequency sweeps

From each resonance curve were measured the 3 dB width and the octave length, and thereafter the dynamic modulus of elasticity and the coefficient of viscosity were calculated for the distinct equivalent intraluminal pressures, as described in a previous work [21], by the formulas (see Eq. (1), Eq. (2)):

(1) $$E' = (2\pi)^2 \frac{f_0^2 mL}{S}$$

(2) $$\beta = 2\pi f_0 \left(2^{\left(\frac{\delta}{\Delta}\right)} - 1\right)$$

where:

E' – dynamic modulus of elasticity [MPa],
f_0 – natural frequency [Hz],
Δ – octave length [mm],
δ – 3 dB width [mm],

S –cross-section area of the preparation [m^2],
β – coefficient of viscosity [Ns/m],
L – length of the preparation [m],
m – weight of the suspended concentrated mass [kg].

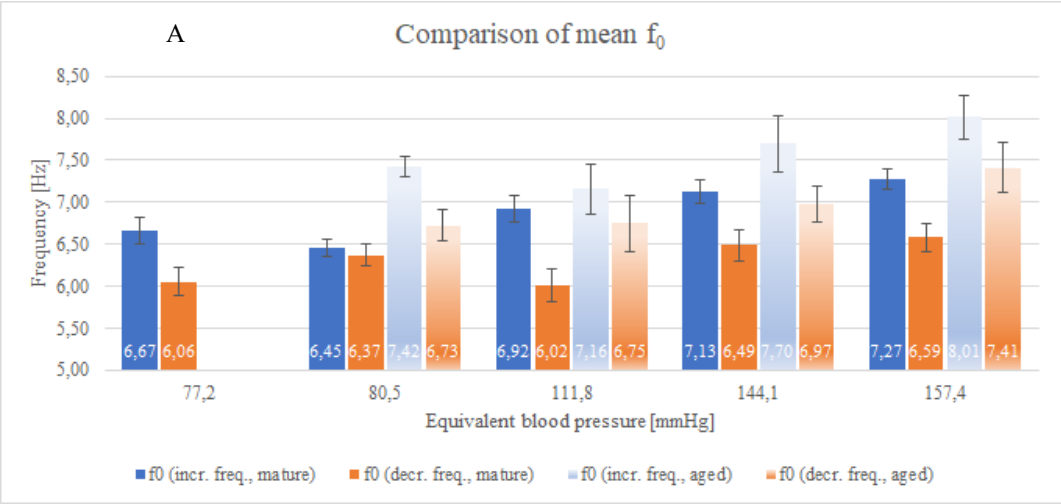
In the next step, the dependence of each viscoelastic characteristic on the equivalent intraluminal pressure was drawn. The resonance curves and therefore the viscoelastic characteristics are usually different at increasing and at decreasing excitation frequency, so this effect was also investigated.

3. Statistical analysis

Data obtained for the three viscoelastic characteristics (f_0, β, E') at each equivalent pressure level, excitation frequency sweep (EFS) and age group were analysed with statistical software Minitab 17. Mean values were calculated ± standard error of the mean (SEM). Regression analysis was performed and best fit models with their 95% confidence intervals (CI) were plotted for each characteristic / pressure relationship (CPR). Additionally, the two age groups and the two EFSs were compared to determine whether there is a statistically significant difference of each CPR with ageing or direction of the EFS. For estimation of the ageing effect, independent samples t-test was used for normally distributed data, as for non-normally distributed data the non-parametric Mann-Whitney U test was used instead. For estimation of EFS effects, paired samples t-test and Wilcoxon signed-rank test were used for normal or non-normal distribution of data, respectively. Sample data at two equivalent pressure levels (80.5 and 157.4 mmHg) were taken and, depending on the applied test, the relevant measure of central tendency (sample mean or median) was used for each comparison between groups. A level of significance for all tests was considered at $p = 0.05$, so only results below this value were accepted as significant.

