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Endothelium-dependent mechanical effects on the beat-to-beat energy dissipation in the arterial wall

Disipación energética latido a latido endotelio-dependiente en la pared arterial

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Abstract
Arterial wall dynamic properties have been found to be stretching rate-dependent. Endothelium modulates the smooth muscle tone, playing an important function in the regulation of the wall dynamics. The aim of this work was to evaluate the role of endothelium on the mechanical and energetic properties of the arterial wall at different stretching rates. Seven ovine brachiocephalic arteries were studied *in vitro*, under pulsatile flow conditions achieved by a pneumatic assist pump. Pressure and diameter signals were assessed in each artery at different frequencies (f) in a physiological range (f_1 = 60, f_2 = 80, f_3 = 100 beats/min) in order to obtain the pressure-diameter relationship. Elastic (E_{dyn}), viscous (η) and mass (M) wall indexes, first order approximation half power bandwidth (f_{C1}), viscous loss modulus ($\eta\omega$), complex viscoelastic modulus (E'), phase angle of $E'(\phi)$, mean elastic work (W_e) and mean viscous work (W_v) were calculated before and after de-endothelization (DE) at each frequency level, using a linear Autoregressive with Exogenous input (ARX) adaptive model of the arterial wall. Values of E', E_{dyn}, and $\eta\omega$ obtained after DE were significantly higher ($p < 0.05$) for all frequencies studied. Values of W_e were significantly higher after DE (from 5.05 ± 1.92 to 6.35 ± 2.53 in f_1; 7.15 ± 2.91 to 8.65 ± 3.23 in f_2 and 8.01 ± 3.21·10⁴ to 9.79 ± 4.19·10⁴ N/s in f_3). Values of W_v for DE arteries were always higher ($p < 0.05$) (from 1.94 ± 0.61 to 2.25 ± 0.77 in f_1; 3.20 ± 1.03 to 4.01 ± 1.19 in f_2 and 2.42 ± 1.50·10⁷ to 4.23 ± 1.50·10⁷ N/s in f_3). Endothelium removal induced significant increases in energy dissipation and viscoelasticity indexes of the arterial wall at identical stretching rate levels. Endothelium could contribute to minimize the beat-to-beat energy dissipation due to arterial wall viscosity in arteries.

Keywords: Endothelium, Arterial wall viscosity, Smooth muscle, Stretching rate, Energy dissipation.

Resumen
La dinámica parietal arterial presenta dependencia con el nivel de frecuencia de estiramiento. El endotelio modula el tono muscular y cumple una importante función en la regulación de la dinámica parietal. El objetivo del presente trabajo fue evaluar el rol del endotelio en las propiedades mecánicas y energéticas de la pared arterial a diferentes frecuencias de estiramiento. Siete arterias braquiocefálicas ovinas fueron estudiadas in vitro, bajo condiciones de flujo pulsátil con un sistema de bombeo neumático. La presión y diámetro arterial se midieron a diferentes frecuencias (f), dentro de un rango fisiológico ($f_1 = 60, f_2 = 80, f_3 = 100$ latidos/min), y se construyó la relación presión-diámetro. Los índices dinámicos elástico (E_{dyn}), viscoso (η) y masa (M), la aproximación de primer orden del ancho de banda (f_{C1}), el módulo de pérdida viscosa ($\eta\omega$), el módulo viscoelástico complejo (E'), el ángulo de fase de $E'(\phi)$, trabajos elástico medio (W_e), y viscoso medio (W_v), fueron calculados para cada nivel de frecuencia, antes y luego de desendotelizar (DE), usando un modelo adaptativo lineal AutoRegresivo con entrada eXógena (ARX). Para cada frecuencia, los valores de E', E_{dyn} y $\eta\omega$, obtenidos tras DE fueron mayores que en las arterias intactas ($p < 0,05$). El W_e fue significativamente superior luego de DE (desde 5,05 ± 1,92 a 6,35 ± 2,53 en f_1; 7,15 ± 2,91 a 8,65 ± 3,23 en f_2 y 8,01 ± 3,21·10⁴ a 9,79 ± 4,19·10⁴ N/s en f_3). El W_v luego de la DE fue siempre superior ($p < 0,05$), (desde 1,94 ± 0,61 a 2,25 ± 0,77 en f_1; 3,20 ± 1,03 a 4,01 ± 1,19 en f_2 y 2,42 ± 1,50·10⁷ a 4,23 ± 1,50·10⁷ N/s en f_3). La remoción del endotelio indujo incrementos significativos en la disipación de energía y los índices viscoleásticos de la pared arterial a idénticas frecuencias de estiramiento. El endotelio podría contribuir a minimizar la disipación de energía por ciclo debido a la viscosidad parietal en las arterias.

