SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

The Role of Plant-Derived Bioactive Compounds and Physical Activity in the Management of Cardiac Dysfunction

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1. Introduction and Aims

Despite the dynamic progress of medical science, cardiovascular diseases remain the leading cause of death worldwide. Regarding the etiology of these conditions, beyond genetically inherited and thus non-modifiable factors, numerous secondary risk factors are well known, many of which could be significantly reduced. A sedentary lifestyle, hypertension, hyperlipidemia, diabetes mellitus, smoking, and excessive alcohol consumption represent modifiable risk factors at the individual level. When identified at an early stage, lifestyle modification, cessation of harmful habits, and the introduction of regular physical exercise may prove sufficient; however, in more advanced cases, pharmacological interventions may be required. In recent years, cardiovascular research has increasingly focused on the investigation of natural compounds, particularly anthocyanins, which are abundant in dark-colored berries. This has been prompted by evidence confirming the significant antioxidant and anti-inflammatory potential, suggesting a possible preventive and therapeutic role.

Advancements in drug research have substantially contributed to increased life expectancy, which rose from 66.2 years in 2000 to 73.3 years in 2024. The demographic aging observed today is not only a result of declining mortality rates and improved survival, but also of decreasing fertility rates and, consequently, fewer births. Increased lifespan and reduced mortality can only be regarded as true progress if these additional years are lived in good health. “Healthy aging” is a multidimensional concept that extends beyond the absence of clinical disease to include preserved cognitive, affective, and social functions. A substantial body of evidence indicates that regular physical activity in older age is one of the most important lifestyle factors for maintaining good health, with pronounced positive effects on physical fitness, flexibility, aerobic capacity, mobility, balance, and the slowing of both mental and cognitive decline.

From a cardiovascular perspective, both unhealthy lifestyle habits and aging can lead to cardiac dysfunction, eventually progressing to heart failure. One of the major current challenges in research is the identification of natural therapies with significant preventive and therapeutic efficacy, thereby improving quality of life and stabilizing health status.

Based on the above, the aims of our study were as follows:

1. In a rabbit model of hypercholesterolemia-induced cardiovascular alterations, to detect these pathological changes, to administer long-term oral anthocyanin treatment, and to evaluate the effects of the applied therapy on atherosclerosis, vascular status, cardiac

functional parameters, and the myocardial HO-1 and eNOS–PKG–SERCA2a signaling pathways.

2. In an aged rat model, to compare—by echocardiographic methods—the effects of voluntary versus forced physical exercise on aging-associated cardiac dysfunction, and, at the molecular level, to analyze the expression of proteins involved in the PKG–STAT3–Opa1 pathway promoting mitochondrial fusion, as well as ATP synthase activity, under the different exercise conditions.

2. Materials and Methods

2.1. Ethical guidelines

Following the arrival of the animals, a 2-week adaptation period was provided. All experiments were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The laboratory animals were treated humanely and all experimental procedures were registered by the Animal Welfare Committee of the University of Debrecen (4/2022/DEMÁB; 3/2022/DEMÁB) and approved by the Food Chain Safety and Animal Health Department of the Agricultural Directorate of the Hajdú-Bihar County Government Office.

2.2. Experimental protocols

2.2.1. Investigation of the PCE treatment in a HC rabbit model

At the beginning of the experiment, following a 2-week adaptation period, the three-year-old male rabbits (Juráskó Kft., Debrecen, Hungary) were randomly assigned into two groups: (1) healthy Control group maintained on a standard rabbit diet; and (2) hypercholesterolemic (HC) group, in which atherosclerosis and the resulting cardiac dysfunction were induced as follows: animals were fed, intermittently, an "atherogenic" diet enriched with 1% cholesterol and 1% triglyceride (Pro Drug Bt., Debrecen, Hungary) for 3 weeks, followed by a standard diet for 1 week; this feeding cycle was repeated 8 times. The development of the diseased animal model required a total of 32 weeks.

After that, each of the two groups was further divided, resulting in the final experimental design with four treatment groups (n = 7 per group): (1) Control: animals maintained on standard diet throughout the entire experimental period; (2) C+PCE: rabbits receiving standard diet supplemented with 9 g/kg Prunus cerasus extract (PCE); (3) HC: animals fed an atherogenic diet; (4) HC+PCE: rabbits receiving an atherogenic diet supplemented with 9 g/ kg PCE. PCE (Prunus cerasus extract, sour cherry extract) treatment was administered for 12 weeks as follows: the calculated dose was dissolved in 200 mL of tap water and placed in the drinking bottle in the evening. The following day, after the entire solution had been consumed, the bottles were refilled with fresh water.

At the endpoint of the experiment, blood samples were collected from the marginal ear vein of the rabbits, followed by echocardiographic measurements under ketamine/xylazine anesthesia (35/3 mg/kg, i.m.). Subsequently, under deep anesthesia, thoracotomy was performed, and the distal part of the thoracic aorta was excised for *ex vivo* vascular studies. The

remaining part of the thoracic aorta was placed in 10% formalin for later histological analysis, while left ventricular samples were frozen in liquid nitrogen and then stored at - 80 °C for Western blot analysis.

