

Original Research

The Effect of Propranolol on Aortic Structure and Function in Normotensive Rats

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Introduction: Beta-blocking agents are widely used for the treatment of many cardiovascular diseases. The effect of these agents, however, on the aortic wall structure and function has not been well defined. The present study was undertaken to investigate the effect of therapy with propranolol on wall structure and aortic function in rats.

Methods: 20 healthy Wistar rats (350-400 g) were assigned to a control group (n=8), with rats receiving only water and food, and an experimental group (n=12), in which 100 mg/kg/day propranolol was administered in the drinking water. Three months after initiation of treatment, aortic pressures and aortic pulse wave velocity (PWV) were measured using high-fidelity Millar catheters. Extensive histopathologic studies were performed in the wall of the descending thoracic aorta.

Results: Systolic, mean, diastolic, and pulse pressure were significantly lower in the propranolol-treated rats compared to controls ($p < 0.05$). For any given systolic, mean, and pulse pressure, PWV was greater in the propranolol-treated animals ($p < 0.05$). The heart rate was lower and the response to isoproterenol infusion was less in the propranolol-treated animals. Smooth muscle content was decreased and collagen content was increased in the aortic wall of the propranolol-treated animals compared to controls.

Conclusions: Long-term propranolol administration elicits an increase in PWV adjusted for aortic pressure. This may be related to accumulation of collagen in the aortic wall at the expense of smooth muscle cells. The aortic stiffening may explain some of the reported data, suggesting that the effect of β -blockade therapy in patients with arterial hypertension may be inferior to other pharmacologic agents.

It is well appreciated today that the aorta not only serves a conduit function, but also plays an important role in modulating left ventricular performance, myocardial perfusion, central hemodynamics, and arterial function throughout the entire cardiovascular system.^{1,2} All these functions of the aorta influence the circulation in a global fashion.

The function of the aorta is largely related to the structure of the arterial wall.³ Beta-blocking drugs have been extensively used for more than half of a century in al-

most all patients with cardiovascular diseases such as arterial hypertension, coronary artery disease, and heart failure. Despite the extensive use of β -blocking drugs in clinical practice for decades, their effect on aortic structure has not been studied. The present study was undertaken to investigate the effect of chronic β -blockade therapy on the structure of the aortic wall and the aortic function in an experimental animal model. Preliminary data from these studies have been reported previously.⁴

Methods

Animal groups and experimental protocol

Twenty healthy male Wistar rats (350-400 g) were used in this study. Animals were individually housed in the animal facility of the Biomedical Research Foundation, Academy of Athens, Athens, Greece, at a constant room temperature (19-21°C) and relative humidity (55%), under an artificial 12-hour light/12-hour dark cycle. During the study, the animals had access to standard rat chow and water *ad libitum*. The animals were randomly assigned to two groups: the propranolol group and the control group. In the propranolol group (n=12), propranolol hydrochloride, 100 mg/kg/day, was included in the drinking water. Animals in the control group (n=8) were age-matched and housed under identical conditions to those of the propranolol group; propranolol was not given to those animals. The protocol was approved by the Veterinary Directorate of the Athens Prefecture and the Review Board of the Biomedical Research Foundation, Academy of Athens, and was conducted in accordance with the guiding principles of the American Physiological Society and Greek Presidential Decree 160/1991, issued after Directive 86/609/EEC.

Three months after the initiation of propranolol therapy or vehicle treatment, and before euthanasia, the following measurements were performed under general anesthesia. Animals were sedated with ketamine (100 mg/kg) and xylazine (5 mg/kg) given intraperitoneally, and were then connected to an anesthetic machine (MDS Matrx, Orchard Park NY, USA) and a volume control respirator (Inspira, Harvard MA, USA). Isoflurane 1-2% (vaporizer setting) in oxygen (1 L/min) was administered for the maintenance of anesthesia. Through appropriate longitudinal incisions, the right common carotid and femoral arteries were identified and dissected. High-fidelity Millar catheters (2F – SPR-612; 1.4F – SPR-671; Millar Instruments, Houston TX, USA) were inserted into both arteries; the tip of the proximal catheter was advanced via the right common carotid artery to the origin of the aortic root. The tip of the second catheter was positioned in the right femoral artery. A catheter was also inserted in the right jugular vein for infusions and blood collection. Continuous electrocardiographic monitoring was performed through appropriate limb leads attached to the animals. Aortic pressures and heart rate were monitored for 10 min, and the average values of aortic pressures (systolic,

