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Effect of camelina oil on the structure of aortas in rats

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Summary

The aim of the present study was to determine whether a disturbance of hormonal homeostasis caused by ovariectomy influences the elasticity and wall structure of the abdominal aorta and whether camelina oil administration has an effect on the properties of the abdominal aorta in rats after ovariectomy. Forty Wistar female rats (220-240 g) were used in the experiment: 10 animals underwent a sham ovary repositioning operation (SHO), and 30 were ovariectomized (OVX). After 7 days of convalescence, the SHO rats and OVX1 rats were given physiological saline intragastrically for 6 weeks, while the treated rats received camelina oil at doses of 5 g/kg/b.w. (OVX2) or 9 g/kg/b.w. (OVX3) once a day. The rats were euthanized 7 weeks after the surgery, and the aorta, before the right and left common iliac arteries, was removed, cut into 5 mm pieces and exposed to a series of step-wise increases in tension. Aortic wall elasticity was measured in duplicate. Samples of the aorta were also prepared for histology (total aortic wall thickness, thickness of tunica intima and media, thickness of adventitia) and immunohistochemistry (volume and mean fluorescence intensity of collagens I and III and elastin), and images were obtained by light and confocal microscopy. Ovariectomy caused a significant decrease in the elasticity of the aorta, as well as in the volume of collagen III and elastin in the tunica intima and media, but no changes in the volume of collagen I, the total wall thickness or the thickness of the tunica intima and media. The elasticity of the aorta, the volume of elastin, and the mean fluorescence intensity of collagens I and III in rats receiving camelina oil at a dose of 9 g/kg/b.w. were similar to or higher than those observed in control animals. In conclusion, our data indicate that ovariectomy induced changes in the aortic wall associated with reduced vessel elasticity. The administration of camelina oil positively influenced the elasticity of the aorta in rats, probably by increasing the elastin content. However, further research is required to verify whether supplementation of diet with oils rich in omega-3 PUFA may prevent negative changes in the structure and function of vessels.

Keywords: camelina oil, artery, rat, collagen, elastin

The main function of large arteries such as the aorta is to serve as a buffer for flow and pressure variation during systole and diastole. Blood pressure is determined mainly by peripheral vascular resistance in young organisms, and by stiffness of large arteries in older organisms. The stiffness of large elastic arteries is determined mainly by the wall thickness. It is a „functional" stiffness because a vessel that is thicker than another vessel made of the same material requires more pressure to distend by the same amount (15). The extracellular matrix components (ECM) in the arterial wall, i.e. elastin, collagens I, III and V, and proteoglycans, determine the mechanical properties of the arterial wall and prevent damage at higher pressures (23, 28, 33, 47). Collagen provides strength and resistance to rupture, elastin determines the elastic properties, proteoglycans contribute to compressibility, and their combination with collagen and elastin prevents irreversible deformation of the vessel against pulsatile blood flow (31). Changes in ECM proteins, such as an increase in the collagen content, crosslinking of elastin and collagen, and fragmentation of elastin fibers, will lead to an increase in arterial stiffness. Moreover, wall thickening alone, without changes in ECM, will also increase arterial stiffness (44). Increased arterial stiffness has been identified as an important and independent predictor of mortality in cardiovascular events (26, 29).

A disturbance of hormonal homeostasis may negatively influence the function and structure of arteries. Studies in rats have shown that the deficiency of sex hormones after ovariectomy causes a decrease in collagen deposition, whereas estradiol therapy reduces the negative effect of the disturbance on the collagen/elastin ratio (10, 13, 44). On the other hand, collagen deposition is reduced by female sex hormones (30). Moreover, female sex hormones stimulated elastin deposition and resulted in a higher elastin/collagen ratio (11, 12). However, some data indicated that postmenopausal women receiving hormonal replacement therapy were characterized by increased arterial stiffness (45), high rates of non-fatal heart attacks, and stroke thromboembolic diseases (37).

Polyunsaturated fatty acids (PUFA) are indispensable supplements of any diet, having an important role in body function. Many experiments confirm the positive effect of PUFA on the bone, skin, and cardiovascular system (3, 19, 34, 38). The American Heart Association Science Advisory Statement supports the benefits of omega-3 fatty acids in reducing the risk of coronary heart disease (25). However, the cardioprotective and vascular-protective role of these fatty acids is still debated (1).