Palabras clave: *Endotelio, Viscosidad parietal arterial, Músculo liso, Frecuencia de estiramiento, Disipación energética.*

Introduction

It has been demonstrated that elastin, collagen and smooth muscle, the quantitatively most important structural components of the arterial wall, are the major determinants of its elastic, viscous and inertial behaviours, and have a frequency-dependence with important consequences in regard to wave propagation (Armentano *et al*., 1995; Cox, 1972; Santana *et al*., 2005). Another constituent of the arterial wall with physiological relevance is the endothelium. It continuously interacts with the blood components and vessel wall constituents, playing a key role in the vascular homeostasis, the maintenance of vessel antithrombotic properties and selective permeability, in the control of the vascular cell growth and in the production, secretion and metabolism of biologically active molecules and extracellular matrix components (Fischer *et al*., 2002; Lévy *et al*., 1993; Recchia *et al*., 1999). Additionally, the vascular endothelium modulates the arterial wall smooth muscle tone and consequently also acts in the regulation of the viscoelastic properties (Fischer *et al*., 2002; Furchgott and Zawadzki, 1980; Lévy *et al*., 1993; Nichols and O'Rourke, 1998; Recchia *et al*., 1999). About this, the physiological role of the endothelial lining would involve the maintenance of the degree of arterial wall viscosity, since it may improve the efficiency of the ventricular-arterial coupling energy balance. It has been suggested that the beating heart stimulates endothelial release of vasoactive substances (Lamontagne *et al*., 1992).

Several models applying theoretical principles have been used in order to characterize the viscoelastic properties of the arterial wall (Bauer *et al*., 1979). The simplest standard mechanical model used for such analysis consists of an elastic element (a spring) and a viscous element (a dashpot) arranged in parallel (Dobrin, 1978; Milnor, 1982). Many years ago, Hardung described the viscoelasticity of blood vessels by a complex expression $E' = E_{dyn} + j \cdot \omega\eta$, where E' is the complex viscoelastic modulus, E_{dyn} is the elastic component, j the imaginary unit and $\omega\eta$ is the viscous loss component of the complex modulus, composed by two terms, the coefficient of viscosity (η) and the angular frequency (ω) (Hardung, 1953; Milnor, 1982). Finally, the mean elastic (W_e) and viscous (W_v) works of the arterial wall show the mean elastic and viscous power dissipation in a quarter of a cardiac cycle.

The aim of this work was to evaluate the role of the endothelium on the mechanical and energetic properties of the arterial wall in an *in vitro* preparation, using a circulating loop at different stretching rates in the physiological range, derived from an adaptive pressure-diameter model.

Material and Methods

Surgical procedure

Seven Corriedale sheep, weighting 25 to 35 kg, aged between 30 and 48 months old were chosen at the beginning of this study. During 14 days before surgery, the animals were appropriately cared and vaccinated. All of them were operated under general anesthesia induced by intravenous thiopental sodium (20 mg/kg) and, after intubation, maintained with 2.5% enfluorane in pure oxygen (4 L/min) through a Bain tube connected to a Bird Mark VIII respirator. Afterwards, each sheep was positioned in left lateral decubitus and a sterile thoracotomy was performed at the left third intercostal space. Then, the brachiocephalic artery was instrumented with a pressure microtransducer and a pair of ultrasonic dimension gauges. The pressure microtransducer (1,200 Hz frequency response, Konigsberg Instruments) was implanted in the arterial lumen through a collateral branch. The arterial pressure was measured with the pressure microtransducer, which had been previously calibrated with a mercury manometer. Two ultrasonic dimension gauges (5 MHz, 4 mm diameter) were sutured on the adventitia of the brachiocephalic trunk. The transit time of the ultrasonic signal (1,580 m/s) was converted into distance through a sonomicrometer, calibrated according to the standard method described in the manual edited by Triton Technology Inc., and it was observed on an oscilloscope (Tektronix 465B) to confirm optimal arterial diameter signal quality. Both, calibrated pressure and diameter signals were displayed on the screen of a four-channel monitor (Gould 51-2341) and registered on a six-channel chart recorder (Gould 2600). All procedures were performed in agreement with previous works (Bia *et al*., 2003; Cabrera *et al*., 2006; Fischer *et al*., 2002; Santana *et al*., 2005).