diastolic, mean) and heart rate were recorded with the aid of a digital acquisition system (Sonometrics System, Sonometrics Co., Ontario, Canada). Average values were obtained for the systolic, diastolic, and mean pressures.

At the end of the experiment, the thoracic and abdominal cavities were entered via a long thoracoabdominal incision, and the exact distance between the tips of the carotid and femoral catheters was measured. Pulse wave velocity (PWV) was calculated as the ratio of the distance between the tips of two catheters to the transit time between the two pressure waves, measured with the foot-to-foot method as previously reported from our group.⁵ The transit time of the pulse wave, between the beginning of the upstroke of the carotid pulse and that of the femoral pulse, was accurately determined using a computer by averaging 30 cardiac cycles.

During anesthesia, blood was collected in chilled tubes containing sodium ethylenedi-aminetetraacetate (EDTA; concentration 1 mg/ml) and immediately centrifuged at 1200 g for 20 min at 4°C. The plasma was removed and stored at -20°C until assay. Plasma renin and aldosterone activities were measured with a modification of a commercially available COBRA-II Gamma counter (Packard, Meriden CT, USA) radioimmunoassay kit.

To evaluate the effectiveness of β -adrenergic receptor blockade therapy, after the baseline recordings, the response to isoprotenerol (0.5 μ g/kg) given intravenously for 1 min was tested.

At the end of these studies, euthanasia was induced with an intravenous overdose of pentobarbital sodium. After animal euthanasia, the descending thoracic aorta was excised with extreme care to avoid damage to the aortic wall from the left subclavian artery to the diaphragm, with the surrounding loose periaortic tissue. The aorta was immediately placed in 10% formalin solution for 24 hours. Then, the specimen was dehydrated in a series of graded ethanol (70%, 96%, 99%) and embedded in paraffin wax. Successive transversal sections of 5 μ m thickness were cut for each specimen with a microtome (Leica RM 2125, Leica, Nussloch, Germany) and the paraffin was cleared from the slides with coconut oil (over 15 min in 60°C). The sections were re-dehydrated in 99%, 96%, and 70% ethanol, followed by a 10 min wash in water, and stained with hematoxylin-eosin for nucleus, Verhoeff's elastica for elastin, and picro-Sirius red for collagen, as previously described by our group.^{4,6,8}

Histopathologic studies

Histomorphometry was performed by a computer-assisted image analysis technique, as previously described.^{6,7,9} In brief, using an optical microscope (Zeiss Axiolab, Carl Zeiss, Oberkochen, Germany) coupled to a video-camera (Sony-iris CCD, SSC-DC38P, Sony Corp., Japan), images were acquired and analyzed using image-processing software (Image-Pro Plus version 4.5, Media Cybernetics Inc, Bethesda MD, USA).

The elastin network was analyzed in terms of the relative area density of elastin, the number of the laminae and the mean thickness of elastin laminae. The collagen network was analyzed in terms of the relative area density of collagen. The smooth muscle cell network was analyzed in terms of the relative area density of smooth muscle cells and the number of smooth muscle nuclei in the media per optical field. The areas occupied by elastin (brown color in Verhoeff's elastica stained sections) and collagen fibers (red color in picro-Sirius red stained sections) were identified by color segmentation of the micrographs using the Image-Pro Plus software; they were then divided by the total media area in the optical field to calculate the relative area density of elastin and collagen in the tunica media. Smooth muscle cell area density was computed using the formula: $100\% - (\text{elastin} + \text{collagen area density})$. Using these calculations, it was hypothesized that ground substance and other components did not contribute to the area of medial layer. Elastin, collagen, and remainder contents of the adventitial layer were similarly analyzed after segmentation in terms of their relative area densities with respect to the area of adventitial layer, assuming that they made up 100% of the material. Area densities for the entire vessel were calculated using the respective ones for the medial and adventitial layers, and their areas. The tunica media was defined between the internal and external elastic lamina, and the tunica adventitia between the external elastic lamina and the external edge of dense adventitia. Measurements were performed in representative optical fields every 10° around the aortic wall circumference in appropriately stained sections. For each animal, three cross-sections were measured for calculating mean values.

