Passive Mechanical Properties of the Guinea Pig Aorta and Portal Vein: a Comparison in the Low-Frequency Range

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Abstract. The study was carried out to determine the differences in the low-frequency dynamic mechanical properties of the guinea pig aorta and portal vein Experiments were performed at five passive load levels in the aorta and at four levels in the portal vein Sudden stretches 65 s in duration were imposed on the muscles, and the resulting force responses were recorded After the experiments, the Fourier transformations of the force responses and the length perturbations were calculated The results of the transformation were used to find the dynamic stiffness values in the range of 0–3 Hz The quotient of the dynamic to the static stiffness (E_d/E_s) obtained for the aorta and portal vein was the higher the higher was the frequency At a given frequency and load level, the quotient E_d/E_s was significantly greater for the portal vein than that for the aorta Furthermore, the quotient value obtained for the portal vein varied with the passive load, whereas the same quotient obtained for the aorta did not vary significantly with the load levels The phase difference between force and length was small and constant over the frequency range of 0–3 Hz, in both vessels The differences in the dynamic characteristics are discussed in relation to the shortening capacity of the muscle cells in the two vessels

Key words: Aorta — Portal vein — Dynamic stiffness — Phase angle — Length perturbation — Force response

Introduction

Evidence has accumulated to show that alterations in the structure of blood vessels affect the external manifestation of the force generated by muscle cells Therefore, the mechanical properties of blood vessels have become the main focus of interest in a great number of studies (Dobrin 1978, Cox 1978, 1980, Langewouters et al 1984, Megerman et al 1986, Hodgkin et al 1992, Kitoh et al 1993) Moreover experiments were performed to find a relationship between the relative contribution

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of various wall constituents of blood vessels and their mechanical properties (Cox 1980, Armentano et al 1991 Barra et al 1993) In this context, the aorta being a vessel subjected to constant pulsatile flow has taken a special interest its dynamic properties at and over the frequencies of the heart rate have been studied widely in addition to its static stiffness properties (Wolinsky and Glakov 1964, Patel and Fry 1964 Patel et al 1970, Busse et al 1981, Toorop et al 1987 Burkhoff et al 1988, Hayashi et al 1994) On the other hand the dynamic mechanical properties of veins are little known (Brown and Heistad 1986)

When structures of the aorta and the portal vein are compared it is seen that the amounts of the wall constituents and their organization differ greatly in the two vessels (Gabella 1984 Thevent and Connat 1995) The aorta has less smooth muscle than the portal vein On the other hand, the aorta contains a greater amount of collagen than the portal vein Therefore, the dynamic mechanical properties of the two vessels can be expected to also differ In our previous study carried out at the very low-frequency range we found that the stiffness of the passive tissue components in the portal vein increased with the increasing frequency (Ozturk and Ungan 1994a) The objective of this study was to assess the low-frequency dynamic properties of the aorta and the portal vein, and to identify the differences in their mechanical properties in the low frequency range

Materials and Methods

Preparation and measuring apparatus

The animals were treated according to the protocol of the grant project supported by the Scientific and Technical Research Council of Turkey (TAG-648) After ether anesthesia the aorta and the portal vein were removed and immediately put into Krebs solution at 36 °C Then they were mounted vertically in an organ bath as described previously (Ozturk and Ungan 1994b) Briefly, the upper ends of the preparations were attached to a force displacement transducer (Grass FT 03) and the lower ends were connected to the lever of a displacement transducer with stiff and light metal rods The other end of the lever of the displacement transducer was attached to the core of an electromagnetic vibrator which was used to impose length perturbations on the muscle strips

The length and force signals from the transducers were first amplified and then digitized by means of an eight channel 12 bit A/D converter and stored in a computer for off-line analysis

The composition of the Krebs solution was (mmol/l) NaCl 118 KCl 4 7 $CaCl_2$ 2 5 $NaHCO_3$ 14 9 KH_2PO_4 1 18 $MgSO_4$ 1 17 glucose 5 5 Throughout the experiments the solutions were maintained at 36 °C and gassed outside of the bath with 95% O_2 and 5% CO_2