2.2.2. Investigation of the effects of physical activity in an aging rat model

In our second experiment, 9 young (12-week-old) and 36 aging (18-month-old) male Wistar rats were used. Following a 2-week acclimatization period after arrival, the animals were assigned into four groups: (1) Young control group (n = 9); (2) Aged control group (n = 12): 18-month-old sedentary animals that remained inactive and did not perform any physical activity throughout the study; (3) Aged voluntary group (n = 12): 18-month-old rats housed in cages equipped with a running wheel (Lafayette Instrument Company, North Lafayette, IN, USA), which they could use 24 hours a day for a period of 6 months according to their needs; (4) Aged forced exercise group (n = 12): 18-month-old rats subjected to a predetermined speed and duration of running using an electronically controlled device, the Walking Wheel System for Rats (Lafayette Instrument Company, North Lafayette, IN, USA), for 6 months on a daily basis, except for Sundays. During the study, in one of the animal facility rooms, the 12:12-hour light–dark cycle was reversed, allowing the nocturnal rats to perform the forced exercise during their active (dark) phase.

The forced exercise protocol began with a habituation phase to familiarize the rats with the running wheel, thereby excluding those unsuitable for the exercise regimen and minimizing stress. Initially, the animals spent 5 minutes in the wheels, after which the system was started at 5 m/min. The speed was then increased by 0.5 m/min each day until the speed of 13 m/min, as recommended in the literature, was reached. Subsequently, the running duration was gradually extended, with a daily increase of 1 minute until the target 20-minute length was achieved.

At the end of the 6-month physical activity period, the aged rats were 24 months old, while the young animals were terminated at 12 weeks of age. At the endpoint of the experiment, echocardiographic measurements were performed under light anesthesia (ketamine/xylazine 50/5 mg/kg, i.m.). Thoracotomy was conducted under deep anaesthesia (100/10 mg/kg ketamine/xylazine, intramuscular injection), followed by blood sample collection via cardiac puncture. Left ventricular tissue samples were frozen in liquid nitrogen and stored at - 80 °C for Western blot analysis and ATP synthase activity measurement, or fixed in 10% neutral buffered formalin for later histological processing. The body weight of all animals was recorded weekly throughout the study.

2.3. Anthocyanins

PCE was produced at the Institute of Food Technology, University of Debrecen. It was prepared from the Hungarian sour cherry cultivar *Érdi bőtermő*, harvested between June and July 2020. The main anthocyanin components of PCE were cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-glucosyl-rutinoside, while several phenolic compounds were present only in negligible amounts.

2.4. Echocardiography

Transthoracic echocardiography was performed in both experimental models. Animals were anesthetized with i.m. ketamine/xylazine combination (rabbit: 35/3 mg/kg; rat: 50/5 mg/kg). After the chest hair removal, the animals were placed into dorsal and lateral decubitus positions with continuous ECG monitoring. Measurements were performed using a GE Vivid E9 system (GE Healthcare, New York, NY, USA). The acquisition protocol followed that used in human studies, in accordance with the recommendations of the American Society of Echocardiography. Firstly, in the parasternal long-axis (PLAX) view, 2D and M-mode imaging were used to determine the anteroposterior diameter of the aortic root and the left atrium, followed by the assessment of the following left ventricular parameters at the level of the papillary muscles: ejection fraction, fractional shortening, left ventricular internal diameters in systole and diastole, and interventricular septal and posterior wall thicknesses in systole and diastole. For greater accuracy, these measurements were repeated in the short-axis (SAX) view. Subsequently, from the apical four-chamber view, the mitral inflow pattern and its early (E) and late atrial (A) components were recorded using pulsed wave (PW) Doppler. From the same view, tissue Doppler imaging (TDI) was used to measure septal and lateral mitral annular velocities: peak systolic velocity (s'), early diastolic (e'), and late diastolic (a') velocities. Using M-mode, the mitral annular plane systolic excursion (MAPSE) and tricuspid annular plane systolic excursion (TAPSE) were determined. From the apical five-chamber view, Doppler was used to measure left ventricular outflow tract (LVOT) velocity and pressure gradients.

All echocardiographic recordings were stored on an external hard drive and analyzed later using EchoPAC PC software (version 112, GE Healthcare, New York, NY, USA). For each parameter, measurements from five consecutive cardiac cycles per animal were averaged. Additional parameters were derived during post-processing: from the mitral inflow pattern the E/A ratio and deceleration time (DecT); from TDI the e'/a' ratio, ejection time, time interval from mitral valve closure to opening, isovolumetric contraction time (IVCT) and isovolumetric

relaxation time (IVRT). Furthermore, the E/e' ratio was calculated to estimate left ventricular filling pressure and the Tei index was determined as a measure of global myocardial performance. In the first experiment, global longitudinal strain (GLS) was also assessed from the apical view using speckle-tracking echocardiography (STE).

2.5. Serum parameters

At the end of both experimental protocols and additionally prior to the 12-week treatment period in the rabbit model, blood samples were collected from the animals into BD Vacutainer SST II Advance tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). In rabbits, samples were obtained from the marginal ear vein, whereas in the rat experiment, following thoracotomy, blood was drawn from the left ventricle. The samples were analyzed at the Department of Laboratory Medicine, Clinical Centre, University of Debrecen. The following parameters were determined: total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP), glucose, urea, and creatinine. In the rabbit model, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and creatine kinase-MB (CK-MB) levels were also measured. Serum parameters were determined using automated analyzers (F. Hoffmann-La Roche AG, Basel, Switzerland).

2.6. *Ex vivo* vascular studies

In the hypercholesterolemic rabbits, following thoracotomy, the distal part of the thoracic aorta was excised and immediately placed into oxygenated Krebs solution. The adventitia was carefully removed and four 2-mm-wide vascular rings were prepared from each animal. The rings were mounted horizontally under a resting tension of 10 mN in vertical organ baths (Experimetria TSZ-04, Experimetria Ltd., Budapest, Hungary) containing 10 ml Krebs solution, continuously aerated with a gas mixture of 95% O_2 and 5% CO_2 at 36°C and pH 7.4. Isometric contractile force of the vascular smooth muscle was recorded using a force transducer (SD-01; Experimetria Ltd., Budapest, Hungary) connected to SPEL Advanced IsoSys software (SOFT 02, version 2.9; MDE Ltd., Budapest, Hungary).