Statistical analysis

Data are given as mean \pm standard error of the mean. For comparisons between propranolol-treated and

control rats, the unpaired Student's t-test was used. A p-value <0.05 was considered statistically significant.

Results

Heart rate was significantly higher in the controls than in the propranolol group (254 ± 7 vs. 233 ± 7 beats per minute, $p < 0.001$).

Systolic (87.0 ± 2.3 vs. 71.4 ± 2.5 mmHg, $p < 0.001$), mean (72.6 ± 2.1 vs. 58.9 ± 2.4 mmHg, $p < 0.001$), diastolic (65.4 ± 2.0 vs. 52.6 ± 2.3 mmHg, $p = 0.001$), and pulse aortic pressure (21.6 ± 0.7 vs. 18.8 ± 0.5 mmHg, $p = 0.003$) were significantly higher in the controls compared to the propranolol group. The heart rate remained unchanged during isoproterenol administration in the propranolol group (233 ± 7 vs. 230 ± 7 beats per minute, $p = 0.5$), but increased significantly in the control group (333 ± 9 vs. 254 ± 7 beats per minute, $p < 0.001$).

Pulse wave velocity

The relationships between PWV, systolic, pulse, and mean aortic pressures are shown in Figure 1. Note the upward displacement of the regression line for propranolol-treated rats compared to controls. For any given pressure, the PWV was greater in the propranolol compared to the control group.

Histopathologic analysis

A detailed comparison of the composition parameters for the descending thoracic aorta between propranolol-treated and untreated rats is shown in Figure 2. A significantly greater relative area density of collagen in the media (30.8 ± 1.2 vs. $27.6 \pm 0.9\%$, $p = 0.05$) and the entire wall (50.5 ± 1.2 vs. $46.5 \pm 0.7\%$, $p = 0.02$) was noted in the propranolol treated rats (Figure 2). A greater elastin area density was observed in the media in the propranolol group compared to controls (24.8 ± 0.3 vs. $23.2 \pm 0.6\%$, $p = 0.03$; Figure 2), although the number of laminae (9.5 ± 0.3 vs. 8.9 ± 0.2 , $p = 0.09$) and their thickness (4.3 ± 0.1 vs. 4.1 ± 0.1 μm , $p = 0.2$) did not differ significantly between the two groups. Elastin density in the entire wall was similar (16.1 ± 0.4 vs. $16.7 \pm 0.6\%$, $p = 0.4$) in the two groups. A lower smooth muscle cell density in the media (44.5 ± 1.4 vs. $49.2 \pm 1.3\%$, $p = 0.03$) and the entire wall (32.8 ± 1.0 vs. $37.4 \pm 0.7\%$, $p = 0.002$) was noted in propranolol-treated compared to control rats (Figure 2). The number of cell nuclei per optical