Camelina oil (*Camelina sativa* L. or false flax oil) is rich in unsaturated fatty acids (about 90%), including 20-24% of linoleic acid (C18:2 omega-6), 36-42% of linolenic acid (C18:3 omega-3), 12-20% of oleic acid (C18:1 omega-9), and 15% of gondoic acid (20:1 omega-9) (16, 17, 32, 51). This composition of PUFA is interesting from the nutritional point of view. Reports show that camelina products are consumed by both humans and animals (8, 16, 17, 32, 40). Indeed, *Camelina sativa*, which until recently has been largely forgotten, was widely cultivated in the past. Currently, given its high content of PUFA and low content of saturated acids, it has again become increasingly popular.

The aim of the present study was to determine whether a disturbance of hormonal homeostasis resulting from ovariectomy influences the elasticity of the abdominal aorta and its wall structure, and whether camelina oil administration affects the properties of the aorta in rats after ovariectomy.

Material and methods

Animals. The study was approved by the Local Animal Welfare Committee. Forty 10-week-old female Wistar rats (initial body mass of approx. 220-240 g) were housed in a room with controlled temperature (22 ± 2°C) and humidity (55 ± 10%) under a 12 : 12 h light–dark cycle. The animals were allowed free access to food and water at all times, except for a period of overnight fasting prior to surgery. After 7 days of acclimatization, the control animals (n = 10) underwent a sham ovary repositioning operation (SHO), and 30 animals were ovariectomized (OVX). General anesthesia for surgery was induced with 10 mg/kg b.w. ketamine (Biowet-Pulawy, Poland), 10 mg/kg b.w. xylazine (SPOFA, Czech Republic), and 0.1 mg/kg b.w. atropinum sulphuricum (Polfa-Warszawa, Poland) (administered intramuscularly). The convalescence of the rats before the experiment lasted for 7 days. During the 6 weeks of the experiment, SHO rats (n = 10) and OVX1 rats (n = 10) were given 1 ml physiological saline intragastrically, whereas the treated rats received camelina oil intragastrically (Semico, Poland; omega-6 19.89%, omega-3 33.95%, omega-6/omega-3 = 0.59) at doses of 5 g/kg/b.w. (OVX2; n = 10) or 9 g/kg/b.w. (OVX3; n = 10) once a day. After the 6 weeks of experimental treatment, the rats were euthanized. Immediately after euthanasia, three parts of the aorta were preserved for experimentation. The aorta before the right and left common iliac arteries was cut into approx. 5 mm pieces (3.9-14.8 mg – median 7.48 mg; diameter at rest 2.00 mm) and kept in PBS Ringer (0.15 M pH 7.4) comprising NaCl 8 g, KCl 0.2 g, $N_2HPO + 2\ H_2O$ 1.44 g, and $NaH_2PO + H_2O$ 0.23 g per liter of dH_2O (SIGMA). These samples were used for measurements of wall elasticity. A second 4 mm piece of the aorta was preserved in formalin for histological analysis, whilst a third 4 mm fragment of the aorta was kept in OCT gel and frozen in liquid nitrogen for immunohistochemical staining.

Measurement of aortic wall elasticity. The 5 mm pieces of the aorta were attached between a FT03 force displacement transducer (Grass Instruments, West Warwick, RI, USA) and a fixed metal pin, and immersed in a 44 ml chamber with PBS Ringer at 37°C. Force was measured with a force transducer connected to a home-built bridge amplifier interfaced with an 8S PowerLab A/D Converter (ADInstruments, Chalgrove, Oxfordshire, UK). The transducer had a functional range of 0-0.05 kg with a reliable force of 2 mg, equivalent to 0.004% of the functional range. The PowerLab 8S A/D Converter was connected to an iBook G4 running Chart5 v.5.4 Software (ADInstruments, Australia.). The data were recorded at a sampling speed of 1.000 data samples per second (1 KHz), and the input impedance of the amplifier was 200 MΩ differential. Duplicate sections were exposed to a series of step-wise increases in tension. Immediately after an increase in tension, the recording trace fell very slightly as the aortic tissue exerted a degree of elastic recoil. This measurement, referred to as elastic recoil, had units of N ms^{-1} mg^{-1} wet wt (wet weight). The elasticity of aortic samples was measured in duplicate.