After a 60-minute equilibration period, concentration-response (E/c) curves to norepinephrine were obtained (1 nmol/l to 10 μmol/l), and half-maximal effective concentrations (EC_{50}) were calculated for each ring. Following a 60-minute washout period, vessels were pre-contracted using norepinephrine at the EC_{50} concentration until contraction

plateaued. Subsequently, acetylcholine-induced relaxation responses were measured by constructing E/c curves (1 nmol/l to 1 mmol/l).

Responses from the aortic rings derived from the same animal were averaged. Norepinephrine-induced contractile responses were expressed as maximal increases in vascular tension relative to the 10 mN resting tension. Acetylcholine-induced vasorelaxation was quantified as the percentage decrease in vascular tension from the norepinephrine pre-contracted state; any contraction observed during acetylcholine administration was recorded as a negative relaxation with its maximum value noted.

2.7. Histology

To detect tissue alterations, various histological stainings were performed. Excised tissue samples were fixed in 10% neutral buffered formalin (pH 7.4) for 24 hours. After that, the samples were rinsed under running tap water for 1 hour and stored in 70% ethanol until further processing. During processing, the samples were dehydrated through an ascending ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5 μm thickness were cut from paraffin blocks using a manual rotary microtome (Leica Biosystems, Nussloch, Germany).

In the hypercholesterolemic rabbit model, hematoxylin and eosin (H&E) staining was used to measure the thickness of the tunica intima and tunica media in aortic sections, from which the average intima/media ratio was calculated (n = 5 per group).

For assessment of fibrotic changes in the left ventricle of rats, Masson's trichrome staining was applied. Perivascular fibrosis ratio (PFR) was determined in five coronary arteries with lumen diameters larger than 50 μm per animal, calculated as the ratio of fibrotic area surrounding the vessel wall to the total vessel area (n = 5 per group).

Stained sections were visualized using a Nikon Eclipse 80i microscope (Nikon Corp., Tokyo, Japan) equipped with Nikon NIS-Elements BR software (ver. 5.41.00; Nikon Corp., Tokyo, Japan).

2.8. Tissue homogenization

In both experimental models, protein expression and ATP synthase (ATPS) activity (in the rat model) were determined from left ventricular myocardial tissue samples. For protein isolation, 300 mg of frozen tissue was homogenized using a blade homogenizer in a lysis buffer containing: 25 mM Tris, 25 mM NaCl, 1 mM sodium orthovanadate, 10 mM sodium fluoride,

10 mM sodium pyrophosphate, 10 nM okadaic acid, 0.5 mM EDTA, 1 mM PMSF, protease inhibitor cocktail and distilled water. The homogenates were centrifuged at 2000 rpm for 10 minutes at 4 °C. The resulting supernatant contained cytosolic and mitochondrial proteins, while the pellet represented the nuclear fraction. All samples were incubated on ice for 1 hour in a homogenization buffer supplemented with 0.1% Triton X-100 (Sigma-Aldrich-Merck KGaA, Darmstadt, Germany). Following a second centrifugation at 14,000 rpm for 10 minutes at 4 °C (after pre-centrifugation of cytosolic + mitochondrial fractions at 10,000 rpm for 20 minutes at 4 °C), the supernatants were aliquoted into three parts: (1) 10 µL of the samples were used for total protein concentration measurement by BCA assay (QuantiPro BCA Assay Kit, Sigma-Aldrich-Merck KGaA, Darmstadt, Germany) using a spectrophotometer (FLUOstar Optima, BMG Labtech, Ortenberg, Germany); (2) 50 µL of the remaining volume was diluted 1:1 with Laemmli buffer (Sigma-Aldrich-Merck KGaA, Darmstadt, Germany); (3) the remaining aliquots were stored at - 80 °C until further analysis.

2.9. Western blot

The calculated loading volumes of the Laemmli-diluted samples were loaded onto 12% SDS-polyacrylamide gels and proteins were separated based on their molecular weight by electrophoresis. Then, proteins were transferred onto nitrocellulose (rabbit experiment) or PVDF (rat experiment) membranes at 25 V for 90 minutes. Membranes were blocked for 60 minutes at room temperature with TBS-T containing 5% BSA to block nonspecific binding sites. Next, membranes were incubated overnight at 4 °C with the following primary antibodies: anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, housekeeping protein; Cat. No: G8795); anti-endothelial nitric oxide synthase (eNOS; Cat. No: ab5589); anti-protein kinase G (PKG; Cat. No: ab97339); anti-sarco/endoplasmic reticulum calcium ATPase 2a (SERCA2a; Cat. No: ab2817); anti-heme oxygenase-1 (HO-1; Cat. No: SAB2108676); anti-histone H3 (housekeeping protein; Cat. No: ab176842); anti-protein kinase G (PKG; Cat. No: ab110124); anti-signal transducer and activator of transcription 3 (STAT3; Cat. No: ab109085); anti-optic atrophy 1 (Opa1; Cat. No: ab42364); and anti-ATP synthase (ATPS; Cat. No: ab181243). Antibodies were obtained from Abcam (Cambridge, UK) or from Sigma-Aldrich-Merck (Darmstadt, Germany). The following morning, membranes were washed 3 times for 10 minutes each with TBS-T, then incubated for 45 minutes at room temperature with appropriate HRP-conjugated anti-rabbit or anti-mouse secondary antibodies. After another 3x10 minute washing with TBS-T, protein bands were visualized using ECL substrate (WesternBright™,

ECL, Advansta Inc., San Jose, CA, USA) and imaged with a LiCor C-Digit® blot scanner (LI-COR Inc., Lincoln, NE, USA). Densitometric analysis of the blots was performed using Image Studio Digits software (ver. 5.2; LI-COR Inc., Lincoln, NE, USA) and protein expression levels were normalized to the housekeeping proteins (GAPDH or Histone H3) detected in the same samples. Results represent the average of three independent experiments, each performed on samples from three animals per group.

