ORIGINAL ARTICLE

Medical Journal of Western Black Sea
Batı Karadeniz Tıp Dergisi

Med J West Black Sea 2024;8(3): 244-256
DOI: 10.29058/mjwbs.1470747

Comparison of Histomorphometric Characteristics of Heart and Aorta in Young Adult and Aged Rats

Genç Erişkin ve Yaşlı Sıçanlarda Kalp ve Aort'un Histomorfometrik Özelliklerinin Karşılaştırılması

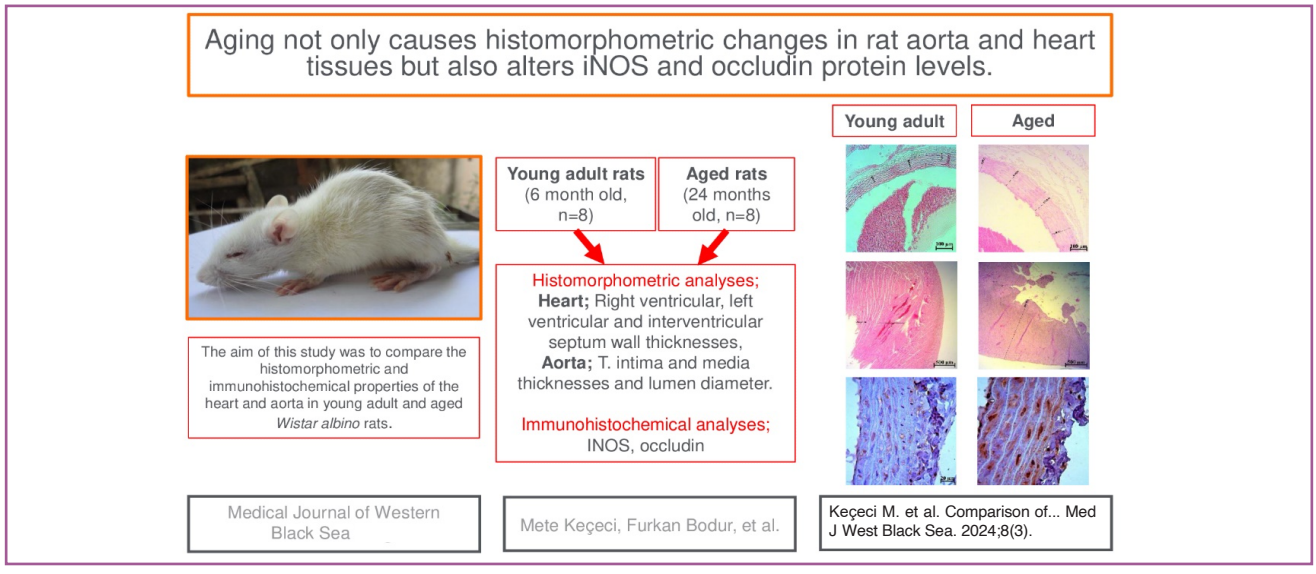
Mete KEÇECİ[1], Furkan BODUR[2], Esra BABAOĞLU[2], Osman CENGİL[1], Cenk Murat ÖZER[2]

[1]Zonguldak Bülent Ecevit University Faculty of Medicine, Department of Histology and Embryology, Zonguldak, Türkiye
[2]Zonguldak Bülent Ecevit University Faculty of Medicine, Department of Anatomy, Zonguldak, Türkiye

ORCID ID: *Mete Keçeci 0000-0002-2144-4730, Furkan Bodur 0000-0002-2495-8315, Esra Babaoğlu 0000-0002-2649-7698, Osman Cengil 0000-0002-0702-6751, Cenk Murat Özer 0000-0002-7813-723X*

Cite this article as: *Keçeci M et al. Comparison of histomorphometric characteristics of heart and aorta in young adult and aged rats. Med J West Black Sea. 2024;8(3):244-256.*

GRAPHICAL ABSTRACT



ABSTRACT

Aim: The aim of this study was to compare the histomorphometric and immunohistochemical properties of the heart and aorta in young adult and aged rats.

Material and Methods: Sixteen female Wistar albino rats, eight young adult (6 months old, female, 233.25±13.85 g) and rats were used in the study. The rats were sacrificed under high dose anaesthesia and heart and aortic tissues were collected. Ventricular and septum interventriculare thicknesses were measured on the heart tissues, tunica media, tunica intima thickness and aortic diameter were measured histomorphometrically on the aortic tissues. In addition, Inducible Nitric Oxide Synthase (iNOS) in the smooth muscle cells of the tunica

Corresponding Author: Furkan Bodur ✉ furkan.bdr81@gmail.com **Received:** 19.04.2024 **Revision:** 09.08.2024 **Accepted:** 10.12.2024

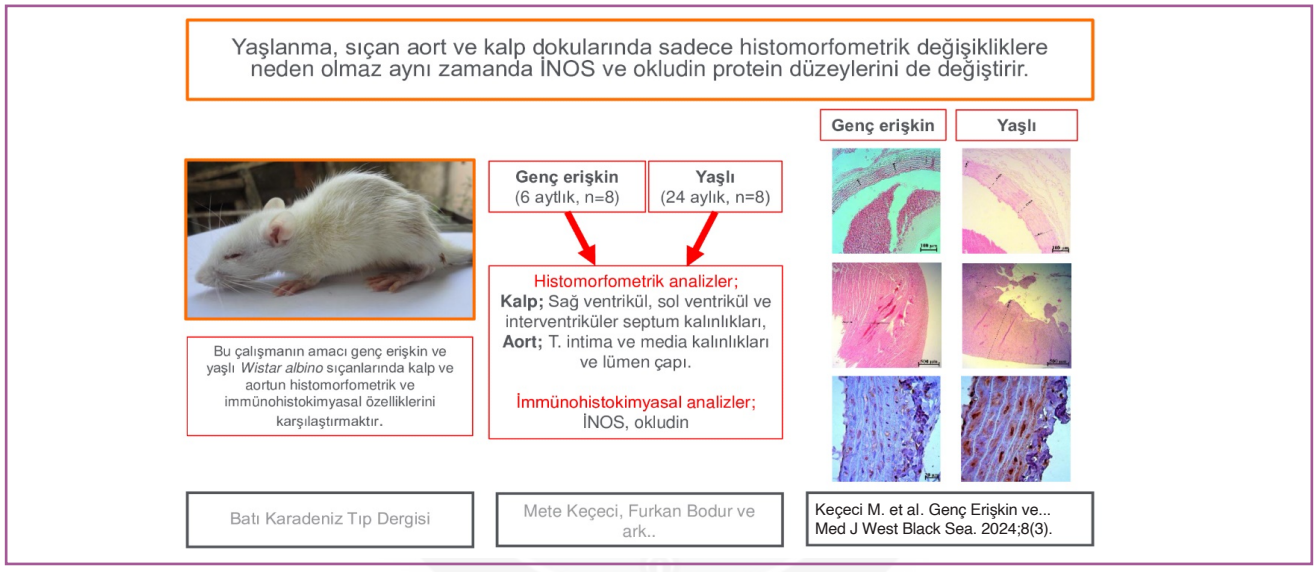
media of the aorta and occludin protein levels in the aorta and heart endothelium were examined by immunohistochemical method and histological scoring was performed.

Results: As a result of statistical analysis, body weight, heart weight, heart weight/body weight ratio, tunica media and intima thicknesses, and aortic diameters were found to be statistically significantly higher in aged rats compared to young adult rats ($p<0.05$). Among the cardiac measurements, only the left ventricle/heart weight ratio was found to be statistically significantly higher in young adults than in aged rats ($p<0.05$). In addition, according to the H scoring of the data obtained as a result of immunohistochemical staining for iNOS protein, iNOS protein level was found to be higher in aged rats than in young rats ($p<0.05$). Immunohistochemical staining for occludin in aorta and heart endothelial cells of aged rats revealed weaker staining compared to young rats.

