Correlation Between Echo-Tracking Parameters and *In Vitro* Measurements of Arterial Contraction and Relaxation in Rats Fed a High-Cholesterol Diet

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 **Yi Zhang**
ABCEF 1 **Wei-Wei Zhan**
BCDE 2 **Yong-Jie Wu**
BCDE 1 **Bo Zhao**
BCE 2 **Wu-Gang Zhou**
BCE 2 **Dong-Rui Chen**
BCE 1 **Wei Zhou**
BCE 1 **Zhen-Hua Liu**
BCE 3 **Wei-Min Jiang**
BCE 3 **Lin Zheng**

1 Department of Ultrasound, Rui Jin Hospital, School of Medicine, Shanghai JiaoTong University, Shanghai, P.R. China
2 Shanghai Institute of Hypertension, Ruijin Hospital, School of Medicine, Shanghai JiaoTong University, Shanghai, P.R. China
3 Department of Pathology, School of Medicine, Shanghai JiaoTong University, Shanghai, P.R. China

Corresponding Author: Wei-Wei Zhan, e-mail: zhanweiweimedsci@163.com
Source of support: None

Background: Echo-tracking (ET) is a new technique that allows the assessment of arterial function and stiffness. This study aimed to ascertain the utility of the echo-tracking (ET) technique to assess vascular stiffness in rats with hypercholesterolemia and atherosclerosis.

Material/Methods: ET was used to measure the arterial stiffness of the aorta in cholesterol-fed Sprague-Dawley rats (group T1, $n=10$, for 4 weeks; group T2, $n=10$, for 12 weeks) and normal control rats (group C1, $n=10$; group C2, $n=10$). *In vitro* isometric tension experiments were used to measure the maximum contractile tension (MCT) and maximum relaxation percentage (MRR%) of aortic rings. Indicators of arterial stiffness and aortic MCT and MRR% were compared between groups using linear regression analysis. Light microscopic evaluation was used to demonstrate atherosclerotic changes in the aorta.

Results: The rat models were successfully induced; pathological examination of the aortas showed significant atherosclerosis in group T2, but not in groups C1, C2, or T1. The arterial stiffness parameters obtained using ET and aortic rings *in vitro* showed significant impairments in T1 and T2 rats compared with C1 and C2 controls (all $P<0.05$ *vs.* controls). In addition, these impairments were greater in the T2 group than in the T1 group (all $P<0.05$). Finally, MRR% correlated with the distensibility coefficient (r=0.396, $P=0.012$), arterial compliance (r=0.317, $P=0.047$), stiffness parameter β (r=−0.406, $P=0.009$) and one-point pulse wave β (r=−0.434, $P=0.005$).

Conclusions: These results suggest that ET could be used to evaluate the changes in arterial wall elasticity associated with atherosclerosis and hypercholesterolemia.

MeSH Keywords: **Arterial Pressure • Atherosclerosis • Hypercholesterolemia • Ultrasonography • Vascular Stiffness**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/894032

3084   5   6   43

Background

Hypercholesterolemia and atherosclerosis are known to be involved in the development of cardiovascular diseases. One of the first steps in atherosclerosis development is endothelial dysfunction, which is a state characterized by decreased arterial responsiveness to stimuli and increased arterial stiffness. Age and smoking, 2 risk factors for cardiovascular diseases, are associated with arterial stiffness [1,2]. Previous studies in humans showed that arterial stiffness could be beneficial for predicting the risk of cardiovascular diseases [3]. Exercise [4] and lipid-lowering treatments have been shown to decrease arterial stiffness in humans [5,6] and in animal models [7].

Echo-tracking (ET) is a new technique that allows the assessment of arterial function and stiffness. ET follows the phase shift in ultrasound frequency generated by vessel wall motion during the cardiac cycle, and automatically calculates indicators of arterial stiffness, such as stiffness parameter α, stiffness parameter β, arterial compliance (AC), distensibility coefficient (DC), and one-point pulse wave velocity (PWVβ). Previous studies have used ET to evaluate arterial function in patients with diabetes [8–10], hypertension [11], cardiovascular disease [12], Grave's disease [13], and progeria [1] in patients receiving hemodialysis [14], in smoking individuals [2], and in normal individuals [15]; ET can also be used in animals.[16]

In addition to previous studies showing an association between pathological states and arterial stiffness measured by ET, it has been reported that arterial stiffness measured using ET is correlated with blood pressure [17]. Using methods other than ET, arterial stiffness has also been shown to correlate with blood pressure [18,19] and noradrenaline [19].

However, few studies have compared the arterial function measured by ET with the *in vitro* arterial isometric tension test or with pathological results. The *in vitro* arterial isometric tension test allows the direct measurement of arterial function in response to different stimuli [20,21]. The aim of the present study was to ascertain the utility of the ET technique in the assessment of vascular stiffness in rats with hypercholesterolemia and atherosclerosis. We used ET and isometric tension tests to assess the aortic properties in normal Sprague-Dawley rats and in rat models of hyperlipidemia and atherosclerosis. We investigated the associations between the arterial properties measured by these 2 methods, as well as their associations with the pathological results.

Material and Methods

Preparation of the cholesterol-fed rat model

All studies were performed with the approval of the animal use and care committee of the Ruijin Hospital, Shanghai Jiaotong University School of Medicine. Male Sprague-Dawley rats, 28 to 32 weeks old and weighing 390 to 600 g, were provided by Slike Experimental Animal Co. Ltd. (Shanghai, China) [SCXK (Shanghai) 2007-0005]. Rats were housed in a temperature- and humidity-controlled environment. Cholesterol-fed rats received 15 g of rat chow per day supplemented with 1.3% (wt./vol.) cholesterol, 10% cane sugar, 16.4% grease, 8.5% casein, 0.3% bile salts, 1.6% premix and 1.9% maltodextrin for 4 weeks to induce hypercholesterolemia (group T1, n=10) [16,22] or for 12 weeks to induce atherosclerosis (group T2, n=10) [16,23]. Previous studies have demonstrated that feeding of rats with a high-fat diet induces a significant increase in plasma cholesterol after 4 weeks [16,22] and atherosclerotic changes in arteries after 12 weeks [16,23]. In the present study, hypercholesterolemia was determined by a biochemical assay and atherosclerosis was confirmed by pathological examination. Control (C1 and C2, n=10 each) rats received 15 g of rat chow daily without supplements. Access to water was unrestricted throughout the study.

