JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2018, 69, 1, 109-116
www.jpp.krakow.pl | DOI: 10.26402/jpp.2018.1.12

M. PAWLOWSKA-OLSZEWSKA[1], I. PUZIO[1], A.P. HARRISON[2], L. BORKOWSKI[3], G. TYMICKI[1], D. GRABOS[1]

SUPPLEMENTATION WITH CAMELINA OIL PREVENTS NEGATIVE CHANGES IN THE ARTERY IN ORCHIDECTOMIZED RATS

[1]Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences, Lublin, Poland; [2]Department of Veterinary Clinical and Animal Science, Faculty of Health and Medical Sciences, Copenhagen University, Frederiksberg C, Denmark; [3]Chair and Department of Biochemistry and Biotechnology, Medical University, Lublin, Poland

The aim of the research was to examine the influence of orchidectomy on the elasticity and wall structure of the abdominal aorta in male rats and to check whether camelina oil treatment has an effect on aorta wall characteristics in orchidectomized rats. Forty 2-month-old male Wistar rats were used in the experiment: 10 animals underwent a sham testis repositioning operation (SHO) and 30 rats were orchidectomized (ORX). After the convalescence period, the SHO and ORX1 rats were given physiological saline intragastrically for 8 weeks; simultaneously, the other rats received camelina oil at the dose of 5 g/kg/b.w. (ORX2) or 9 g/kg/b.w. (ORX3) once a day. At the end of experiment, the animals were euthanized and fragments of the aorta were sampled for elasticity measurement and for histomorphometric and immunohistochemistry analysis. Orchidectomy caused a significant increase in the thickness of the total wall and its particular layers, mean intensity of elastin fluorescence in the tunica intima-media, and the volume of collagen I in tunica adventitia of the abdominal aorta in comparison to the other groups. The mean intensity of collagen I fluorescence in the tunica adventitia and tunica intima-media was significantly lower in the aorta of the orchidectomized rats. The values of the histomorphometric parameters of animals receiving camelina oil were lower than in the ORX1 group and higher than in the SHO rats. The values of the other parameters analyzed after the camelina oil treatment were similar to those in the SHO rats. In conclusion, our study showed that orchidectomy induced changes in the abdominal aorta wall characteristic for aging. Supplementation with camelina oil prevents negative consequences in the vessel wall structure in males with impaired endocrine function.

Key words: *camelina oil, abdominal aorta, collagen, elastin, sex hormones, orchidectomy, tunica adventitia, tunica intima-media, polyunsaturated fatty acids*

INTRODUCTION

Sex hormones, androgens and estrogens, have an important influence on the cardiovascular system. Moreover, studies suggest that the male sex hormones play a role in the sexual differentiation of cardiovascular diseases/hypertension (1). The level of testosterone in males declines progressively with advancing age and causes different physiological changes in the organism. However, early-onset androgen deficiency may cause similar changes. More studies have documented association of lower testosterone levels with cardiovascular disease events (2, 3). The low level of testosterone is associated with increased stiffness of the large artery, reduced arterial compliance and endothelium-dependent dilatation, hypertension, and atherosclerosis (4, 5). Moreover, testosterone therapy positively influences blood pressure (6) and cardiovascular status, e.g. reducing arterial stiffness (7). However, these data are the opposite to those reported in studies in hypertensive rats (8). No association between the testosterone level and cardiovascular disease events was indicated as well (9).

Diet has been investigated as one of the most important environmental factors associated with cardiovascular health. For a few years, studies have focused on nutritional intervention in prevention and treatment of cardiovascular diseases. Much attention in this field is paid to polyunsaturated fatty acids (PUFAs), especially from n-3 family. Anti-inflammatory, antithrombotic, antioxidative, antiatherogenic, and vasoprotective effects of n-3 PUFAs on the cardiovascular system have been reported (10, 11). Some studies have also indicated that n-3 PUFAs may influence arterial wall structure but there are opposite results available as well (12, 13).

Recent studies suggest that *Camelina sativa spp* may be a good source of PUFAs for human and animals. This is an oilseed crop from the Brassicaceae family. Its seeds have been found in archeological excavations from the Bronze Age. For many years, this plant has been forgotten, but it has aroused more interest in recent years. Camelina oil, also known as false flax or gold-of-pleasure, is rich in unsaturated fatty acids (about 90%) in a proportion of 20 – 24% linoleic acid (LA, C18:2 n-6), 36 – 42% α-linolenic acid (ALA, C18:3 n-3), 12 – 20% oleic acid (OA, C18:1 n-9), and 15% gondoic acid (GA, 20:1 n-9) (14, 15). Generally, camelina oil has qualitatively more PUFAs and monounsaturated fatty acids (MUFAs) and less saturated fatty

acids (SFAs) than other vegetable oils like rape oil, soya oil, olive oil *etc*. Moreover, camelina oil is more oxidatively stable than other oils due to the high content of phenolic compounds and γ-tocopherol (15). The high content of ALA is a good source for synthesis of other long chain PUFAs, eicosapentaenoic acid (EPA, C20:5), and docosahexaenoic acid (DHA, C22:6). This characteristic gives camelina oil a unique nutritional advantage over common vegetable oils that have been used in animal and human nutrition. The positive effects of camelina bioproduct intake have been previously reported in humans and different animals (16, 17).