Conclusion: In general, our study emphasises the importance of understanding the histomorphometric changes that occur in heart and aortic tissue as a result of aging. Further research is needed to better understand the mechanisms underlying these age-related changes and to identify potential therapeutic targets for the prevention and treatment of cardiovascular diseases in elderly individuals.

Keywords: Ageing, aorta, heart, histomorphometry, iNOS

GRAFİKSEL ÖZET



ÖZ

Amaç: Bu çalışmanın amacı, genç erişkin ve yaşlı sıçanlarda kalp ve aortun histomorfometrik ve immünohistokimyasal özelliklerini karşılaştırmaktır.

Gereç ve Yöntemler: Çalışmada sekiz genç erişkin (6 aylık, dişi, 233,25±13,85 g) ve sekiz yaşlı (24 aylık, dişi, 257,12±17,48 g) olmak üzere toplam on altı dişi Wistar albino sıçan kullanıldı. Deneye dahil edilen sıçanlar yüksek doz anestezi altında sakrifiye edilerek kalp ile aort dokuları toplandı. Kalp dokuları üzerinden ventrikül ve septum interventiculare kalınlıkları ölçülürken aort dokusu üzerinde tunica media ve tunica intima kalınlığı, aort çapı histomorfometrik olarak ölçüldü. Ayrıca aortaların tunica medialarının düz kas hücrelerinde Inducible Nitric Oxide Synthase (iNOS) ve aorta ve kalp endotelinde okludin protein düzeyleri immünohistokimyasal yöntemle incelendi ve histolojik skorlama yapıldı.

Bulgular: Yapılan istatistiksel analiz sonucunda yaşlı sıçanlarda vücut ağırlığı, kalp ağırlığı, kalp ağırlığı/ vücut ağırlığı oranı, tunica media ve intima kalınlıkları, aort çapları genç erişkin sıçanlara göre istatistiksel olarak anlamlı seviyede yüksek olduğu tespit edildi ($p<0.05$). Kalp ölçümlerinden sadece sol ventrikül/ kalp ağırlığı oranının genç erişkinlerde yaşlı sıçanlara göre istatistiksel olarak anlamlı seviyede yüksek olduğu belirlendi ($p<0.05$). Ayrıca iNOS proteini için yapılan immünohistokimyasal boyama sonucunda elde edilen verilerin H skorlamasına göre yaşlı sıçanlarda iNOS protein düzeyi gençlere göre yüksek bulundu ($p<0.05$). Yaşlı sıçanların aorta ve kalp endotel hücrelerinde okludin için yapılan immünohistokimyasal boyama sonucunda genç sıçanlara göre daha zayıf boyanma gözlendi.

Sonuç: Genel olarak çalışmamız, yaşlanmanın bir sonucu olarak kalp ve aort dokusunda meydana gelen histomorfometrik değişikliklerin anlaşılmasının önemini vurgulamaktadır. Yaşa bağlı bu değişikliklerin altında yatan mekanizmaları daha iyi anlamak ve yaşlı bireylerde kardiyovasküler hastalıkların önlenmesi ve tedavisine yönelik potansiyel terapötik hedefleri belirlemek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Sözcükler: Aort, histomorfometri, iNOS, kalp, yaşlanma

INTRODUCTION

Aging is a universal phenomenon expressing the complexity and variability of biological processes (1). The effects of the aging process, especially on the cardiovascular system, have been a major focus of interest in the fields of health and medicine. It is known that some structural and morphometric changes occur in the main cardiovascular structures such as the heart and aorta with age (2-4). These changes start in the first decades and continue to increase over the years and may increase the risk of heart failure, atherosclerosis and other cardiovascular diseases (2,5).

Histomorphometric characteristics of the cardiovascular system are of great importance in understanding the functioning of this system in health and disease states (6). For example, when the relationship between age-related changes in cardiovascular structures and diseases was analysed, increased tunica intima thickness in vascular structures was associated with the early stages of atherosclerosis (2,3). Arteriosclerosis has been associated with systolic hypertension, left ventricular wall thickening, stroke and atherosclerosis, whereas increased left ventricular wall thickness has been associated with delayed early diastolic heart filling and increased likelihood of heart failure (2). Therefore, in-depth examination and understanding of these histomorphometric changes associated with aging is a critical step for the prevention and treatment of aging-related diseases.

The main aim of this study was to compare the histomorphometric and immunohistochemical properties of the heart and aorta in young adult and old female rats. The reason for the selection of female rats in our study was to reveal the histopathological changes in the cardiovascular system of rats at 108 weeks of age, which corresponds to the postmenopausal period (human age equivalent to 60 years), when the protective effect of estrogen disappears (7). This comparison aims to provide basic information to understand the changes in the aging process and to identify potential strategies for the prevention of cardiovascular diseases. In particular, the differences in ventricle, septum interventriculare, aortic wall thickness, vessel diameters and iNOS expression in the aortic wall of young adult and aged rats were evaluated to compare how cardiovascular structures are affected anatomically and histologically during the aging process.

MATERIAL and METHODS

This study was conducted between 9 September and 30 December 2023 at the Department of Histology and Embryology, Zonguldak Bülent Ecevit University (ZBEU). Ethics committee approval for the study was obtained from ZBEU Animal Experiments Local Ethics Committee on 07.09.2023 with protocol number 2023-16-07/09. A total of sixteen female Wistar albino rats, eight young adult (6 months old, female, 233.2 g) and eight aged (24 months old, female, 257.12 17.48 g), produced in the laboratory of ZBEU Experimental Research Application and Research Centre were used in our study. All rats were maintained under optimum laboratory conditions (temperature 22 ± 1 °C, humidity 55 ± 8%, 12 h light/dark cycle), daily drinking water and 21% crude protein pellet feed ad libitum throughout the experimental period.

The rats were sacrificed under high dose anaesthesia and heart and aortic tissues were collected. Body weights of the rats just before euthanasia and heart weights just after euthanasia were measured using a precision balance. Each heart was then divided in half with a longitudinal incision from the apex to the base. The dissection plane passed through the deepest points of the right and left ventricles and divided the septum interventriculare and septum interatriale into two (8). The aorta was excised at the end of the arcus aorta and the beginning of the aorta thoracica descenens (9). The heart tissues divided into right and left halves and the aorta thoracica were fixed with neutral formalin and blocked by applying routine tissue tracing procedures. From the prepared paraffin blocks, 5 μm thick sections were taken using ShandonFinesse 325 brand cylinder microtome and stained with haematoxylin+eosin (H+E) staining method. In addition, occludin expression among endothelial cells in the tunica intima of aortas of aged and young adult rats was demonstrated by immunohistochemistry and semiquantitative histological scoring (h-score) was performed.

For immunohistochemical examination of heart and aortic tissues, 3 μm thick sections obtained from paraffin blocks were cut on positively charged slides. After deparaffinization and immersion, the sections in citrate buffer were kept just below the boiling point for 15 minutes in a microwave oven to reveal the antigenic binding sites. The sections were then placed in distilled water and allowed to cool at room temperature for 20 minutes. After 3 washes with PBS, sections were treated with 3% H_2O_2 for 10 minutes to block endogenous peroxidase activity. After 7 minutes of Ultra V block (LabVision, TA-015-UB) to block nonspecific binding sites, sections were treated with INOS (1/1000 dilution, Thermo scientific, Rabbit polyclonal IgG, (PA1-036)) in aortic tissue and Occludin in aortic and heart tissue (1/1500, Thermo scientific, Rabbit polyclonal IgG, (71-1500)) primary antibody. (24 hours at +4 ºC). After washing with PBS, secondary antibody (Biotinylated Link, Dako, K0609) and streptavidin-peroxidase (Streptavidin HRP, Dako, K0609) were applied for 30 and 10 minutes, respectively, at room temperature. Then, sections were treated with diaminobenzidine (DAB) chromogen solution (Vector, SK-4100) until the desired staining intensity was obtained under the light

microscope and counterstained with hematoxylin. During all incubations, tissues were kept in a humid environment to prevent drying and avoid background staining. Sections covered using entellan and omit were examined under Zeiss Axio Lab A1 light microscope. In photographing all findings, Zeiss Axio Lab. A1 brand photomicroscope was used. All immunohistochemical analyzes were performed within the protocols recommended by the manufacturer.