2.10. ATPS activity measurement

In the rat experiment, left ventricular ATP synthase (ATPS, also known as Complex V) activity was measured using the ATP Synthase Activity Assay Kit (ab109716; Abcam, Cambridge, United Kingdom). Myocardial samples were applied to a 96-well microplate provided by the manufacturer. During the 3-hour incubation period, the ATPS bound to the wells of the plate. The enzyme activity was assessed by monitoring the oxidation of NADH to NAD+, which was detected as a decrease in absorbance at 340 nm using a spectrophotometer (Varioskan LUX Multimode Microplate Reader; Thermo Fisher Scientific, Waltham, MA, USA). The enzyme activity was calculated as the ratio of the difference in absorbance values from the linear portion of the reaction curve to the elapsed time between these two points. ATP synthase activity was determined from myocardial samples obtained from five animals per group. Each sample was analyzed in triplicate and the results were averaged.

2.11. Statistical analysis

Data analysis was performed using GraphPad Prism software (version 10; GraphPad Software Inc., La Jolla, CA, USA). Gaussian distribution was assessed using the D'Agostino–Pearson test for the rabbit experiment and the Shapiro-Wilk test for the rat experiment. In the rabbit model, group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for normally distributed data. If the normality test was not passed, the Kruskal-Wallis test followed by Dunn's post hoc analysis was applied. For the rat study, comparisons between the young control and aged sedentary groups were performed using an unpaired t-test. Differences among the three aged groups (sedentary, voluntary and forced exercise) were analyzed by one-way ANOVA followed by Tukey's post hoc test. Results were considered statistically significant at $p < 0.05$. Data are presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Atherosclerotic rabbit model

3.1.1. Serum parameters

Total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels were significantly elevated in the hypercholesterolemic (HC) group compared to the control group (both $p < 0.0001$). A similar trend was observed for high-density lipoprotein cholesterol (HDL-C) levels (Control vs. HC: $p = 0.0022$). The plasma atherogenic index (AIP) was also significantly higher in the HC rabbits than in the healthy controls ($p < 0.0001$).

Treatment of the HC rabbits with PCE (HC+PCE) resulted in a significant improvement in the lipid profile compared to the untreated HC animals. Specifically, PCE significantly reduced total cholesterol ($p = 0.0013$) and LDL-C levels ($p < 0.0001$). Moreover, anthocyanin-treated HC rabbits exhibited significantly lower AIP values compared to the untreated HC counterparts ($p = 0.0028$).

No significant differences were observed in serum triglyceride levels among the experimental groups. The inflammatory marker C-reactive protein (CRP) concentration was approximately fourfold higher in the serum of the HC rabbits compared to the healthy controls, whereas it was reduced fourfold in the treated group relative to the untreated HC animals. Additionally, no significant changes were detected in the cardiac necroenzyme marker CK-MB levels. Blood glucose levels and liver enzymes (AST and ALT) also showed no significant differences across the groups.

A limitation of our study is that NT-proBNP levels remained below the detection limit in all treatment groups.

3.1.2. Echocardiography

Analysis of the echocardiographic data revealed that the HC rabbits exhibited signs of diastolic dysfunction compared to the control group. In the HC group, the left atrium to aortic root diameter ratio (LA/Ao) was significantly increased ($p < 0.0001$), while the deceleration time (DecT) and isovolumetric relaxation time (IVRT) were prolonged (DecT: $p < 0.0001$; IVRT: $p = 0.0148$). Moreover, both the E/A ratio and the e'/a' ratio were reduced (E/A: $p = 0.0234$; e'/a': $p = 0.0002$) relative to the healthy controls. The elevated E/e' ratio observed in the HC animals ($p < 0.0001$) indicated increased left ventricular filling pressures.

Significant improvements in diastolic function parameters were observed in the PCE-treated group (HC+PCE) compared to the untreated HC rabbits: left atrial enlargement was attenuated (LA/Ao ratio: $p < 0.0001$), the prolongation of DecT was reduced ($p < 0.0001$) and a significantly higher e'/a' ratio was detected via tissue Doppler imaging ($p = 0.0002$). Furthermore, the E/e' ratio was significantly decreased in the PCE-treated animals compared to the untreated hypercholesterolemic rabbits ($p < 0.0001$).

No significant differences were found in the Tei index among the experimental groups. Speckle-tracking echocardiography (STE) demonstrated reduced global longitudinal strain (GLS) in the HC group compared to controls, whereas GLS values improved in the HC+PCE group. Heart rate (HR), ejection fraction (EF), and fractional shortening (FS) remained within the normal ranges in all groups. Myocardial systolic velocity (s') and mitral annular plane systolic excursion (MAPSE) were unaffected by either the atherogenic diet or the anthocyanin treatment. Lastly, no significant differences were detected in left ventricular outflow tract (LVOT) parameters, including average and peak flow velocities (Vmean, Vmax) and pressure gradients (maxPG, meanPG).