Histological analysis of the aortic structure. Tissue samples (4 mm pieces of the aorta) were fixed in 4% formalin buffer, dehydrated in a series of a graded ethanol solutions, and embedded in paraffin. Paraffin-embedded tissue was cut with a microtome (Microm HM 360) into 4.5 μm thick sections. The sections were stained with orcein and indigo carmine (the traditional Frankel method). Microscopic images (10-12 from each sample) were collected by means of a Nikon Eclipse E800-light microscope (magnification 200 ×) and a Nikon D70-digital camera.

Picture analysis and measurements were performed by Microimage v. 4.0 for Windows 95/NT/98. The histomorphometric analysis consisted of measurement of the total aortic wall thickness, the thickness of the tunica intima and media, and the thickness of the tunica adventitia.

Immunohistochemical staining. Pieces (4 mm) of the aortas were washed in phosphate-buffered saline (PBS), embedded in OCT gel (Tissue-Tek® OCT™ Compound),

frozen in liquid nitrogen, and stored at –80°C until use. Serial sections (10 µm) were cut by a cryostat (Leyca, DE) at 20°C and transferred to silane-coated slides. After rehydrating in phosphate-buffered saline (PBS), the samples were fixed in 4% paraformaldehyde, blocked in 8% bovine serum albumin (BSA), and incubated with a primary antibody (Sigma, mouse monoclonal anti-collagen I and III at a dilution of 1: 100 and rabbit monoclonal anti-elastin at a dilution of 1: 100, Abcam) and a secondary antibody Alexa fluor 647 and Alexa fluor 405 (diluted 1 : 500).

Microphotography and image analysis. In order to show aortic wall structures, microphotography was performed by an Olympus FV1000 confocal microscope (600 × magnification, 60 × oil lens). The microphotographs were taken using the predefined settings of wavelength and filters that corresponded with the type of fluorochrome stain used (Alexa Fluor 647 and Alexa Fluor 405).

Four sites on the opposite sides of the aorta slice were photographed. The microscope settings used to photograph the control aortic slices, especially detector sensitivity and excitation light intensity, were set in order to avoid overexposure. All photographs analyzed were taken with the same microscope settings, which made it possible to obtain lighter or darker images of aortic walls from the experimental group compared to the images of aortic walls from the control group. This procedure enabled us to compare the quantity of type I and III collagen in the aortic walls examined.

A semiquantitative analysis of type I and III collagen in the entire volume of the aortic walls was performed on previously obtained confocal microphotographs. An analysis of mean fluorescence and of type I and III collagen in the aorta slices was performed. The fluorescence intensity of every slice was encoded to 12-bit grayscale separately for every channel. The fluorescence intensity value was converted to 1 µm^3.

Type I and III collagen and elastin contents were analyzed, where the greater quantity of antibodies bound specifically to the volume of a single slide. Since the collagen-binding antibodies were conjugated to the fluorescent stain, one can safely assume that fluorescence intensity was dependent on the collagen I and III content of the analyzed slice. In every slide of the control and experimental groups, the area (in µm^2) marked with antibodies was analyzed. Every optic slice was analyzed.