Measurement of abdominal aorta stiffness

Blood pressure was measured by a BP-98A device (SOFTRON, Japan). After an intraperitoneal injection of chloral hydrate (1 mL/300 g) for anesthesia, transabdominal grayscale ultrasound with aortic stiffness measurement was performed on the first day (baseline), and at 4 weeks (groups T1 and C1) (Figure 1), or 12 weeks (groups T2 and C2) (Figure 2). A longitudinal section of the abdominal aorta 2 to 3 cm distal to the iliac artery was obtained using an ESAOTE MyLab 90 ultrasound scanner with a 7.5–10 MHz linear array probe (ProSoundMyLab 90, ESAOTE Co., Italy). The ultrasound system has an embedded vessel wall ET mode that can automatically measure the diameter of the vessel and dynamic changes in the diameter during the cardiac cycle (CC). The sampling gate was placed at the middle of the vessel after setting the B/M mode (grayscale imaging with motion mode). Efforts were made to obtain the maximum inner diameter of the vessel and to optimize the images by setting the ultrasound beam vertical to the arterial wall. The waveforms reflecting the changes in the arterial inner diameter, including systolic diameter (Ds) and diastolic diameter (Dd), were continuously collected for more than 12 CCs. Five steady waveforms within 12 CCs were selected manually, and the systolic pressure (Ps) and diastolic pressure (Pd) data were entered into the ultrasound system. Then, the stiffness values were calculated automatically by the system according to the following equations:
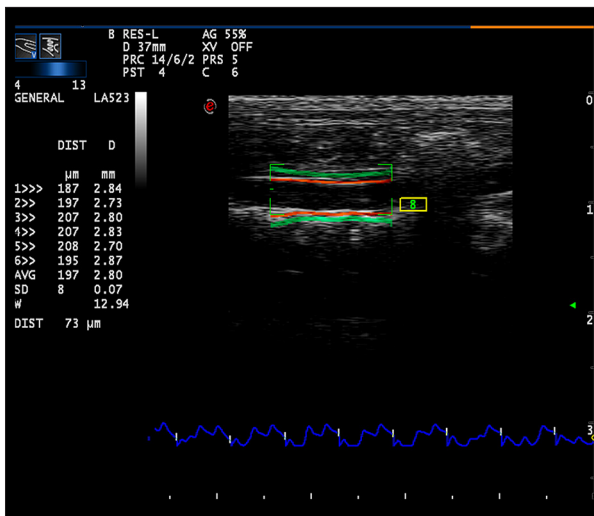
Figure 1. Echo-tracking in a rat with hypercholesterolemia at 4 weeks and atherosclerosis at 12 weeks.
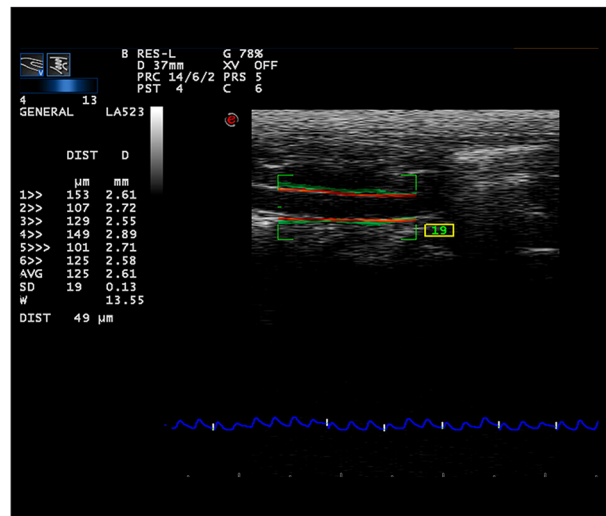

Figure 2. Echo-tracking in a control rat at 4 weeks and control rat at 12 weeks.

Stiffness parameter $\alpha = \ln (Ps/Pd)/[(As-Ad)/Ad]$

Stiffness parameter $\beta = \ln (Ps/Pd)/[(Ds-Dd)/Dd]$

$AC\ (cm/mmHg) = \pi\ (Ds \times Ds - Dd \times Dd)/[4 \times (Ps-Pd)]$

$DC\ (mmHg^{-1}) = (As-Ad)/(As \times \Delta P)$

$PWV\beta\ (m/s) = \sqrt{(\beta pd/2\rho)}$

where Ps and Pd are systolic and diastolic blood pressures, As and Ad are the systolic and diastolic area of the artery, Ds and Dd are the systolic and diastolic diameter of the artery, ρ is the density of blood (1050 kg/m³), and ΔP is the difference of the closed position between the culmination of Ps, extroversion pulse wave and backward wave [24].

Tension test

The abdominal aorta was dissected under anesthesia. Ring segments of 2–3 mm in length were dissected free of fat and connective tissue, and were mounted in a Multi Wire Myograph System (Danish Myo Technology Model 610M, Denmark) for measurement of isometric tension. The remaining rings were sent to the Pathology Department. Two steel wires (40-µm diameter) were introduced through the lumen of the segments, which were mounted according to the method described by Mulvany and Halpern [25]. Vessels were maintained at 37°C in physiological Krebs solution containing 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgCl_2$, 11 mmol/L D-glucose, 25 mmol/L $NaHCO_3$, 2.5 mmol/L $CaCl_2$, and 0.026 mmol/L EDTA that was bubbled with 95% O_2 and 5% CO_2 to maintain the buffer at pH 7.4. Data were recorded using a PowerLab/8sp data acquisition system (A.D. Instruments, Castle Hill, Australia). After being mounted, the arteries were allowed to equilibrate for 90 minutes before they were stretched stepwise to characterize the passive elastic properties, as previously described [25]. Contractility of the

segments was then tested by exposing them 2 times to a depolarizing 60 mmol/L KCl solution. After recording the maximum contractile tension (MCT), the segment was washed with Krebs solution. Contractility of segments was then tested by an exposure to phenylephrine (Phe, 1 µmol/L) and the MCT value was recorded. Maximum relaxation responses (MRR) to acetylcholine (Ach, 1 µmol/L) were recorded. The curves of the contractile and relaxation responses were saved. The MCT induced by Phe or KCl was set as 100%. The ratio of the vascular tone range under Ach to the MCT indicated the variation in the vascular tone (Figure 3).