Experimental procedure and data analysis

After mounting, the portal vein or aorta preparation was stretched to the length at which force was first manifest Then they were allowed to accommodate for 30 minutes in normal Krebs solution After that period, the Krebs solution was replaced with Ca^{2+}-free Krebs solution containing 2 mmol/l EGTA, and the preparation was maintained in that solution for additional 30 minutes During that time the muscle fully relaxed Subsequently, the muscle length increased until the passive force was 2 5 mN, and it was kept under that passive force level for additional 10 minutes At the end of that period, the length of the muscle was measured and denoted L_r Thereafter, length perturbations in the form of step function (extensions) were imposed on the muscle and the resulting force responses were recorded The amplitude of the length perturbations was maintained below 5% of the muscle length L_r The rise time of the length change was 10 ms The duration of the perturbation was adjusted to 65 s so that force responses could reach steady-state level The force and length perturbations were sampled with a frequency of 50 Hz In order to eliminate variations of the force responses with time, the force responses to ten consecutive length perturbations were averaged on-line In order to assess the effect of the applied passive load (force) on the dynamic stiffness properties, experiments were repeated at the load levels of 5, 7 5, 10 mN for the portal vein, and at 5, 7 5, 10, 12 5, 15 mN for the aorta, in a random order

After the experiments, the Fourier transforms of the average force response and length perturbation were calculated using an FFT algorithm Fourier transformation was performed over 1024 data points Since the sampling frequency during the experiment was 50 Hz, before transformation, the data were rearranged by taking every third point in order to reduce the data points to 1024 This enabled us to include in the analysis the steady state part of the force responses

The dynamic stiffness values were calculated as the ratio of the modulus of the force response ($|F(j\omega)|$) to the modulus of length perturbation ($|L(j\omega)|$) In order to assess the variations in the dynamic stiffness with respect to the static stiffness, the dynamic stiffness values were normalized by dividing them by the stiffness at 0 Hz The variation of the stiffness ratio with the frequency was plotted on a semilogarithmic paper Phase values ($\Phi(\omega)$) were obtained by taking the difference between the phase angles of the force and length signals

The mean values were expressed as means ± S D with the number of experiments shown in brackets Statistical comparisons were made using Student's t-test on paired data $p < 0\ 05$ was taken as the confidence limit

Results

Figs 1*A* and 1*B* illustrate typical force responses to step length perturbations

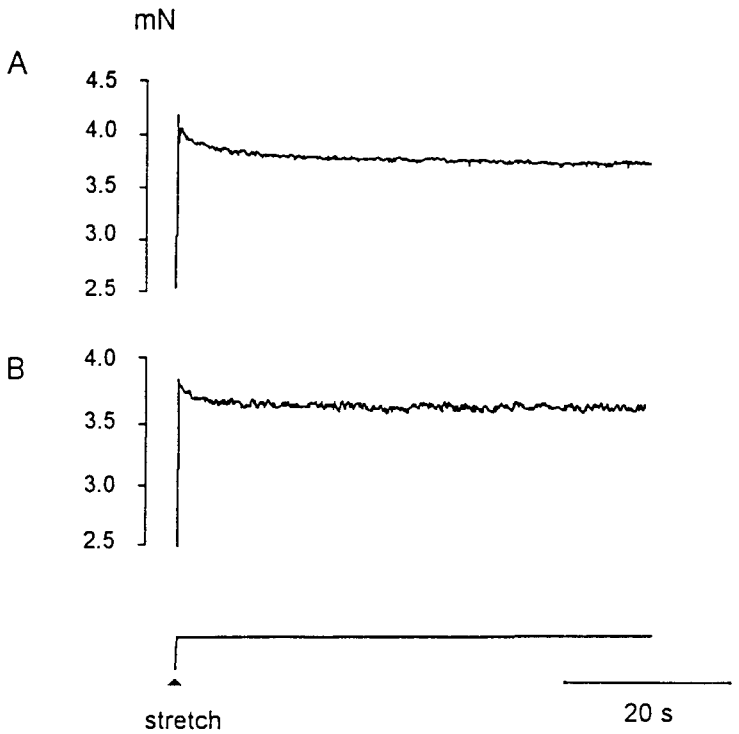

Figure 1. Force responses of an aorta (A) and a portal vein (B) to step length perturbations (stretches) of 0.4 mm in amplitude The rise time of the length perturbations was 10 ms The passive load for both muscles was 2.5 mN. Each response is the on-line average of the force responses to ten successive stretches. (for the aorta: length = 7 mm, mass = 12 mg; for the portal vein length = 6 mm, mass = 4 mg)