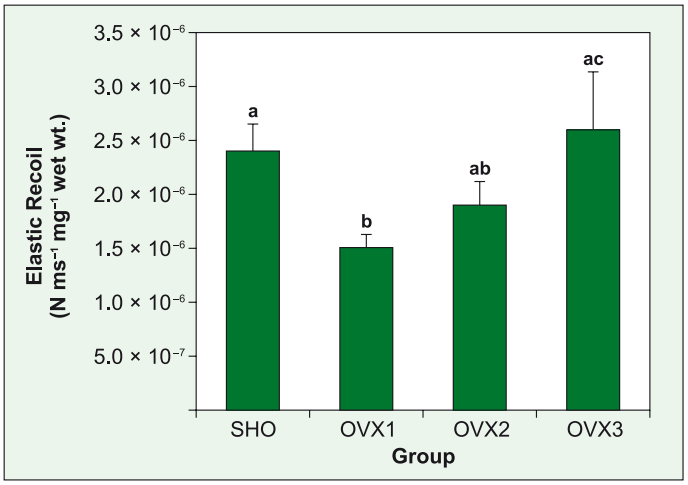

Fig. 1. Elastic recoil recordings for abdominal aortic sections from the rats (first repeat of two measurements). Recordings were made at 1,000 data samples per second (means ± SEM) Explanation: a, b, c – values with different superscripts are significantly different (P ≤ 0.05)

Statistical analysis. Data, which are presented as the mean ± SEM for the elastic recoil and thickness of the aorta and for the volume and fluorescence intensity of collagens I and III and elastin, were normally distributed and of equal variance. A Two-Tailed Unpaired analysis was performed on the elastic recoil data (GraphPad InStat 3 for Mac – Version 3.0 b, 2003). Measurements of tissue and filament thickness, which were normally distributed and of equal variance for both light microscope and light confocal microscope sections, were analyzed by a one-way ANOVA followed by Tukey's test. P values ≤ 0.05 were considered significant (Instat vs. 3.0a for Mac).

Results and discussion

Of considerable interest was the finding that the administration of camelina oil proved capable of restoring some (OVX2 group) or all (OVX3 group) of the original properties of the aortic sections of the control sham-operated rats (Fig. 1). In a repeat measurement, the same trend was observed. The sham-operated rats (SHO group) had elastic recoil of $2.4 \times 10^{-6} \pm 2.5 \times 10^{-7}$ N ms^{-1} mg wet wt., which was significantly higher than that recorded for the ovariectomized rats administered physiological saline (OVX1 group): $1.5 \times 10^{-6} \pm 1.3 \times 10^{-7}$ N ms^{-1} mg wet wt. (P ≤ 0.05). Ovariectomized rats administered 5 mg/kg b. wt. of camelina oil (OVX2 group) had elastic recoil of $1.9 \times 10^{-6} \pm 2.1 \times 10^{-7}$ N ms^{-1} mg wet wt., which was not significantly different from the elastic recoil of the OVX1 group. However, when a higher dose of oil was administered to ovariectomized rats (OVX3 group), elastic recoil was higher than the value for the ovariectomized rats from the OVX1 group (Fig. 1).

The histological analysis showed that the total wall thickness of the abdominal aorta in the untreated ovariectomized rats (OVX1 group) was similar to that observed in the control rats. The total aortic wall thickness in rats receiving camelina oil was significantly higher than that in the untreated ovariectomized rats (P ≤ 0.05) (Tab. 1). Camelina oil administration at

Tab. 1. The total wall thickness of the abdominal aorta and the thickness of the tunica intima and media, and of the tunica adventitia in the rats (means ± SEM)

Treatment group	Thickness of the abdominal aorta (µm)		
	Total wall thickness	Tunica intima and media	Tunica adventitia
SHO	140.8 ± 7.9^{ab}	76.5 ± 11.5^{a}	60.5 ± 4.9^{a}
OVX1	136.2 ± 0.3^{a}	70.2 ± 4.5^{ab}	64.4 ± 4.8^{b}
OVX2	157.6 ± 14.4^{cb}	74.5 ± 10.1^{ab}	78.4 ± 1.1^{c}
OVX3	162.6 ± 0.5^{c}	95.5 ± 1.6^{c}	61.7 ± 1.8^{abd}

Explanation: a, b, c, d – values in the column with different superscripts are significantly different (P ≤ 0.05)

a dose of 9 g/kg/b.w. significantly increased the total wall thickness as well as the thickness of the tunica media and intima, compared with those in the SHO and OVX1 groups. The abdominal aorta of the OVX3 rats was also characterized by a greater thickness of the tunica intima and media compared with that of the OVX2 rats ($P \leq 0.05$). The tunica adventitia had a significantly greater thickness in the OVX2 rats compared with that in the other groups ($P \leq 0.05$) (Tab. 1).