MRR percentage (MRR%) = (MCT value to Phe – MRR value to Ach)/MCT value to Phe ×100%.

Pathological evaluation

After completion of the experiment, the remaining aortas were removed for gross pathological evaluation. For light microscopic examination, the specimens were fixed in 4% formalin for 24 hours, embedded in paraffin, and cut into 5-µm tissue sections for hematoxylin-eosin (HE) staining.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Continuous data are expressed as the mean ±SD. Comparisons of means were performed using analysis of variance (ANOVA) followed by LSD post-hoc tests. To assess covariates and confounders, we performed analysis of covariance and multiple linear regression. P-values <0.05 were considered significant.
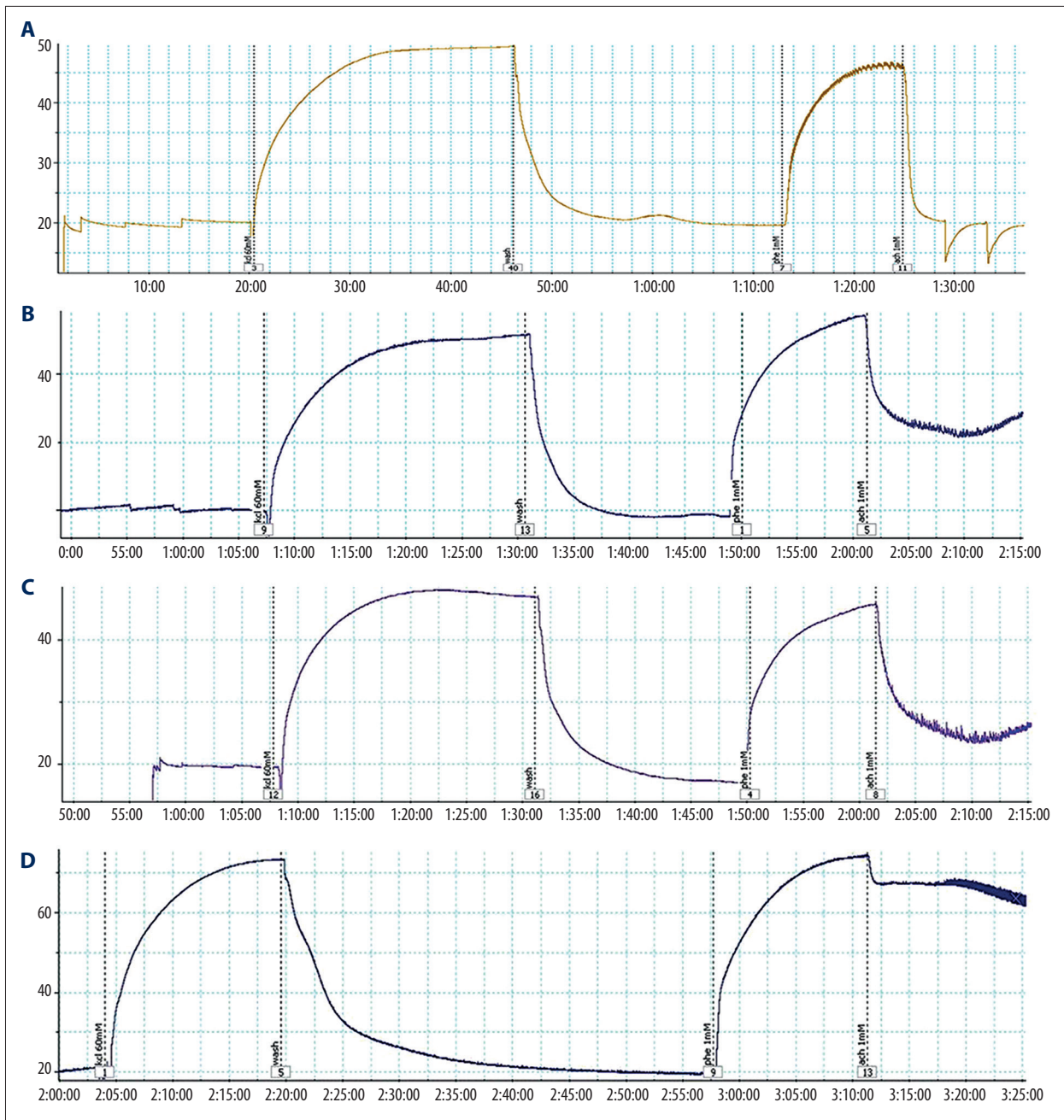
Figure 3. Contraction and relaxation response curves of aortic rings. (**A**): contraction response curve to KCl and Phe, and the relaxation response curve to Ach in the aortic ring of a rat in group C1. (**B**): contraction response curve to KCl and Phe, and the relaxation response curve to Ach in the aortic ring of a rat in group T1. (**C**): contraction response curve to KCl and Phe, and the relaxation response curve to Ach in the aortic ring of a rat in group C2. (**D**): contractile response curve to KCl and Phe, and the relaxation response curve to Ach in the aortic ring of a rat in group T2.

Results

Baseline parameters

At baseline, there were no significant differences in weight, height, Ps, Pd, DC, AC, α, β, or PWVβ between groups T1 and C1 or between groups T2 and C2 (all $P>0.05$) (Table 1).

Echo-tracking detected differences between the groups

At the end of the 4- or 12-week period, Ps was higher in the T2 group than in the C2 group ($P<0.001$). Ps and Pd were higher

Table 1. Parameters at baseline.

