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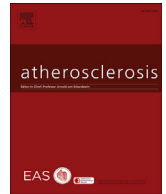
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## A non-hypocholesterolemic atorvastatin treatment improves vessel elasticity by acting on elastin composition in WHHL rabbits



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### ABSTRACT

**Background and aims:** Statins are prescribed for their preventative effects within atherosclerosis development. To our knowledge, no study focusing on very low-dose (non-hypolipidemic effect) and long-term atorvastatin treatment *in vivo* was available. Our aim was to assess the effect of such atorvastatin treatment on the mechanical and functional characteristics of arteries in the context of primary prevention.

**Methods:** An atorvastatin treatment (2.5 mg/kg/day) was tested against controls on 34 male 3 to 12 month-old WHHL rabbits. No effect on total cholesterol, triglycerides, HDL or LDL was observed. The arterial stiffness was evaluated on vigil animals by pulse wave velocity (PWV) measurement. Then, *in vitro* measurements were made to evaluate (1) the endothelial and vascular smooth muscle function, (2) the elasticity of the arterial wall and (3) the composition in collagen and elastin in the aorta.

**Results:** The PWV increasing observed with age in control group was canceled by treatment, creating a significance difference between groups at 12 months ( $5.17 \pm 0.50$  vs  $2.14 \pm 0.34$  m s<sup>-1</sup> in control and treated groups respectively). Vasoreactivity modifications can't explain this result but maintain of elasticity with treatment in large arteries was confirm by a static tensile test. A first possible explanation is the change of wall composition with treatment, validated by the percentage of elastin at 12 months, 4.4% lower in the control group compared to the treated group ( $p < 0.05$ ).

**Conclusions:** This study shows that a non-hypocholesterolemic statin treatment could improve vessel elasticity in the atherosclerotic WHHL model. The great novelty of this work is the vessel wall composition changing associated. This first approach in animal opens the reflection on the use of these low doses in humans. This could be interesting in the context of arterial stiffening with aging, non-hyperlipidemic atherosclerosis or with cholesterol reduce by another therapy or lifestyle modification.

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### 1. Introduction

The use of statins is recommended to prevent clinical

complications of atherosclerosis like myocardial infarction, stroke or peripheral arterial diseases [1]. Indeed, these molecules reduce the development of atherosclerosis [2] and the occurrence of cardiovascular events [3] through mechanisms dependent or independent of their lipid lowering effects [4,5] like their antithrombotic effects [6], the reduction of inflammation [7], or oxidative stress [8]. If the use of statins in secondary prevention is well established, their interest in primary prevention is still to be

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fully evaluated. Several studies have shown that lipid-lowering doses of statins in individuals with high circulating lipid level reduced the atherosclerotic cardiovascular risk [9]. Moreover, statins were shown to contribute to plaque stabilization [10] as well as to improve the endothelial function [11]. However, high-doses and long-term treatments can induce adverse effects. The most common adverse effect described is a myotoxicity [12] but others, less frequent, could appear such as hepatotoxicity [13], an increase in the prevalence of diabetes [14] or an increased risk of intracerebral hemorrhage [15]. Thus, in order to limit these phenomena, low doses of statins could be used. To our knowledge, no study focusing on very low-dose (non-hypolipidemic effect) and long-term treatment *in vivo* was available. Hence, our work aims at analyzing the impact of a very low dose of statins on the vascular system in primary prevention of atherosclerosis. A study in Watanabe heritable hyperlipidemic (WHHL) rabbits was performed to determine if atorvastatin (the most prescribed statin) could improve the functionality of the vascular system, at a non-hypocholesterolemic dose. The WHHL rabbit was chosen as a model of hypercholesterolemia and spontaneous development of atherosclerosis (similarly to that developed in humans) [16].

## 2. Materials and methods

### 2.1. Animals

This study was performed in accordance with the European Council revised guidelines on animal care for experimental and other scientific purposes. Experiments were approved by the local ethic committee for animal experimentation (Authorization number: CEEA.2012.189). This study was performed on male homozygous WHHL rabbits (3165 ± 489 g) produced by CPA Jeanrot (FRANCE, 45160 Olivet).

During the experiment, animals were kept at the veterinary school of Nantes (Oniris, France) in an animal house under a 12-h light-dark-cycle at 21 °C. Food (Lapin Clapier PDC; Le Pré du Clocher; 44155 Ancenis). Water and food were given *ad libitum*.

### 2.2. Experimental design

Thirty four 3 month-old WHHL rabbits from the age of 3 to 12 months were randomized in two groups: control (C) and treated (T).

Treated group received 2.5 mg/kg body weight (bw)/day of atorvastatin (atorvastatin calcium PF-00079943-07 Pfizer) diluted in drinking water. When all daily water with treatment was drunk, fresh water was given *ad libitum*. Animals were weighted every week to adjust the treatment. Every month, pulse wave velocity and blood sampling were performed on each animal. Other analyses were made on anaesthetized and euthanized animals: on 4 control 3 month-old rabbits then, on 5 control and 5 treated animals at the ages of 6, 9 and 12 months.

### 2.3. Measured parameters

#### 2.3.1. Blood sampling and analysis

Blood sampling was performed on vigil animals at 10, 11 and 12 months from the median artery of the ear. Serum was removed after spontaneous coagulation, total cholesterol, triglycerides and HDL were measured on a Siemens dosage chain (CA12340063) with ADVIA reactivities. LDL was measured with a specific kit (LDL quantitation kit MAK045; Sigma-Aldrich). Circulating Elastin was quantified with specific ELISA kit (E-EL-RB0506; Elabscience).

#### 2.3.2. Pulse wave velocity (PWV)

Every month, PWV measurements were made on vigil animals as previously described. Briefly, a piezo electric sensor (AD instruments) was placed on the auricular artery and another on the caudal one. The distance (*d*) between the two sensors was measured. Recordings of pulses were made during 10 min with the software LabChart 7 (AD instruments). 30 measurements of the time interval ( $\Delta t$ ) between the wave front of the auricular waveform and that of the caudal waveform were taken. The PWV was calculated using the formula  $PWV (m.s^{-1}) = d/\Delta t$ .

#### 2.3.3. Arterial blood pressure and heart rate measurements

Arterial blood pressure and heart rate were measured on 12 month-old rabbits from control and treated groups after anaesthesia with intramuscular injection of ketamine (100 mg/kg bw) and xylazine (10 mg/kg bw). The arterial blood pressure was measured with a catheter inserted in the carotid (connected to a pressure sensor and a MP35, Biopac Systems, Inc.). The analysis of the recording was made on the BSL Pro 3.7 software and allowed to measure, systolic, diastolic, differential and mean blood pressures; heart rate was also calculated.