The volume of collagen I in all wall layers did not show significant differences. No significant differences in the values of the mean fluorescence intensity of collagen I were observed between the SHO and untreated ovariectomized rats (Tab. 2). A higher intensity of fluorescence for collagen I in the tunica intima and media, as well as in the tunica adventitia, was observed in rats receiving camelina oil at a dose of 9 g/kg b.w., compared with the SHO and OVX1 groups ($P \leq 0.05$) (Tab. 2).

The analysis of the tunica intima and media showed a reduced volume of collagen III in all ovariectomized rats compared with the SHO animals (Tab. 3). Moreover, in rats from the OVX2 group, the volume of collagen III was significantly greater than in the other groups. The mean fluorescence intensity of collagen III in the tunica intima and media was lower in the OVX1 and OVX2 groups, compared with the control and OVX3 groups ($P \leq 0.05$). The mean fluorescence intensity of collagen III in the aortas of rats receiving camelina oil at a dose of 9 g/kg b.w. was similar to that of the SHO group. No changes in the volume of collagen III in the tunica adventitia were observed. In both groups receiving camelina oil, the mean fluorescence intensity of collagen III in the adventitia was higher than that in the aortas of the SHO and OVX1 groups (Tab. 3).

The volume of elastin in the tunica intima and media of the untreated rats after ovariectomy (OVX1) was reduced compared with that in the control group and both groups receiving camelina oil ($P < 0.05$) (Tab. 4). However, the mean fluorescence intensity of elastin was similar in all ovariectomized animals and lower than that in the control animals, but these differences were not statistically significant (Tab. 4, Fig. 2).

Tab. 2. The mean fluorescence intensity and volume of collagen I in the tunica intima and media, and in the tunica adventitia in the abdominal aorta of the rats (means ± SEM)

Treatment group	Tunica intima and media		Tunica adventitia	
	Volume of collagen I (mm^3)	Mean fluorescence intensity of collagen I in 1 μm^3	Volume of collagen I (mm^3)	Mean fluorescence intensity of collagen I in 1 μm^3
SHO	$34.2 \times 10^{-3} \pm 1 \times 10^{-3}$	797.4 ± 10.1^{a}	$50.7 \times 10^{-3} \pm 12 \times 10^{-3}$	739.6 ± 32.7^{a}
OVX1	$32.3 \times 10^{-3} \pm 19 \times 10^{-3}$	751.9 ± 17.4^{a}	$50.3 \times 10^{-3} \pm 2 \times 10^{-3}$	703.1 ± 15.6^{a}
OVX2	$57.2 \times 10^{-3} \pm 15 \times 10^{-3}$	1507.3 ± 47.7^{ab}	$64.0 \times 10^{-3} \pm 20 \times 10^{-3}$	998.7 ± 31.9
OVX3	$40.2 \times 10^{-3} \pm 8 \times 10^{-3}$	1558.3 ± 185.0^{b}	$50.9 \times 10^{-3} \pm 5 \times 10^{-3}$	1247.2 ± 27.7^{b}

Explanation: a, b – values within a column with different superscripts are significantly different ($P \leq 0.05$)

Tab. 3. The mean fluorescence intensity and volume of collagen III in the tunica intima and media, and in the tunica adventitia in the abdominal aorta of the rats (means ± SEM)

Treatment group	Tunica intima and media		Tunica adventitia	
	Volume of collagen III (mm^3)	Mean fluorescence intensity of collagen III in 1 μm^3	Volume of collagen III (mm^3)	Mean fluorescence intensity of collagen III in 1 μm^3
SHO	$61 \times 10^{-6} \pm 6 \times 10^{-6\ a}$	308.8 ± 2.5^{a}	$33 \times 10^{-6} \pm 2 \times 10^{-6}$	403.64 ± 0.13^{a}
OVX1	$42 \times 10^{-6} \pm 3 \times 10^{-8\ b}$	250.7 ± 18.6^{b}	$30 \times 10^{-6} \pm 5 \times 10^{-7}$	366.12 ± 31.19^{a}
OVX2	$45 \times 10^{-6} \pm 2 \times 10^{-7\ c}$	243.4 ± 10.7^{b}	$32 \times 10^{-6} \pm 4 \times 10^{-6}$	560.70 ± 45.33^{b}
OVX3	$41 \times 10^{-6} \pm 2 \times 10^{-7\ bd}$	313.5 ± 12.2^{ac}	$32 \times 10^{-6} \pm 1 \times 10^{-6}$	507.86 ± 3.1^{b}