Taking into consideration the significance of androgens in the cardiovascular function, the significance of PUFAs for cardiovascular system health, and the interesting composition of camelina oil, we have designed an experiment to explain (1) if orchidectomy influences the aortic elasticity and structure, (2) whether the camelina oil treatment has an effect on the properties of abdominal arteries in orchidectomized rats.

MATERIALS AND METHODS

Animals

The study was approved by the Local Animal Welfare Committee of the University of Life Sciences in Lublin, Poland (No 43/2010). Forty 2-month-old male Wistar rats (initial BW approximately 220 – 250 g) were used and housed in a room with controlled temperature (22 ± 2ºC) 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.

Surgery

After 7-day of acclimatization, the control animals (n = 10) underwent a sham testis repositioning operation (SHO) and thirty animals were orchidectomized (ORX). General anesthesia for the 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 lasted for 7 days prior to the experiment. During the 8 weeks of the experiment, the SHO rats and ORX1 rats (n = 10) were given 1 ml physiological saline intragastrically, while the treated rats received camelina oil (Semico, Poland) intragastrically at doses of 5 g/kg/b.w. (ORX2; n = 10) and 9 g/kg/b.w. (ORX3; n = 10).

The fatty acid composition of the camelina oil was analyzed using a gas chromatography system with Flame Ionization Detection (GC-FID). Fatty Acid Methyl Esters (FAME) were prepared according to regulation PN-ISO 5509:1996 (18). Analyses of FAME were performed with an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a Flame Ionization Detector and a capillary column HP-SSAP (30 m × 0.53 mm ×1 μm) according to PN-EN ISO 5508:1996 (19). The fatty acid content is presented in *Table 1*.

After euthanasia, three parts of the aorta were preserved for experimentation. An aorta fragment of 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 used for measurement of wall elasticity. A second 4-mm piece of the aorta was preserved in formalin (Chempura, Poland) for histological analysis, whilst a third 4-mm fragment of the aorta was kept in Criomatrix gel (Thermo Fisher Scientific, UK) and frozen in liquid nitrogen for immunohistochemical staining.

Measurement of aorta wall elasticity

Two fragments of the aorta were exposed to a series of step-wise increases in tension as described previously (20, 21). A parameter referred to as elastic recoil' was determined (N ms^{-1} mg^{-1} wet wt.).

Histological analysis of the aorta structure

Fragments of the aortas (4-mm pieces) were fixed in 4% formalin buffer dehydrated through ascending grades in ethyl alcohol (Chempura, Poland), cleared in xylene (Chempura, Poland), and infiltrated in paraffin (Paraplast Plus, USA). The paraffin-embedded tissue was cut into 4.5-μm thick sections using a microtome (Microm HM 355, Microm International GmbH, Germany). The sections were stained with the Frankel method with orcein (Sigma-Aldrich, USA) and indigo carmine (Sigma-Aldrich, USA). Microscopic images were collected using a Ziss FL-40-light microscope (USA), magnification 200×, and a camera (Zeiss-Axiocom ERc5s, USA). The pictures were analyzed and measured using Microimage v.4.0 for Windows 95/NT/98. The histomorphometric analysis consisted of measurement of the total aorta wall thickness and the tunica intima-media and adventitia thickness.

Immunohistochemical staining, microphotography, and image analysis

Fragments of aortas (4 mm) were kept in phosphate-buffered saline (PBS), embedded in Criomatrix gel, and frozen in liquid nitrogen. Sections were cut at 10 μm using a cryostat (Cryotome FSE, Thermo Shandon Limited, UK) at 20ºC and mounted on adhesion glass slides (Super Frost Pus, Menzel GmbH & CoKG, Germany). The slides were re-hydrated in phosphate-buffered-saline (PBS), fixed in 4% paraformaldehyde (15,812-7, Sigma-Aldrich, USA), blocked in 8% bovine serum albumin (BSA-A4503, Sigma-Aldrich, USA), and incubated with an anti-elastin rabbit polyclonal primary antibody (ab 21610, Abcam, USA), anti-collagen I mouse monoclonal antibody (C2456, Sigma USA), and anti-collagen III mouse monoclonal antibody (C7805, Sigma, USA) at a dilution of 1:100, as well as an Alexa fluor 647 (A21236, Life Technologies, USA) and Alexa fluor

Table 1. Fatty acid content in camelina oil.

Fatty acids	**Content** (as % of total FAME)
C14:0	0.06
C15:0	0.02
C16:0	5.10
C16:1	0.11
C17:0	0.06
C18:0	2.43
C18:1	14.96
C18:2	18.46
C18:3	33.95
C20:0	1.35
C20:1	14.33
C20:2	2.04
C20:4	1.43
C22:0	0.3
C22:1	2.93
C24:0	0.19
C24:1	0.66
Total n-6 PUFA	21.93
Total n-3 PUFA	33.95

405 secondary antibodies (A31556, Life Technologies, USA) (diluted 1:500).

The aorta preparations were examined using an Olympus FV1000 confocal microscope (Japan, 600 × magnification, 60 × oil lens) and photographed. Four sites on opposite sides of the analyzed aorta slice were photographed. The microphotographs were performed using predefined settings of the wavelength and filters that corresponded with the type of the fluorochrome stain used (Alexa Fluor 647 and Alexa Fluor 405). The microphotography was prepared in a procedure presented previously. The microphotographs of collagen I and III and elastin in the aorta were analyzed by the Image J 1.47v program (volume - mm^3, mean fluorescence intensity - in 1 μm^3).