In vitro measurements

After *in vivo* pressure-diameter measurements, the sheep was sacrificed by an overdose of sodium pentobarbital, and segments of brachiocephalic artery were excised and mounted in the organ chamber of a specially manufactured flow loop that was previously reported (Fischer *et al*., 2002; Zócalo *et al*., 2006).

During the experiments, the segments of ovine arteries were perfused with anticoagulated blood, maintaining a viscosity level within 2-2.5 mPa·s and a shear stress within 140-162 mPa. Viscosity of lamb blood samples (2 ml), anticoagulated with EDTA

(1.5 mg/mL), was measured using a rotational viscometer (Brookfield Digital Viscometer, LVDT-II+) at a shear-rate range of 0.6-200 s^{-1} (Brookshier and Tarbell, 1991).

The *in vivo* length of the segments ranged from 5 to 7 cm. The measurement was always performed putting two suture references in the adventitial tissue of the artery. Arteries were excised at the level of the suture references and were mounted in the *in vitro* circulation loop, preserving the *in vivo* length (Cabrera *et al*., 2006).

The organ reservoir was filled with a buffered HEPES-Tyrode solution, which composition was (mM): NaCl 140, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.0, HEPES 10, glucose 11.1. The pH of the solution was adjusted to 7.4 using NaOH and the solution was oxygenated during the experiments. The perfusion line was composed of polyethylene tubing and a Windkessel reservoir powered by a pneumatic pump (Jarvik Model 5, Kolff Medical Inc.). The pneumatic device was regulated with a Utah heart driver that allows fine adjustments at different levels of heart rate, length of systolic and diastolic periods of each cycle and pressure values (Figure 1). After being placed in the organ reservoir, the arterial segment was allowed to equilibrate for a period of 20 min under steady flow conditions of 160 ml/min, at a stretching rate of 60 beats/min and maintaining pressures levels within physiological range (11.3 ± 0.6 kPa).

All experiments were performed on segments of ovine arteries perfused with a constant hematocrit of 30.17 ± 7.7%, obtained by separating the whole ovine blood through sedimentation and remixing the plasma and red cells. Mean value of blood viscosity was 2.33 mPa·s. Arterial diameter and pressure were measured as described above at three consecutively stretching rates of 60, 80 and 100 beats/min (1.00, 1.34 and 1.67 Hz respectively), considering 10 min between successive stretching rates.

Arterial pressure and diameter signals were digitized using a specific program manufactured in our laboratory (Armentano *et al*., 1995). *In vivo* pressure and diameter signal shapes were reproduced in the *in vitro* experiments (Cabrera *et al*., 2006; Fischer *et al*., 2002; Zócalo *et al*., 2006). The similarity criteria of the *in vivo* and *in vitro* curves were the morphology given by the maximum value of cross-correlation function between absolute values of both curves. Besides, *in vitro* instantaneous pressure-diameter loops were monitored during all experiments.