#### 2.3.4. Aorta dimensions

Aorta dimensions were measured every 3 months using optical coherence tomography (OCT). Previously anaesthetized rabbits were heparinized (0.5 ml at 5000 U/ml) and then, euthanized with an intra-arterial injection of ketamine (100 mg/kg bw) and xylazine (10 mg/kg bw). OCT imaging was performed with Terumo FastView op-16p2613 from the iliac bifurcation to the aorta arch for each rabbit. Images were acquired and stored digitally for subsequent analysis with OCT Lunawave (Terumo).

#### 2.3.5. *In vitro* measurements on vessel samples

Vessels were sampled every three months on euthanized rabbits to determine the mechanical properties of the aorta, the vaso-reactivity and the elastin/collagen *ratio*.

**2.3.5.1. Mechanical properties of the aorta.** They were assessed using a static uniaxial tensile test. The first 2 cm segment after the aortic arch was removed as quickly as possible after euthanasia. Vessels were placed into a saline solution (in mM: NaCl 137; KCl 5.4; CaCl<sub>2</sub> 2.5; Na<sub>2</sub>HPO<sub>4</sub> 1.2; MgCl<sub>2</sub> 1.2; Hepes 20; D Glucose 15, pH 7.4) at 4 °C. Aorta was opened to form a planar surface and two rectangular pieces were cut (6 mm in the longitudinal direction of the aorta, 4 mm in the transversal one). Tensile tests were performed in the longitudinal direction of the aorta. Sample ends were fixed between the grips; the saline solution was used to maintain the samples wet during the whole test. A static uniaxial tensile test was carried out with a dynamic mechanical thermal analyser (DMTA) VA4000 testing device (01 db-Mettravib) at ambient temperature. All samples were tested at a constant rate of 50 μm/s, and the nominal stress, that is the force at time *t* divided by the initial sample section, was measured as a function of the Cauchy strain, that is the sample length between the lower and upper grips at time *t*, divided by its initial value. All samples were extended up to a Cauchy strain of 2.5. The mechanical property of aortas discussed in the paper is the linear elastic modulus in the aorta longitudinal direction, characterized by the Young's modulus determined from the linear part of the stress/strain curves, at low strains.

**2.3.5.2. Vasoreactivity.** Vessels were sampled from three different territories (aorta, carotid, mesenteric) and washed with saline solution.

Vessels were carefully dissected under microscope to remove surrounding connective tissues and cut into rings of 2 mm. Two

rings from each vessel were mounted between two 0.025 stainless-steel wires in a 5 ml organ bath containing saline solution maintained at 37 °C. Rings were progressively stretched to a resting tension of 6 g for aorta and 2 g for others arteries. Isometric tension was recorded using a force displacement transducer (MP150, Biopac systems Inc., EMKA Technologies), and displayed on a computer with LabChart 7 software (ADInstruments). Rings were allowed to equilibrate for a 45 min period and washed with saline solution every 15 min. Thereafter, rings were contracted with  $2.5 \cdot 10^{-4}$  M phenylephrine (PE). Response to acetylcholine cumulative doses (Ach,  $10^{-9}$  to  $3.10^{-4}$  M) was made. Between trials, the rings were allowed to recover for 45 min with washes every 5–10 min. After another pre-contraction with PE  $2.5 \cdot 10^{-4}$  M, cumulative doses of sodium nitroprusside (SNP  $10^{-9}$  to  $3.10^{-4}$  M to aorta and carotids, SNP  $10^{-10}$  to  $3.10^{-5}$  M to mesenteric) were tested.

The dilating responses were expressed as percentages of the PE-induced precontractile force. The logarithm of the concentration of Ach (respectively SNP) that elicited 50% of the maximal response ( $E_{max}$ ) was designated as the  $EC_{50}$ . These values were ascertained for each animal by logistic curve fitting of the semilogarithmic dose-response relationship with TableCurve software 2D (5.01.01 version).

**2.3.5.3. Elastin and collagen quantification.** Aorta samples were fixed in Bouin's solution for 48 h and then, included in paraffin. Section of 5  $\mu$ m from 12 months rabbits was deparaffined and mounted with Neo-Mount<sup>®</sup> (Merck Millipore 109016).

Two-photon and second harmonic generation microscopy was performed in parallel, using a modified confocal microscope (FV300/BX51WI, Olympus) and a femtosecond Ti:Sapphire laser (Verdi-V5/Mira, Coherent) tuned at  $\lambda = 830$  nm as the light source. In order to avoid photobleaching, the laser power was limited to about 100 mW at the objective of the microscope (20X, 0.75NA, UPlanSApo, Olympus). Endogenous two-photon excitation fluorescence (TPEF) from elastin was epi-collected and descanned, then filtered by the dichroic mirror (DCRXU675, 675 nm cut-off wavelength, Chroma) of the scanning unit before to be detected by the internal photomultiplier tube (PMT) of the microscope (R928, Hamamatsu). The voltage of the internal PMT was set to 640 V for all experiments. Second harmonic generation (SHG) from collagen was collected in transmission by the condenser of the microscope, then passed through a 10 nm-FWHM band-pass filter centered on the second harmonic of the laser at 415 nm (NT65-680, Edmund Optics) in order to reject the TPEF light. The SHG signal was then detected by an external PMT module (H5783, Hamamatsu) connected to a transimpedance amplifier (C7319, Hamamatsu,  $10^6$  V/A gain). The voltage of the external PMT was set to 810 V for all experiments. IR blocking filters (Schott BG39 model, Edmund Optics) were inserted on both TPEF and SHG detection pathways to remove the light from the laser. The lateral and axial resolutions of the microscope were estimated to be respectively about 0.5  $\mu$ m and 3  $\mu$ m for the 0.75NA objective lens used [17]. It is worthwhile to notice that the axial resolution was closed to the thickness of the samples (5  $\mu$ m), which means that the TPEF and SHG signals result from almost the whole thickness of the samples. 12 bit intensity resolution and 2048  $\times$  2048 definition TPEF and SHG images were acquired in parallel at the slower scanning speed of the microscope, corresponding to a pixel dwell-time of about 10  $\mu$ s. Images were exported in tif format to be analyzed with ImageJ software.

#### 2.4. Statistical analysis

Statistical tests were performed using SIGMA STATS 10 (StatSoft). Time effect and differences between groups were tested with an all-pairwise multiple comparison procedures (Student-

Newman-Keuls or Kruskal Wallis method) including matched samples when possible. Results were presented as Medians  $\pm$  IQR or Mean  $\pm$  SD depending on n. Differences were considered as significant when  $p < 0.05$ .

### 3. Results

#### 3.1. Blood parameters

The plasmatic concentrations measured at the end of the experimentation ( $n = 5$  rabbits per month per group on months 10, 11 and 12 ie 15 animals per group for each parameter) for the control and treated groups were presented in Table 1. No significant effect of the treatment was observed for any of these parameters.

#### 3.2. Pulse wave velocity

PWV measurements are presented in Fig. 1 for the two experimental groups ( $n = 34$  animals). Aging led to a significant increase of PWV in control group (from  $2 \text{ m}\cdot\text{s}^{-1}$  at 3 months to  $5 \text{ m}\cdot\text{s}^{-1}$  at 12 months). No change was observed in the treated group throughout the experiment: PWV was maintained close to  $3 \text{ m}\cdot\text{s}^{-1}$  and was significantly lower when compared to the control group at 12 months.