4. Results

Results for the three CPRs, at increasing and decreasing EFS, are presented on Fig. 3. Each bar represents a mean value ± SEM. Blue bars represent mean values at increasing EFS and orange bars represent mean values at decreasing EFS. Dark and light colour bars represent the mature group and the aged group, respectively.



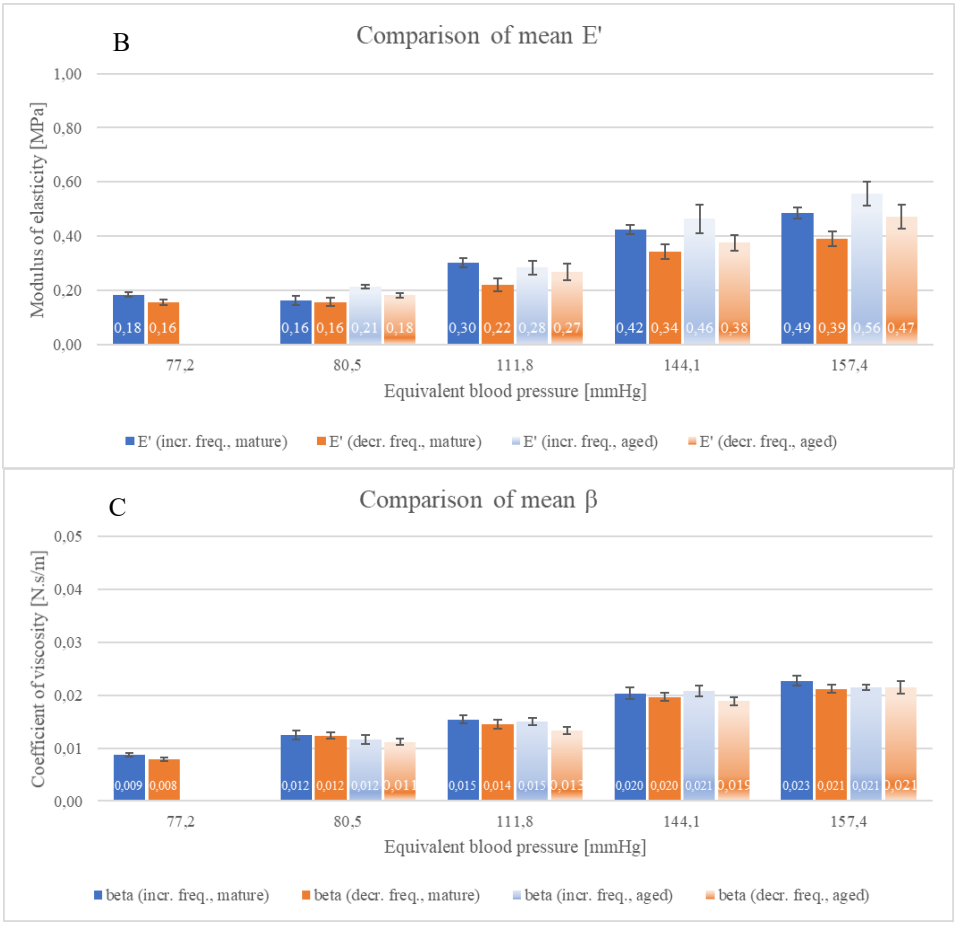
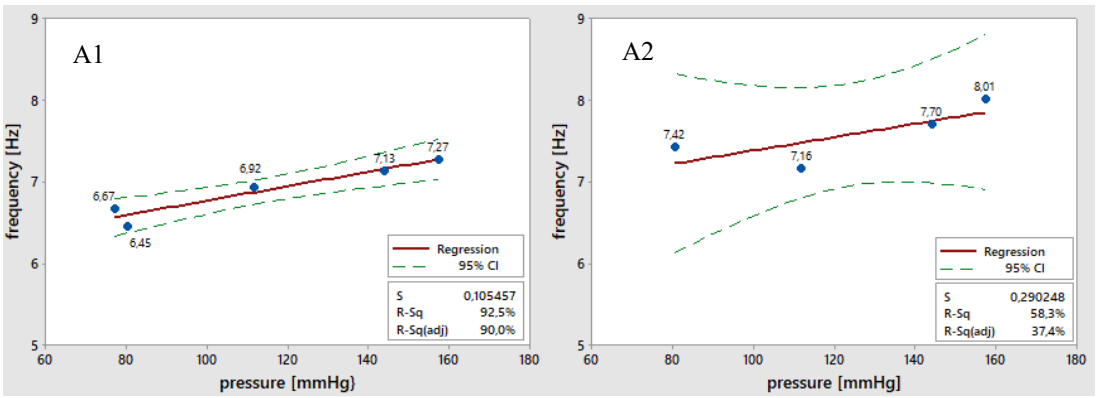