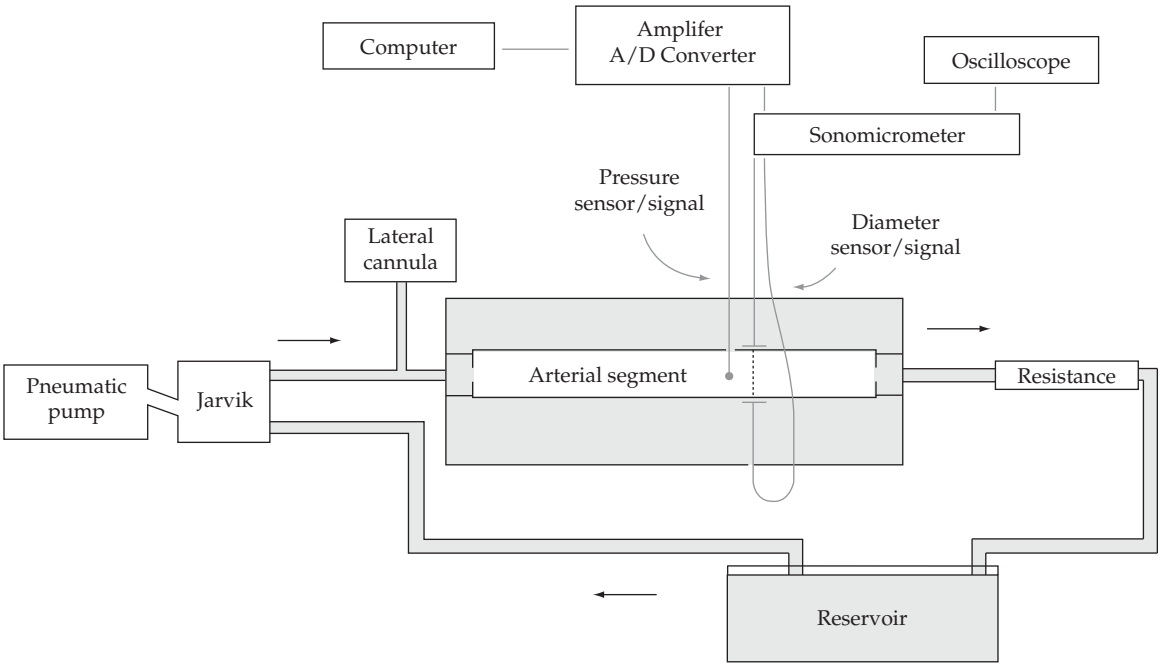

Figure 1. Schema showing the circulation mock used to test arterial segments. The perfusion line was powered by a pneumatic pump, connected to a Jarvik (artificial heart). Each arterial segment was mounted in the perfusion line and immersed in a thermally regulated and oxygenated Tyrode's solution. Pressure (Konisberg) and diameter (sonomicrometer) signals were displayed on a computer. The lateral cannula was used to insert the catheter employed to perform the endothelial rubbing (de-endothelization procedure).

All measurements were performed in each arterial segment before and after de-endothelization (DE), obtained by repeated gentle rubbing with a partially inflated 7F Fogarty embolectomy catheter (Fischer *et al*., 2002). In all cases, we infused 2 cm of saline solution in the balloon of the Fogarty catheter. This determined a mean inflating pressure of 17.33 kPa, which was the mean arterial systolic pressure level observed in *in vivo* conditions. As a distending pressure in the Fogarty balloon determined a given diameter value, we put special attention on checking that the *in vivo* systolic external diameter was always higher than the maximum balloon diameter, previous to DE, in each experimental session. In this way, we assured the integrity of the arterial wall structure.

This investigation complies with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH publication #85-23, revised 1996).

Data analysis

The data analysis was performed using an approach previously published in an extensive form (Armentano *et al*., 2006; Zócalo *et al*., 2006). A computerized procedure was used to determine the pressure-diameter loop and to calculate the mechanical parameters using an original system developed in our laboratory. A 3rd order linear autoregressive with exogenous input model (ARX) was used to fit the pressure and diameter data and the dynamic range, i.e. its corner frequency f_C (Armentano *et al*., 2006; Zócalo *et al*., 2006). In the continuous time domain, the model is better understood and a physical meaning can be given to the model coefficients. Taking into account that a 2nd order differential equation characterizes the wall dynamics, the transfer function would be:

$$H(s)=\frac{D(s)}{P(s)}=\frac{1}{Ms^2+\eta s+E_{dyn}} \quad (1)$$

The elastic (E_{dyn}), viscous (η) and inertial (M) indexes were derived as previously reported (Armentano *et al*., 2006; Zócalo *et al*., 2006). The compliance (C) was quantified as E^{-1} (Zócalo *et al*., 2006).