Statistical analysis

Data, which are presented as the mean ± SE, were found to be normally distributed and exhibited 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 exhibited equal variance for both light microscope and light confocal microscope sections, were analyzed using Statistica 10.0 software followed by a Tukey test. P values ≤ 0.05 were considered significant.

RESULTS

At the end of the experiment, the elastic recoil of the abdominal aorta did not vary significantly between all groups (data not shown). The same trend was observed for aorta elasticity in two repeat measurements. The results were in the range of $1.5 – 2.4 \times 10^{-6}$ N ms^{-1} mg^{-1} wet wt.

The histological analysis of the aorta structure showed that the total wall thickness of the abdominal aorta was increased in the orchidectomized animals administered physiological saline (ORX1), compared to the control group ($P \leq 0.05$). It was also observed that the wall thickness was greater in both groups of animals receiving camelina oil than in the SHO group ($P \leq 0.05$), but lower than in ORX1 ($P \leq 0.05$). A similar tendency was observed for the tunica intima-media and tunica adventitia, as their thickness was significantly higher in the orchidectomized rats (ORX1) compared to the SHO group ($P \leq 0.05$). The administration of camelina oil led to a significant increase in the thickness of the tunica intima-media in the ORX2 group and

Table 2. Thickness of the total wall, tunica intima-media, and tunica adventitia in the abdominal aorta in sham-operated rats (SHO) and orchidectomized rats that did not receive camelina oil (ORX1) or received camelina oil at doses of 0.5 g/kg/b.w. (ORX2) or 9 g/kg/b.w. (ORX3) (mean ± SE).

Treatment group	**Thickness of the abdominal aorta** (μm)		
	Total wall thickness	Tunica intima-media	Tunica adventitia
SHO	213.7 ± 5.6	148.2 ± 1.8	65.1 ±3.9
ORX1	242.7 ± 4.0*	165.9 ± 1.3*	77.4 ±2.5*
ORX2	225.4 ± 2.1*§	157.1 ± 2.4*§	68.3 ± 0.4§
ORX3	229.3 ± 1.4*§	155.6 ± 0.6§	73.7 ± 1.8*§†

Statistical significance is indicated as *P < 0.05 versus SHO; § versus ORX1; † versus ORX2.

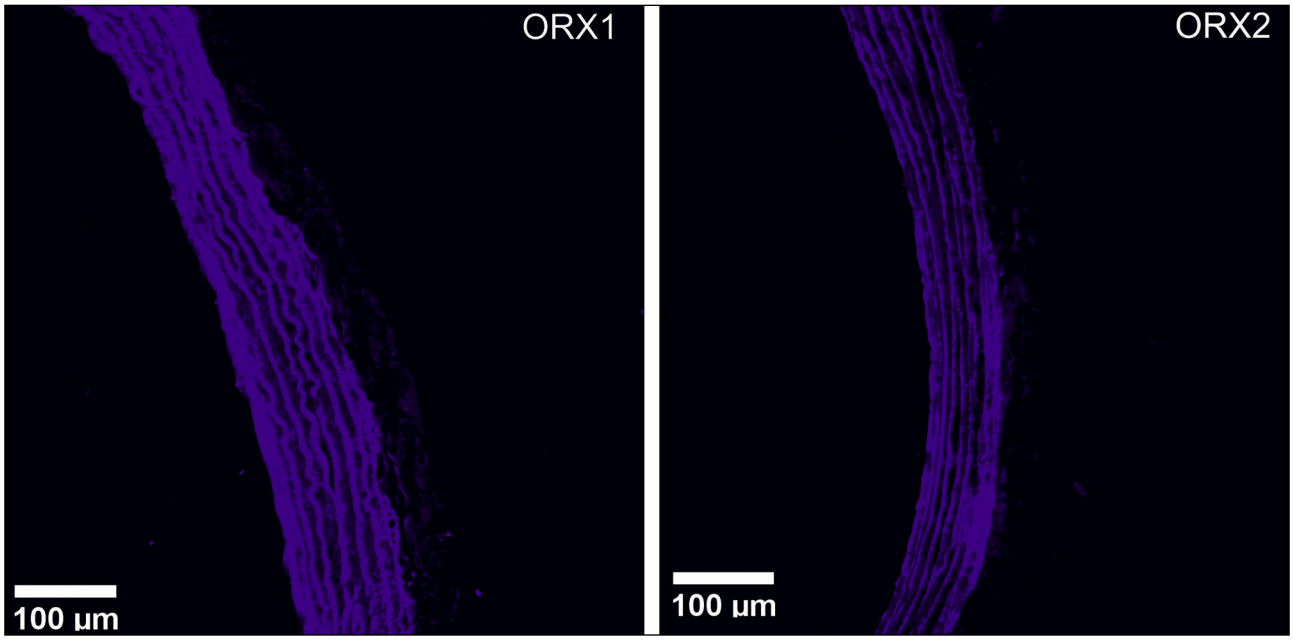

Fig. 1. Confocal microscope images of elastin fibers in the media of the abdominal aortic wall of orchidectomized rats that did not receive camelina oil (ORX1) and received camelina oil at the dose of 5 g/kg/b.w. (ORX2).

Table 3. Volume and mean intensity of elastin fluorescence in the tunica intima-media in the abdominal aorta in sham-operated rats (SHO) and orchidectomized rats that did not receive camelina oil (ORX1) or received camelina oil at doses of 0.5 g/kg/b.w. (ORX2) or 9 g/kg/b.w. (ORX3) (mean ± SE).