Histological scoring (h score) was performed manually using the following criteria to define the immunohistochemical results; 0; no staining, 1+; weak but detectable staining, 2+; medium or pronounced staining, 3+; intense staining. The H-SCORE value for each section was obtained by multiplying the percentage of stained cells for each density category by its density. Scoring was done under the light microscope at x40 objective magnification on 20 randomly selected fields on each section and mean scores were used for statistical analysis. H-score = ∑i i xPi, i; density score, Pi; cell percentage (10).

Morphometric Measurements

H+E stained sections were evaluated under a light microscope and analysed using AxioVisionRel. 4.8 programme and ImageJ software. Thirty-two preparations of heart tissue and sixteen preparations of aortic tissue of each subject were subjected to quantitative studies. The thicknesses of the right ventricle (LVW), left ventricle (RVW) and septum interventriculare (ISW) were measured in three different regions: basal (close to the atrioventricular valves), middle and apex (8). These measurements were made at X2.5 objective magnification of the microscope and the average of these measurements made on the right and left heart were recorded.

The thickness of tunica media and tunica intima, tunica intima/tunica media ratio, lumen diameter, total diameter including tunica media and tunica intima were measured from the aorta thoracica tissue sections. For tunica media thickness (MW), the distance between the innermost and outermost elastic laminae was measured (11). For tunica intima thickness (IW), the distance between the endothelial layer (apical surface of endothelial cells) and the inner elastic lamina was measured (12). The thickness of tunica media was measured at X10 objective magnification of the microscope and the thickness of tunica intima was measured at X100 objective magnification of the microscope from twenty-four different areas in the transverse section of the aorta thoracica and the average of these measurements was taken and recorded. In addition, tunica intima/ tunica media ratio was calculated using these measurements. For lumen diameter, the three farthest distances between the apical surfaces of the endothelial cells were marked. These three furthest distances and the three longest distances perpendicular to them were measured at X2.5 objective magnification of the microscope. These measurements were averaged and evaluated as lumen diameter. For the total diameter, the three farthest distances between the outer faces of the tunica median and the three longest distances cutting them perpendicularly were also measured at X2.5 objective magnification of the microscope. These measurements were averaged and evaluated as total diameter.

Statistical Analysis

Statistical analysis of the data was performed using Jamovi 2.3.21 package programme. The conformity of continuous variables to normal distribution was analysed by Shapiro Wilk test. Descriptive data of quantitative variables were given as mean, standard deviation, median, minimum and maximum values. Mann Whitney U test was used for comparisons of quantitative independent variables between two groups. As a result of statistical analysis, $p<0.05$ was accepted as significant level.

RESULTS

Histomorphometric Results

A statistically significant difference was found between the groups in the comparison of body weights and heart weights of the subjects belonging to young adult and aged groups (mean±SD for body weight 233.25±13.85 and 257.13±17.48, p=0.005, mean±SD for heart weight 0.73±0.068 and 1.00±0.25, p=0.002, respectively) (Table 1). A statistically significant difference was found between young adult and aged rat groups in the comparison of heart weight/body weight ratios obtained by using body weights and heart weights obtained from all subjects (mean±SD 0.31±0.02 and 0.39±0.11, respectively, p=0.015) (Table 1). There was no statistically significant difference between young adult and aged groups in terms of left ventricular basal, middle and apical level measurements and mean left ventricular thicknesses obtained from the mean of these measurements (p=0.382, p=0.956, p=0.505, p=1.00, respectively) (Table 1, Figure 1). Again, no statistically significant difference was found between the young adult and aged groups in terms of right ventricular basal, middle and apical level measurements and mean right ventricular thicknesses obtained from the mean of these measurements (p=0.878, p=0.956, p=0.798, p=0.956, respectively) (Table 1, Figure 2). Similarly, there was no statistically significant difference between the young adult and aged groups in terms of septum interventriculare basal, middle and apical level measurements and mean septum interventriculare thicknesses obtained from their averages (p=0.083, p=0.721, p=0.645, p=0.442, respectively) (Table 1, Figure 3). When left ventricular thickness/heart weight, right ventricular thickness/heart weight and septum interventriculare thickness/heart weight ratios were compared, there was a statistically significant difference between the young adult

Table 1. Body composition and histomorphometric analyses of basal, middle and apical thickness of the right ventricular (RVW), left ventricular (LVW) and interventricular septum (ISW) walls of the heart of aged and young adult rats.

		Aged (n=8) Median (Min.-Max.)	Young adult (n=8) Median (Min.-Max.)	p
Body weight (BW) (g)		251.50 (241-286)	233.50 (208-259)	**0.005**
Heart weight (HW) (g)		0.96 (0.76-1.61)	0.72 (0.67-0.87)	**0.002**
HW/BW		0.35 (0.31-0.66)	0.31 (0.28-0.36)	**0.015**
Left ventricular (LV) thickness (µm)	Basal	2533.70 (1724.04-3031.82)	2345.01 (1644.28-2962.71)	0.382
	Middle	2397.02 (2175.54-2737.30)	2334.68 (1998.74-3373.73)	0.956
	Apical	1984.56 (1784.05-2329.58)	2246.86 (1748.40-2913.52)	0.505
	Total	2348.87 (2049-2519)	2296.80 (1948-3049)	1.00
Right ventricular (RV) thickness (µm)	Basal	844.84 (716.97-1490.54)	830.89 (775.45-1530.35)	0.878
	Middle	1055.10 (451.33-1356.21)	936.57 (684.45-1590.65)	0.956
	Apical	732.85 (493.21-987.78)	647.11 (359.67-1226.96)	0.798
	Total	846.98 (567-1258)	789.40 (618-1332)	0.956
Septum interventriculare (SIV) thickness (µm)	Basal	1836.02 (1269.44-3532.82)	1595.84 (1082.75-1924.72)	0.083
	Middle	2053.16 (1396.14-3592.31)	1949.21 (1263.78-2731.13)	0.721
	Apical	1636.15 (1128.13-2242.79)	1510.21 (1249.46-2433.96)	0.645
	Total	1865.15 (1445-3123)	1714.08 (1199-2140)	0.442

and aged groups only in terms of left ventricular thickness/heart weight, but there was no significant difference between the groups in terms of the other parameters. The mean±SD for left ventricular thickness/heart weight ratio was 3253±596 and 2405±463, p=0.007, mean±SD for right ventricular thickness/heart weight ratio was 1251±415 and 927±194, p=0.2779, p=0.2779, mean±SD for septum interventriculare thickness/heart weight ratio was 2329±447 and 2049±790, p=0.083, respectively in the young adult and aged groups (Table 2).

In the statistical analysis of the data obtained from the measurements of tunica media thickness (µm), tunica intima thickness (µm), lumen diameter (µm) and total diameter (µm) on the thoracic aorta tissues obtained from the subjects belonging to the young adult and aged groups, a significant difference was found between the groups for all parameters. Mean±SD and p values of young adult and aged groups were 78.80±4.16 and 114.95±7.73, p<0.01 for tunica media thickness, 3.70±0. 55 and 7.48±1.75, p<0.01, for lumen diameter 1269.93±31.44 and 1597.81±168.33, p<0.01 and for total diameter 1420.01±27.04 and 1827.18±180.17, p<0.01, respectively (Table 3, Figure 4).