The vascular wall, modelled as a second order mechanical system (equation 1), could be associated to a low-pass filter whose cut-off frequencies (f_{C1} and f_{C2}) are calculated as the roots of the denominator of equation 1 (Peterson *et al*., 1960):

$$f_{C1,2}=\frac{1}{2\pi}\left[\frac{\eta}{2M}\pm\sqrt{\left(\frac{\eta}{2M}\right)^2-\frac{E_{dyn}}{M}}\right] \quad (2)$$

Assuming that f_{C2} (related to inertial effects) is larger than f_{C1}, we can accept that $2\omega\cdot f_{C1}$ would be the dominant pole of the transfer function $H(\omega)$. Consequently, the first order system, obtained from the reduction of our model agrees with other arterial models that represent the arterial wall mechanics (Peterson *et al*., 1960). Taking into account this reduction, the dynamic range of the low pass filter would have the dynamical range:

$$f_{C1}=\frac{1}{2\pi}\frac{E_{dyn}}{\eta} \quad (3)$$

with a flat amplitude in the band pass of the compliance frequency response.

The loss modulus ($\eta\omega$) was calculated as:

$$\eta\omega=\eta\cdot 2\pi f \quad (4)$$

where f is the stretching rate (Hz).

The complex viscoelastic modulus E' was calculated in intact and DE arteries, at three consecutively stretching rates (f_1 = 60, f_2 = 80 and f_3 = 100 beats/min) as:

$$E'=\sqrt{E_{dyn}^2+\left(\eta\bar{\omega}\right)^2} \quad (5)$$

The phase angle (ϕ) of E' was calculated as:

$$\phi=\mathrm{tg}^{-1}\left(\frac{\eta\bar{\omega}}{E_{dyn}}\right) \quad (6)$$

The mean elastic power dissipation (W_e) was calculated in intact and DE arteries (Midoux, 1993), at three consecutively stretching rates (f_1 = 60, f_2 = 80 and f_3 = 100 beats/min) as:

$$W_e=E_{dyn}\frac{\bar{\omega}\cdot D_m^2}{\pi} \quad (7)$$

where D_m is the mean arterial diameter measured.

The mean viscous power dissipation (W_v) was calculated in intact and DE arteries (Midoux, 1993), at the three consecutively stretching rates (f_1 = 60, f_2 = 80 and f_3 = 100 beats/min) as:

$$W_v=\eta\frac{\bar{\omega}^2\cdot D_m}{2} \quad (8)$$

All measurements and calculated values were expressed as mean ± SD. Values of $p < 0.05$ were considered statistically significant. Results were subjected to one-way analysis of variance (ANOVA) for repeated measurements. Statistical analysis was performed with Stat View 4.11 software (Abacus Concepts, Inc.).

Results

In intact arteries D_m value was 10.95 ± 0.96 mm and mean pressure level was 11.3 ± 0.6 kPa for all stretching rates studied.

In intact arteries values of E_{dyn} corresponding to the stretching rate levels studied (f_1 = 60, f_2 = 80 and f_3 = 100 beats/min) were virtually independent of the frequency. Similar behavior was observed in E' values (Table 1). Values of η and ηω showed significant decrease at the highest level of frequency analyzed ($p < 0.05$) as can be seen in Table 1. Values of M and f_{C1} for intact arteries did not show significant differences among the stretching levels studied. The phase angle ϕ of E' showed no statistically significant differences at the increasing stretching rate levels studied (0.071 ± 0.048, 0.085 ± 0.057 and 0.057 ± 0.013 radians).

Values of W_e calculated for intact arteries at the three stretching rate levels (5.05 ± 1.92, 7.15 ± 2.91 and 8.01 ± 3.21·10⁴ N/s) showed a soft tendency to an (non-significant) increase as can be seen in Figure 2. Maximum W_v value, calculated for intact arteries was located at the center of the physiological stretching rate (1.94 ± 0.61, 3.20 ± 1.03 and 2.42 ± 1.50·10⁷ N/s).