Treatment group	Tunica intima-media	
	Volume of elastin (mm^3)	Mean intensity of elastin fluorescence in 1 μm^3
SHO	$50 \times 10^{-5} \pm 2 \times 10^{-5}$	243.82 ± 40.3
ORX1	$46 \times 10^{-5} \pm 3 \times 10^{-5}$	316.94 ± 11.0*
ORX2	$51 \times 10^{-5} \pm 3 \times 10^{-7}$§	186.73 ± 15.7§
ORX3	$52 \times 10^{-5} \pm 5 \times 10^{-6}$§	192.59 ± 36.2§

Statistical significance is indicated as *P < 0.05 versus SHO; § versus ORX1.

Table 4. Volume and mean intensity of collagen I fluorescence in the tunica intima-media and tunica adventitia in the abdominal aorta in sham-operated rats (SHO) and orchidectomized rats that did not receive camelina oil (ORX1) or received camelina oil at doses of 0.5 g/kg/b.w. (ORX2) or 9 g/kg/b.w. (ORX3) (means ± SE).

Treatment group	Tunica intima-media		Tunica adventitia	
	Volume of collagen I (mm^3)	Mean intensity of collagen I fluorescence in 1 μm^3	Volume of collagen I (mm^3)	Mean intensity of collagen I fluorescence in 1 μm^3
SHO	$81.45 \times 10^{-6} \pm 86.46 \times 10^{-7}$	176.71 ± 56.22	$38.75 \times 10^{-6} \pm 65.89 \times 10^{-7}$	1135.40 ± 498.45
ORX1	$84.74 \times 10^{-6} \pm 87.44 \times 10^{-7}$	86.84 ± 45.83 *	$47.95 \times 10^{-6} \pm 92.93 \times 10^{-7}$*	696.88 ± 97.81*
ORX2	$73.21 \times 10^{-6} \pm 78.12 \times 10^{-7}$ *	192.70 ± 84.49§	$40.47 \times 10^{-6} \pm 99.29 \times 10^{-7}$§	1574.35 ± 829.57§
ORX3	$80.09 \times 10^{-6} \pm 86.34 \times 10^{-7}$ §	189.87 ± 95.63§	$40.120 \times 10^{-6} \pm 92.26 \times 10^{-7}$§	1020.07 ± 613.70†

Statistical significance is indicated as * P < 0.05 versus SHO; § versus ORX1; † versus ORX2.

Table 5. Volume and mean intensity of collagen III fluorescence in the tunica intima-media and tunica adventitia in the abdominal aorta in sham-operated rats (SHO) and orchidectomized rats that did not receive camelina oil (ORX1) or received camelina oil at doses of 0.5 g/kg/b.w. (ORX2) or 9 g/kg/b.w. (ORX3) (means ± SE).

Treatment group	Tunica intima-media		Tunica adventitia	
	Volume of collagen III (mm^3)	Mean intensity of collagen III fluorescence in 1 μm^3	Volume of collagen III (mm^3)	Mean intensity of collagen III fluorescence in 1 μm^3
SHO	$30.3 \times 10^{-5} \pm 36.9 \times 10^{-6}$	22.2 ± 11.3	$17.0 \times 10^{-5} \pm 43.3 \times 10^{-5}$	162.5 ± 102.9
ORX1	$32.4 \times 10^{-5} \pm 61.5 \times 10^{-6}$	18.0 ± 12.9	$20.2 \times 10^{-5} \pm 75.3 \times 10^{-5}$	102.4 ± 75.9
ORX2	$26.6 \times 10^{-5} \pm 37.7 \times 10^{-6}$	33.2 ± 14.3*	$19.2 \times 10^{-5} \pm 60.1 \times 10^{-5}$	210.4 ± 106.8§
ORX3	$30.4 \times 10^{-5} \pm 54.4 \times 10^{-6}$	29.1 ± 16.9§†	$16.8 \times 10^{-5} \pm 51.1 \times 10^{-5}$	142.9 ± 91.1†

Statistical significance is indicated as * P < 0.05 versus SHO; § versus ORX1; † versus ORX2.

tunica adventitia in the ORX3 group, compared to the SHO group (P ≤ 0.05) (*Table 2*). However, the values of these parameters were significantly lower than in the ORX1 group.

There were no significant differences in the volume of elastin in the rats after orchidectomy (group ORX1) in comparison to the sham-operated rats (group SHO) (*Table 3*, *Fig. 1*). The groups receiving camelina oil were characterized by a significantly higher volume of elastin in the tunica intima-media in comparison to the ORX1 group (P ≤ 0.05). However, in the ORX1 rats, the mean intensity of elastin fluorescence in the tunica intima-media in the

abdominal aorta was higher than in the sham-operated group (SHO) and camelina oil treated groups ($P \leq 0.05$) (*Fig. 1*).

The volume of collagen I in the tunica intima-media of the aorta wall in the orchidectomized rats (ORX1) was higher compared to the other groups, wherein statistically significant differences were noted between the ORX1 rats and the treated rats (*Table 4*). The lowest values of collagen I the in tunica intima-media were observed in rats supplemented with camelina oil at a dose of 5 g/kg b.w. The volume of collagen I in the adventitia was significantly higher in the ORX1 group in comparison to the treated groups and the SHO group ($P \leq 0.05$). However, the lowest mean intensity of collagen I fluorescence in the tunica intima-media and in the tunica adventitia was noted in the untreated orchidectomized rats (ORX1). The values of this parameter in rats treated with camelina oil did not differ from that noted in the SHO rats. No significant differences in the value of collagen III in the aorta walls were observed (*Table 5*). Significantly higher values of the mean intensity of collagen III fluorescence, in comparison to the orchidectomized rats treated with physiological saline, were observed for the tunica-intima in the ORX2 and ORX3 groups and for the tunica adventitia in the ORX2 group (*Table 5*).