Explanation: a, b, c, d – values in the column with different superscripts are significantly different ($P \leq 0.05$)

Tab. 4. The mean fluorescence intensity and volume of elastin in the tunica intima and media in the abdominal aorta of the rats (means ± SEM)

Treatment group	Tunica intima and media	
	Volume of elastin (mm^3)	Mean fluorescence intensity of elastin in 1 μm^3
SHO	$333 \times 10^{-6} \pm 1 \times 10^{-5\ ac}$	270.22 ± 10.6
OVX1	$296 \times 10^{-6} \pm 1 \times 10^{-5\ b}$	230.15 ± 1.7
OVX2	$370 \times 10^{-6} \pm 2 \times 10^{-5\ c}$	227.94 ± 17.5
OVX3	$336 \times 10^{-6} \pm 2 \times 10^{-5\ c}$	229.84 ± 10.8

Explanation: a, b, c – values in the column with different superscripts are significantly different ($P \leq 0.05$)

The level of estrogens declines progressively with age, causing various physiological changes in the female organism. Indeed, early-onset estrogen deficiency produces similar changes. Ovariectomy affects, among other things, the steroid hormone balance of an individual, and this could also be expected to contribute to inducing a change in the arterial wall, perhaps through changes at the level of the smooth muscle collagen, fibrin or elastin content (6, 27). Estrogenic effects on collagen I were demonstrated in the arteries and other tissues of ovariectomized animals (44). In our experiment, ovariectomy caused a decrease in the collagen III volume and mean fluorescence intensity in the tunica intima and media, but no changes in collagen I parameters in the vessel wall. A reduction in interstitial collagen staining was found in the hearts of ovariectomized rats, which corresponded to the