(extensions) of 0.4 mm in amplitude measured for an aorta and a portal vein, respectively. Each response is the average of ten responses to successively applied length perturbations, and they were measured under a passive load of 2.5 mN. As it is seen from the Figures, the force responses of the aorta and the portal vein were characterized by two phases. The first phase appeared as an immediate increase in the force coincident with the applied length perturbation. The second phase consisted of a slow decay to a steady-state. Similar two-phase responses were observed in both vessels with an increased passive load imposed on the muscle; however, the initial increase in force and the steady state reached higher force levels as the load increased.

The quotient of the dynamic to the static stiffness (E_d/E_s) obtained for the aorta and the portal vein displayed an increase with the increasing frequency (Fig. 2). The variations of the quotient with the increasing frequency seemed to be

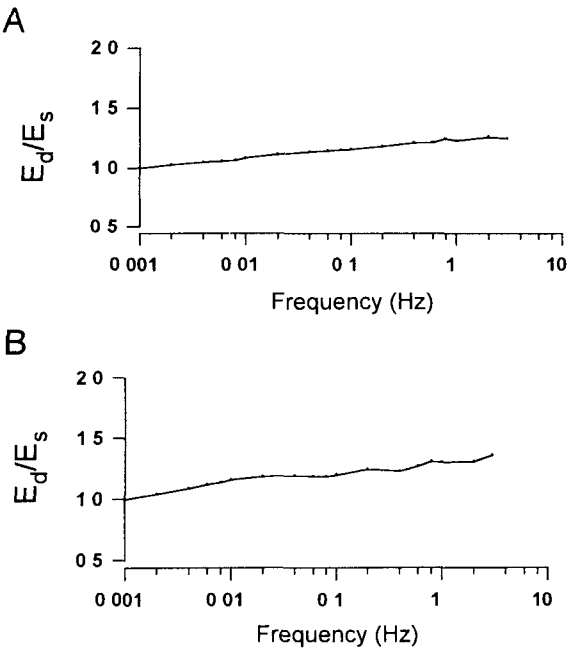

Figure 2. Variations of the stiffness quotient E_d/E_s with the frequency for the aorta (A) and the portal vein (B) calculated from the force responses in Fig 1 4 and 1B respectively E_d is the dynamic stiffness, E_s is the stiffness at 0 Hz (i e static stiffness)

steeper for the portal vein than for the aorta Similar curves were obtained under other passive load levels In order to illustrate the variations of the E_d/E_s with the passive load levels, the dynamic stiffness values at 1 Hz were divided by the static stiffness values, and the quotients (E_d(1Hz)/E_s) were plotted against the passive load levels (Fig 3) The quotient calculated for the portal vein displayed a slight increase with the increasing load levels (Fig 3B) The increases between 2 5 and 7 5 mN and between 2 5 and 10 mN were found to be statistically significant ($p < 0\ 02$, $p < 0\ 05$, respectively) On the other hand, the quotient obtained for the aorta remained almost constant within the same load levels (Fig 3 4) In addition, at each load level the quotient obtained for the portal vein was greater than that for the aorta The quotients obtained for the aorta and the portal vein were significantly different when the load was 7 5 or 10 mN ($p < 0\ 02$)

The phase difference between force and length obtained for both vessels were almost constant and small within the frequency band of 0 3 Hz In both vessels the independence of the phase difference on frequency persisted even at higher loads, and there was no significant difference in the phase angles calculated at various