DISCUSSION

Mechanical loads from blood pressure, surrounding tissue, and body movement affect arterial walls. The strength, compliance, and stability of arteries under these loads are essential to the maintenance of adequate arterial function. The mechanical properties of the arterial wall are dependent on extracellular matrix components (ECM) produced by smooth muscle cells (SMCs) and fibroblasts. The major parts of ECM of large arteries are collagen and elastin fibers, which determine the strength and the extensibility and elasticity of vessels, respectively. Elastin fibers are more flexible, whereas collagen fibers are stiffer (22). The ratio of these proteins plays an important role in determining the elasticity and stiffness of the arterial wall. Elastin fibers are organized in the medial layer of large arteries and they are associated with circumferentially associated smooth muscle cells and collagen. These fibers are considered to contribute in major part to the elastic properties of the aorta within the normal range of arterial pressure. Aortic collagen comprises types I and III, which account for 80 – 90% of total collagen, together with minor amounts of types IV, V, VI and VIII (23). Types I, III, and VI are present in the intimal, medial, and adventitial layers (24). Collagen fibers protect the aortic wall from rupturing when exposed to abnormally high pressure. An alteration in the structure, quantity, and ratio of ECM components can lead to mechanical and functional changes associated with different diseases (25).

Arterial stiffness has been reported as an independent predictor of cardiovascular morbidity and mortality in humans (26). It can be influenced by passive mechanisms that involve the mechanical properties of the wall and active mechanisms resulting from cellular and molecular functions of endothelial cells, SMCs and ECM (27). The stiffness of large arteries is determined mainly by ECM compounds and wall thickness. Different factors that increase the stiffness of the vessel wall, i.e. additional collagen amounts, degradation of elastin fibers, and crosslinking of elastin and collagen fibers, have been discussed (28). Moreover, wall thickening alone leads to an increase in stiffness during aging. Some authors (29) reported that reduced androgen levels and a lower testosterone/17β-estradiol ratio were related to the degree of carotid artery media thickening. However, in the present study, the changes in the wall structure connected with wall thickening were not associated with changes in the wall elasticity, because orchidectomy did not influence significantly the elastic recoil of the abdominal aorta in the rats.

Vascular aging is associated with structural and functional changes in arteries, even in healthy elderly subjects. These changes include an increase in the intima-media thickness resulting from the structural modification and accumulation of ECM components and disorganization of SMCs (30). Increased expression of matrix metalloproteinases (MMPs) and decreased expression of MMP inhibitors lead to fragmentation of elastin (31). Moreover, quite recently, the relationship between the MMPs concentration, MMP-9/TIMP-1 ratio, and abdominal aortic aneurysm was confirmed again (32). According to Siennicka *et al.*, the presence of thrombi with thin segments in the aneurysm sac, associated with higher proteolytic activity, could possibly be used as a potential indicator of a aneurism rupture site (32).

Decreased elastin content associated with increased collagen content affects the mechanical properties of wall arteries, mainly contributing to the stiffening of arteries (33). It has already been shown that the collagen content was increased, whereas the elastin content was decreased with age in different arteries of humans (34). The studies indicate that thickening of arterial walls is caused mainly by increased collagen content (35). In left ventricular hypertrophy, an increase in collagen content is also associated with an increase in the wall thickness and a decrease in the internal diameter of the left ventricle chamber (36). The beneficial role of L-arginine treatment and nitric oxide (NO) in the function and stiffness of heart tissues was demonstrated as well (36).

In conditions of early-onset androgens deficiency, we observed that the total wall thickness and the thickness of particular layers were higher in the orchidectomized rats compared to the SHO animals. These changes are similar to those mentioned above. However, the intima-media thickening was been associated with significant changes in the volume of ECM components in the abdominal aortas of the orchidectomized rats. However, a trend towards an increase in the volume of collagen I and III in the tunica intima-media was observed in the orchidectomized rats in comparison to the SHO rats. In contrast, the thickening of the tunica adventitia after orchidectomy was connected with a significant increase in the collagen I volume. Moreover, orchidectomy reduced significantly the mean intensity of collagen I fluorescence in all analyzed wall layers compared to the SHO group. However, a downward trend was only observed for collagen III in the adventitia.

On the other hand, we noted that the orchidectomy did not influence significantly the volume of elastin in the inner wall layer in comparison to the control animals. Instead, significantly higher mean intensity of elastin fluorescence was observed but these changes are difficult to explain. Elastin is one of the most stable components of the extracellular matrix, but impairment of its mechanical function is associated with aging and diseases such as atherosclerosis. Elastic fibers are degraded and fragmented during aging and diseases leading to increased stiffness of the arterial wall (37). Thus, loss of tissue elasticity is one of the signs of aging. It is considered that elastin fibers, which are damaged during aging, are generally not replaced because elastin expression is then switched off, whereas more collagen is produced. This may cause reduction of the elastin to collagen ratio and changes in the mechanical properties of arteries (38). However, in our study, no significant changes were noted in the volume of elastin and the elastic properties of the abdominal aorta between the SHO and ORX1 rats.