3.1.3. Endothel-dependent vasorelaxation

Noradrenaline (NA)-induced vasoconstrictive responses were significantly attenuated in the HC rabbits compared to Controls at a concentration of 1 μmol/L NA. In all experimental groups, acetylcholine (Ach) administration up to 1 μmol/L elicited vasorelaxation, whereas higher concentrations of Ach induced vasoconstriction in the vascular rings. The atherogenic diet significantly impaired Ach-mediated relaxation in the aortic rings, while the Ach-induced contraction remained preserved. Notably, treatment with PCE did not affect the vascular responses to Ach. Based on the *ex vivo* vascular assays, we concluded that the cholesterol-rich diet caused marked endothelial dysfunction in the rabbits, which was not ameliorated by the oral PCE administration.

3.1.4. Histology

No significant differences were observed in the thickness of the tunica intima or tunica media between the Control and the Control+PCE groups. In contrast, animals fed the atherogenic diet exhibited a marked increase in intimal thickness due to the presence of foam cell-rich atherosclerotic plaques. The intima-to-media ratios were significantly elevated in both the HC and HC+PCE groups compared to the healthy Control group ($p < 0.0001$). However, no

significant difference was detected between the aortic samples from the untreated and the PCE-treated HC animals ($p > 0.05$).

3.1.5. Western blot

Myocardial eNOS levels were significantly lower in the HC rabbits compared to the healthy Control group ($p = 0.0190$). Similarly, significant differences were observed in the expression levels of PKG and SERCA2a between the Control and untreated HC groups (PKG: $p = 0.0005$; SERCA2a: $p < 0.0001$). In the HC+PCE group, the expression of the eNOS, PKG, and SERCA2a proteins was significantly increased compared to the HC group fed only the cholesterol-rich diet (HC vs. HC+PCE: $p < 0.0001$ for all three proteins).

Western blot analysis revealed that the HO-1 levels were markedly elevated in the myocardium of the atherogenic diet-fed rabbits compared to the healthy Control group ($p < 0.0001$). This elevated cardiac HO-1 expression was significantly attenuated following the PCE treatment ($p < 0.0001$).

Finally, no significant differences were observed in the expression of any of the investigated proteins between the two healthy groups (Control vs. Control+PCE).

3.2. „Aging" rat model

3.2.1. Body weight gain

Statistical analysis revealed that the body weight gain was lower in animals subjected to forced exercise compared to those in the sedentary and voluntary activity groups (Aged sedentary vs. Aged forced: $p = 0.0038$; Aged recreational vs. Aged forced: $p = 0.018$).

3.2.2. Serum parameters

Total cholesterol, LDL-C, and HDL-C levels were significantly elevated in the aged sedentary group compared to the young control group (all $p < 0.0001$). Neither voluntary nor forced exercise reduced the levels of these parameters. No significant differences were observed in triglyceride concentrations or calculated AIP values among the experimental groups. Regarding hepatic enzymes, AST and ALT levels were significantly increased in the aged sedentary group compared to the young rats (AST: $p < 0.0001$; ALT: $p = 0.0042$). Blood glucose

levels showed a similar trend ($p = 0.0005$). No changes were detected in the CRP, urea and creatinine levels.

3.2.3. Echocardiography

In the aged sedentary rats, marked signs of age-related diastolic dysfunction were observed, including increased LA/Ao ratio and reduced E/A ratio compared to the young control animals (LA/Ao ratio: $p = 0.0089$; E/A ratio: $p < 0.0001$). A decrease in the e′/a′ ratio, as well as prolongation of the DecT and IVRT, was also detected in the aged sedentary group (e′/a′ ratio: $p < 0.0001$; DecT: $p = 0.0003$; IVRT: $p = 0.0006$ vs. young group). Furthermore, these animals exhibited a significantly elevated Tei index and E/e′ ratio (Tei index: $p = 0.0003$; E/e′ ratio: $p = 0.0004$). Regarding the systolic function, EF and FS values were significantly reduced in aged sedentary rats compared with the young group (EF: $p = 0.001$; FS: $p = 0.0008$).

Left ventricular diastolic function improved in both exercise groups, however, the changes were more significant in the forced running animals. Left atrial enlargement was attenuated in both active groups compared with the aged sedentary rats (both $p = 0.0037$). Moreover, physical activity significantly reduced the mean E/e′ ratio relative to the sedentary aged group (recreational: $p = 0.0083$; forced: $p = 0.0006$). In the forced exercise group, the DecT and IVRT prolongation was diminished (DecT: $p = 0.0032$; IVRT: $p = 0.0002$) and the tissue Doppler imaging revealed a marked increase in the e′/a′ ratio ($p < 0.0001$) compared with the aged sedentary animals. This exercise modality also decreased the Tei index ($p = 0.0013$), indicating an improvement in the global ventricular performance.

Notably, significant differences between the two exercise groups were also detected in three echocardiographic parameters: forced exercise rats exhibited a higher e′/a′ ratio, as well as lower Tei index and IVRT values compared with the voluntary group (e′/a′ ratio: $p = 0.0084$; Tei index: $p = 0.0018$; IVRT: $p = 0.0028$).

Among the three aged groups, no significant differences were observed in heart rate, ejection fraction, or fractional shortening values. Finally, no substantial differences were detected in the left ventricular outflow tract (LVOT) parameters between any of the experimental groups.

3.2.4. Histology

Aging induced perivascular fibrosis in the rat myocardium, as evidenced by a significantly higher perivascular fibrosis ratio (PFR) in aged sedentary animals compared with

the young controls ($p < 0.0001$). One of the key findings of our study was that both voluntary and forced exercise significantly attenuated perivascular collagen deposition (voluntary: $p = 0.0294$; forced: $p = 0.0005$ vs. sedentary group). No significant difference in PFR was observed between the two exercise groups.