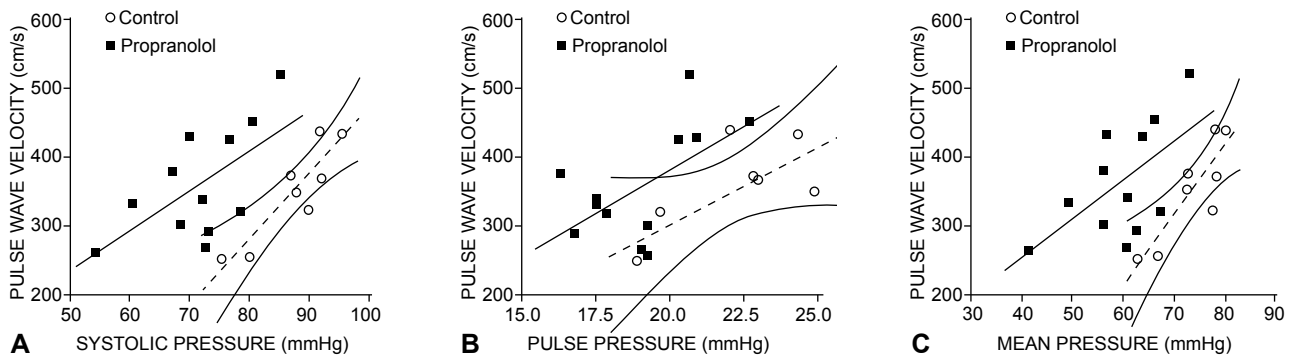


Figure 1. Relationship between pulse wave velocity and (a) systolic aortic pressure, (b) pulse pressure, and (c) mean aortic pressure in controls and propranolol-treated rats. The linear regressions lines for the two groups are shown.

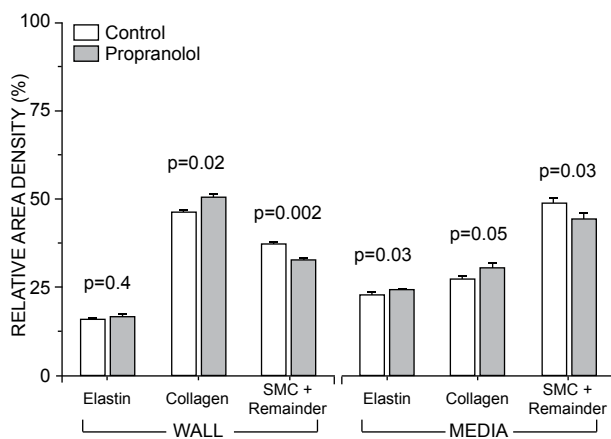


Figure 2. Effect of chronic therapy with propranolol on the composition of the aortic wall. Mean values \pm 1 standard error of the mean. SMC – smooth muscle cells. p-values for comparisons between the two groups are shown.

field was similar (89.3 ± 9.6 vs. 95.1 ± 7.4 , $p=0.3$) in the controls and the propranolol treated rats.

Representative histologic sections of the thoracic aortic wall from an animal treated with propranolol and from a control animal are shown in Figure 3.

There was no significant difference between the two groups in plasma renin (0.41 ± 0.02 vs. 0.38 ± 0.03 ng/ml, $p=0.4$) or aldosterone levels (120 ± 10 vs. 130 ± 15 pg/ml, $p=0.5$).

Discussion

The present study shows that therapy with propranolol produces changes in the structure of the wall of the thoracic aorta. Specifically, an increase in collagen and a decrease in smooth muscle contents were observed. These histologic changes were associated

with functional changes of the aorta. For any given pressure, the aortic PWV was greater in the propranolol than in the control group. Previous studies from our laboratory⁸ demonstrated decreased elastic properties of the aortic wall *in vitro* in animals treated with propranolol. The present study correlates the structure of the aortic wall with aortic function *in vivo*.

The normal mammalian aortic wall contains smooth muscle cells, collagen, and elastin. Smooth muscle proteins account for approximately 20% of the dry weight of the media, while collagen and elastin together account for about 60%. There is usually more elastin than collagen in the thoracic aorta and more collagen than elastin in the abdominal aorta. The total elastin plus collagen is approximately the same in all parts of the aorta and it is constant for adult mammalian aortas, regardless of species.