Fig. 3. Mean f_0 (A), E′ (B) and β (C) values at each equivalent blood pressure levels, compared for age and EFS.

Regression analysis was performed and equations are presented in Table 1 below. Analysis of the data shows that f_0 and β increased linearly with increasing intraluminal mean-pressure for both age groups. The natural frequency was estimated to be higher in the aged aortas than in the mature ones. Mean values were between 6.02 and 7.27 Hz for the mature, and between 6.73 and 8.01 Hz for the aged preparations, in the range of equivalent intraluminal pressure (80-160 mmHg). The coefficient of viscosity was estimated between 0.008 N.s/m and 0.023 N.s/m for the mature and between 0.011 N.s/m and 0.021 N.s/m for the aged preparations, and instead of the tendency for increasing with the age it was not found significantly different between the two groups. The modulus of elasticity was higher for the aged aortas, increasing from 0.16 MPa at 80 mmHg up to 0.49 MPa at 160 mmHg. The lowest value for the aged aortas was about 0.18 MPa at 80 mmHg, increasing up to 0.56 MPa at 160 mmHg (see Fig.4 and Fig.5).



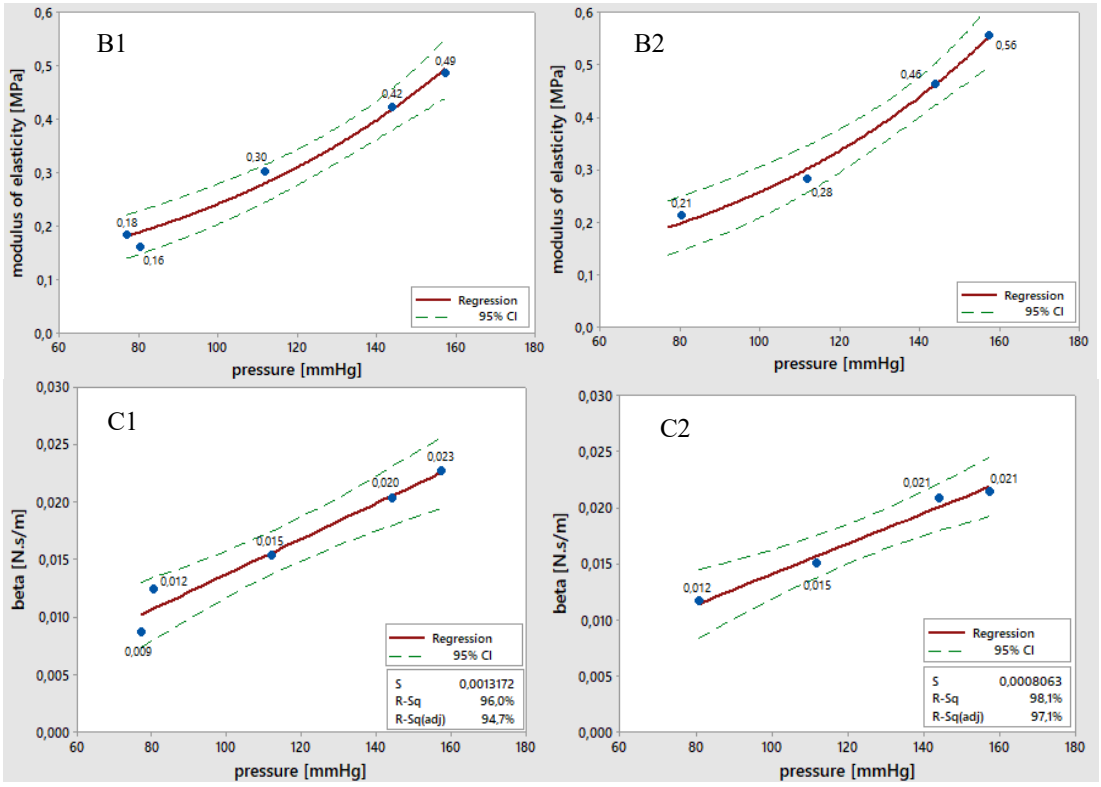
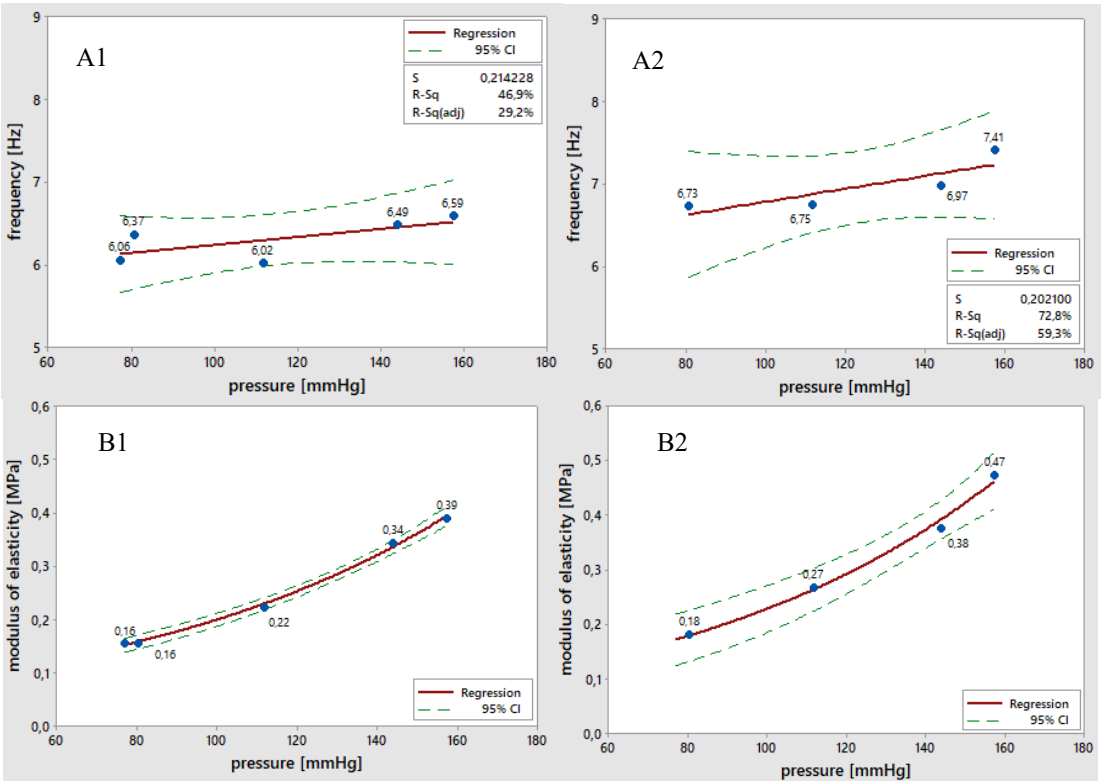