3.2.5. Western blot

Western blot analysis revealed an age-related downregulation of the PKG–STAT3–Opa1 axis. The myocardial expression of these proteins was reduced in the aged sedentary group compared with the young control rats (PKG: $p = 0.0092$; STAT3: $p < 0.0001$; Opa1: $p = 0.0005$). This signaling pathway impairment appeared to be reversible with physical activity, as the expression levels of all three proteins were significantly higher in both the forced and the voluntary exercise groups than in the aged sedentary animals (PKG: sedentary vs. voluntary: $p = 0.0060$; sedentary vs. forced: $p = 0.0007$; STAT3: sedentary vs. voluntary: $p = 0.0002$; sedentary vs. forced: $p < 0.0001$; Opa1: $p < 0.0001$ for both comparisons).

Furthermore, changes in the ATP synthase expression were noted. Interestingly, no significant difference was observed between the healthy young and aged sedentary groups ($p = 0.3686$). In contrast, both the voluntary and the regular forced running significantly increased ATPS levels (sedentary vs. voluntary: $p = 0.0285$; sedentary vs. forced: $p = 0.0002$).

Notably, no significant differences in the expression of any of the examined proteins were detected between the two exercise groups.

3.2.6. ATPS activity

A significant decrease in ATPS activity was observed in the aged sedentary rats compared with the young group ($p = 0.0026$). The most striking finding from the data comparison was that the initiation of forced physical activity at an advanced age markedly increased the ATPS activity relative to the aged sedentary animals ($p = 0.0086$). In contrast, voluntary activity did not exert a significant effect on ATPS activity during aging.

4. Discussion

Cardiac dysfunction (especially diastolic dysfunction) and the consequent development of heart failure remain unresolved clinical challenges. Due to the globally prevalent unhealthy dietary habits and the progressive aging of the population, the incidence of these conditions is expected to continue rising, imposing a substantial burden on the global economy and healthcare systems. The primary aim of our research group was to investigate the cardiovascular effects of these two societal challenges in experimental animal models, and to explore the potential of natural interventions, such as physical exercise and anthocyanin administration, in mitigating the underlying pathophysiological processes. It is important to emphasize that both physical activity and anthocyanins were examined not as preventive strategies, but as therapeutic interventions targeting pre-existing cardiovascular alterations. In both studies, functional assessments were performed first, followed by molecular and histological analyses to support the findings. Our experiments focused on molecular pathways whose modulation may hold therapeutic potential in the treatment of atherosclerosis- and aging-related cardiac dysfunction.

In our first experiment, we investigated the effects of the long-term oral anthocyanin treatment on cardiac dysfunction secondary to hypercholesterolemia and consequent atherosclerosis, using a rabbit model. Rabbits are highly sensitive to atherogenic diet, as they naturally exhibit high cholesterol ester transfer protein (CETP) activity and, similar to humans, display a lipid profile characterized by elevated LDL-C and low HDL-C levels. Furthermore, the electrophysiological, mechanical and structural properties of the rabbit myocardium, the architecture of the ion channels, the coronary anatomy and its responses to ischemia and various pharmacological agents more closely resemble those observed in humans than in other rodent species. Finally, in both rabbits and humans, the β isoform of myosin heavy chain is predominant.

The echocardiographic assessment of the rabbit model revealed the development of diastolic dysfunction in the hypercholesterolemic group. We detected significantly reduced E/A and e′/a′ ratios, along with prolonged deceleration time (DecT) and isovolumetric relaxation time (IVRT), indicating impaired left ventricular relaxation and, consequently, restricted ventricular filling. The significantly higher E/e′ ratio observed in HC animals indicated elevated left ventricular filling pressures, thereby confirming the diagnosis of diastolic dysfunction. In response to the increased filling pressure, left atrial enlargement - reflected by an elevated LA/Ao ratio - was also observed. In such cases, the left atrial pressure rises in order to maintain

adequate ventricular filling, leading to stretching of the atrial wall and subsequent chamber dilation. This, in turn, increases the risk of atrial fibrillation and thromboembolic events, while the elevated pressure may retrogradely propagate into the pulmonary veins, potentially causing dyspnoea and reduced exercise tolerance. Although the ejection fraction (EF), fractional shortening (FS) and peak systolic myocardial velocity (s′) remained within the normal ranges, the reduction in global longitudinal strain (GLS) - a more sensitive parameter of systolic performance - suggests the potential for subsequent deterioration in systolic function over time.

The 12-week-long anthocyanin treatment led to an improvement in cardiac function, as evidenced by the increased e′/a′ ratio, reductions in LA/Ao and E/e′ ratios and normalization of both deceleration time and GLS, indicating that the applied sour cherry extract (Prunus cerasus extract, PCE) exerted a substantial beneficial effect on myocardial performance. Interestingly, in our study, no notable difference was observed in the Tei index, a parameter of global myocardial performance. This observation is in line with the report of Bruch et al., who noted that the diagnostic value of the Tei index is limited in individuals with coronary artery disease (CAD) primarily presenting with diastolic dysfunction. Taken together, our echocardiographic findings suggest that anthocyanin-rich Prunus cerasus extract effectively attenuates cardiac dysfunction induced by atherogenic diet.