In the statistical analysis of the Tunica media thickness/Lumen diameter, Tunica intima thickness/Lumen diameter, Tunica media thickness/Total diameter and Tunica intima thickness/Total diameter ratios calculated using the above data, a statistically significant difference was found between

Table 2. Histomorphometric examination of the ratio of left ventricular thickness (LVT), right ventricular thickness (RVT) and interventricular septum thickness (IST) to heart weight in aged and young adult rats.

	Aged (n=8) Median (Min.-Max.)	Young adult (n=8) Median (Min.-Max.)	p
LVT/HW	2331 (1556-3156)	3146 (2350-4234)	**0.007**
RVT/HW	858 (644-1175)	1102 (858-1962)	0.279
IST/HW	1735 (1174-3548)	2331 (1787-2931)	0.083

Table 3. Histomorphometric examination of tunica media thickness, tunica intima thickness, lumen diameter and total diameter of the aorta thoracica in aged and young adult rats.

	Aged (n=8) Median (Min.-Max.)	Young adult (n=8) Median (Min.-Max.)	p
Tunica media thickness (µm)	116.97 (98.96-124.41)	79.52 (71.92-84.04)	**<0.001**
Tunica intima thickness (µm)	7.18 (5.60-11.35)	3.63 (3.12-4.61)	**<0.001**
Lumen diameter (µm)	1653.43 (1339.78-1769.04)	1261.52 (1234.08-1330.13)	**<0.001**
Total diameter (µm)	1903.14 (1558.21-1987.19)	1421.64 (1381.00-1472.90)	**<0.001**

the young adult and aged groups (p<0.01 for all comparisons, mean±SD for Tunica media thickness/Lumen diameter in the young adult and aged groups were 0.062±0.004 and 0.072±0.005 for Tunica media thickness/Lumen diameter, 0.002±0.0004 and 0.004±0.0016 for Tunica intima thickness/Lumen diameter, 0.055±0.003 and 0.063±0.040 for Tunica media thickness/Total diameter, 0.0026±0.0003 and 0.0041±0.0013 for Tunica intima thickness/Total diameter (Table 4.).

Immunohistochemical findings

In the statistical analysis of h-score values obtained as a result of immunohistochemical staining to determine iNOS protein levels in aorta tissues of young adult and aged female rats, a statistically significant difference was found between the two groups (p<0.005 and median (min-max) values were 107 (75-121) and 132 (98.7-161.7), respectively (Figure 5,6).

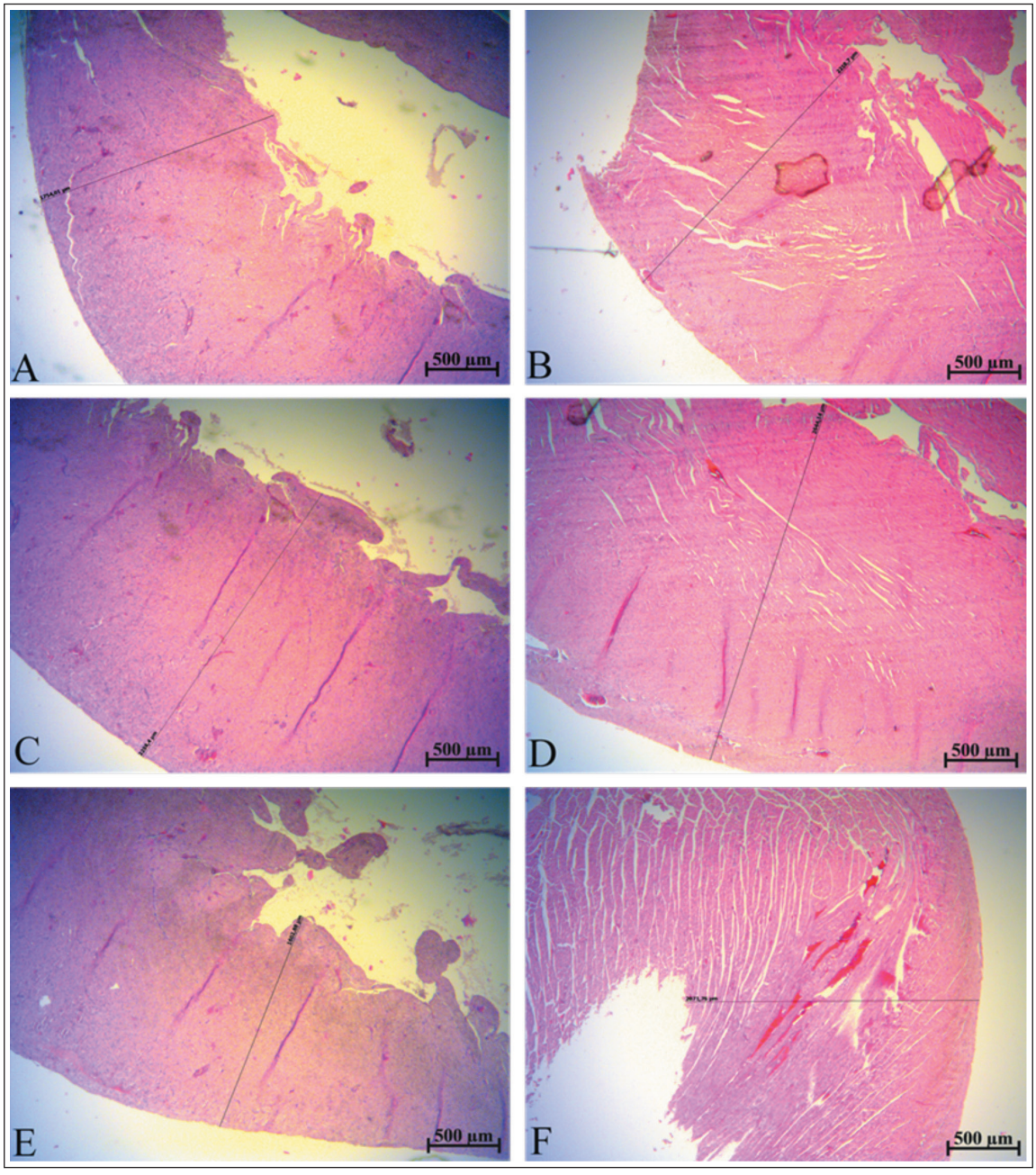


Figure 1: Histomorphometric images of aged and young adult rat left ventricles. **A,B)** left basal, **C,D)** middle left, **E,F)** left apical. **A,C,E)** aged group, **B,D,F)** young adult group. Scale bar: 500 µm.

Table 4. Examination of the ratio of tunica media thickness (TMT) and tunica intima thickness (TIT) of the aorta thoracica to heart weight (HW), lumen diameter (LD) and total diameter (TD) in aged and young adult rats.