#### 3.3. Blood pressure and cardiac frequency

At 12 months ( $n = 5$  per group), no difference was observed between control and treated group on mean arterial pressure ( $114 \pm 22$  mmHg), systolic pressure ( $143 \pm 17$  mmHg), diastolic pressure ( $92 \pm 18$  mmHg), differential blood pressure ( $42 \pm 3$  mmHg) and heart rate ( $163 \pm 36$  beat per min).

#### 3.4. Aorta dimensions

Artery wall thickness was significantly thinning from the aortic arch to iliac bifurcation. No statistical difference was observed between linear regression curve of control and treated groups at 12 months ( $n = 5$  per group). Moreover, no difference in total aorta length was observed over time ( $191.85 \pm 26.55$  mm).

#### 3.5. Static tensile test

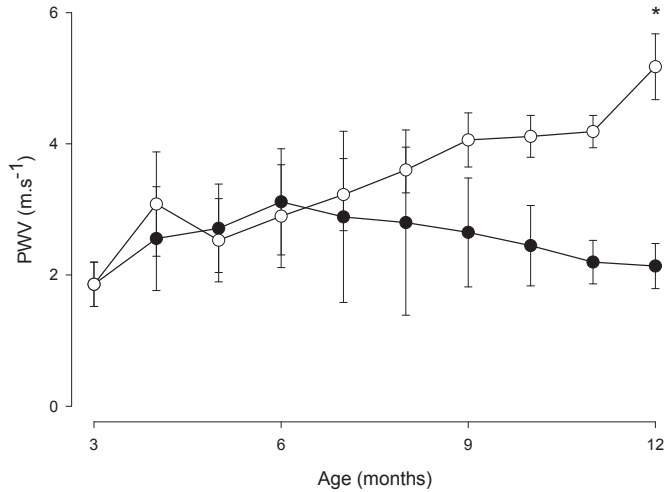
For each rabbit, a stress/strain curve of the aorta was plotted. All stress/strain curves are non-linear over the whole investigated elongational strain range, but exhibit a linear portion at low strains. A linear fit of the low strain part of the stress/strain curve allowed us to determine the Young's modulus E.

The evolution of E with age was presented in Fig. 2. In control group, E increased significantly with time, from 50 kPa at 3 months to 110 kPa at 12 months. In the treated group E ranged between 50 and 65 kPa, and was significantly different from the control at 12 months.

**Table 1**

Plasmatic concentrations of lipids and elastin in control and treated groups at the end of the experiment.  $n = 15$  per group for each parameter. Data are expressed as Mean  $\pm$  SD.

	Control group	Treated group	<i>p</i> value
Total cholesterol	$17.76 \pm 2.01$ mM	$15.58 \pm 4.43$ mM	$p = 0.099$
Triglycerides	$5.33 \pm 1.56$ mM	$3.95 \pm 1.83$ mM	$p = 0.202$
HDL	$0.57 \pm 0.25$ mM	$0.55 \pm 0.19$ mM	$p = 0.514$
LDL	$14.48 \pm 2.68$ mM	$14.93 \pm 6.68$ mM	$p = 0.984$
Elastin	$0.76 \pm 0.53$ ng/ml	$0.82 \pm 0.44$ ng/ml	$p = 0.520$



**Fig. 1.** Evolution of pulse wave velocity (PWV). PWV during time in the control (○) and the treated groups (●), from 15 animals per group at the beginning of the experiment to 5 animals per group at 12 months. Data are expressed as Medians  $\pm$  IQR. \* indicate significant difference at 12 months ( $p < 0.05$ ).

### 3.6. Vasoreactivity

E<sub>max</sub> and EC<sub>50</sub> in response to PE, Ach and SNP in the three different arterial territories (aorta, carotid and mesenteric) are presented in Table 2.

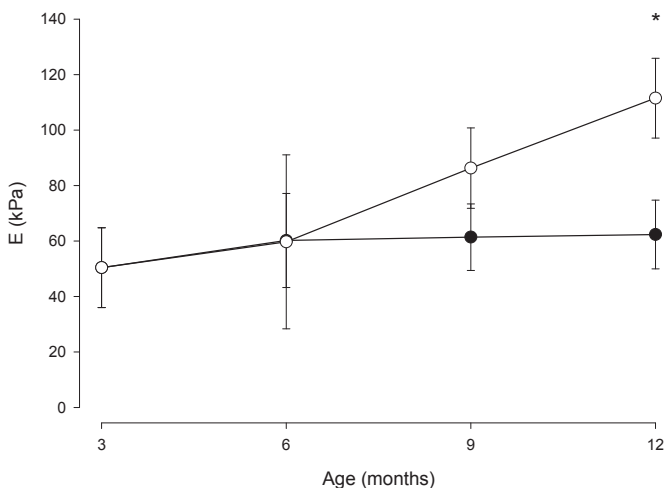
#### 3.6.1. PE-induced vasoconstriction

A single dose of PE ( $10^{-4}$  M) allowed to obtain a maximal ring contraction (E<sub>max</sub>). No statistical difference in E<sub>max</sub> was observed with time or treatment.

#### 3.6.2. Endothelial-dependent relaxation induced by Ach

ACh relaxation of aorta and carotid rings from the treated group were not different from the control group and no time effect was observed.

At 12 months, for mesenteric in the control group, EC<sub>50</sub> and E<sub>max</sub> could not be measured due to a complete absence of response of this artery to Ach. On the contrary, the treated group exhibited at 12



**Fig. 2.** Young's modulus (E). E as a function of age in the control (○) and the treatment groups (●),  $n = 4$  at 3 months and  $n = 5$  per group at the other ages. Data are expressed as Medians  $\pm$  IQR. \* Indicates a significant difference between control and treated groups at 12 months ( $p < 0.05$ ).

months a response similar to those previously observed (Fig. 3).

#### 3.6.3. Endothelium-independent relaxation to SNP

Whatever the vessel, no change in E<sub>max</sub> and EC<sub>50</sub> values was observed.

### 3.7. Elastin and collagen quantification

Combined TPEF/SHG images were made. Elastin, is the main source of endogeneous TPEF signal in vessel walls, and collagen is the exclusive source of SHG signal owing to its non-centrosymmetric organization. TPEF image reveals the typical lamellar structure of elastin fibers. However, a non-negligible background remains and could be attributed to the residual emission of cellular NAD(P)H and fibroblasts at this excitation wavelength [18]. In order to isolate the elastin fibers as much as possible, an intensity threshold was applied to the TPEF image, taking advantage that TPEF signal from elastin is generally stronger than the remaining fluorescence background from the non-fibrous component of the vessel. The threshold image was then binarized to quantify elastin by counting the number of pixels over the preset threshold (pixels "ON") within selected ROIs. The result, which is given in % of the total area of the ROIs, represents elastin fibers density within the vessel.