Serum parameters were analyzed to evaluate indicators suitable for assessing the severity of cardiovascular diseases. Marked alterations in lipid status were observed as a result of the atherogenic diet, with the HC group showing significant increases in the atherogenic index as well as total cholesterol, LDL-C and HDL-C levels compared to healthy controls. These findings are consistent with previous literature and represent the pathophysiological basis underlying the study, as hypercholesterolemia plays a fundamental role in the endothelial damage and the development of atherosclerosis. Conversely, in the group treated with Prunus cerasus extract (PCE), normalization of the lipid profile was observed, particularly regarding total cholesterol, LDL-C and the atherogenic index, indicating a pharmacological modulation of the existing lipid metabolism disorder. Several earlier studies have reached similar conclusions, confirming the beneficial effect of oral anthocyanin intake on hypercholesterolemia. Although further investigations are needed to elucidate the exact mechanism of action, it is hypothesized that the applied sour cherry extract exerted an inhibitory effect on CETP activity. CK-MB levels showed no significant differences between the experimental groups, suggesting that the investigated process did not progress to the extent of myocardial injury and consequent necroenzyme release.

Vascular alterations resulting from high cholesterol intake were identified in thoracic aorta samples using *ex vivo* functional assays and histological staining. In HC rabbits, acetylcholine (Ach)-induced endothelium-dependent vasorelaxation was significantly impaired, indicating endothelial dysfunction and concomitant disruption of nitric oxide synthesis. This finding aligns with the classic observation by Ludmer et al., who reported that arterial responsiveness to Ach stimulation is markedly reduced in atherosclerosis. Histological examination of the thoracic aorta confirmed the functional impairment by revealing a widened tunica intima and extensive atheromatous plaques. Taken together, these results demonstrate that hypercholesterolemia and the resultant endothelial dysfunction are closely interrelated processes that jointly contribute to the disruption of vascular homeostasis.

It is important to mention, as a limitation of our study, that although atherosclerosis was confirmed in the thoracic aorta in this model, literature data suggest that the pathological process likely extended to the coronary arteries, implying that endothelial dysfunction also developed in the coronaries. While PCE treatment exerted beneficial effects on the lipid profile and cardiac dysfunction, it proved to be ineffective at the level of vascular structures. The fact that anthocyanin treatment did not reduce the extent of atherosclerosis and therefore did not improve vascular status points to the lack of reversibility of the structurally severely damaged arterial wall integrity. Based on these observations, it can be hypothesized that the effects mediated by PCE are tissue-specific, primarily manifesting at the myocardial level, whereas its therapeutic efficacy is limited in vascular walls characterized by advanced atheromatous lesions.

The impairment of the NO–cGMP–PKG signaling pathway is known as a central pathophysiological mechanism underlying diastolic dysfunction. Therefore, in our study, we analyzed the expression of the key components of this cardioprotective molecular axis using Western blot technique. SERCA2a, one of the main downstream targets of this pathway, plays a fundamental role during diastole by removing Ca^{2+} from the cytoplasm, thereby ensuring optimal relaxation. Huang and colleagues demonstrated that hypercholesterolemia-induced cardiac functional alterations are associated with decreased myocardial SERCA2a levels and activity. According to our results, the expression of eNOS, PKG, and SERCA2a in left ventricular samples from HC animals was significantly lower than in the healthy control group, indicating atherosclerosis-associated myocardial downregulation of the NO–cGMP–PKG pathway. These molecular-level changes support the diastolic dysfunction identified by the echocardiographic assessment.

When studying the molecular effects of PCE on the myocardium, we observed that in the left ventricles of the anthocyanin-treated HC group, the expression levels of eNOS, PKG, and SERCA2a proteins were significantly increased compared to the untreated HC group, which molecularly confirms the improvement in diastolic function. The eNOS-inducing effect of anthocyanins has also been supported by previous studies. Our results highlight that PCE enhances eNOS expression, thereby stimulating the activity of the NO–cGMP–PKG pathway and contributing to the development of the cardioprotective effects. This hypothesis is further supported by findings from Quintieri and colleagues, who demonstrated that anthocyanins protect against ischemia/reperfusion injury through mediation of the NO–cGMP–PKG pathway. Notably, the PCE-induced improvement in diastolic function was also accompanied by an increase in SERCA2a expression, opening a new perspective in understanding the myocardial mechanisms of anthocyanins. Overall, the results of our study clearly support our research group's proposition that the applied sour cherry extract exerts beneficial effects in atherosclerosis-associated cardiac dysfunction, primarily through upregulation of the myocardial NO–cGMP–PKG signaling pathway and enhancement of the downstream target SERCA2a expression.

We also examined the changes in myocardial HO-1 expression using Western blot, as this stress protein plays a key role among the cellular defense mechanisms responsible for restoring homeostasis, primarily through its cytoprotective effects against oxidative stress and inflammatory processes. According to the literature, hypercholesterolemia is closely associated with increased oxidative stress in the myocardium, which several studies have linked to the induction of HO-1 expression. Accordingly, in our study we observed an elevated HO-1 expression pattern in the left ventricles of rabbits fed a cholesterol-rich diet. One surprising finding of our study was that HO-1 expression decreased in the myocardium of hypercholesterolemic animals treated with PCE. This is partly contradictory to previous experimental observations suggesting that flavonoid-rich plants act as HO-1 inducers and thereby exert favorable cardiovascular effects. However, our observations align with a more recent scientific perspective which calls for a reevaluation of the previously considered unequivocally protective role of the HO-1 enzyme, as both the extent and context of its expression play a crucial role. For example, it has been demonstrated that excessive myocardial HO-1 expression - especially in aging or under increased pressure load - may contribute to the worsening of heart failure. The exact mechanism behind the reduction of HO-1 expression detected in our experiment is currently unclear; however, we hypothesize that it can be explained by the complex interactions between anthocyanins and cholesterol metabolism. It is

plausible that the anthocyanin treatment-induced reduction in myocardial oxidative stress - attributable to improved lipid profiles and a significant decrease in LDL cholesterol levels - made the excessive induction of HO-1 unnecessary. Considering that HO-1 expression is closely related to the duration and severity of hypercholesterolemia, the favorable modulation of the lipid profile alone could result in decreased expression. As an alternative explanation, it is also possible that PCE modulated other secondary signaling pathways in the myocardium that influence HO-1 expression, thus elucidating the precise mechanism of action requires further targeted investigation.