	Hypercholesterolemia at 4 weeks (T1)	Control at 4 weeks (C1)	Atherosclerosis at 12 weeks (T2)	Control at 12 weeks (C2)
n	10	10	10	10
Height (cm)	44.90±1.85	45.70±1.83	44.87±1.79	46.35±1.71
Weight (g)	498.7±46.99	501.60±47.92	499.54±45.44	502.01±45.65
Ps (mmHg)	102.14±2.85	99.5±6.42	100.07±3.54	98.37±4.43
Pd (mmHg)	76.71±6.47	70.90±8.01	74.54±7.43	75.43±7.22
DC	0.04±0.02	0.06±0.02	0.04±0.01	0.05±0.02
AC	0.15±0.08	0.35±0.11	0.28±0.10	0.29±0.09
α	2.87±2.23	1.64±0.65	2.24±2.01	2.20±1.98
β	5.87±4.48	3.50±1.17	4.98±4.01	4.59±2.44
PWVβ	5.35±1.84	4.12±0.65	5.03±0.98	4.98±1.23

Ps – systolic blood pressure; Pd – diastolic blood pressure; DC – distensibility coefficient; AC – arterial compliance; α – stiffness parameter α; β – stiffness parameter β; PWVβ – one-point pulse wave velocity. All data are presented as mean ±SD. All $P>0.05$ in each row.

Table 2. Blood pressure, stiffness parameters, arterial compliance, distensibility coefficient, and one-point pulse wave velocity after treatment.

	Hypercholesterolemia at 4 weeks (T1)	Control at 4 weeks (C1)	Atherosclerosis at 12 weeks (T2)	Control at 12 weeks (C2)
Ps (mmHg)	101.00±5.44	98.5±7.62	108.80±4.39[###,$]	102.5±2.42
Pd (mmHg)	73.40±8.48	68.90±9.04	80.20±1.62[$]	77.50±5.44
DC	0.04±0.01*	0.06±0.03	0.03±0.01[#]	0.05±0.02
AC	0.15±0.07*	0.31±0.20	0.12±0.05[#]	0.18±0.04
α	2.58±0.81*	1.67±0.67	3.31±1.17[#]	2.14±0.96
β	5.32±1.66*	3.47±1.38	6.88±2.18[#]	4.57±1.60
PWVβ	5.06±0.71*	4.08±0.83	7.06±1.39[###,$]	4.54±0.77

Ps – systolic blood pressure; Pd – diastolic blood pressure; DC – distensibility coefficient; AC – arterial compliance; α – stiffness parameter α; β – stiffness parameter β; PWVβ – one-point pulse wave velocity. All data are presented as mean ±SD. * $P<0.05$ T1 vs. C1; # $P<0.05$, ### $P<0.001$ T2 vs. C2; $ $P<0.001$ T1 vs. T2.

in the T2 group than in the T1 group ($P<0.05$). The DC and AC values of the T1 and T2 groups were decreased compared with C1 and C2 (all $P<0.05$). Furthermore, α, β, and PWVβ values were increased in groups T1 and T2 compared with C1 and C2 (all $P<0.05$). PWVβ was higher in the T2 group compared with T1 ($P<0.001$) (Table 2).

Atherosclerosis affected aortic ring responses

At the end of the 4- or 12-week period, MCT to KCl, MCT to Phe and MRR% were decreased in the T1 group compared with C1 ($P<0.001$, $P<0.01$ and $P<0.001$, respectively). MCT to KCl and Phe were increased in the T2 group compared with C2 ($P<0.05$ and $P<0.05$, respectively). However, MRR% was decreased in the T2 group compared with C2 ($P<0.001$). MCT to KCl and MCT to Phe were higher in the T2 group compared with T1 ($P<0.001$ and $P<0.05$, respectively) (Table 3).

Table 3. Aortic ring tension parameters.

	Hypercholesterolemia at 4 weeks (T1)	Control at 4 weeks (C1)	Atherosclerosis at 12 weeks (T2)	Control at 12 weeks (C2)
KCl	32.65±5.61***	40.85±1.85	43.66±4.28[#,$$$]	39.21±3.52
Phe	35.04±6.74**	40.13±1.98	41.84±3.46[#,$]	38.48±3.82
MRR%	0.34±0.05***	0.65±0.20	0.29±0.09[###]	0.67±0.22

KCl – the maximum contractile tension value of the ring to high-K solution; Phe – the maximum contractile tension value of the ring to phenylephrine; MRR% – maximum relaxation response percentage. All data are presented as mean ±SD. ** $P<0.01$,*** $P<0.001$ T1 vs. C1; [#] $P<0.05$, [###] $P<0.001$ T2 vs. C2; [$] $P<0.05$, [$$$] $P<0.001$ T1 vs. T2.

Table 4. Correlations between blood pressure, stiffness parameter β, arterial compliance, distensibility coefficient, and one-point pulse wave velocity.

	β		DC		AC		PWVβ	
	r	P	r	P	r	P	r	P
Ps	0.373	0.018	−0.438	0.005	−0.492	0.001	0.471	0.002
Pd	0.377	0.017	−0.417	0.007	−0.530	<0.001	0.387	0.014
DC	−0.798	<0.001			0.872	<0.001	−0.720	<0.001
AC	−0.667	<0.001	0.872	<0.001			−0.641	<0.001
β			−0.798	<0.001	−0.667	<0.001	0.838	<0.001
PWVβ			−0.720	<0.001	−0.641	<0.001		

Ps – systolic blood pressure; Pd – diastolic blood pressure; DC – distensibility coefficient; AC – arterial compliance; α – stiffness parameter α; β – stiffness parameter β; PWVβ – one-point pulse wave velocity.

Table 5. Correlation analysis between maximum contractile tension value and relaxation percentage.

	Phe		MRR%	
	r	P	r	P
DC	0.030	0.855	0.396	0.012
AC	0.138	0.395	0.317	0.047
β	0.076	0.640	−0.406	0.009
PWVβ	0.046	0.779	−0.434	0.005
KCl	0.707	<0.001		

Phe – the maximum contractile tension value of the ring to phenylephrine, MRR%=maximum relaxation response percentage; KCl – the maximum contractile tension value of the ring to high-K solution; DC – distensibility coefficient; AC – arterial compliance; α – stiffness parameter α; β – stiffness parameter β; PWVβ – one-point pulse wave velocity.