In DE arteries values of E_{dyn} corresponding to the stretching rate levels studied (f_1 = 60, f_2 = 80 and f_3 = 100 beats/min) were also virtually independent of the frequency. Similar behavior was observed in E' values (Table 1). Values of ηω showed non-significant changes in the three levels of frequency analyzed. Obtained values of η showed a significant ($p < 0.05$) decrease at the highest level of stretching rate level

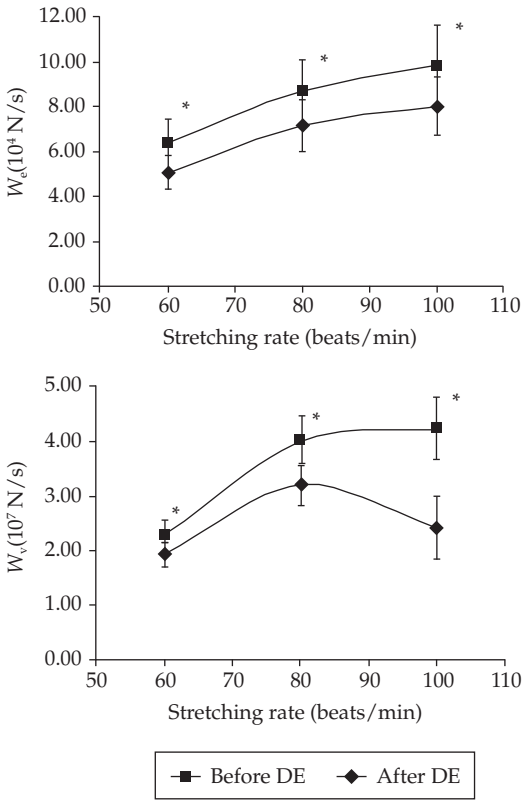

Figure 2. Superior and inferior panels show the calculated values of mean elastic work (W_e) and mean viscous work (W_v) respectively, obtained for three different stretching rate levels in intact and de-endothelized arteries (DE). The mean values were obtained considering the seven animals. During each experimental condition, twenty beats were recorded to obtain each animal's mean value. W_e and W_v values after DE were significantly higher (*) than those corresponding to the intact artery ($p < 0.05$).

Table 1. Arterial wall biomechanical parameters behavior at physiological stretching rates before and after de-endothelization.

	N = 7	f_1 (60 beats/min)	f_2 (80 beats/min)	f_3 (100 beats/min)
E_{dyn} (10^6 Pa)	Intact arteries	2.01 ± 0.81	2.08 ± 0.81	1.85 ± 0.79
	DE arteries	2.46 ± 1.01*	2.45 ± 0.71*	2.27 ± 0.85*
η (10^4 Pa·s)	Intact arteries	2.27 ± 0.63	2.12 ± 0.62	1.01 ± 0.5#
	DE arteries	2.62 ± 0.73*	2.63 ± 0.61*	1.91 ± 0.85#*
M (10^2 Pa·s²)	Intact arteries	5.84 ± 1.14	5.32 ± 2.01	5.54 ± 1.87
	DE arteries	4.72 ± 1.58	4.98 ± 1.54	5.15 ± 0.98
f_{C1} (Hz)	Intact arteries	14.12 ± 3.15	16.46 ± 2.85	18.15 ± 5.26
	DE arteries	14.94 ± 2.84	15.03 ± 3.42	16.14 ± 4.12
ηω (10^5 Pa)	Intact arteries	1.42 ± 0.39	1.77 ± 0.52	1.06 ± 0.52
	DE arteries	1.65 ± 0.46	2.20 ± 0.51	2.01 ± 0.49*
E' (10^6 Pa)	Intact arteries	2.01 ± 0.81	2.08 ± 0.91	1.85 ± 0.80
	DE arteries	2.46 ± 0.99*	2.46 ± 0.91	2.28 ± 1.04*

E_{dyn}: Arterial wall dynamic elastic modulus, η: wall viscous modulus, M: wall inertial modulus, f_{C1}: first order corner frequency. Wall loss modulus (ηω) and complex viscoelastic modulus (E') values are mean ± SD. *$p < 0.05$, respect to the intact arteries group using ANOVA for repeated measurements, for three different stretching rates (f). # $p < 0.05$, between f_1 and f_3 using ANOVA for repeated measurements.

analyzed (Table 1). Values of M and f_{C1} for DE arteries did not show significant differences in the stretching levels studied. The phase angle ϕ of E' showed no statistically significant differences at the three increasing stretching rate levels studied (0.070 ± 0.046, 0.089 ± 0.055 and 0.087 ± 0.034 radians).