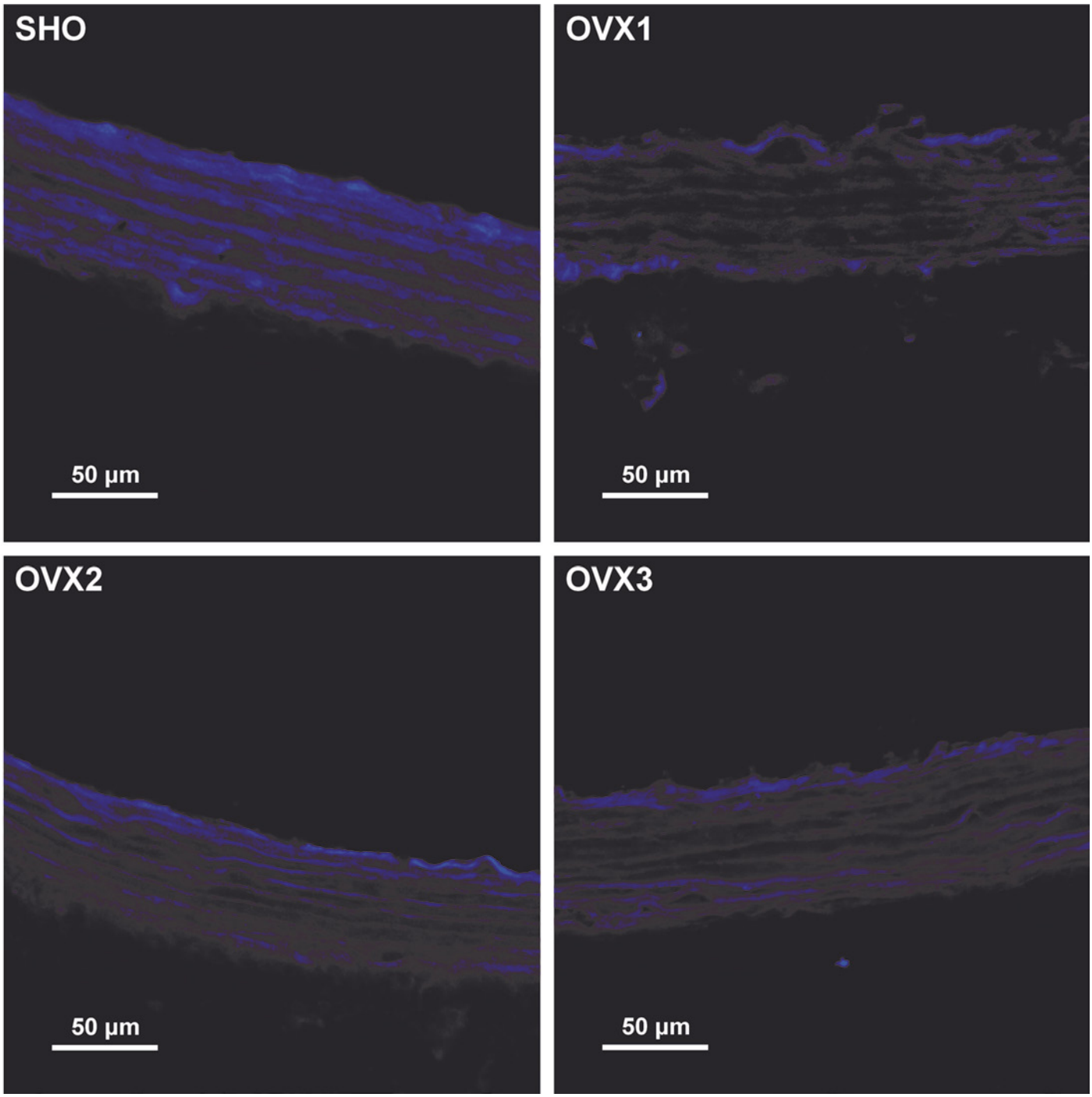

Fig. 2. Confocal microscope images of elastin fibers in the media of the abdominal aortic wall of sham-operated (SHO) and ovariectomized rats that did not receive camelina oil (OVX1) or received camelina oil at doses of 5 g/kg/b.w. (OVX2) or 9 g/kg/b.w. (OVX3)

lowest protein expression of collagen type I, the highest expression of matrix metalloprotease-9 (MMP-9), and the greatest activation of MMP-2 and MMP-9 (44). Moreover, an increased left ventricle mass and the onset of cardiac fibrosis after ovariectomy in aged rat hearts is associated with an increased collagen type I/III ratio and with a decreased expression of both estrogen receptors α and β (49). On the other hand, estrogen treatment prevented the loss of collagen I peptides and increased collagen III expression (20, 44), as well as stimulated collagen III turnover of an overloaded left ventricle in ovariectomized rats (7). These data indicate that estrogens attenuate fibrosis in an overloaded heart by increasing the activity of matrix metalloprotease (MMPs) and decreasing ECM accumulation (7).

In the female organism, ovariectomy causes changes similar to the progressive diminution of estrogens with age. One of the hallmarks of ageing is the loss of tissue elasticity. In the cardiovascular system, it is associated with the fragmentation and thinning of elastin fibers. In the aging process, elastic fibers suffer fragmentation and damage, and mechanical load is transferred to collagen fibers, which are 100-1000 times as stiff as elastic