Correlations

β was positively correlated with Ps (r=0.373, P=0.018) and Pd (r=0.377, P=0.017), and negatively correlated with DC (r=−0.798, P<0.001) and AC (r=−0.667, P<0.001). DC was negatively correlated with Ps (r=−0.438, P=0.005) and Pd (r=−0.417, P=0.007). AC was negatively correlated with Ps (r=−0.492, P=0.001) and Pd (r=−0.530, P<0.001). PWVβ was positively correlated with β (r=0.838, P<0.001), Ps (r=0.471, P=0.002) and Pd (r=0.387, P=0.014). PWVβ was negatively correlated with DC (r=−0.798, P<0.001) and AC (r=−0.667, P<0.001) (Table 4).

MRR% was negatively correlated with α (r=-0.390, P=0.013), β (r=-0.406, P=0.009) and PWVβ (r=-0.434, P=0.005). MRR% was positively correlated with DC ((r=0.396, P=0.012) and AC (r=0.317, P=0.047) (Table 5).

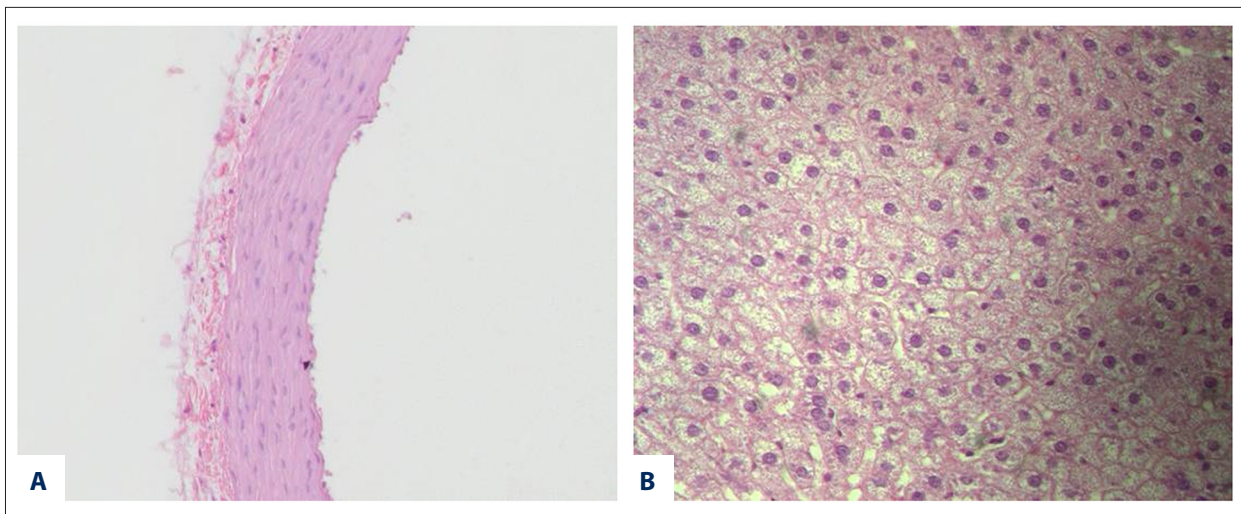**Figure 4.** HE staining of the aorta of a control rat at 4 weeks and control rat at 12 weeks. (**A**): 200× magnification; (**B**): 400× magnification.
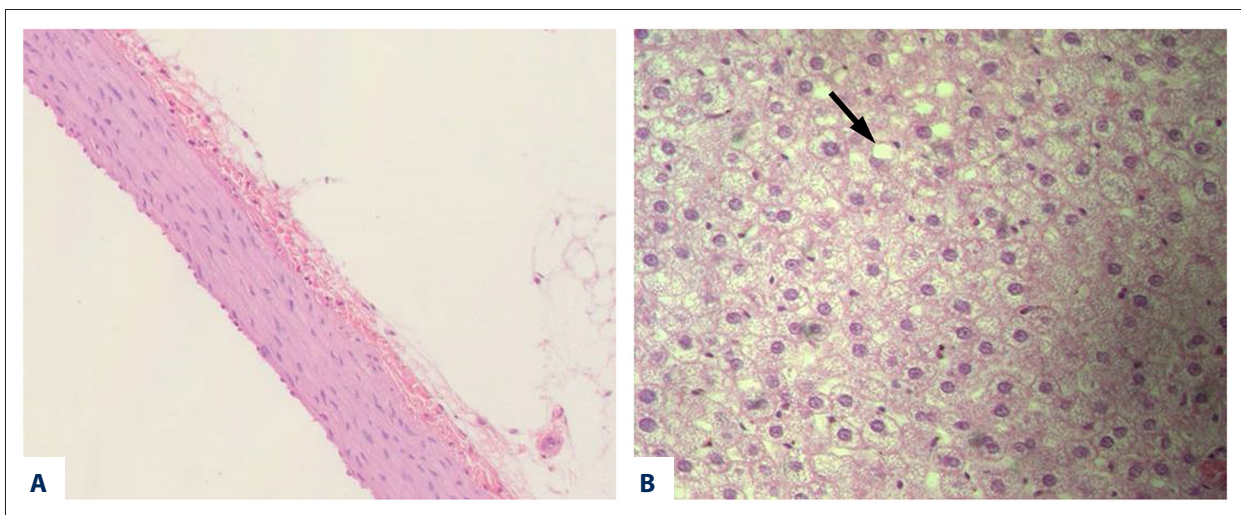

Figure 5. HE staining of the aorta of a rat with hypercholesterolemia at 4 weeks. (**A**): 200× magnification; (**B**): 400× magnification. Foam cells are indicated by a black arrow.