Many studies present a beneficial effect of n-3 PUFAs on the cardiovascular system (13, 16, 20). The first information about

the health-promoting effect of diet rich in PUFAs was recorded a few decades ago (39). It should be emphasized that the cardioprotective role of PUFAs, especially n-3, is multifactorial. Thus, it was observed that n-3 fatty acid significantly affected stiffness, arterial blood pressure, and blood lipids, which reduced atherosclerosis changes in the cardiovascular system (10, 11, 16). Diet rich in fish and fish oil has a positive influence on the heart, i.e. prevention of ventricular fibrillation or sustained arrhythmias during ischemia and reperfusion (40, 41). Beneficial effects of n-3 fatty acids in the therapy and prevention of rheumatoid arthritis, memory deficiency, and cancer, especially breast, colon, and prostate cancer, have been reported as well (41).

Many studies show the important role of the n-6/n-3 fatty acid ratio on health. Some of them demonstrate that a high ratio of n-6/n-3 fatty acids is connected with pathogenesis of cardiovascular disease, but their lower ratio is associated with suppressed levels of vascular endothelial growth factor and inflammatory biomarkers, as well as with reduced rates of platelet aggregation (42).

However, little is known about the influence of PUFA supplementation on the aorta structure in humans and animals. In a study conducted by Losurdo *et al.* (13), n-3 PUFA treatment led to significant thickening of the aorta in ovariectomized female rats, wherein positive effects on arterial stiffness and hemodynamic parameters were observed. In the present study, significant diminution of artery thickening was visible in the camelina oil-treated groups compared to the orchidectomized untreated group. Furthermore, the tunica intima-media and adventitia of ORX rats receiving camelina oil were characterized by a lower volume of collagen I. These changes were accompanied by a simultaneous increase in the mean intensity of collagen I fluorescence in these layers. Additionally, the camelina oil supplementation caused an increase in the volume of elastin compared to that in the untreated ORX rats. However, this increase is not connected with changes in the elasticity of the aortic wall. This may be explained by some data suggesting that elastin expression is retained in old organisms but synthesis of new elastin fibers is not adequate (38).

The changes mentioned above indicate that camelina oil, which is a good source of n-3 PUFAs, prevented negative changes in the aorta structure caused by orchidectomy. Notwithstanding, an ambiguous effect of n-3 PUFA administration on the intima-media thickness was also observed (43). As previously discussed, camelina oil is a good source of LA from the n-6 family and ALA from the n-3 family. These fatty acids are required but cannot be synthesized by humans and mammals. However, LA and ALA are able to elongate and desaturate long-chain fatty acids synthesized in the organism. Moreover, LA and ALA compete for the same enzymes involved in their metabolism. The products of elongation and desaturation of LA and ALA are arachidonic acid (AA, 20; 4 n-6) and EPA, respectively. Both AA and EPA are precursors for the synthesis of different regulatory molecules e.g. prostaglandins, thromboxanes, and leukotriens (44). As indicated in earlier studies, collagen I and III synthesis is inhibited by an increasing level of PGE_2 (45). The reduction of the androgen level in males is associated with an increase in the synthesis of PGE_2 as a metabolite of AA, while n-3 PUFA supplementation decreases PGE_2 release. In an *in vitro* study, EPA-treated fibroblasts produced more collagen than AA-treated cells and that was due to regulation *via* PGE_2 (46). These statements can be confirmed by the lower mean intensity of collagen I and III fluorescence for the intima-media and adventitia in the ORX rats in our study. On the other hand, an increase in these parameters was observed in the rats receiving camelina oil. Thus, administration of the ALA-rich camelina oil to orchidectomized rats may exert an impact on the synthesis of collagen because ALA competes with n-6 acids for the same enzymes in metabolic pathways. PUFAs can also modify collagen formation by altering gene expression in the nuclear factor-κB (NF-κB) pathway. As shown in an *in vitro* study, NF-κB seems to be an essential mediator of collagen formation in fibroblasts and SMCs influenced by PUFAs (47). Moreover, some data indicate that dietary supplementation with n-3 PUFAs can alter MMPs and their tissue inhibitors (TIMPs, tissue inhibitors of metalloproteinases) in vessel tissue (48, 49). MMPs cause proteolysis of elastin and collagen, which leads to changes in the mechanical properties of the aortic wall. Reduced MMP-2 and MMP-9 and increased TIMP-1 expression was observed after high n-3 PUFA diet (48, 49). These results were also associated with a trend for reduced elastin fragmentation (49). The influence of other biologically active factors on the MMP/TIMP balance toward the anti-proteolytic direction was also observed (50). In the study conducted by Maghrebi and Renno, genistein treatment lowered MMP-2 and MMP-9 and increased TIMP-1 and TIMP-2 in testicular tissue (50). Altogether, the above data suggest a protective effect of dietary supplementation with n-3 PUFAs against tissue degradation. In our study, a higher volume of elastin was observed in the camelina oil-treated rats in comparison to the untreated ORX rats; yet, no significant changes in vessel elasticity was noted.

In summary, orchidectomy in the rats led do similar changes in the aorta wall structure e.g. aging. These changes were manifested in an increase in the total wall thickness and in particular layers or the arterial wall. However, the changes in the wall structure were not associated with changes in aorta wall elasticity. The present study has also shown that camelina oil prevents negative changes in the vascular system associated with disturbed homeostasis of sex hormones in male rats.