	Aged (n=8) Median (Min.-Max.)	Young adult (n=8) Median (Min.-Max.)	p
TMT/HW (µm/gr)	121.28 (77.27-134.16)	106.80 (96.60-120.07)	**0.028**
TIT/HW (µm/gr)	7.52 (3.70-14.93)	4.97 (4.05-6.02)	**0.015**
TMT/LD (µm/µm)	0.072 (0.065-0.082)	0.062 (0.054-0.067)	**<0.001**
TIT/LD (µm/µm)	0.004 (0.0032-0.0083)	0.002 (0.0023-0.0035)	**<0.001**
TMT/TD (µm/µm)	0.062 (0.058-0.070)	0.055 (0.048-0.058)	**<0.001**
TIT/TD (µm/µm)	0.0037 (0.0028-0.0072)	0.0025 (0.0021-0.0032)	**<0.001**

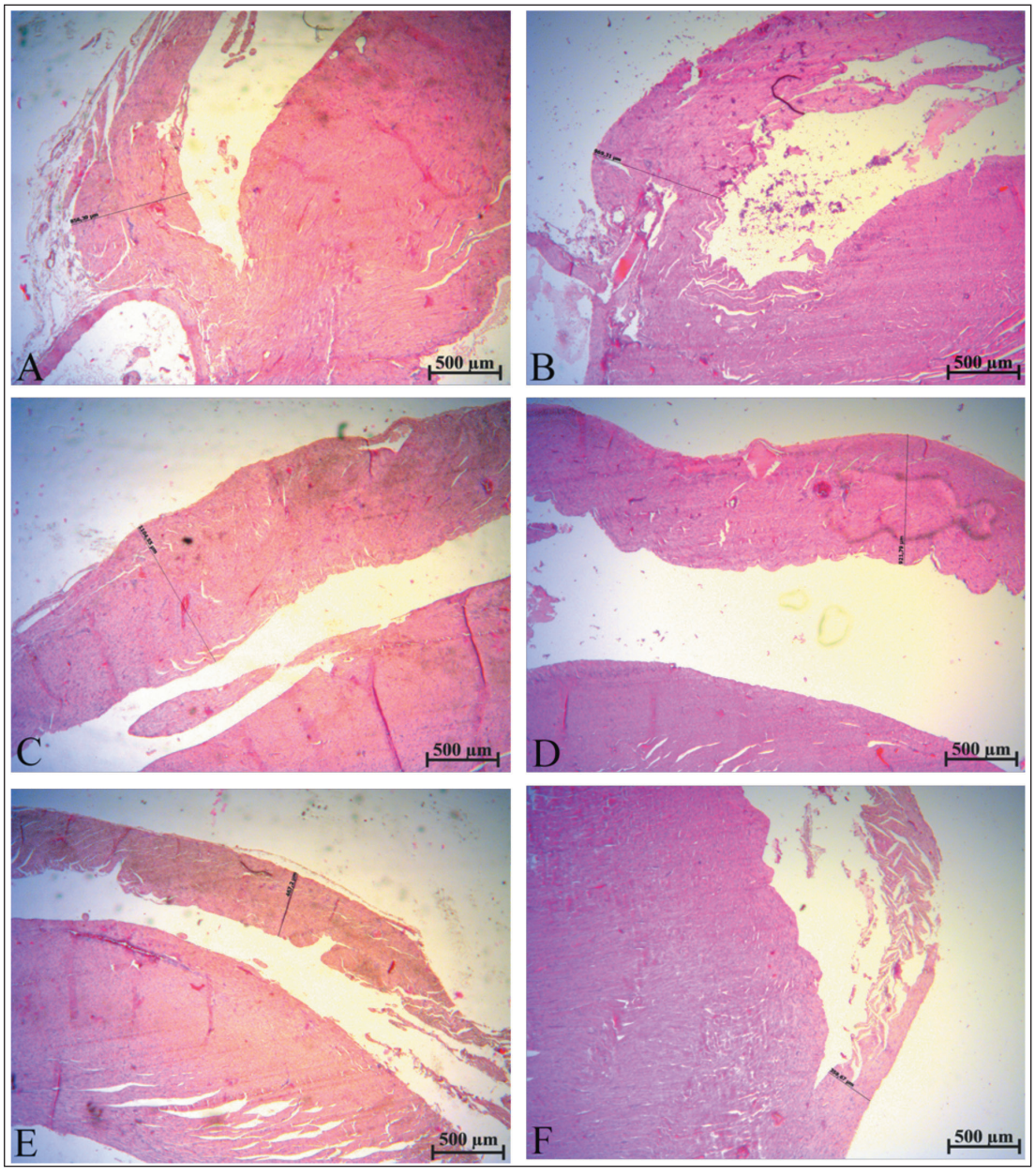


Figure 2: Histomorphometric images of aged and young adult rat right ventricles. **A,B)** right basal, **C,D)** middle right, **E,F)** right apical. **A,C,E)** aged group, **B,D,F)** young adult group. Scale bar: 500 µm.

As a result of immunohistochemical staining performed to show occludin protein levels in the endothelial cells of the heart and aortic tissues, weak immunostaining was observed in the endothelial cells of both the aorta and heart tissue of elderly subjects, indicating a decrease in the occludin protein level (Figure 6)

DISCUSSION

This study investigates the comparison of young adult and aged female rat groups in terms of body composition and histomorphometric measurements of the heart and aorta. Results show that aged rats have higher body weight and heart weight than young adults. This difference may reflect the effects of ageing on metabolism and the cardiovascular system. Aging is usually accompanied by a decrease in physical activity levels and a decrease in metabolic rate, leading to an increase in body weight (13). Increased body weight in aged rats may be attributed to decreased energy expenditure and changes in nutrient metabolism (14,15).

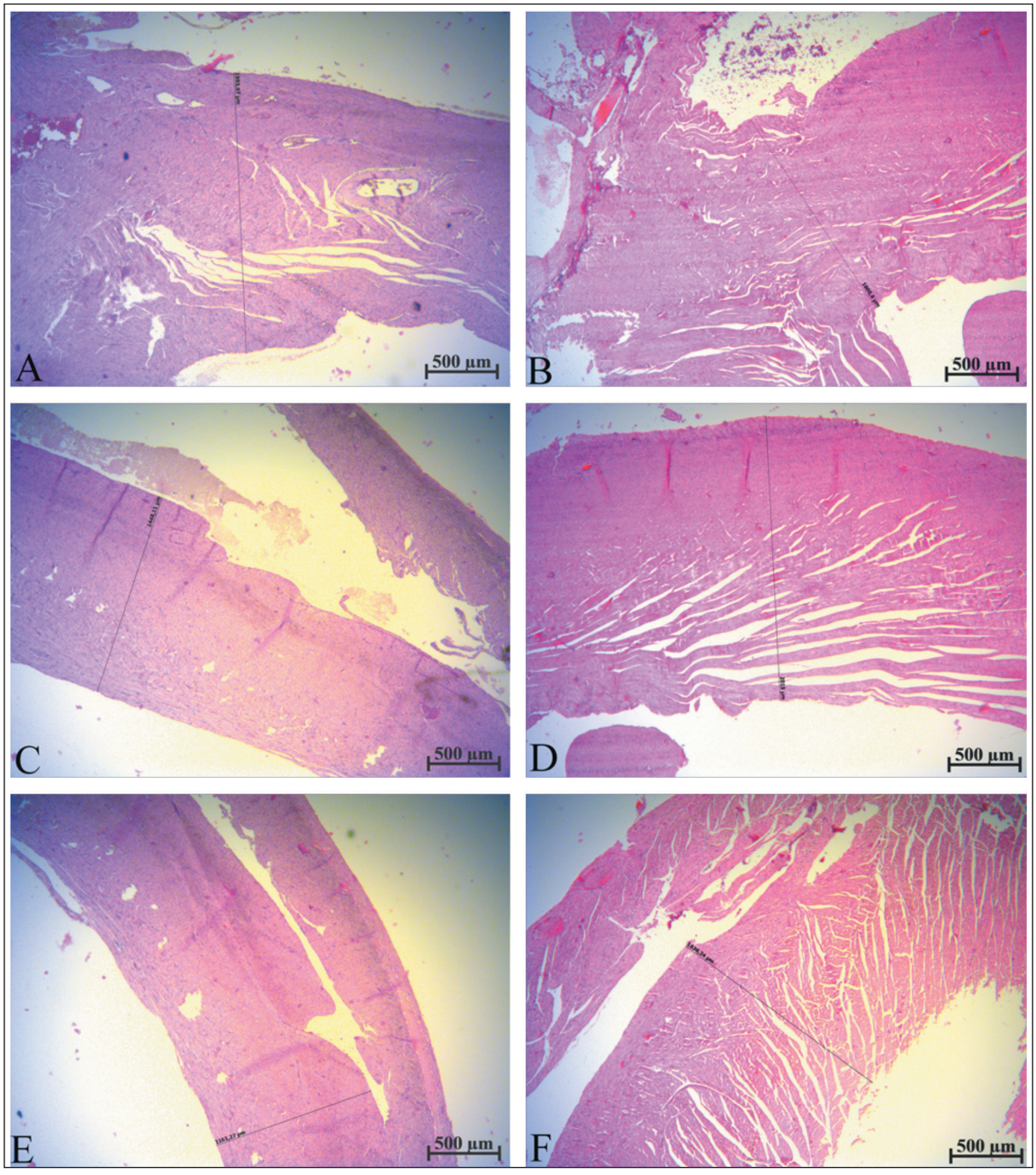


Figure 3: Histomorphometric images of aged and young adult rat septum interventriculare. **A,B)** septum basal, **C,D)** septum middle, **E,F)** septum apical. **A,C,E)** aged group, **B,D,F)** young adult group. Scale bar: 500 µm.