Fig. 4. Fitted line plots of f_0 (A), E' (B) and β (C) vs pressure at 4 months (left, 1) and 14 months (right, 2), at increasing excitation frequency sweep



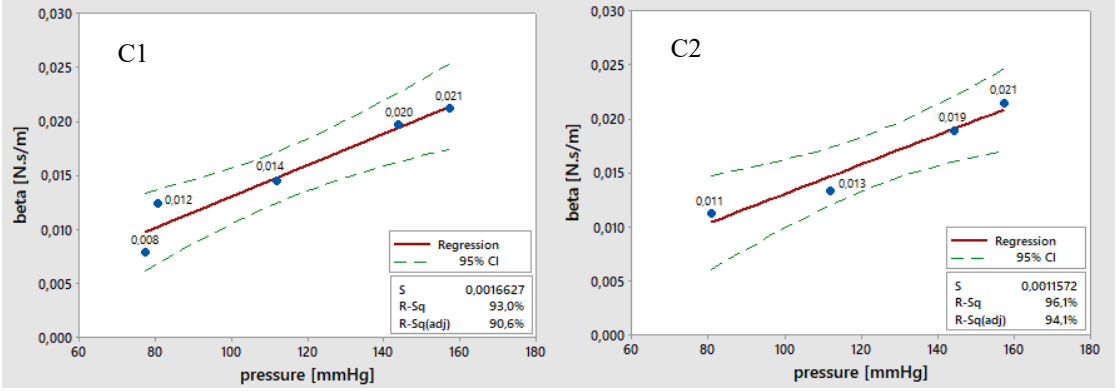


Fig. 5. Fitted line plots of f_0 (A), E′ (B) and β (C) vs pressure at 4 months (left, 1) and 14 months (right, 2), at decreasing excitation frequency sweep

Table 1
Regression equations and significance

	Age group	Excitation frequency	Regression equation	r^2 (S)	p-value
f_0	Mature	Increasing	$f_0 = 5.879 + 0.008852\ p$	0.925	0.009
		Decreasing	$f_0 = 5.754 + 0{,}004804\ p$	0.469	0.202
	Aged	Increasing	$f_0 = 6.567 + 0.008134\ p$	0.583	0.236
		Decreasing	$f_0 = 5.996 + 0.007846\ p$	0.728	0.147
E′	Mature	Increasing	$E' = 0{,}0686698 \exp(0{,}0125435\ p)$	(0.0212)	-
		Decreasing	$E' = 0.0604052 \exp(0.0119088\ p)$	(0.0069)	-
	Aged	Increasing	$E' = 0.067413 \exp(0.0133685\ p)$	(0.0162)	-
		Decreasing	$E' = 0.\ 0660337 \exp(0.0123695\ p)$	(0,0144)	-
β	Mature	Increasing	$\beta = -\ 0.001673 + 0.000154\ p$	0.947	0.003
		Decreasing	$\beta = -\ 0.001365 + 0.000144\ p$	0.930	0.008
	Aged	Increasing	$\beta = 0.000485 + 0.000136\ p$	0.971	0.010
		Decreasing	$\beta = -\ 0.000540 + 0.000136\ p$	0.961	0.020

After comparing the two age groups, the natural frequency (f_0) was found to be significantly different between the mature and the aged aortas regardless of frequency sweep and pressure level. On the other hand, the modulus of elasticity (E′) was found significantly different for both EFS only at the lowest pressure level – 80.5 mmHg. No significant differences were found between the two age groups for both EFS at the highest pressure – 157.4 mmHg. In contrast, the coefficient of viscosity (β) was not found significantly different between the two age groups for both frequency sweeps and at each pressure level (see Table 2).