The contrast of the collagen fiber in the SHG image is very high since type I fibrillar collagen exhibits a strong SHG response [19] and is particularly abundant in vessel walls. Furthermore a recent analysis of SHG images of type I collagen showed that the relative number of pixels "ON" is a good indicator of the collagen amount in the tissue, although SHG images are often partly saturated due to the huge dynamic of the SHG response of fibrillar collagen [20]. Collagen in our SHG images of vessels was then quantified by counting the relative number of pixels over a preset threshold, whose value was chosen to remove the background noise of the images. Representative TPEF and SHG images are shown on Fig. 4.

The result of the quantification is expressed in % of the total area of selected ROIs and summarized on Fig. 4. At 12 months, no significant difference was observed for collagen between control and treated groups. However, a significant difference can be observed for elastin between control and treated groups at 12 months.

## 4. Discussion

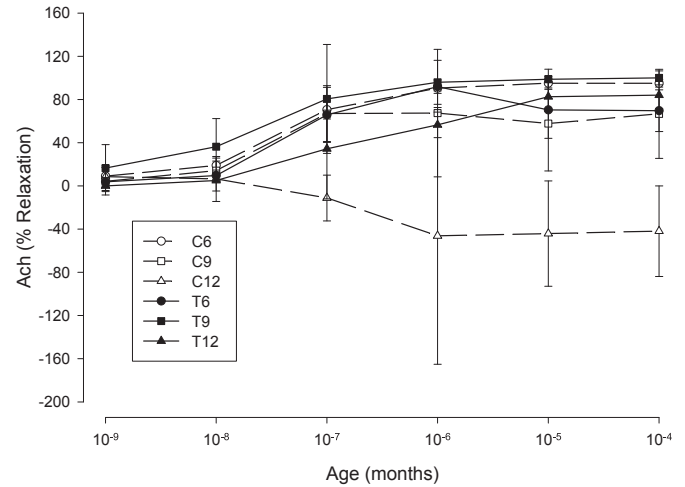
The aim of this study was to examine the potential effects of a very low-dose atorvastatin treatment on the vascular function in a high cardiovascular risk population. For this, 34 WHHL rabbits were submitted or not to an atorvastatin treatment.

When considering the hypolipidemic effect of the treatment, the dose used in this study may be considered as a very low-dose since no change in circulating levels of total cholesterol, HDL, LDL and triglycerides could be observed. In fact, in human studies statin treatments are considered as "low-dose statin therapy" when doses are below those currently prescribed but still with a lipid lowering effect [21–23].

The overall effect of the treatment on vigil animals was evaluated by measuring the pulse wave velocity [24]. This measure evidence progressive stiffening in non-treated WHHL conductance arteries. This arterial pathological aging is completely prevented in the presence of atorvastatin, at least until the end of the study on 12 month old animals. It is important to remind that the pulse wave velocity is a cardiovascular risk indicator whose predictive value has been largely demonstrated [25]. PWV is due essentially to the deformability of the large arteries. In humans, a low lipid-lowering dose of atorvastatin (10 mg/day) can reduce PWV in hypertensive and hypercholesterolemic subjects [26]. In our study, heart rate and

**Table 2** Response to acetylcholine (ACh), sodium nitroprusside (SNP) and phenylephrine (PE) exposition of aorta, carotid and mesenteric rings. n = 5 per group per sampling date. Data are expressed as Medians ± IQR.

Age (months)	Aorta			Carotid			Mesenteric		
	6	9	12	6	9	12	6	9	12
<b>ACh EMax(%)</b>									
Control	68.01 ± 0.00	51.86 ± 13.56	54.94 ± 8.56	75.97 ± 0.00	88.64 ± 3.15	70.48 ± 14.38	94.04 ± 2.62	65.81 ± 46.05	ND
Treated	70.55 ± 12.55	51.61 ± 6.35	41.26 ± 5.76	79.07 ± 7.11	72.58 ± 0.84	80.90 ± 15.07	84.12 ± 6.87	98.12 ± 35.92	83.79 ± 7.78
<b>EC<sub>50</sub>(M)</b>									
Control	2.54.10 <sup>-7</sup> ± 0.00	1.57.10 <sup>-7</sup> ± 3.60.10 <sup>-8</sup>	1.61.10 <sup>-7</sup> ± 2.33.10 <sup>-7</sup>	8.04.10 <sup>-8</sup> ± 0.00	4.77.10 <sup>-8</sup> ± 2.84.10 <sup>-8</sup>	5.56.10 <sup>-8</sup> ± 3.70.10 <sup>-9</sup>	4.54.10 <sup>-8</sup> ± 2.22.10 <sup>-8</sup>	2.96.10 <sup>-8</sup> ± 7.38.10 <sup>-8</sup>	ND
Treated	2.09.10 <sup>-7</sup> ± 2.25.10 <sup>-8</sup>	1.13.10 <sup>-7</sup> ± 4.46.10 <sup>-8</sup>	1.75.10 <sup>-7</sup> ± 5.1.10 <sup>-8</sup>	3.84.10 <sup>-8</sup> ± 1.35.10 <sup>-7</sup>	3.99.10 <sup>-8</sup> ± 1.03.10 <sup>-8</sup>	4.12.10 <sup>-7</sup> ± 9.62.10 <sup>-8</sup>	7.52.10 <sup>-8</sup> ± 7.10.10 <sup>-9</sup>	4.23.10 <sup>-8</sup> ± 6.61.10 <sup>-8</sup>	4.95.10 <sup>-7</sup> ± 5.16.10 <sup>-7</sup>
<b>SNP EMax(%)</b>									
Control	115.17 ± 18.0	136.65 ± 37.2	79.45 ± 29.9	77.72 ± 8.21	93.98 ± 3.48	98.86 ± 20.41	100.96 ± 2.51	98.78 ± 96.45	90.81 ± 9.57
Treated	109.25 ± 60.7	87.11 ± 9.72	86.12 ± 3.07	83.08 ± 11.12	91.51 ± 1.72	82.69 ± 5.72	95.73 ± 0.68	102.99 ± 2.0	98.12 ± 14.08
<b>EC<sub>50</sub>(M)</b>									
Control	5.72.10 <sup>-5</sup> ± 5.67.10 <sup>-5</sup>	2.75.10 <sup>-4</sup> ± 2.74.10 <sup>-4</sup>	1.48.10 <sup>-6</sup> ± 1.78.10 <sup>-6</sup>	2.96.10 <sup>-7</sup> ± 2.8.10 <sup>-8</sup>	2.15.10 <sup>-7</sup> ± 1.35.10 <sup>-7</sup>	3.61.10 <sup>-7</sup> ± 6.06.10 <sup>-7</sup>	1.03.10 <sup>-7</sup> ± 8.77.10 <sup>-8</sup>	8.80.10 <sup>-7</sup> ± 8.08.10 <sup>-7</sup>	5.46.10 <sup>-7</sup> ± 7.15.10 <sup>-7</sup>
Treated	1.48.10 <sup>-5</sup> ± 8.53.10 <sup>-5</sup>	1.21.10 <sup>-6</sup> ± 8.43.10 <sup>-7</sup>	1.51.10 <sup>-6</sup> ± 2.52.10 <sup>-6</sup>	6.28.10 <sup>-7</sup> ± 8.82.10 <sup>-7</sup>	1.7.10 <sup>-7</sup> ± 3.73.10 <sup>-7</sup>	3.26.10 <sup>-7</sup> ± 3.5.10 <sup>-7</sup>	3.29.10 <sup>-7</sup> ± 1.28.10 <sup>-6</sup>	5.88.10 <sup>-9</sup> ± 1.59.10 <sup>-8</sup>	3.89.10 <sup>-8</sup> ± 5.03.10 <sup>-7</sup>
<b>PE EMax(g)</b>									
Control	6.48 ± 0.635	4.52 ± 2.205	6.34 ± 0.32	8.33 ± 1.49	6.11 ± 2.61	0.18 ± 4.18	1.35 ± 0.50	0.79 ± 0.21	0.82 ± 0.07
Treated	7.55 ± 2.38	7.61 ± 1.31	5.68 ± 2.35	5.81 ± 1.09	6.54 ± 1.78	4.62 ± 0.69	0.97 ± 0.08	1.39 ± 0.12	1.04 ± 0.35