Values of W_e calculated for DE arteries at the three stretching rate levels (6.35 ± 2.53, 8.65 ± 3.23 and 9.79 ± 4.19·10^4 N/s) showed a soft tendency to an (non-significant) increase as can be seen in Figure 2. Values of W_v calculated for DE arteries showed a similar behavior (2.25 ± 0.77, 4.01 ± 1.19 and 4.23 ± 1.50·10^7 N/s) as can be seen in Figure 2.

In DE arteries E_{dyn} showed higher values after DE than in intact arteries at the three frequency levels studied (isofrequencial analysis, $p < 0.05$). Similar behavior was observed analyzing E' values as can be seen in Table 1. Values of η and $\eta\omega$ were always higher in DE than those obtained in intact arteries ($p < 0.05$) as can be seen in Table 1. Values of ϕ showed no statistically significant differences between intact and DE arteries in the three stretching rate levels studied.

Values of W_e calculated for intact arteries were always lower than those obtained in DE arteries ($p < 0.05$) in the three stretching rate levels evaluated. Values of W_v calculated for intact arteries were always lower than those obtained in DE arteries ($p < 0.05$) in the three stretching rate levels evaluated. Intact and DE arteries showed significant differences in terms of W_v at higher values of stretching rate (Figure 2).

Discussion

In this *in vitro* experiment, endothelium removal induced significant increases in arterial wall viscosity and stiffness at identical stretching rate levels. Consequently arterial wall energy dissipation was influenced by endothelial function.

Our *in vitro* model allowed performing experimental sessions with arteries submitted to physiological ranges of pressure, diameter, stretching rate and blood hematocrit values. This is an important issue since the biomechanical properties, and the smooth muscle activity have shown dependence on the beating activity of the heart, blood viscosity, blood flow and hematocrit values (Jackson *et al*., 1991; Lamontagne *et al*., 1992; Levenson *et al*., 1990; Melkumyants *et al*., 1989; Pohl *et al*., 1986). Our results showed that the presence of endothelium could contribute to minimize the beat-to-beat energy dissipation caused by arterial wall viscosity in systemic circulation. This endothelial control of arterial wall viscosity through smooth muscle function modulation would be more important in the smallest precapillary vessels where myogenic activity is strong (Pohl *et al*., 1986). Arterial wall viscosity parameters were calculated applying a method developed in our laboratory, using an autoregressive with exogenous input adaptive model, which can be used with either intact or de-endothelized arteries (Armentano *et al*., 1995; Zócalo *et al*., 2006). E' and ϕ calculi were performed from the stress-strain model (Gow and Taylor, 1968).

Viscosity is the force required to overcoming a lack of slipperiness and it has been called "internal friction" by Newton (Folkow and Neil, 1971). Arterial wall viscosity can be defined as the lag of pulsatile diameter change behind applied pressure (O'Rourke, 1982). Therefore, it can be represented as the hysteresis loop when pressure and diameter (or stress and strain) are plotted against each other (Armentano, *et al*., 1995; Armentano *et al*., 1998; Bauer *et al*., 1979; O'Rourke, 1982; Wetterer *et al*., 1978). Arterial viscoelasticity can be quantitatively analyzed using instantaneous pressure-diameter recordings, both in experimental preparations and in clinical non-invasive studies, as previously reported (Armentano *et al*., 1995; Armentano *et al*., 1998; Bauer *et al*., 1982). The arterial wall viscosity has been reported increased in vessels of both hypertensive animals and patients (Armentano *et al*., 1998; Barra *et al*., 1997). Therefore, the non-invasive clinical measurement of arterial wall viscosity could be highly relevant to diagnose arterial diseases and/or to identify high-risk populations.