Pathological analysis

Gross examination showed a smooth aortic intima with no atherosclerotic plaque in groups C1, C2, and T1, whereas obvious atherosclerotic plaque formation was observed in the T2 group. Microscopy showed that the endothelial cells were smooth and that the intima was level. The elastic fibers of the tunica media showed a smooth structure. The tunica adventitia showed loose connective tissues in groups C1 and C2 (Figure 4). However, in the T1 group, some foam cells were observed under the endothelial cells (Figure 5). In the T2 group, the endothelial cells were reduced and the intima was clearly thickened. The surface of the plaques showed a fibrous cap with lipid deposits. The deep part was necrotic. The tunica media was compressed and atrophied (Figure 6).

Discussion

The aim of the present study was to ascertain the utility of the ET technique for the assessment of vascular stiffness in rats with hypercholesterolemia and atherosclerosis. The results showed an increase in stiffness parameters and a decrease in dilation parameters evaluated using ET in rat models of hypercholesterolemia and atherosclerosis. Importantly, the stiffness changes detected by ET in rats with hypercholesterolemia were evident in the absence of gross pathological changes (such as the presence of plaque), suggesting that ET may detect functional abnormalities reflecting the underlying disease that might not be identified with morphological techniques such as angiography. Therefore, ET represents a viable method for detecting changes in arterial stiffness in rats
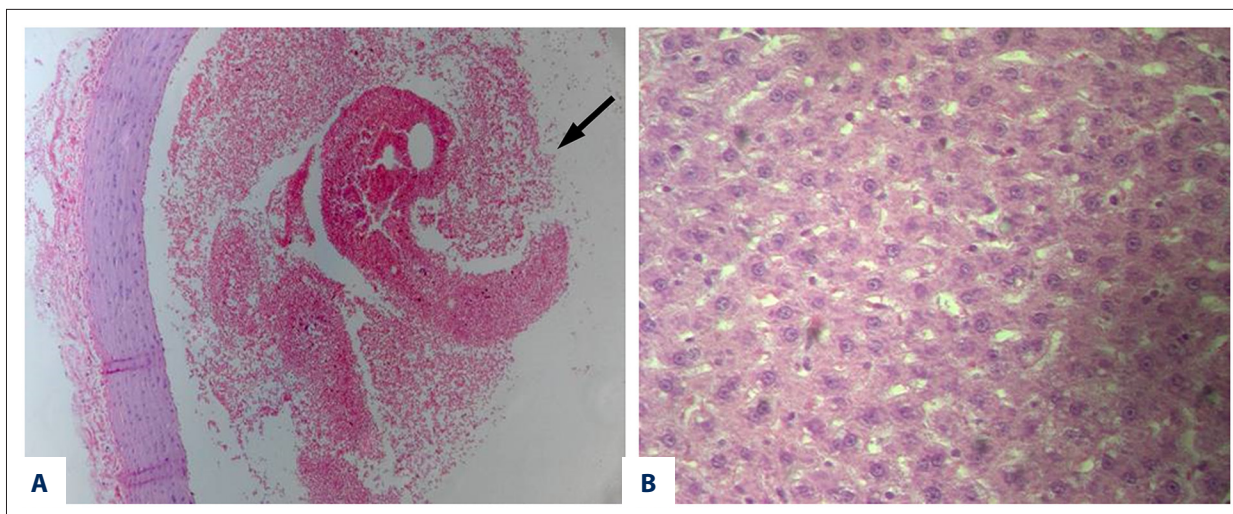
Figure 6. HE staining of the aorta of a rat with atherosclerosis at 12 weeks. (**A**): 200× magnification; (**B**): 400× magnification. A plaque is indicated by a black arrow.

that occur secondary to hypercholesterolemia and/or atherosclerosis. On the basis of these findings, further studies are merited to determine whether ET could be used as a noninvasive technique in the clinic for monitoring pathological changes in arterial function in patients with hypercholesterolemia and/or atherosclerosis.

Previous studies have shown that rabbit models of atherosclerosis had higher arterial stiffness than controls [26–28]. These results were also observed in humans with increasing levels of atherosclerosis [3,29,30]. These results could be because high atherosclerosis induces structural stiffness in arteries due to calcification and hypertrophy. In the present study, pathological changes of atherosclerosis were observed in the aortas of the T2 group, and these aortas showed decreased reactivity to the different stimuli used to assess their responsiveness, suggesting an association between atherosclerosis and arterial stiffness. However, assays were not performed to assess the changes in collagen associated with atherosclerosis and their association with arterial stiffness parameters. Future studies will have to address this issue. Nevertheless, a recent study has shown that arterial stiffness predicted cardiovascular death independently from arterial thickness [31], causing some controversy regarding the subject.

In the present study, β, AC, DC, and PWVβ were selected to assess arterial stiffness by ET. Among these, β and PWVβ are stiffness parameters, and AC and DC are dilation parameters. As might be expected, the present study showed that stiffness parameters were negatively correlated with dilation parameters. In addition, β and PWVβ were higher in the T1 and T2 groups compared with controls, while DC and AC were lower. These results are supported by previous studies. Indeed, a study in rabbits showed that ET parameters were associated

with atherosclerosis [16]. A previous study in humans has shown that there was an association between aortic stenosis and arterial compliance [32]. Another study in humans has shown that arterial stiffness could detect the early changes induced by atherosclerosis [28]. In addition, an autopsy study revealed that there was a relation between pathological findings of carotid atherosclerosis and pre-death ET β parameter [33].

PWVβ represents the vascular wall's bionomics, vascular geometry characteristics, and blood density. PWVβ reflects vascular stiffness because the variations in vascular geometry and blood density are usually small. A cross-sectional study indicated that aortic PWVβ was correlated with the cardiovascular risk and that the stroke risk was 1.72-fold higher when PWVβ was increased by 4 m/s [34]. Arterial stiffness was correlated with cardiovascular mortality in terminal-stage nephritis. Furthermore, PWVβ can predict the risk of cardiovascular diseases in people over 70 years old [35,36]. In the present study, PWVβ was increased in the atherosclerosis groups, but no differences were observed in β, AC, and DC, suggesting that PWVβ can objectively reflect the degree of arterial stiffness and that PWVβ is more sensitive than the other indexes. It is possible that these parameters are sensitive indexes of vascular function but that they are not related to variations in morphology. However, these findings need to be validated in humans. Nevertheless, PWV has been shown to predict cardiovascular events in humans [37,38].