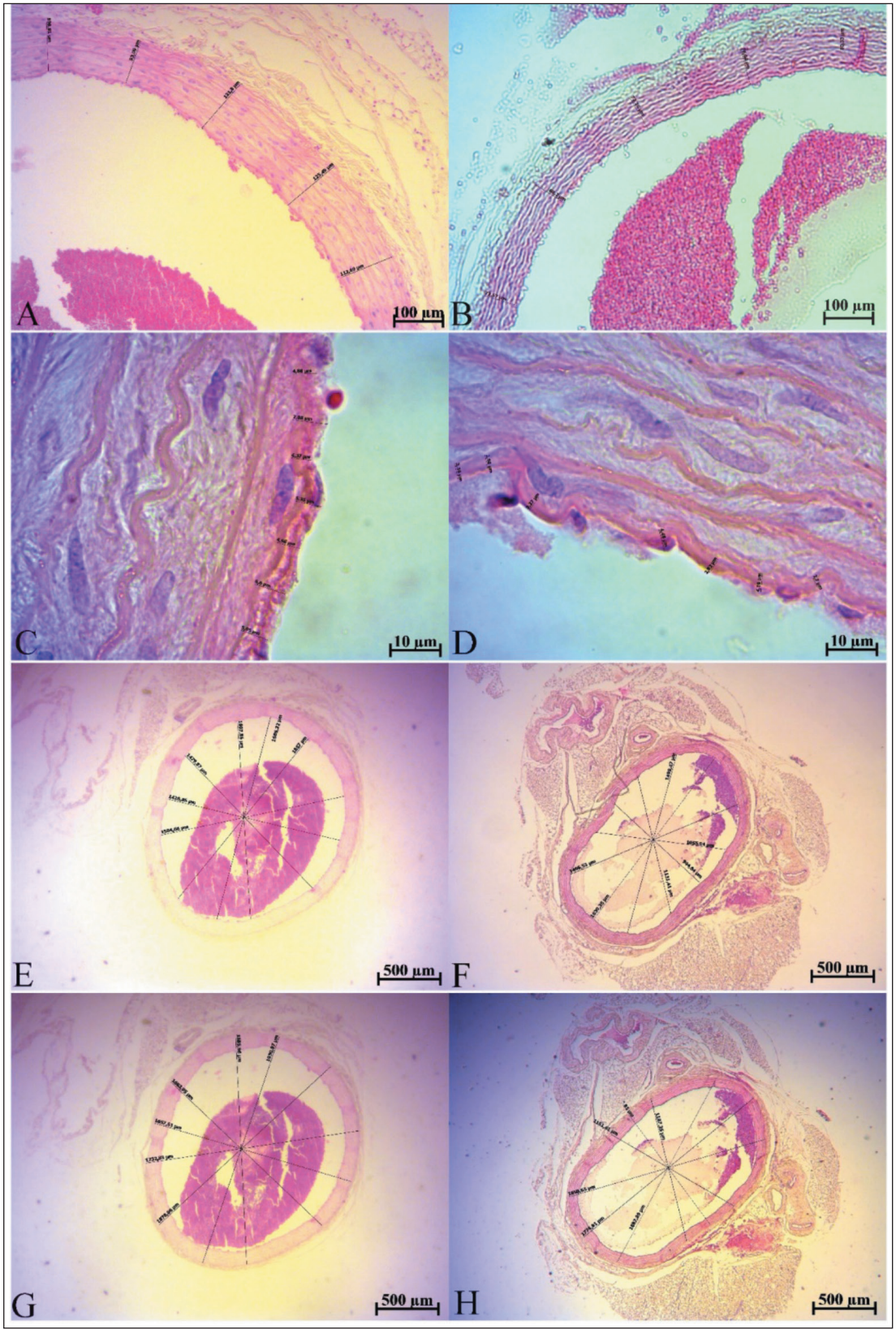


Figure 4: Histomorphometric images of aged and young adult rat aortas. **A,B)** Tunica media, **C,D)** Tunica intima, **E,F)** Lumen diameter, **G,H)** Total diameter. **A,C,G,E)** Aged group; **B,D,H,F)** young adult group. Scale bar: **A,B)** 100 μm, **C,D)** 10 μm, **E,F,G,H)** 500μm.

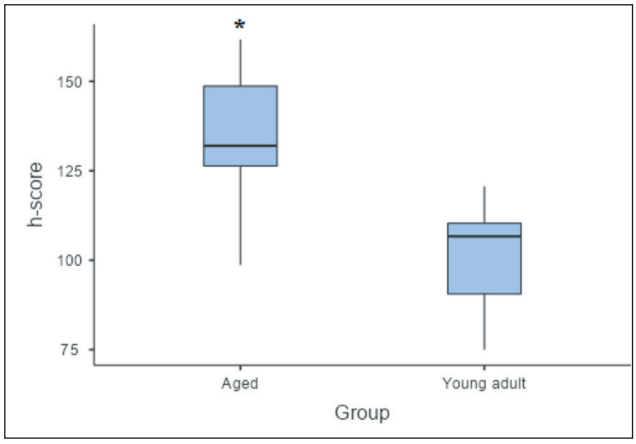


Figure 5: Comparison of h-score values obtained as a result of immunohistochemical staining performed to determine iNOS protein levels in aortic tissues of aged and young adult rats. Values are given as median (min-max). *p<0.05.