REFERENCES

1. Natoli AK, Medley TL, Ahimastos AA, *et al.* Sex steroids modulate human aortic smoth muscle cell matrix protein deposition and matrix metalloproteinase expression. *Hypertension* 2005, 46: 1129-1134.
2. Morgentaler A, Feibus A, Baum N. Testosterone and cardiovascular disease - the controversy and the facto. *Postgrad Med* 2014; 127: 159-165.
3. Ohlsson C, Barrett-Connor E, Bhasin S, *et al.* High serum testosterone is associated with reduced risk of cardiovascular events in elderly men. The MrOS (osteoporotic fractures in men) study in Sweden. *J Am Coll Cardiol* 2011; 58: 1674-1681.
4. Malkin CJ, Channer KS, Jones TH. Testosterone and heart failure. *Curr Opin Endocrinol Diabetes Obes* 2010; 17: 262-268.
5. Carrero JJ, Qureshi AR, Parini P, *et al.* Low serum testosterone increases mortality risk among male dialysis patients. *J Am Soc Nephrol* 2009; 20: 613-620.
6. Zitzmann M, Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long term intramuscular testosterone undecanoate therapy in hypogonadal men. *J Clin Endocrin Metab* 2007; 92: 3844-3853.
7. Yaron M, Greenman Y, Rosenfeld JB, *et al.* Effect of testosterone replacement therapy on arterial stiffness in older hypogonadal men. *Eur J Endocrinol* 2009; 160: 839-846.
8. Reckelhoff JF, Zhang H, Granger JP. Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension* 1998; 31: 435-439.
9. Vikan T, Schirmer H, Njolstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular

disease and mortality in men: the Tromso study. *Eur J Endocrinol* 2009; 161: 435-442.
10. Abeywardena MY, Head RJ. Long chain n-3 polyunsaturated fatty acids and blood vessel function. *Cardiovasc Res* 2001; 52: 361-371.
11. Chang CL, Torrejon C, Jung UJ, Graf K, Deckelbaum RJ. Incremental replacement of saturated fats by n- 3 fatty acids in high-fat, high-cholesterol diets reduces elevated plasma lipid levels and arterial lipoprotein lipase, macrophages and atherosclerosis in LDLR$^{-/-}$mice. *Atherosclerosis* 2014; 234: 401-409.
12. Engler MM, Engler MB, Pierson DM, Molteni LB, Molteni A. Effects of docosahexaenoic acid on vascular pathology and reactivity in hypertension. *Exp Biol Med* (*Maywood*) 2003; 228: 299-307.
13. Losurdo P, Grillo A, Panizon E, *et al.* Baroreflex sensitivity and central hemodynamics after omega-3 polyunsaturated fatty acids supplementation in an animal model of menopause. *Vascul Pharmacol* 2015; 71: 65-69.
14. Flachowsky G, Schaarmann G, Jahreis G, *et al.* Influence of feeding of oil seeds and by products from oilseeds on vitamin E concentration of animal products. *Fett Lipid* 1997; 99: 55-60.
15. Zubr J, Matthaus B. Effects of growth conditions on fatty acids and tocopherols in Camelina sativa oil. *Ind Crop Prod* 2002; 15: 155-162.
16. Karvonen HM, Aro A, Tapola NS, Salminen I, Uusitupa MI, Sarkkinen ES. Effect of a linolenic acid-rich Camelina sativa oil on serum fatty acid composition and serum lipids in hypercholesterolemic subjects. *Metabolism* 2002; 51: 1253-1260.
17. Peiretti PG, Mussa PP, Prola L, Meineri G. Use of different levels of false flax (Camelina sativa L.) seed in diets for fattening rabbits. *Livestock Sci* 2007; 107: 192-198.
18. PN-ISO 5509: 1996 - Analysis of fatty acid methyl esters by gas chromatography [in Polish].
19. PN-EN ISO 5508: 1996 - Determination of fatty acid composition. [in Polish].
20. Harrison A, Pawlowska M, Bartels M, Valverde Piedra JL, Skrzypek H, Pierzynowski S. The effect of oral administration of alfa ketoglutarate on stomach-by-pass induced stiffness in rats. *Baltic J Comp Clin Syst Biol* 2011; 1: 24-34.
21. Pawlowska M, Harrison A, Piersiak T, *et al.* Effect of camelina oil on the structure of aortas in rats. *Med Weter* 2016; 72: 240-246.
22. Van Bavel E, Siersma P, Spaan JA. Elasticity of passive blood vessels: a new concept. *Am J Physiol Heart Circ Physiol* 2003; 285: H1986-H2000.
23. Kielty CM, Hopkinson I, Grant ME. The collagen family: structure, assembly, and organization in the extracellular matrix. In: Connective Tissue and Its Hereditable Disorders, Molecular. Genetic and Medical Aspects, P.M. Royce, B. Steinmann (eds.). New York, Wiley Liss, 1993, 103-148.
24. Aguila MB, Mandarim-de-Lacerda CA. Aorta wall quantitative alterations due to different long-term high-fat diet in rats. *Food Chem Toxicol* 2003; 4: 1391-1397.
25. Tsamis A, Rachev A, Stergiopulos N. A constituent-based model of age-related changes in conduit arteries. *Am J Physiol Heart Circ Physiol* 2011; 301: H1286-H1301.
26. McEniery CM, Wilkinson IB, Avolio AP. Age, hypertension and arterial function. *Clin Exp Pharmacol Physiol* 2007; 34: 665-671.
27. Avolio A. Arterial stiffness. *Pulse* (*Basel*) 2013; 1: 14-28.
28. Wagenseil JE, Mecham RP. Elastin in large artery stiffness and hypertension. *J Cardiovasc Transl Res* 2012; 5: 264-273.
29. Makinen J, Jarvisalo MJ, Pollanen P, *et al.* Increased carotid atherosclerosis in andropausal middle-aged men. *J Am Coll Cardiol* 2005; 45: 1604-1608.
30. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardi-ovascular disease enterprises: Part III: Cellular and molecular clues to heart and arterial aging. *Circulation* 2003; 107: 490-497.
31. Li Z, Froehlich J, Galis ZS, Lakatta EG. Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats. *Hypertension* 1999; 33: 116-123.
32. Siennicka A, Zuchowski M, Kaczmarczyk M, Cnotliwy M, Clark JS, Jastrzebska M. Spatial differences of matrix metalloproteinase-2 and matrix metalloproteinase-9 within abdominal aortic aneurysm wall and intraluminal thrombus. *J Physiol Pharmacol* 2016; 67: 902-910.
33. Kovacic JC, Moreno P, Nabel EG, Hachinski V, Fuster V. Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly. *Circulation* 2011; 17: 1900-1910.
34. Avolio A, Jones D, Tafazzoli-Shadpour M. Quantification of alterations in structure and function of elastin in the arterial media. *Hypertension* 1998; 32: 170-175.
35. Vimani R, Avolio AP, Mergener WJ, *et al.* Effect of aging on aortic morphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. *Am J Pathol* 1991; 139: 1119-1129.
36. Ahmad A, Sattar MA, Rathore HA, *et al.* Enhanced expression of endothelial nitric oxide synthase in the myocardium ameliorates the progression of left ventricular hypertrophy in L-arginine treated Wistar-Kyoto rats. *J Physiol Pharmacol* 2016; 67: 31-44.
37. Green EM, Mansfield JC, Bell JS, Winlove CP The structure and micromechanics of elastic tissue. *Interface Focus* 2014; 4: 20130058. doi: 10.1098/rsfs.2013.0058
38. Todorovich-Hunter L, Johnson DJ, Ranger P, Keeley FW, Rabinovitch M. Altered elastin and collagen synthesis associated with progressive pulmonary hypertension induced by monocrotaline. A biochemical and ultrastructural study. *Lab Invest* 1988; 58: 184-195.
39. Bang HO, Dyeberg J, Hjoorne N. The composition of food consumed by Greenland Eskimos. *Acta Med Scand* 1976; 200: 69-73.
40. McLennan PL. Cardic physiology and clinical efficacy of dietary fish oil clarified through cellular mechanisms of omega-3 polyunsaturated fatty acid. *Eur J Appl Physiol* 2014; 114: 1333-1356.
41. Asif M. Chemical characteristics and nutritional potentials on unsaturated fatty acids. *Chemistry Int* 2015; 1: 118-133.
42. Hammad S, Pu S, Jones PJ. Current evidence supporting the link between dietary fatty acid and cardiovascular disease. *Lipids* 2016; 51: 507-517.
43. Sekikawa A, Kadowaki T, El-Saed A, *et al.* Differential association of docosahexaenoic and eicosapentaenoic acids with carotid intima-media thickness. *Stroke* 2011; 42: 2538-2543.
44. Tapiero H, Ba GN, Couvreur P, Tew KD. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomed Pharmacother* 2002; 56: 215-222.
45. Varga J, Diaz-Perez A, Rosenbloom J, Jimenez SA. PGE2 causes a coordinate decrease in the steady state levels of fibronectin and types I and III procollagen mRNAs in normal human dermal fibroblasts. *Biochem Biophys Res Commun* 1987; 147: 1282-1288.
46. Jia Y, Turek JJ. Polyenoic fatty acid ratios alter fibroblast collage production via PGE2 and PGE receptor subtyperesponse. *Exp Biol Med* 2004; 229: 676-683.