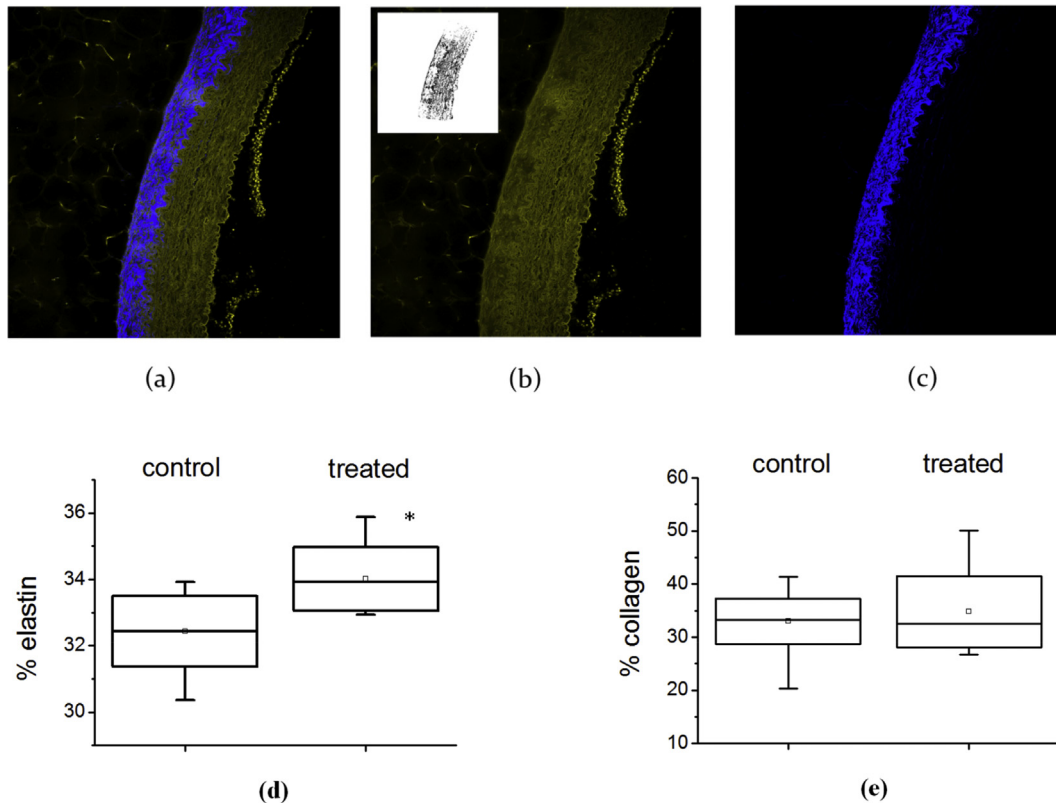
**Fig. 3.** Dose-response curves of mesenteric rings to acetylcholine (ACh). n = 5 per group per sampling date. Data are expressed Medians ± IQR.

blood pressure are however not impacted at 12 months. The difference in PWV observed between treated and control animals could result from either a functional or a structural alteration of the conductance arteries.

Functional aspects were evaluated on isolated rings of aorta, carotid and mesenteric arteries. Statins, used at lowering-cholesterol dose, could have an impact on endothelial function in different territories such as aorta [27], cerebral arteries [28,29] or cutaneous micro vessels [30]. In this study, after a PHE contraction of the vessel, the endothelial-dependent relaxation was tested by cumulative concentration of ACh and the endothelial-independent relaxation by SNP. On aorta and carotid, no change in endothelial function and vascular smooth muscle activity with time or treatment were observed. So, the difference observed in PWV is not the consequence of a modification of functionality of these large caliber vessels. On the mesenteric artery, the treatment prevents the occurrence of endothelial dysfunction at 12 months, demonstrating a protective effect of statin on the endothelial function in this experiment but this result does not explain by itself the change of PWV.

Arterial stiffening, particularly in the context of atherosclerosis, is often attributed to an increase in intima-media thickness [31]. This hypothesis is not supported by the measurements of the arterial wall: no thickness modification was observed when comparing control and treated groups. However, a structural modification of the arteries has been suggested by the static tensile tests. The arteries were shown to exhibit a non-linear elastic behavior, which is observed in many biological network forming systems [32]. In this study, we focused on the linear elastic properties which were characterized by the Young's modulus at low strains. The increase of the Young's modulus with time in the control group reflects a loss of elasticity of the arterial vascular tissue. This result differs from the Young's modulus value of the treated group that seems to be maintained over time. Such a statin effect has previously been described. In healthy subjects, a non-cholesterol-lowering dose might improve the vascular elasticity [33], whereas high doses would have no effect on elasticity [34]. This difference of elasticity evolution with time between treated and non-treated groups could result from differences in the composition or in the topology of the extracellular matrix networks responsible for the elasticity of the tissues.