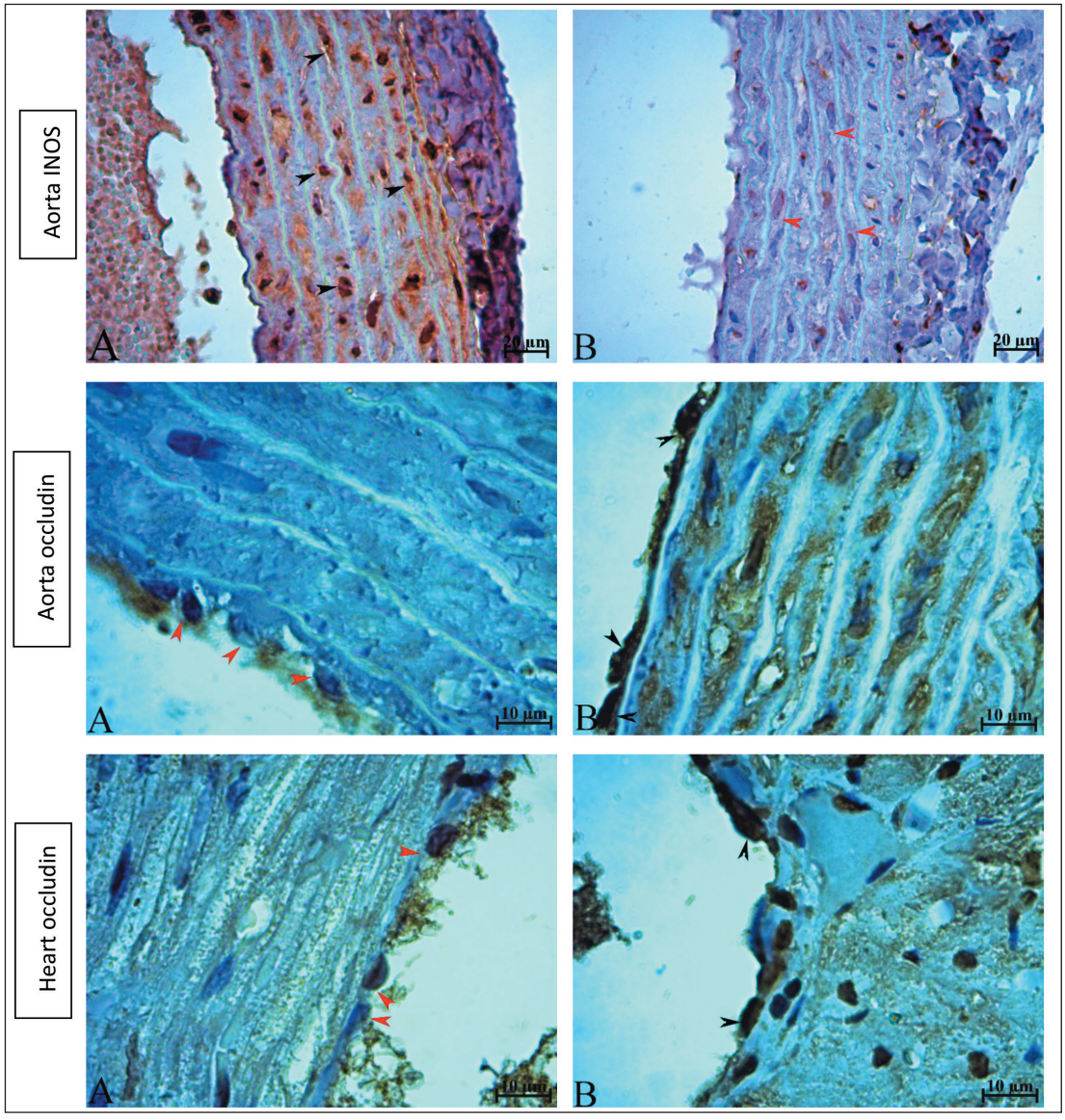


Figure 6: Immunohistochemical staining results for iNOS protein in aortic tissues and occludin protein in heart and aorta tissues in aged and young adult groups. Cells showing distinct staining are marked with a black arrowhead. Weakly stained or unstained cells are marked with a red arrowhead. **A)** Aged, **B)** Young adult.

Histomorphometric measurements revealed significant differences in the structure of the arterial wall between young adult and aged groups. Significant increases in parameters such as tunica media and intima thickness, lumen diameter and total diameter were observed in aged rats compared to young adults. In studies related with tunica intima-media thickness, it was reported that tunica intima and media thickness increased with aging (16,17). However, some studies have suggested that intimal-medial thickness is mainly the result of intimal thickening (18), whereas other studies have found that normal vascular ageing causes more medial thickening in the absence of atherosclerosis (19). These differences may be due to analyses of different regions of the aorta. Histomorphometric evaluations provide important information to better understand the biological basis of these differences. Stefanadis et al. reported a significant increase in the thickness of the tunica media of the aorta in elderly individuals, which is one of the main changes caused by the aging process in the arterial wall structure (20). Guzik and Touyz reported that this increase is directly linked to atherosclerosis and may be an indicator of increased cardiovascular risk with age (21). At the same time, North and Sinclair suggested that aging accelerates arterial wall thickening and loss of elasticity, facilitating the formation of atherosclerotic plaques and thus playing an important role in the pathogenesis of cardiovascular diseases (22). In these studies, it has been reported that elastin and collagen fibres, which are structural components of the arterial wall, decrease markedly with age, resulting in a stiffer and more fragile arterial wall. At the same time, proliferation and migration of vascular smooth muscle cells and increased accumulation of extracellular matrix (ECM) have been reported to be one of the main causes of arterial wall thickening. These biological processes reduce the elasticity of the arterial wall, increase arterial stiffness and consequently lead to haemodynamic imbalances that play an important role in the regulation of blood pressure. In addition, statistically significant differences were observed in Tunica media/Lumen diameter, Tunica intima/Lumen diameter, Tunica media/Total diameter and Tunica intima/Total diameter ratios between young adult and elderly groups. These ratios indicate that aging has a specific age-related effect on arterial wall remodelling. These changes may be an important predisposition for the development of cardiovascular diseases such as arterial stiffness and stenosis. In particular, increased tunica media thickness may be an indicator of arterial stiffness, which may be associated with decreased cardiovascular function with age (19). These findings play a critical role in understanding the effects of normal vascular aging processes on arterial remodelling, and in this context, histomorphometric analyses have emerged as an important tool for evaluating the effects of aging on cardiovascular risk.

When the wall thicknesses of the ventricles and septum interventricular were analysed, no statistically significant difference was found between the groups. However, when the left ventricular thickness/heart weight ratio was analysed, a statistically significant difference was found between the young adult and elderly groups. Although there was no significant difference in terms of wall thickness, we think that the significant differences in left ventricular thickness/heart weight ratio may indicate left ventricular dilatation and this may indicate an increase in preload, although this study is not a physiological study. It is thought that future physiological studies may reveal the cause of the current situation.

Immunohistochemical staining results showed a statistically significant difference in iNOS protein levels between the young adult and elderly groups. This suggests that morphological and functional changes may occur in aortic tissues of elderly individuals compared with young adults. iNOS protein is known to play a role in the regulation and remodelling of cardiovascular function (23). Studies have shown that aging may lead to progressive aortic stiffening and changes in vascular smooth muscle cells and changes in elastin content contribute to aortic stiffening (24,25). Higher iNOS protein levels in the elderly group have been associated with cardiovascular ageing processes and may indicate a potential increased risk of cardiac remodelling and dysfunction in elderly individuals. Furthermore, immunohistochemical staining to demonstrate occludin protein levels in endothelial cells of heart and aortic tissues revealed weak immunostaining in endothelial cells of both aortic and heart tissues of elderly individuals, indicating a decrease in occludin protein levels. Occludin protein plays a critical role in the maintenance of tight junctions in endothelial cells and it has been suggested that a decrease in the level of this protein may contribute to increased vascular permeability and endothelial dysfunction with the aging process (26,27). This decrease in occludin expression may be associated with impaired vascular wall integrity and consequently increased cardiovascular risk. Studies have also shown that iNOS plays a role in cardiovascular pathophysiology, with increased iNOS expression contributing to cardiac dysfunction and remodelling in conditions such as heart failure and myocardial infarction (23,28). iNOS expression has been associated with metabolic remodelling, cytokine production and proinflammatory cascades within macrophages, all of which may affect cardiovascular health and function (29). We believe that these findings emphasise the importance of considering age-related changes in cardiac function and remodelling in the development and treatment of cardiovascular diseases in elderly individuals.