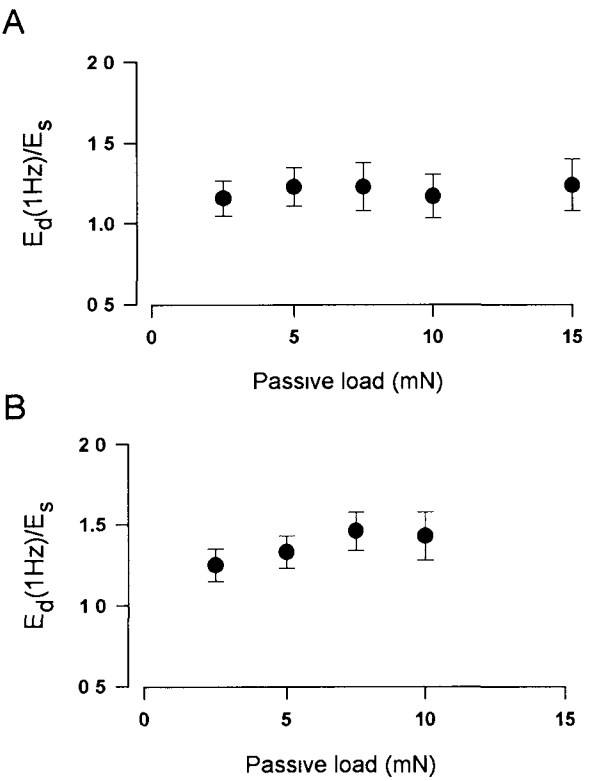

Figure 3. Changes in the stiffness quotient $(E_d(1\text{Hz})/E_s)$ with the imposed passive load, obtained for the aorta (A) and the portal vein (B) The values are means of seven experiments, and the bars show the standard deviations E_d (1Hz) is the dynamic stiffness at 1 Hz, E_s is the static stiffness

load levels either The mean phase differences were $2\,1 \pm 1\,1°$ $(n = 8)$ for the aorta, $3\,1 \pm 1\,9°$ $(n = 8)$ for the portal vein

The dynamic stiffness is known to have two components, one of them being the viscous modulus, the other one being the elastic modulus These two components are related to the dynamic stiffness by the equations (Bergel 1961),

$$E_e(\omega) = |E_d(j\omega)| \cos\theta$$
$$E_i(\omega) = |E_d(j\omega)| \sin\theta$$

where θ is the phase difference between force and length, $E_d(j\omega)$ is the dynamic stiffness, $E_e(\omega)$ is the elastic modulus component of the dynamic stiffness, and $E_i(\omega)$ is the component of the viscous modulus When the dynamic stiffness was

resolved into its components by means of the above equations, we found that the viscous components of both vessels were small, and their stiffness was mainly determined by their elastic properties. According to our results, the relative contribution of the viscous component to the dynamic stiffness was $3.6 \pm 1.9\%$ ($n = 8$) in the aorta and $5.4 \pm 3.3\%$ ($n = 8$) in the portal vein. This implies that the frequency-dependent changes in stiffness mainly reflect alterations in the elastic properties of the aorta and the portal vein.

Discussion

A noteworthy result obtained in this study is that the quotient of the dynamic to the static stiffness obtained for both vessels increases as the frequency rises and that the increase is more marked in the portal vein than in the aorta. While stiffness properties of the aorta have been studied widely, little information is available on the portal vein (Patel et al. 1970, Busse et al. 1981, Langewouters et al. 1984, Chu and Reddy 1992, Matsuda et al. 1993, Hayashi et al. 1994). Moreover, previous studies on the aorta in the low frequency range have given conflicting results. Learoyd and Taylor (1966) observed increases in the elastic modulus of the human aorta with the increasing frequency, in the range of 0–2 Hz. Similarly, Bergel (1961), Patel et al. (1970) and Gow (1972) observed a dependence of the elastic modulus on the frequency in the low frequency range. On the other hand, Busse et al. (1981) and Bauer et al. (1982) found that the dynamic stiffness of the rat aorta was almost constant within a frequency range of 0.1–20 Hz. As it is seen, our results support the observations that the dynamic stiffness of the aorta increases with the increasing frequency of perturbations.