Table 2
Comparison between the two age groups

	Equivalent pressure [mmHg]	Age group	Increasing excitation frequency			Decreasing excitation frequency		
			Group mean or median	Difference estimate	p-value	Group mean or median	Difference estimate	p-value
f_0	80.5	Mature Aged	$\bar{x}_1 = 6.45$ $\bar{x}_2 = 7.42$	- 0.964	$p < 0.01$	$\bar{x}_1 = 6.06$ $\bar{x}_2 = 6.73$	- 0.666	$p < 0.01$
	157.4	Mature Aged	$\bar{x}_1 = 7.27$ $\bar{x}_2 = 8.01$	- 0.738	$p < 0.01$	$\bar{x}_1 = 6.59$ $\bar{x}_2 = 7.41$	- 0.825	$p = 0.024$
E’	80.5	Mature Aged	$\bar{x}_1 = 0.176$ $\bar{x}_2 = 0.213$	- 0.037	$p < 0.01$	$\bar{x}_1 = 0.156$ $\bar{x}_2 = 0.182$	- 0.0252	$p = 0.044$
	157.4	Mature Aged	$\bar{x}_1 = 0.486$ $\bar{x}_2 = 0.556$	- 0.070	$p = 0.10$	$\bar{x}_1 = 0.390$ $\bar{x}_2 = 0.473$	- 0.0834	$p = 0.11$
β	80.5	Mature Aged	$M_1 = 0.012$ [a] $M_2 = 0.011$ [a]	0.0014	$p = 0.49$	$\bar{x}_1 = 0.012$ $\bar{x}_2 = 0.011$	0.0012	$p = 0.25$
	157.4	Mature Aged	$M_1 = 0.021$ [a] $M_2 = 0.022$ [a]	0.0002	$p = 0.74$	$M_1 = 0.0203$ [a] $M_2 = 0.0209$ [a]	- 0.0009	$p = 0.62$

[a] sample median was used due to non-normally distributed data and subsequent utilization of a non-parametric test

After comparing the two excitation frequency sweeps, significant differences were found in both age groups with respect to f_0 at 80.5 mmHg and 157.4 mmHg. The elastic modulus was found significantly different between all but the mature group at 80.5 mmHg. There was no statistically significant difference for the coefficient of viscosity among all tested groups (see Table 3).

Table 3
Comparison between the two excitation frequency sweeps

Equivalent pressure		Groups tested	Difference	95% CI for the difference	p-value
80.5 mmHg	f_0	mature, increasing EFS vs. mature, decreasing EFS	0.392	0.047; 0.738	0.029
		aged, increasing EFS vs. aged, decreasing EFS	0.691	0.253; 1.129	0.006
	E’	mature, increasing EFS vs. mature, decreasing EFS	0.0162	-0.0117; 0.0441	0.226
		aged, increasing EFS vs. aged, decreasing EFS	0.0312	0.0097; 0.0527	0.010
	β	mature, increasing EFS vs. mature, decreasing EFS	0.00015 [a]	-	0.906
		aged, increasing EFS vs. aged, decreasing EFS	0.00047	-0.0006; 0,0016	0.360

157.4 mmHg	f_0	mature, increasing EFS vs. mature, decreasing EFS	0.710	0.375; 1.046	0.000
		aged, increasing EFS vs. aged, decreasing EFS	0.600	0.362; 0.838	0.000
	E′	mature, increasing EFS vs. mature, decreasing EFS	0.1019	0.0576; 0.1463	0.000
		aged, increasing EFS vs. aged, decreasing EFS	0.0825	0.0563; 0.1087	0.000
	β	mature, increasing EFS vs. mature, decreasing EFS	0.00167 [a]	-	0.060
		aged, increasing EFS vs. aged, decreasing EFS	0.00065 [a]	-	0.636

[a] sample median was used due to non-normally distributed data and subsequent utilization of a non-parametric test

5. Discussion and conclusions

It should be noticed that similar studies were conducted on strips and on cylindrical segments of rat aorta by some of co-authors of the present paper [21, 23, 24]. Here, the experimental device is improved, especially its recording system and data-processing procedure (follows a new paper), that claiming more precise experimental data and results. Nevertheless, the herein reported results are close to the previous led on strip preparations, leading to the conclusion that the rat aorta represents the same as the reported up to now viscoelasticity and biomechanical behaviour.