In the case of the aorta, the mechanical behavior is classically due to the mechanical response of the different components of the



**Fig. 4. Elastin and collagen quantification.** (A) Combined TPEF/SHG image. (B) TPEF image: the signal mainly arises from elastin fibers. The inset displays the resulted threshold and binarized image used for quantification of elastin only. (C) SHG image: the signal exclusively arises from collagen fibers. Quantification of elastin (D) and collagen (E) in control and treated groups of 12 month-old WHHL rabbits (n = 5 animals per group). Data are presented as Medians, IQR and ranges. \*p < 0.05.

network forming the artery wall: collagen and elastin [35]. In particular, elastin plays a crucial role in vessel elasticity, and its degradation, or disarray, could promote aortic stiffness [36]. The inhibition of enzymes responsible for elastin degradation such as cathepsins G and S, have a protective effect from atherosclerosis [37,38]. In this study, the percentage of collagen was not modified by the treatment. However, changes in collagen types or orientation of its fibers can't be excluded [39]. In contrast, the percentage of the second component, elastin, was significantly higher at 12 months in treated group when compared to the control group. In WHHL rabbit, a decrease of the amount of elastin in time (from 100 mg/g of insoluble elastin at 6months to 60 mg/g at 2.5 years) has been previously shown [40]. Thus, the atorvastatin used in this study could have prevented this elastin reduction via a lower degradation by proteases or as a consequence of its action on inflammatory processes [41–43]. However, no modification of circulating elastin witch could be a marker of elastin degradation was observed in this study. Elastin fragmentation could also be due to an attenuation of TGF $\beta$  pathway [44]. An exploration of TGF $\beta$  pathway would go further in the study of this important preservative protein against atherosclerosis and vessel degradation [45,46]. It is nevertheless important to point out that atherosclerosis can induce an increase of the number of smooth muscle cells and their migration [47] and that statins have been shown to decrease the number of these cells [48]. Thus, in our model which spontaneously develops atherosclerosis, the reduced percentage of elastin observed in the control group could be due to a relative increase in smooth muscle cell number. Further investigations are necessary to test this hypothesis. Moreover, additional measures would be useful to reduce some limitations of this study. Even if lipid levels were evaluated in this study, additional measurements

on cardiovascular biomarkers concerning lipid particle functionality [49,50] could be quantify to show eventual modification by statin. Similarly, a confirmation of elastin concentration difference between controls and treated by another method than TPEF should be performed. Immunohistochemical method for example, would also quantify elastin and bring the first evidence about the spatial organization of fibers.

In conclusion, this study is a contribution to the analysis of the effects of a very low-dose of statin used at concentrations below its lipid-lowering effect. It clearly shows a beneficial effect of such a treatment on the vascular system of the atherosclerotic WHHL rabbit. The improvement observed *in vivo* appears to be due to compositional changes of the arterial wall consecutive to this long term treatment and independent to functional vascular effects. If further studies confirm compositional and durable changes in vessel wall of animal models, improving elasticity, a translation to clinical study could be considered, particularly in the context of aging and patient with high/increasing PWV.

#### Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

#### References

- [1] N.J. Stone, J.G. Robinson, A.H. Lichtenstein, C.N.B. Merz, C.B. Blum, R.H. Eckel, A.C. Goldberg, D. Gordon, D. Levy, D.M. Lloyd-Jones, P. McBride, J.S. Schwartz, S.T. Shero, S.C. Smith, K. Watson, P.W.F. Wilson, 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults a report of the american college of cardiology/american heart association task force on practice guidelines, *Circulation* 129 (2014) S1–S45,