Despite the wide use of β -blocking drugs in clinical practice, there have been no studies to define aortic wall structure and to correlate aortic structure with function. It has been suggested recently that therapy with β -blocking drugs in patients with arterial hypertension may be inferior to the use of other pharmacologic agents, such as calcium channel blockers or angiotensin converting enzyme inhibitors,^{10,11} despite the fact that the effect on brachial artery pressure was the same. This may be attributed to differences in central aortic pressure: i.e. the central aortic pressure in patients treated with β -blockers was higher compared to the aortic pressure of patients treated with other pharmacologic agents.¹²

The ejection of blood from the left ventricle during systole generates a pressure wave that is perceived in peripheral vessels as the arterial pressure. The PWV, defined as the speed with which the pulse wave travels in the aorta, is directly related to the elas-

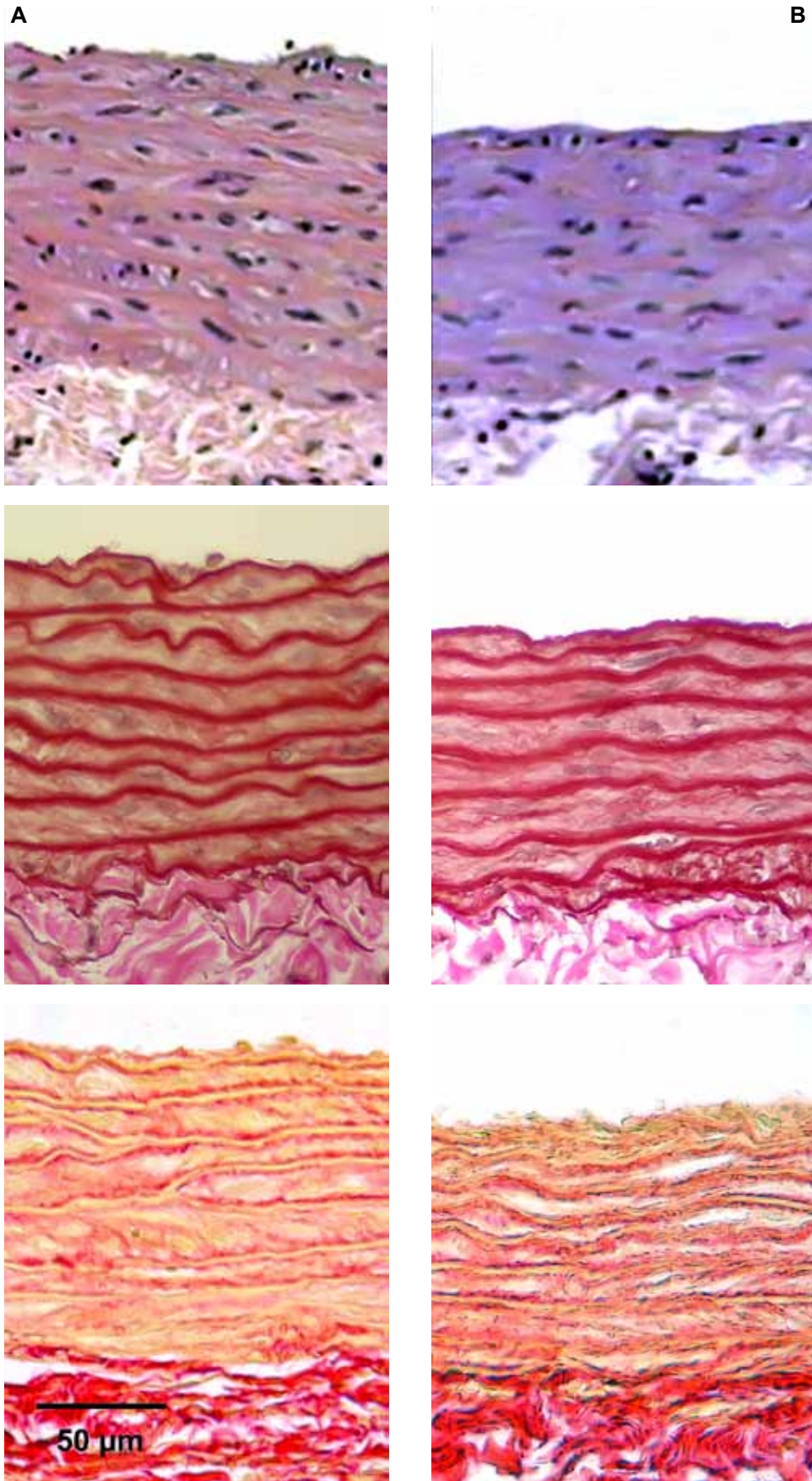


Figure 3. Representative histologic sections of the thoracic aorta from an untreated (A) and a propranolol-treated rat (B). Hematoxylin-eosin stain was used to identify smooth muscle cell nucleus (black, upper panel), Verhoeff's elastica was used to identify elastin (brown, middle panel), and Picro-Sirius red to identify collagen (red, lower panel) ($\times 40$).

tic properties of the aortic wall. A decrease in aortic wall elasticity causes an increase in PWV, while an increase in aortic elasticity causes a decrease in PWV. When the pulse reaches the periphery, it returns to the ascending aorta. Normally the reflecting waves reach the ascending aorta early in diastole, which results in the formation of the diastolic wave. When the elastic properties of the aortic wall are diminished and the PWV increases, the reflecting waves from the periphery return earlier into the ascending aorta, fuse with the systolic part of the pulse and result in an increase in pulse pressure, a late systolic peak of aortic pressure, and the disappearance of the diastolic wave. Thus, the aortic wave reflections are significant determinants of the central aortic pressure.³ These effects of the aortic stiffening on PWV and reflected wave velocity may explain the differential effects of pharmacologic agents on cardiovascular events in patients with arterial hypertension.