In conclusion, this study provided valuable information on histomorphometric changes in heart and aortic tissue as a consequence of aging. Our findings show that ageing leads

to significant changes in heart weight, heart weight/body weight ratio and left ventricular thickness/heart weight ratio. These changes suggest that ageing may increase the risk of cardiac remodelling and dysfunction, consistent with previous research. Our analysis of aortic tissue also revealed significant changes in tunica media thickness, tunica intima thickness, lumen diameter and overall diameter. These findings suggest that aging may also lead to structural and functional changes in the aorta, which may contribute to the development of cardiovascular diseases. Differences in iNOS protein levels in aortic tissues of young adult and aged rats further support this hypothesis. Higher iNOS protein levels in the elderly group suggest a potential increased risk of cardiac remodelling and dysfunction in elderly individuals. This study may be an important small step towards understanding the complex effects of aging on the structure and function of the cardiovascular system. However, further research is needed on how these findings can be applied in clinical practice and how potential strategies for the prevention or treatment of cardiovascular diseases during the ageing process can be developed. This study may provide an important basis for identifying cardiovascular risk factors associated with ageing and developing more effective measures for ageing populations.

Acknowledgment

None.

Author Contributions

Concept, design, data collection, analysis and interpretation: Mete Keçeci, Furkan Bodur, Cenk Murat Özer, Osman Cengil, Literature search: Mete Keçeci, Cenk Murat Özer, Esra Babaoğlu, Writing: Mete Keçeci, Furkan Bodur, Cenk Murat Özer, Esra Babaoğlu, Approval: Cenk Murat Özer

Conflicts of Interest

No conflict of interest is reported by authors.

Financial Support

None to declare.

Ethical Approval

Ethics committee approval numbered 2023-16-07/09 was obtained from Zonguldak Bulent Ecevit University Animal Experiments Local Ethics Committee for the study.

Review Process

Extremely and externally peer-reviewed and accepted.

REFERENCES

1. Bruce R Troen. The biology of aging. Mt Sinai J Med. 2003;70(1):3-22.
2. Strait JB, Lakatta EG. Aging-Associated Cardiovascular Changes and Their Relationship to Heart Failure. Heart Fail Clin. 2012 January;8(1):143-64.
3. North BJ, Sinclair DA. The Intersection Between Aging and Cardiovascular Disease. Circ Res. 2012 April 13;110(8):1097-108.
4. Bentov I, Reed MJ. The effect of aging on the cutaneous microvasculature. Microvasc Res. 2015 July;100:25-31.
5. Özerkan Çakan F. Changes in cardiovascular physiology in the elderly. Turk Kardiyol Dernegi Arsivi-Arch Turk Soc Cardiol [İnternet]. 2017 [a.yer 23 Mart 2024]; Erişim adresi: https://archivestsc.com/jvi.aspx?un=TKDA-89856
6. Fonseca DA, Antunes PE, Antunes MJ, Cotrim MD. Histomorphometric analysis of the human internal thoracic artery and relationship with cardiovascular risk factors. Pizzi C, editör. PLOS ONE. 25 Ocak 2019;14(1):e0211421.
7. Sengupta, Pallav. The laboratory rat: relating its age with human's. International journal of preventive medicine, 2013, 4.6: 624.
8. Borges RSDM, Medeiros ADM, Silva JGD, Paiva RRLT, Costa HDS, Oliveira MFD, vd. Morphometry of the heart of greater rhea (Rhea americana americana , Linnaeus, 1758). Anat Histol Embryol. 2021 March;50(2):345-9.
9. Altaweel R, Shatarat A, Badran D, Abu Tarboush NM. The effects of irisin on the rat thoracic aorta: a histological study. Folia Morphol. 2022 December 08;81(4):923-30.
10. Inan M, Uz YH, Kizilay G, Topcu-Tarladacalisir Y, Sapmaz-Metin M, Akpolat M, vd. Protective effect of sildenafil on liver injury induced by intestinal ischemia/reperfusion. J Pediatr Surg. 2013 August;48(8):1707-15.
11. Mompeó-Corredera B, Hernández-Morera P, Castaño-González I, Quintana-Montesdeoca MDP, Mederos-Real N. Regions of the human renal artery: histomorphometric analysis. Anat Cell Biol. 2022 September 30 ;55(3):330-340.
12. Miura K. Tunica intima compensation for reduced stiffness of the tunica media in aging renal arteries as measured with scanning acoustic microscopy. Yu J, editör. PLOS ONE. 2020 November 04;15(11):e0234759.
13. Westerterp KR. Changes in physical activity over the lifespan: impact on body composition and sarcopenic obesity. Obes Rev. 2018 December;19(S1):8-13.
14. Miyasaka K, Ichikawa M, Kawanami T, Kanai S, Ohta M, Sato N, vd. Physical activity prevented age-related decline in energy metabolism in genetically obese and diabetic rats, but not in control rats. Mech Ageing Dev. 2003 February;124(2):183-90.
15. Rising R, Lifshitz F. Energy expenditures & physical activity in rats with chronic suboptimal nutrition. Nutr Metab. 2006 December;3(1):11.
16. Nagai Y, Metter EJ, Earley CJ, Kemper MK, Becker LC, Lakatta EG, vd. Increased Carotid Artery Intimal-Medial Thickness in Asymptomatic Older Subjects With Exercise-Induced Myocardial Ischemia. Circulation. 1998 October 13 ;98(15):1504-9.
17. Virmani R, Avolio AP, Mergner WJ, Robinowitz M, Herderick EE, Guo SY, vd. Effect of Aging on Aortic Morphology in Populations with High and Low Prevalence of Hypertension and Atherosclerosis. 1991;139(5).
18. Laurent S. Defining vascular aging and cardiovascular risk. J Hypertens. 2012 June ;30:S3-8.

19. Xu X, Wang B, Ren C, Hu J, Greenberg DA, Chen T, vd. Age-related Impairment of Vascular Structure and Functions. Aging Dis. 2017;8(5):590.
20. Stefanadis C, Karayannacos PE, Boudoulas H, et al. Age-related changes in the aortic root structure and function in normal humans. J Am Coll Cardiol. 2014;20(5):1044-1050. doi:10.1016/S0735-1097(02)02159-8.
21. Guzik TJ, Touyz RM. Oxidative stress, inflammation, and vascular aging in hypertension. Hypertension. 2017;70(4):660-667. doi:10.1161/HYPERTENSIONAHA.117.07887.
22. North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. Circ Res. 2012;110(8):1097-1108. doi:10.1161/CIRCRESAHA.111.246876.
23. Soskic SS. Regulation of Inducible Nitric Oxide Synthase (iNOS) and its Potential Role in Insulin Resistance, Diabetes and Heart Failure. Open Cardiovasc Med J. 2011 July 07 ;5(1):153-63.
24. Radu-Ionita F, Ţintoiu IC, Rosu A, Bontas E, Cochior D, Bolohan R, vd. Aging Aorta—Cellular Mechanisms. Içinde: New Approaches to Aortic Diseases from Valve to Abdominal Bifurcation [Internet]. Elsevier; 2018 [a.yer 2024 March 25]. s. 3-23. Erişim adresi: https://linkinghub.elsevier.com/retrieve/pii/B9780128099797000018
25. De Moudt S, Hendrickx JO, Neutel C, De Munck D, Leloup A, De Meyer GRY, vd. Progressive aortic stiffness in aging C57Bl/6 mice displays altered contractile behaviour and extracellular matrix changes. Commun Biol. 2022 June 17 ;5(1):605.
26. Lee JG, Takemoto DJ. Role of claudin-5 in blood-brain barrier integrity during aging. Neurobiol Aging. 2021;107:196-203.
27. Jia W, Lu R, Yin L, et al. The role of tight junctions in cardiovascular diseases: from mechanism to clinical manifestation. J Cardiovasc Transl Res. 2022;15(3):570-581.
28. Feng Q, Lu X, Jones DL, Shen J, Arnold JMO. Increased Inducible Nitric Oxide Synthase Expression Contributes to Myocardial Dysfunction and Higher Mortality After Myocardial Infarction in Mice. Circulation. 2001 August 07 ;104(6):700-4.
29. Muniyappa R, Sowers JR. Role of nitric oxide and oxidative stress in cardiovascular disease. Curr Hypertens Rep. 2013;15(5):408-415.