- <http://dx.doi.org/10.1161/01.cir.0000437738.63853.7a>.
- [2] G.C. Makris, A. Lavidá, A.N. Nicolaides, G. Geroulakos, The effect of statins on carotid plaque morphology: a LDL-associated action or one more pleiotropic effect of statins? *Atherosclerosis* 213 (2010) 8–20, <http://dx.doi.org/10.1016/j.atherosclerosis.2010.04.032>.
  - [3] P. Amarenco, J. Labreuche, P. Lavallée, P.-J. Touboul, Statins in stroke prevention and carotid atherosclerosis systematic review and up-to-date meta-analysis, *Stroke* 35 (2004) 2902–2909, <http://dx.doi.org/10.1161/01.STR.0000147965.52712.f>.
  - [4] A. Blum, HMG-CoA reductase inhibitors (statins), inflammation, and endothelial progenitor cells—New mechanistic insights of atherosclerosis, *BioFactors* 40 (2014) 295–302, <http://dx.doi.org/10.1002/biof.1157>.
  - [5] M. Satoh, Y. Takahashi, T. Tabuchi, Y. Minami, M. Tamada, K. Takahashi, T. Itoh, Y. Morino, M. Nakamura, Cellular and molecular mechanisms of statins: an update on pleiotropic effects, *Clin. Sci.* 129 (2015) 93–105, <http://dx.doi.org/10.1042/CS20150027>.
  - [6] A.P. Owens III, N. Mackman, The antithrombotic effects of statins, *Annu. Rev. Med.* 65 (2014) 433–445, <http://dx.doi.org/10.1146/annurev-med-051812-145304>.
  - [7] A.S. Antonopoulos, M. Margaritis, R. Lee, K. Channon, C. Antoniades, Statins as anti-inflammatory agents in atherosclerosis: molecular mechanisms and lessons from the recent clinical trials, *Curr. Pharm. Des.* 18 (2012) 1519–1530, <http://dx.doi.org/10.2174/138161212799504803>.
  - [8] J.R. Morrow, S. Sher, S. Ali, I. Uphoff, R. Patel, M. Porkert, N.-A. Le, D. Jones, A.A. Quyumi, The differential effect of statins on oxidative stress and endothelial function: atorvastatin versus pravastatin, *J. Clin. Lipidol.* 6 (2012) 42–49, <http://dx.doi.org/10.1016/j.jacl.2011.08.006>.
  - [9] E. Bruckert, J. Ferrières, Evidence supporting primary prevention of cardiovascular diseases with statins: gaps between updated clinical results and actual practice, *Arch. Cardiovasc. Dis.* 107 (2014) 188–200, <http://dx.doi.org/10.1016/j.acvd.2014.01.011>.
  - [10] P. Libby, M. Aikawa, Mechanisms of plaque stabilization with statins, *Am. J. Cardiol.* 91 (2003) 4–8, [http://dx.doi.org/10.1016/S0002-9149\(02\)03267-8](http://dx.doi.org/10.1016/S0002-9149(02)03267-8).
  - [11] S. de Jongh, M.R. Lilien, J. op't Roodt, E.S. Stroes, H.D. Bakker, J.J. Kastelein, Early statin therapy restores endothelial function in children with familial hypercholesterolemia, *J. Am. Coll. Cardiol.* 40 (2002) 2117–2121, [http://dx.doi.org/10.1016/S0735-1097\(02\)02593-7](http://dx.doi.org/10.1016/S0735-1097(02)02593-7).
  - [12] R.C. Pasternak, C. Smith Sidney, C.N. Bairey-Merz, S.M. Grundy, J.J. Cleeman, C. Lenfant, ACC/AHA/NHLBI clinical advisory on the use and safety of statins 12, *J. Am. Coll. Cardiol.* 40 (2002) 567–572, [http://dx.doi.org/10.1016/S0735-1097\(02\)02030-2](http://dx.doi.org/10.1016/S0735-1097(02)02030-2).
  - [13] M.W. Russo, J.H. Hoofnagle, J. Gu, R.J. Fontana, H. Barnhart, D.E. Kleiner, N. Chalasani, H.L. Bonkovsky, Spectrum of statin hepatotoxicity: experience of the drug-induced liver injury network, *Hepatology* 60 (2014) 679–686, <http://dx.doi.org/10.1002/hep.27157>.
  - [14] P.M. Ridker, E. Danielson, F.A.H. Fonseca, J. Genest, A.M.J. Gotto, J.J.P. Kastelein, W. Koenig, P. Libby, A.J. Lorenzatti, J.G. MacFadyen, B.G. Nordestgaard, J. Shepherd, J.T. Willerson, R.J. Glynn, Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein, *N. Engl. J. Med.* 359 (2008) 2195–2207, <http://dx.doi.org/10.1056/NEJMoa0807646>.
  - [15] M. Westover, M.T. Bianchi, M.H. Eckman, S.M. Greenberg, Statin use following intracerebral hemorrhage: a decision analysis, *Arch. Neurol.* 68 (2011) 573–579, <http://dx.doi.org/10.1001/archneurol.2010.356>.
  - [16] M. Shioimi, T. Ito, The watanabe heritable hyperlipidemic (WHHL) rabbit, its characteristics and history of development: a tribute to the late Dr. Yoshio Watanabe, *Atherosclerosis* 207 (2009) 1–7, <http://dx.doi.org/10.1016/j.atherosclerosis.2009.03.024>.
  - [17] W.R. Zipfel, R.M. Williams, W.W. Webb, Nonlinear magic: multiphoton microscopy in the biosciences, *Nat. Biotechnol.* 21 (2003) 1369–1377, <http://dx.doi.org/10.1038/nbt899>.
  - [18] T. Boulesteix, A.-M. Pena, N. Pagès, G. Godeau, M.-P. Sauviat, E. Beaurepaire, M.-C. Schanne-Klein, Micrometer scale ex vivo multiphoton imaging of unstained arterial wall structure, *Cytom. A* 69A (2006) 20–26, <http://dx.doi.org/10.1002/cyto.a.20196>.
  - [19] R.M. Williams, W.R. Zipfel, W.W. Webb, Interpreting second-harmonic generation images of collagen I fibrils, *Biophys. J.* 88 (2005) 1377–1386, <http://dx.doi.org/10.1529/biophysj.104.047308>.
  - [20] D. Sevrain, M. Dubreuil, G.E. Dolman, A. Zaitoun, W. Irving, I.N. Guha, C. Odin, Y. Le Grand, Evaluation of area-based collagen scoring by nonlinear microscopy in chronic hepatitis C-induced liver fibrosis, *Biomed. Opt. Express* 6 (2015) 1209–1218, <http://dx.doi.org/10.1364/BOE.6.001209>.
  - [21] A. Dirisamer, N. Hachemian, R.A. Bucek, F. Wolf, M. Reiter, P.K. Widhalm, The effect of low-dose simvastatin in children with familial hypercholesterolemia: a 1-year observation, *Eur. J. Pediatr.* 162 (2003) 421–425, <http://dx.doi.org/10.1007/s00431-003-1181-3>.
  - [22] A. Tawakol, Z.A. Fayad, R. Mogg, A. Alon, S. Subramanian, A. Abdelbaky, I. Nunes, G. Herman, C. Beals, S.S. Shankar, Abstract 18242: high dose statin results in a further reduction in atherosclerotic inflammation compared with low dose statin: results of a multi-center PET-CT study, *Circulation* 124 (2011) A18242.
  - [23] A. Kunt, S. Özcan, A. Küçüker, D. Odabaşı, A. Sami Kunt, Efficacy of chronic statin therapy on major cardiac events after coronary artery bypass grafting: low-dose versus high-dose, *Med. Glas. Off. Publ. Med. Assoc. Zenica Doboj Cant. Bosnia Herzegovina* 12 (2015) 196–201, <http://dx.doi.org/10.17392/795-15>.
  - [24] S. Laurent, J. Cockcroft, L.V. Bortel, P. Boutouyrie, C. Giannattasio, D. Hayoz, B. Pannier, C. Vlachopoulos, I. Wilkinson, H. Struijker-Boudier, Expert consensus document on arterial stiffness: methodological issues and clinical applications, *Eur. Heart J.* 27 (2006) 2588–2605, <http://dx.doi.org/10.1093/eurheartj/ehl254>.
  - [25] C. Vlachopoulos, K. Aznaouridis, C. Stefanadis, Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis, *J. Am. Coll. Cardiol.* 55 (2010) 1318–1327, <http://dx.doi.org/10.1016/j.jacc.2009.10.061>.
  - [26] A.I. Kanaki, P.A. Sarafidis, P.I. Georgianos, K. Kanavos, I.M. Tziolas, P.E. Zebekakis, A.N. Lazaridis, Effects of low-dose atorvastatin on arterial stiffness and central aortic pressure augmentation in patients with hypertension and hypercholesterolemia, *Am. J. Hypertens.* 26 (2013) 608–616, <http://dx.doi.org/10.1093/ajh/hps098>.
  - [27] X. Gong, Y. Ma, Y. Ruan, G. Fu, S. Wu, Long-term atorvastatin improves age-related endothelial dysfunction by ameliorating oxidative stress and normalizing eNOS/iNOS imbalance in rat aorta, *Exp. Gerontol.* 52 (2014) 9–17, <http://dx.doi.org/10.1016/j.exger.2014.01.015>.
  - [28] K. Sander, U. Hof, H. Poppert, B. Conrad, D. Sander, Improved cerebral vasoreactivity after statin administration in healthy adults, *J. Neuroimaging* 15 (2005) 266–270, <http://dx.doi.org/10.1111/j.1552-6569.2005.tb00320.x>.
  - [29] S. Giannopoulos, A.H. Katsanos, G. Tsiygoulis, R.S. Marshall, Statins and cerebral hemodynamics, *J. Cereb. Blood Flow. Metab. Off. J. Int. Soc. Cereb. Blood Flow. Metab.* 32 (2012) 1973–1976, <http://dx.doi.org/10.1038/jcbfm.2012.122>.
  - [30] L.A. Holowatz, L. Santhanam, A. Webb, D.E. Berkowitz, W.L. Kenney, Oral atorvastatin therapy restores cutaneous microvascular function by decreasing arginase activity in hypercholesterolemic humans, *J. Physiol.* 589 (2011) 2093–2103, <http://dx.doi.org/10.1113/jphysiol.2010.203935>.
  - [31] N.M. van Poepele, D.E. Grobbee, M.L. Bots, R. Asmar, J. Topouchian, R.S. Reneman, A.P.G. Hoeks, D.A.M. van der Kuip, A. Hofman, J.C.M. Witteman, Association between arterial stiffness and atherosclerosis in the rotterdam study, *Stroke* 32 (2001) 454–460, <http://dx.doi.org/10.1161/01.STR.32.2.454>.
  - [32] A.V. Dobrynin, J.-M.Y. Carrillo, Universality in nonlinear elasticity of biological and polymeric networks and gels, *Macromolecules* 44 (2011) 140–146, <http://dx.doi.org/10.1021/ma102154u>.
  - [33] M. Lunder, M. Janić, S. Habjan, M. Šabović, Subtherapeutic, low-dose fluvastatin improves functional and morphological arterial wall properties in apparently healthy, middle-aged males – a pilot study, *Atherosclerosis* 215 (2011) 446–451, <http://dx.doi.org/10.1016/j.atherosclerosis.2010.12.041>.
  - [34] K.D. Ballard, B.A. Taylor, J.A. Capizzi, A.S. Grimaldi, C.M. White, P.D. Thompson, Atorvastatin treatment does not alter pulse wave velocity in healthy adults, *Int. Sch. Res. Not.* 2014 (2014) e239575, <http://dx.doi.org/10.1155/2014/239575>.
  - [35] N. Assoul, P. Flaud, M. Chaouat, D. Letourneur, I. Bataille, Mechanical properties of rat thoracic and abdominal aortas, *J. Biomech.* 41 (2008) 2227–2236, <http://dx.doi.org/10.1016/j.jbiomech.2008.04.017>.
  - [36] J. Zhang, X. Zhao, D.E. Vatner, T. McNulty, S. Bishop, Z. Sun, Y.-T. Shen, L. Chen, G.A. Meininger, S.F. Vatner, Extracellular matrix disarray as a mechanism for greater abdominal versus thoracic aortic stiffness with aging in primates, *Arterioscler. Thromb. Vasc. Biol.* 36 (2016) 700–706, <http://dx.doi.org/10.1161/ATVBAHA.115.306563>.
  - [37] J.-L. Figueiredo, M. Aikawa, C. Zheng, J. Aaron, L. Lax, P. Libby, J.L. de Lima Filho, S. Gruener, J. Fingerle, W. Haap, G. Hartmann, E. Aikawa, Selective cathepsin S inhibition attenuates atherosclerosis in apolipoprotein E-deficient mice with chronic renal disease, *Am. J. Pathol.* 185 (2015) 1156–1166, <http://dx.doi.org/10.1016/j.ajpath.2014.11.026>.
  - [38] J. Wang, S. Sjöberg, T.-T. Tang, K. Öörni, W. Wu, C. Liu, B. Secco, V. Tia, G.K. Sukhova, C. Fernandes, A. Lesner, P.T. Kovanen, P. Libby, X. Cheng, G.-P. Shi, Cathepsin G activity lowers plasma LDL and reduces atherosclerosis, *Biochim. Biophys. Acta BBA Mol. Basis Dis.* 1842 (2014) 2174–2183, <http://dx.doi.org/10.1016/j.bbadis.2014.07.026>.
  - [39] A. Karimi, M. Navidbakhsh, A. Shojaei, A combination of histological analyses and uniaxial tensile tests to determine the material coefficients of the healthy and atherosclerotic human coronary arteries, *Tissue Cell* 47 (2015) 152–158, <http://dx.doi.org/10.1016/j.tice.2015.01.004>.
  - [40] K.M. Dalessandri, P. Eisele, T.S. Wong, J. Parker, R.B. Rucker, Elastin degradation in the aorta of Watanabe hereditary hyperlipidemic rabbits, *Mech. Ageing Dev.* 74 (1994) 117–120, [http://dx.doi.org/10.1016/0047-6374\(94\)90103-1](http://dx.doi.org/10.1016/0047-6374(94)90103-1).
  - [41] S. Abisi, K.G. Burnand, J. Humphries, M. Waltham, P. Taylor, A. Smith, Effect of statins on proteolytic activity in the wall of abdominal aortic aneurysms, *Br. J. Surg.* 95 (2008) 333–337, <http://dx.doi.org/10.1002/bjs.5989>.
  - [42] A.M. Briones, N. Rodríguez-Criado, R. Hernanz, A.B. García-Redondo, R.R. Rodríguez-Díez, M.J. Alonso, J. Egado, M. Ruiz-Ortega, M. Salas, Atorvastatin prevents angiotensin II-induced vascular remodeling and oxidative stress, *Hypertension* 54 (2009) 142–149, <http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.133710>.
  - [43] S. Blankier, B.W. McCrindle, S. Ito, R.S.M. Yeung, The role of atorvastatin in regulating the immune response leading to vascular damage in a model of Kawasaki disease, *Clin. Exp. Immunol.* 164 (2011) 193–201, <http://dx.doi.org/10.1111/j.1365-2249.2011.04331.x>.
  - [44] S.M. Krishna, S.W. Seto, R.J. Jose, E. Biros, C.S. Moran, Y. Wang, P. Clancy, J. Golledge, A peptide antagonist of thrombospondin-1 promotes abdominal aortic aneurysm progression in the angiotensin II-infused apolipoprotein E-deficient mouse, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 389–398, <http://dx.doi.org/10.1161/ATVBAHA.114.304732>.