fibers (46). Instead, more collagen is produced, which decreases the ratio of elastin to collagen and shifts the arterial mechanical properties into a stiffer range of collagen fibers (46). However, in the untreated OVX rats we did not observe significant changes in the total wall thickness of the abdominal aorta, the tunica intima and media thickness, or the collagen I volume and its mean fluorescence intensity in all layers of the wall that are characteristic of ageing vessels. Our experiment indicated a negative influence of ovariectomy on the volume of elastin in the tunica intima and media. Therefore, the changes in the structure of the arterial wall found in our experiment may have been the cause of disturbances in the elasticity of the arterial wall. It is clear from the results presented in Fig. 1 that aortic sections from the untreated ovariectomized rats exhibited much less elastic recoil than those from the SHO rats. Unfortunately, there are no clearly documented investigations on the effect of estrogen deficiency on elastin synthesis and its content in blood vessels. There are, however, some reports showing the effect of estrogen deficiency on the structure of skin. According to those reports, estrogen deficiency in young women with premature menopause accelerated degenerative changes in dermal elastic fibers (5, 41). Elastin is a component of elastic fibers, which is synthesized in arteries mostly by smooth muscle cells, while adventitial fibroblasts and endothelial cells also display some elastogenic abilities (4). The collagen/elastin ratio has significance for the mechanical properties of arterial walls. It seems that matrix metalloproteases are the main factor in the collagen/elastin relation. An increased expression of matrix metalloproteases, as well as a decreased expression of tissue inhibitors of MMPs, contributes to the fragmentation of elastic fibers (30, 50). Increased collagen deposition and reduction in the elastin content due to elastin fiber degradation, often associated with vascular calcifications, contribute to the development of arterial stiffening (24).

Supplementation with omega-3 acids is associated with reduced cardiovascular mortality and post-infarction death (2, 14, 25). Long-term omega-3 supplementation reduces total peripheral resistance due to an increase in cardiac output without modification of arterial pressure, decreases left ventricle systolic and diastolic diameters, left ventricle weight and collagen density, improves left ventricle hemodynamics and function, and prevents left ventricle remodeling and glutathione deficiency (9, 39). However, the impact of individual acids and oils on aortic structure and properties is still little known. In our study, the administration of camelina oil in ovariectomized rats was associated with an increase in aortic elasticity, despite an increase in the total wall thickness, as well as in the thickness of the tunica intima and media, and of the tunica adventitia. This increase in the wall thickness is physiological because the walls of larger arteries thicken with age, even without changes in ECM proteins (46). Therefore, the above-mentioned results are in this line. However, the lack of changes in these parameters in the untreated OVX rats is difficult to explain. Perhaps, this was due to the too short a period after ovariectomy. It should be expected that the synthesis of extracellular matrix components is more sensitive to lower estrogen levels. We observed an increase in the mean fluorescence intensity of collagens type I and III in all layers, and of collagen type III in the tunica adventitia of the ovariectomized rats receiving camelina oil at a dose of 9 g/kg b. w. These results indicate that camelina oil administration may influence the structure of fibers. Moreover, supplementation with this oil positively influenced blood vessels by maintaining the volume of elastin similar to that of the control rats. These findings suggest that camelina oil probably protected and stimulated the vessel wall cells to synthesize elastin. The positive effect of omega-3 fatty acids on the function of fibroblasts and the synthesis of collagen and elastin has already been demonstrated (18, 22). However, a previous study has shown a negative influence of linoleic acids on the synthesis of collagen in chondrocytes (48).

Many products of the metabolism of fatty acids are a source of regulatory molecules, e.g. prostaglandins, thromboxanes and leukotriens (42). An increasing level of PGE_2 is a factor that inhibits the formation of collagens type I and III (36, 43). Because the reduction of the estrogen level in females is associated with an increase in PGE_2 synthesis, the decrease in the collagen III volume observed in our experiment may have been a consequence of these changes. A significant negative correlation between the percentage of collagen and the amount of synthesized PGE_2 was previously confirmed (22). PGE_2 is a metabolite of arachidonic acid (C20:4) from the omega-6 PUFA family. Thus, the administration of camelina oil, rich in linolenic acid (C18:3 omega-3), which competes with the omega-6 acids for the same enzymes in metabolic pathways, may affect the synthesis and ratio of collagens I and III in ovariectomized rats. Moreover, it seems that another pathway of PUFA action is the nuclear factor-κB (NF-κB). As reported by some authors, NF-κB is an essential mediator of collagen formation in fibroblasts influenced by PUFA. Data in this field are not conclusive, but they show the importance of a particular class of fatty acids (21, 35).

In conclusion, our study indicates that ovariectomy induced changes in the aortic wall associated with reduced vessel elasticity. The administration of camelina oil positively influenced the elasticity of the aorta in rats, probably by increasing the elastin content. However, further research is required to verify whether supplementation of diet with oils rich in n-3 PUFA may prevent negative changes in vessel structure and function.

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