Arterial stiffening, as demonstrated in our recent study,⁸ is the net effect of many interactions among steady and dynamic modifications in the cellular and fibrous elements of arterial wall, along with the effects of hemodynamic stresses.¹³ The vascular remodeling in the present study is expressed by a reduction of smooth muscle mass and an accumulation of extracellular matrix, i.e. collagen fibers, a setting promoting wall stiffening. The increase in collagen demonstrated in this study may be the basic factor contributing to aortic stiffening in response to prolonged therapy with propranolol. A partially different rearrangement of arterial wall material, via hypertrophy and accumulation of collagen fibers in tunica media, has been shown to be associated with arterial stiffening in arterial hypertension.¹⁴ Aldosterone is explicitly implicated in hypertensive remodeling with increased collagen formation, matrix remodeling, and vascular hypertrophy, causing increased arterial stiffness.¹⁵⁻¹⁷ In the present study, however, aldosterone and renin did not differ between the two groups.

Propranolol has been found to delay aortic root dilatation in patients with the Marfan syndrome, when administered in doses that lower left ventricular dp/dt.¹⁸ Long-term β -blockade therapy may also protect against expansion of aortic aneurysms by minimizing the hemodynamic stresses on the aortic wall. Importantly, studies have shown that the lower rate of aneurysm expansion among patients receiving β -blockade therapy was not associated with decreases in mean, systolic or diastolic blood pressure.¹⁹ Accumulation of collagen may protect the aorta from aor-

tic dilatation. This effect of propranolol may be important in the treatment of patients with aortic dilatation, regardless of etiology. Propranolol was found to delay aortic aneurysm formation in turkeys fed with β -aminopropionitrile,²⁰ this effect being independent of changes in heart rate, blood pressure, or left ventricular dp/dt.²¹ Additional analysis indicated that propranolol directly increased the strength of aortic wall by stimulating lysyl oxidase activity and promoting collagen and elastin cross-linking,²² so that its effect in turkeys fed with β -aminopropionitrile could be dependent on direct interaction with β -receptors located in vascular tissues. These findings support the results of the present study and together suggest that the potential mechanisms for the aortic stiffening found here are due to a direct metabolic effect of propranolol on the aortic wall, irrespective of its hemodynamic effects.

In conclusion, prolonged administration of β -blockers modifies the structure of the aortic wall. The aortic wall compositional variations observed here, namely the accumulation of collagen fibers at the expense of smooth muscle cells, produced stiffening of the aortic wall, which has a negative impact on the function of the cardiovascular system.

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