AC and DC reflect the vascular abilities of contraction and relaxation. We performed aortic ring testing according to the method by Mulvany and Halpern [25] and tested the validity and confidence of vascular stiffness parameters measured by ET with the parameters using aortic rings. Cai et al. [21] used a similar approach in high-fat-fed rats and controls and

measured the response of thoracic aorta to norepinephrine (10^{-8}–10^{-4} mmol/L) and Ach (10^{-6}–10^{-4} mmol/L) after 5 weeks. The results showed that the response to norepinephrine or Ach was weaker in the high-fat diet group and that arterial contraction and relaxation were decreased. These results were similar to those observed in the T1 and C1 groups. The present study showed that the MRR% of aorta rings in the T2 group was obviously lower than in C2, but that the MCT to KCl and Phe were obviously higher than in the C2 group. Furthermore, MCT was higher in the T2 group compared with T1. These results suggest that atherosclerosis has a direct impact on the reactivity of arteries to contraction/dilation stimuli.

Indeed, some studies have shown that the impairment of vascular endothelial cells and abnormal vascular relaxation function was due to the impairment of the usual vasoactive factors released by the endothelium [39]. Indeed, blood vessel endothelium releases vasoactive substances like prostacyclin PGI_2, NO, and endothelin, which act together to maintain a proper vascular tone [40,41]. Factors involved in the pathogenesis of atherosclerosis are toxic to endothelial cells [42], damaging arterial endothelial cells, and decreasing NO and biological function of the cells. A previous study showed that there was a relation between arterial stiffness and flow-mediated dilation in patients with or without cardiovascular diseases [43]. Imbalances in dilation mediators result in endothelial dysfunction, a state promoting the development of arteriosclerosis plaque and the deregulation of vascular tone. In the present study, the MCT in the T2 group was obviously higher than in T1 and controls. We suppose that the aortic contractile response to KCl and Phe would be strengthened, possibly because of serious endothelial dysfunction and induced production of the vasoconstrictor endothelin by the endothelium. However, in-depth biochemical studies are necessary to examine this point.

MRR% reflects the relaxation function of aortic rings. In rings confirmed to have serious atherosclerosis, β and PWVβ were higher, representing a higher degree of impairment of the endothelial cells. Consequently, the relaxation ability of the aortic rings was much lower and AC and DC were much lower. The *in vitro* results correlated with those obtained using ET. Indeed AC, DC, and MRR% in the T1 and T2 groups were lower than in C1 and C2. However, no difference was observed between the T1 and T2 groups. Furthermore, MRR% was negatively correlated with Ps, β, and PWVβ, and positively correlated with DC and AC. These results strongly suggest that the parameters obtained using ET and the tension test are representative of each other, and that ET correctly assesses arterial stiffness. In addition, the pathological changes were confirmed in the atherosclerosis rat models. The changes in the aortas were confirmed by pathological examination of the specimens, strengthening our results.

The results of the present study were obtained in an animal model; therefore, further studies should be performed in humans. In addition, in-depth immunohistochemical analyses should be performed to characterize the changes in aortas in relation to ET parameters.

Conclusions

ET and *in vitro* aortic ring tension are correlated with the pathological evaluation. Therefore, ET represents a suitable technique for evaluating the changes in arterial wall elasticity associated with atherosclerosis and hypercholesterolemia in rats. Further studies are merited to explore whether ET could have clinical utility in the assessment of arterial wall elasticity in patients with hypercholesterolemia and/or atherosclerosis.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

References:

1. Varady E, Feher E, Levai A, Battyany I: Estimation of vessel age and early diagnose of atherosclerosis in progeria syndrome by using echo-tracking. Clin Hemorheol Microcirc, 2010; 44: 297–301

2. Zhang P, Guo R, Li Z et al: Effect of smoking on common carotid artery wall elasticity evaluated by echo tracking technique. Ultrasound Med Biol, 2014; 40: 643–49

3. Vincze M, Der H, Kerekes G et al: Decreased flow-mediated dilatation with increased arterial stiffness and thickness as early signs of atherosclerosis in polymyositis and dermatomyositis patients. Clin Rheumatol, 2014; 33(11): 1635–41

4. Madden KM, Lockhart C, Cuff D et al: Short-term aerobic exercise reduces arterial stiffness in older adults with type 2 diabetes, hypertension, and hypercholesterolemia. Diabetes Care, 2009; 32: 1531–35

5. Kanaki AI, Sarafidis PA, Georgianos PI et al: Effects of low-dose atorvastatin on arterial stiffness and central aortic pressure augmentation in patients with hypertension and hypercholesterolemia. Am J Hypertens, 2013; 26: 608–16

6. Ohbayashi H, Miyazawa C, Miyamoto K et al: Pitavastatin improves plasma pentraxin 3 and arterial stiffness in atherosclerotic patients with hypercholesterolemia. J Atheroscler Thromb, 2009; 16: 490–500

7. Chatterjee S, Bedja D, Mishra S et al: Inhibition of glycosphingolipid synthesis ameliorates atherosclerosis and arterial stiffness in apolipoprotein E–/– mice and rabbits fed a high-fat and -cholesterol diet. Circulation, 2014; 129: 2403–13

8. Liu YP, Zhan WW, Zhang YF et al: Carotid intima-media thickness and stiffness in relation to type 2 diabetes in Chinese. Endocrine, 2007; 31: 289–93

9. Araki T, Emoto M, Teramura M et al: Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. Metabolism, 2006; 55: 996–1001

10. Giannattasio C, Failla M, Capra A et al: Increased arterial stiffness in normoglycemic normotensive offspring of type 2 diabetic parents. Hypertension, 2008; 51: 182–87

11. Jaroch J, Loboz Grudzien K, Bociaga Z et al: The relationship of carotid arterial stiffness to left ventricular diastolic dysfunction in untreated hypertension. Kardiol Pol, 2012; 70: 223–31

12. Vriz O, Bossone E, Bettio M et al: Carotid artery stiffness and diastolic function in subjects without known cardiovascular disease. J Am Soc Echocardiogr, 2011; 24: 915–21