Relaxed vessels are known to become stiffer at higher pressure levels. Taking into account that fact, we performed experiments at various passive load levels and explored whether the low-frequency dynamic stiffness characteristics of the aorta and the portal vein would change with the load levels. Our results showed that the two vessels differed greatly in that respect, the dynamic stiffness at a given frequency was dependent on the passive load for the portal vein and remained almost unchanged for the aorta (Fig. 3). At each load level, the quotient $E_d(1\text{Hz})/E_s$ was greater for the portal vein than for the aorta. Previous experiments have shown an association between the contents of the wall constituents of the blood vessels and their mechanical properties (Cox 1980, Armentano et al. 1991, Cabrera Fischer et al. 1991). Structural changes in aging, exercise training or calcification of the internal elastic membrane cause alterations in the compliance of the arterial system (Learoyd and Taylor 1966, Burattini et al. 1992, Matsuda et al. 1993, Michel et al. 1994, Cameron and Dart 1994, Atkinson et al. 1994). Since the aorta and the portal vein have different structures in regard to the muscle and connective tissue contents, and to their organizations on the wall, their structural differences would

reflect their dynamic stiffness properties However, our results showed that the differences in their mechanical characteristics are not only quantitative, but that the variations of the dynamic characteristics with different loads have different features in both vessels This result suggests that the relation between the mechanical properties and the geometrical organization of the wall constituents and their relative contents is of a complex nature

The quotient of dynamic stiffness at 1 Hz to static stiffness calculated for the portal vein in the present study varied between 1 25–1 46, depending on the load, it was around 1 16–1 24 for the aorta The same quotient reported in the literature varies between 1 1 and 2 1 (Learoyd and Taylor 1966, Dobrin 1978, Busse et al 1981) In those studies, however, experiments have mainly been carried out by applying pressure as the input and measuring diameter changes as the output Besides, in those studies the dynamic elasticity was mostly examined in the circumferential direction, however experiments have shown that the vessel mechanics differs depending on the direction in which the stiffness characteristics are explored (Patel et al 1970, Dobrin 1978) Therefore our study differs from those cited in the experimental approach and in the way that the elastic modulus values were calculated Regardless of these differences, the quotients we obtained for the aorta and the portal vein were within the range reported in the studies referred to Our results also confirm the view that the quotient value increases as the smooth muscle content increases (Bergel 1961, Busse et al 1981)

The differences we could observe in the dynamic properties of the aorta and the portal vein should be expected to affect the transmission of force along the two vessels and the extent to which smooth muscles can shorten Recent experiments on rabbit mesotubarium superius muscles have shown that during isometric contractions, more crossbridges ought to attach in a shorter muscle in order to generate the same force (Meiss 1992) Similar results were obtained in various smooth muscle tissues too (Pfitzer et al 1982) The reason for the decrease in the efficiency of force generation as the muscle shortens was suggested to be the constraints imposed by the tissue elements on the muscle cells (Meiss 1990, 1992) Meiss (1992) also observed a length-dependent active stiffness increase during isotonic shortening Based on these results, he proposed that, as muscle shortens crossbridges should overcome more radial and axial forces arising from the connective tissue elements In line with this interpretation, experiments have also shown that the velocity of shortening decreases as the muscle becomes shorter According to this view an increase in the compliance of the tissue elements would facilitate the extent of the shortening of the muscle Our results show that frequency is another parameter which introduces limitations on the internal shortening and, in turn, determines the shortening capacity of the muscle cells According to our results as the muscle cells contract at higher frequencies the shortening occurs in a smaller amplitude since the dynamic stiffness has increased In addition since the quotient of dy-

namic stiffness to static stiffness has greater values for the portal vein, the relative resistive force of the tissue elements would be higher in the portal vein compared to that in the aorta The shortening capacity of smooth muscle cells in blood vessels are important in relation to the blood flow, by the reciprocal relation between the vessel resistance and the fourth power of the vessel radius With its smooth muscle content being low, the aorta usually does not display spontaneous diameter oscillations under normal physiological conditions, however, it is subjected to pulsatile flow constantly Therefore it exhibits diameter variations at a frequency of the heart rate The portal vein, in contrast, is a vessel which has well developed spontaneous contractions with frequencies ranging between 0 01 and 1 Hz (Johansson and Bohr 1966) Therefore the low-frequency dynamic properties of the tissue elements would affect the shortening capacity of smooth muscle cells in a wider frequency range in the portal vein On the other hand, our results showed that the viscous moduli of the two vessels relative to their total stiffnesses are small This result indicates that the viscous property of the tissue elements would not interfere with the transmission of force in both vessels

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