Previous studies have demonstrated that arterial wall viscosity plays a significant role in the mechanical behavior of muscular arteries both in animal preparations and humans (Gow and Taylor, 1968; Imura *et al*., 1990). The major effect of arterial wall viscosity on arterial mechanical properties is the alteration of the arterial pulse wave. We are concerned with the way in which it has been described in literature: as an attenuation similar to that caused by blood within the vessel, or as a dampening higher to that due to the viscosity of the blood alone (Nichols and O'Rourke, 1998), or as an element with a minor role in the vessel wall mechanics (Giezeman *et al*., 1994). The location of viscous elements in the arterial wall is largely represented by the smooth muscle component (Armentano *et al*., 1995; Armentano *et al*., 1998; Barra *et al*., 1997; Boutouyrie *et al*., 1997; O'Rourke, 1982), and arterial properties can be modified by the state of the wall muscle, as smooth muscle is strong enough to alter the properties of the vessel wall (Bergel, 1961; Nichols and O'Rourke, 1998). Furthermore, in 1902, Bayliss had already proposed

that contraction was the reaction of arterial wall smooth muscle to stretching force (Bayliss, 1902).

Our results are consistent with previous reports, which show that in blood vessels the stress-strain relationships are frequency-dependent because of the viscous properties of the wall, besides a complete description involves consideration of the spectrum of complex viscoelastic moduli *versus* frequency (Milnor, 1982). Changes observed in W_v and $\eta\omega$ at high stretching rates are relevant findings since there is experimental evidence concerning the frequency dependent nature of the mechanical properties of the arterial wall (Cox, 1972). Our *in vitro* model used physiological ranges of stretching rate and this is a limitation, since some statistically significance could be obtained at higher frequencies. However, as we performed the same beating smooth muscle stimulation twice, in intact arteries and after DE, and taking into account calcium and sodium dynamics across the cell membrane, we assure the complete recovering of basal arterial intrinsic properties not using frequencies beyond physiological ranges. No doubt, experiment preconditioning could exist using a stretching rate of 60 beat/min in the initial equilibrium phase. However, at this stretching rate, viscous and elastic wall indexes showed the least changes. Consequently, from this starting point and at time intervals of 10 min, we obtained instantaneous pressure and diameter values with consecutive stretching rates of 60, 80 and 100 beats/min in order to avoid bias in the measures and ensure homogeneity in the treatment of the vessels. An additional limitation of our experimental protocol is the use of random stretching rates that could avoid the experimental preconditioning.

The filtering function, the capacity of the arterial wall to store, transmit and dissipate energy, characterized by the first order approximation half power bandwidth f_{C1}, is key to the supplementary pumping action of the arterial wall, as well as to avoid early mechanical failure or disruption (Bia *et al*., 2003; Santana *et al*., 2005; Zócalo *et al*., 2006). As a rule, high frequency vibrations produce structure injures. The aim of filtering is reducing accelerating oscillations. This filtering function depends on the mechanical properties of the arterial wall, and so, the process of de-endothelization or the stretching frequency did not change this filter capability.

Obtained values of E' and ϕ were similar to those previously reported in the same arteries (Bergel, 1961; Learoyd and Taylor, 1966). Values of η were also similar to those reported by Bauer in *in vitro* experiments with abdominal aortic arteries obtained from normotensive rats (Bauer *et al*., 1982). Mean elastic and viscous works, evaluated by W_e and W_v respectively, showed higher values at all frequencies under study after endothelium removal denoting that the presence of endothelial cell could contribute to minimize the energy loss of the arterial wall.

There is a substantial difference between our experimental study and Recchia's work (Recchia *et al*., 1999). Recchia's work deals with *in vitro* porcine carotid artery segments and the endothelium influence. In the mentioned work, in all segments under study, adventitia was removed before starting the recordings. As was previously reported by our group (Cabrera *et al*., 2006) and other authors (Souza *et al*., 2006), the adventitia removal provokes vascular smooth muscle constriction and so, determines viscoelastic changes. Consequently, we think that in the mentioned work the vessels were pre-contracted (by the adventitia elimination) before starting the experiment. Consequently, this initial constriction could be the cause of the large standard deviation and the lack of statistical differences when the viscous properties were evaluated before and after the endothelium removal (Recchia *et al*., 1999).

Conclusion

We conclude that the stretching rate related arterial wall viscoelastic behavior has endothelium-dependence in the physiological range in this *in vitro* experiment, with pressure-diameter curves adjusted to physiological morphologies. Endothelial cells might play a role in regulating arterial wall viscosity levels at physiological stretching rates. Endothelium could contribute to minimize the beat-to-beat energy dissipation due to arterial wall viscosity in arteries.

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