- [45] S. Redondo, C.G. Santos-Gallego, T. Tejerina, TGF- $\beta$ 1: a novel target for cardiovascular pharmacology, *Cytokine Growth Factor Rev.* 18 (2007) 279–286, <http://dx.doi.org/10.1016/j.cytogfr.2007.04.005>.
- [46] S. Redondo, C.G. Santos-Gallego, P. Ganado, M. García, L. Rico, M.D. Rio, T. Tejerina, Acetylsalicylic acid inhibits cell proliferation by involving transforming growth factor- $\beta$ , *Circulation* 107 (2003) 626–629, <http://dx.doi.org/10.1161/01.CIR.0000045664.75269.A5>.
- [47] J.H. Ip, V. Fuster, L. Badimon, J. Badimon, M.B. Taubman, J.H. Chesebro, Syndromes of accelerated atherosclerosis: role of vascular injury and smooth muscle cell proliferation, *J. Am. Coll. Cardiol.* 15 (1990) 1667–1687, [http://dx.doi.org/10.1016/0735-1097\(90\)92845-S](http://dx.doi.org/10.1016/0735-1097(90)92845-S).
- [48] Y. Fukumoto, P. Libby, E. Rabkin, C.C. Hill, M. Enomoto, Y. Hirouchi, M. Shiomi, M. Aikawa, Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits, *Circulation* 103 (2001) 993–999, <http://dx.doi.org/10.1161/01.CIR.103.7.993>.
- [49] C.G. Santos-Gallego, C. Giannarelli, J.J. Badimón, Experimental models for the investigation of high-density lipoprotein-mediated cholesterol efflux, *Curr. Atheroscler. Rep.* 13 (2011) 266–276, <http://dx.doi.org/10.1007/s11883-011-0177-0>.
- [50] C.G. Santos-Gallego, J.J. Badimon, R.S. Rosenson, Beginning to understand high-density lipoproteins, *Endocrinol. Metab. Clin. North Am.* 43 (2014) 913–947, <http://dx.doi.org/10.1016/j.ecl.2014.08.001>.