47. Ringseis R, Gahler S, Eder K. Conjugatedlinoleic acid isomers inhibit platelet-derived growth factor-induced NF-κB transactivation and collagen formation in human vascular smooth muscle cells. *Europ J Nutr* 2008; 47: 59-67.
48. Wang JH, Eguchi K, Matsumoto S, *et al.* The ω-3 polyunsaturated fatty acid, eicosapentaenoic acid, attenuates abdominal aortic aneurysm development via suppression of tissue remodeling. *PLoS One* 2014; 9: e96286. doi: 10.1371/journal.pone.0096286
49. Kavazos K, Sci KB, Nataatmadja M, Hartland E, Williams C, Russel FD. Dietary supplementation with omega-3 polyunsaturated fatty acids modulate matrix metalloproteinase immunoreactivity in a mouse model of pre-abdominal aortic aneurysm. *Heart Lung Circ* 2015; 24: 377-385.
50. Al-Maghrebi M, Renno WM. Genistein alleviates testicular ischemia and reperfusion injury-induced spermatogenic damage and oxidative stress by suppressing abnormal testicular matrix metalloproteinase system VIA the Notch 2/Jagged 1/Hes-1 and caspase-8 pathways. *J. Physiol Pharmacol* 2016; 67: 129-137.

Received: August 2, 2017
Accepted: February 15, 2018

Author's address: Dr. Marta Pawlowska-Olszewska, Department of Animal Physiology, University of Life Sciences, 12 Akademicka Street, 20-033 Lublin, Poland.
E-mail: martapaw@autograf.pl