13. Wei W, Wang J, Zhao Q, Yang J: [Echo-tracking technology for evaluating femoral artery endothelial function in patients with Grave's disease]. Nan Fang Yi Ke Da Xue Xue Bao, 2012; 32: 1478–81 [in Chinese]

14. Yu ZX, Wang XZ, Guo RJ et al: Comparison of ultrasound echo-tracking technology and pulse wave velocity for measuring carotid elasticity among hemodialysis patients. Hemodial Int, 2013; 17: 19–23

15. Varady E, Feher E, Marko L, Battyany I: Determination of normal beta values via radio frequency echo-tracking technique in a healthy Central European population. Clin Hemorheol Microcirc, 2014; 57(2):185–89

16. Wen C, Zhang L, Wang Y et al: Sonographic assessment of atherosclerotic and hypercholesterolemic artery using the echo-tracking technique in an animal model. Vascular, 2010; 18: 178–84

17. Qin YQ, Chen AH, Tang XM: [Echo-tracking technology for evaluating the impact of blood pressure on vascular endothelial function]. Nan Fang Yi Ke Da Xue Xue Bao, 2012; 32: 396–99 [in Chinese]

18. Carretta R, Bardelli M, Cominotto F et al: Relationship between mechanical properties of the carotid artery wall and baroreflex function in acutely treated hypertensive patients. J Hypertens, 1996; 14: 1105–10

19. Duprez DA, De Buyzere ML, Verloove HH et al: Influence of the arterial blood pressure and nonhemodynamic factors on regional arterial wall properties in moderate essential hypertension. J Hum Hypertens, 1996; 10: 251–56

20. Garcia-Redondo AB, Briones AM, Beltran AE et al: Hypertension increases contractile responses to hydrogen peroxide in resistance arteries through increased thromboxane A2, Ca2+, and superoxide anion levels. J Pharmacol Exp Ther, 2009; 328: 19–27

21. Cai B, Zheng HZ: [Effects of high fat diet on arterial tention in rats]. Journal of Guangdong Medical College, 2000; 18: 239–40 [in Chinese]

22. Yan LP, Chan SW, Chan AS et al: Puerarin decreases serum total cholesterol and enhances thoracic aorta endothelial nitric oxide synthase expression in diet-induced hypercholesterolemic rats. Life Sci, 2006; 79: 324–30

23. Wu J, Zhao MY, Zheng H et al: Pentoxifylline alleviates high-fat diet-induced non-alcoholic steatohepatitis and early atherosclerosis in rats by inhibiting AGE and RAGE expression. Acta Pharmacol Sin, 2010; 31: 1367–75

24. Liu LS, Caguioa ES, Park CG et al: Reducing stroke risk in hypertensive patients: Asian Consensus Conference recommendations. Int J Stroke, 2006; 1: 150–57

25. Mulvany MJ, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res, 1977; 41: 19–26

26. Band W, Goedhard WJ, Knoop AA: Comparison of effects of high cholesterol intake on viscoelastic properties of the thoracic aorta in rats and rabbits. Atherosclerosis, 1973; 18: 163–71

27. Pynadath TI, Mukherjee DP: Dynamic mechanical properties of atherosclerotic aorta. A correlation between the cholesterol ester content and the viscoelastic properties of atherosclerotic aorta. Atherosclerosis, 1977; 26: 311–18

28. Mokhtari-Dizaji M, Nikanjam N, Saberi H: Detection of initial symptoms of atherosclerosis using estimation of local static pressure by ultrasound. Atherosclerosis, 2005; 178: 123–28

29. Richter HA, Mittermayer C: Volume elasticity, modulus of elasticity and compliance of normal and arteriosclerotic human aorta. Biorheology, 1984; 21: 723–34

30. Roos CJ, Delgado V, de Koning EJ et al: Associations of atherosclerosis in the descending thoracic aorta on CTA with arterial stiffness and chronic kidney disease in asymptomatic patients with diabetes mellitus. Int J Cardiovasc Imaging, 2014; 30: 1151–59

31. Shoji T, Maekawa K, Emoto M et al: Arterial stiffness predicts cardiovascular death independent of arterial thickness in a cohort of hemodialysis patients. Atherosclerosis, 2010; 210: 145–49

32. Antonini-Canterin F, Rosca M, Beladan CC et al: Echo-tracking assessment of carotid artery stiffness in patients with aortic valve stenosis. Echocardiography, 2009; 26: 823–31

33. Wada T, Kodaira K, Fujishiro K et al: Correlation of ultrasound-measured common carotid artery stiffness with pathological findings. Arterioscler Thromb, 1994; 14: 479–82

34. Ni YB, Zhang WZ, Wang HZ: The relationship between pulse wave velocity and pulse pressure in Chinese patients with essential hypertension. Chin J Cardiol, 2003; 31: 257–59

35. Blacher J, Pannier B, Guerin AP et al: Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. Hypertension, 1998; 32: 570–74

36. Meaume S, Rudnichi A, Lynch A et al: Aortic pulse wave velocity as a marker of cardiovascular disease in subjects over 70 years old. J Hypertens, 2001; 19: 871–77

37. Cruickshank K, Riste L, Anderson SG et al: Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation, 2002; 106: 2085–90

38. Sutton-Tyrrell K, Najjar SS, Boudreau RM et al: Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation, 2005; 111: 3384–90

39. Brandes RP, Behra A, Lebherz C et al: N(G)-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation in the rabbit renal artery: effect of hypercholesterolemia. Atherosclerosis, 1997; 135: 49–55

40. Britten MB, Zeiher AM, Schachinger V: Clinical importance of coronary endothelial vasodilator dysfunction and therapeutic options. J Intern Med, 1999; 245: 315–27

41. Inagami T, Naruse M, Hoover R: Endothelium as an endocrine organ. Annu Rev Physiol, 1995; 57: 171–89

42. Vogel RA: Cholesterol lowering and endothelial function. Am J Med, 1999; 107: 479–87

43. Nigam A, Mitchell GF, Lambert J, Tardif JC: Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. Am J Cardiol, 2003; 92: 395–99