In the range of equivalent intraluminal pressures between 80 and 160 mmHg, the natural frequency was found between 6.02 and 7.27 Hz for the mature, and between 6.73 and 8.01 Hz for the aged rat aorta. This observation may lead to the conclusion that resonance amplitudes caused by excitation frequencies between 6 and 8 Hz could threaten the rat aorta if continuously maintained. Moreover, the natural frequency of aged rat aorta was found to be significantly higher than the mature aorta. This ageing effect was found not only at increasing excitation frequency sweep, but also at decreasing. Nevertheless, the aged aorta was estimated to be relatively less dependent on the level of intraluminal pressure, compared to the mature aorta. Previous studies on strip preparations from mature rats [21] found natural frequency between 8 and 5 Hz for equivalent intraluminal pressures between 120 and 200 mmHg. Our current results are in accordance with these previous findings. Although three of four applied linear regression models for f_0 did not have sufficiently high coefficients of determination and/or low enough p-values ($r^2 > 0.8$; $p < 0.05$), we assume that this is due to the small sample sizes in the both age groups and larger samples would provide the required significance in future experiments.

In a paper describing the results of studying rat aorta segments, the natural frequency of mature rat aorta was found to be over 15 Hz and lifting, while for aged aortas the natural frequency was found diminishing with the intraluminal pressure [12, 25]. The type of the preparations could explain the difference – the structure of the strip preparation is particularly injured, although facilitating the experiment. On this stage of work, such preparations are preferred and are sufficiently easy for making. Unfortunately, we found little in the literature about similar biomechanical studies. In the works of Apter & Marquez (1968) [19], and Hardung (1970) [16] some of the characteristics such as the resonance curve and natural frequency of dog aorta [19] or oscillatory loading on arterial preparations [16] were only applied. In a recent study [1], human descending thoracic aorta strips and individual layer preparations were loaded by sinusoidal oscillations at physiological and small amplitudes but different information was drawn by the experiments. Viscoelastic parameters such as dynamic stiffness ratio and loss factor were obtained at three different levels of pre-stretch, and at physiological conditions, providing further knowledge about the arterial wall properties [1].

We found that the dynamic modulus of elasticity is significantly higher in aged compared to mature preparations, characterising the aged rat aorta as less distensible than the mature one. This observation confirms our previous findings on rat aorta cylindrical segments for both mature and aged groups [12]. The

elder cylindrical preparations have shown a different behaviour but we have no experimental data about that group. The raising modulus of elasticity could be explained by the adaptive structural and functional changes that develop with age, leading to a stiffer behaviour of the arterial tissue. These values are difficult to be directly compared with literature data because the modulus of elasticity was measured by others mostly in vitro and only at a chosen value of the intraluminal pressure – usually 100 mmHg, and the dependence on the intraluminal pressure was neglected.

Despite the small sample size of the studied preparations presented here, we found no significant change in the coefficient of viscosity with progression of age. This conclusion is contrary to the results from previous findings for increasing values in aged group [12, 25]. As it was noticed, the cited studies were conducted on cylindrical segments. We have no data for elder rat aorta investigated on strip preparations.

A significant effect of the direction of excitation frequency sweep was observed in both age groups regarding natural frequency and elastic modulus. Mean values of these two viscoelastic characteristics were lower at decreasing excitation frequency sweep. However, one exception was elastic modulus independence of frequency sweep in the mature group. In terms of coefficient of viscosity, both age groups showed behaviour independent of the direction of excitation frequency sweep.

In conclusion, the present work adds complementary experimental data for strip preparations of healthy untreated rat aorta at different ages. It contributes for more precise characterisation of the biomechanical behaviour of rat aorta wall in dynamics – closely to the conditions in the organism but not affected by any neural or humoral regulation in the organism.

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