In summary, the results of our first experiment indicate that a 12-week-long treatment with anthocyanin-rich Prunus cerasus extract (PCE) significantly alleviated pre-existing cardiac dysfunction induced by an atherogenic diet through activation of the NO–PKG signaling pathway and increased expression of the SERCA2a protein in myocardial tissue. The absence of significant improvement in vascular status suggests that the effect of PCE is organ-specific, primarily targeting cardiac tissue. Based on these protective effects, dietary anthocyanins may offer a targeted therapeutic option for modulating heart function impairments associated with atherosclerosis. Furthermore, our findings support the increasingly recognized view that excessive HO-1 expression - while fundamentally important for maintaining cellular homeostasis - can act as a "double-edged sword" and may have harmful consequences under certain pathological conditions.

In our second experiment, we used an aging rat model to compare the long-term cardiac effects of voluntary versus forced exercise initiated in old age. Research over the past decade has revealed a significant association between mitochondrial dysfunction, aging and the development of heart failure. Based on this, our aim was to study a molecular pathway whose proteins allow investigation of both cardiac dysfunction and mitochondrial impairment within the context of aging.

In this study, serum parameter analysis revealed a significant deterioration of the lipid profile in the aged groups, characterized by increased levels of total cholesterol, LDL-C and HDL-C. These observations are consistent with previous literature documenting the presence of multifactorial hypercholesterinemia associated with aging. Surprisingly, neither voluntary nor forced physical activity proved to be sufficient to reduce the elevated cholesterol levels associated with aging. This is likely due to the lipid metabolism alterations induced by the aging process, resulting in a diminished response of the elderly organism to lifestyle interventions. At this point, it is important to highlight one limitation of our experiment, namely that the rat, as a laboratory animal model, can not be considered fully representative for studying the

physiological and pathological aspects of dyslipidemia. The evaluation of morphometric data also confirmed previous research findings: weight gain was significantly lower in animals undergoing the forced exercise program compared to inactive aged rats and those engaged in voluntary activity. Based on these results, it can be suggested that regular, controlled physical exercise in old age may be more effective for body weight regulation than spontaneous forms of movement.

When analyzing the cardiac status, our aim was to identify functional and structural myocardial alterations that develop solely as a consequence of aging, without the presence of other risk factors. To determine these changes, we first compared the 12-week-old young group with the 24-month-old aged group. Based on echocardiographic examinations, the 2-year-old rats exhibited significantly reduced E/A and e'/a' ratios, as well as prolonged deceleration time (DecT) and isovolumetric relaxation time (IVRT), collectively indicating impaired left ventricular relaxation. Due to insufficient myocardial relaxation, left ventricular filling pressure increases, as reflected by an elevated E/e' ratio, which is a key hemodynamic feature of diastolic dysfunction. Ultimately, this leads to elevated end-diastolic pressure, subjecting the left atrium to increased pressure load, resulting in structural remodeling, primarily atrial dilation (higher LA/Ao ratio). The observed changes in these parameters provide convincing evidence that diastolic dysfunction developed in the 24-month-old sedentary aged animals. Our results are consistent with findings by Rowe et al., who observed progressive deterioration of diastolic function with advancing age in comparisons between 3- and 24-month-old rats.

When evaluating the effects of physical activity, both voluntary and forced exercise protocols resulted in improvements in two diastolic parameters by reducing left ventricular filling pressure (E/e' ratio) and left atrial enlargement (LA/Ao ratio). However, it is noteworthy that an increase in the e'/a' ratio, as well as normalization of IVRT and DecT, were only observed in the structured, forced exercise group, indicating that improvement in myocardial relaxation capacity was detectable exclusively in this group. A previous study similarly reported significant improvement in diastolic heart function in 24-month-old rats following 12 weeks of treadmill training. Furthermore, regarding the Tei index, an integrated measure of the global left ventricular systolic and diastolic function, the decrease observed in response to forced exercise aligns with findings by Cho et al., who detected similar favorable changes during forced physical activity in aged mouse models. Based on our echocardiographic results, it can be concluded that age-related diastolic dysfunction can be significantly improved through regular physical exercise, particularly via forced training programs.

In the aging rat model, the significant myocardial collagen accumulation observed during histological analysis - especially in the perivascular regions of the vessels - further substantiates the presence of the age-associated structural remodeling. This is supported by the complete absence of these alterations in the young control group. The resulting left ventricular stiffness and prolongation of the relaxation phase due to fibrosis are also confirmed by the echocardiographic parameters detailed above. Our histological findings align with observations by Reed et al., who demonstrated a correlation between increased cardiac fibrosis and diastolic dysfunction in an aging mouse model. Horn and Trafford similarly highlighted in their study the relationship between perivascular fibrosis and cardiac aging, suggesting that perivascular connective tissue accumulation may contribute to coronary microcirculation impairment, which can further exacerbate cardiomyocyte aging in the present context.

Based on our histological results, both forms of exercise reduced the extent of perivascular fibrosis, suggesting an improvement in microcirculatory perfusion. A 2017 study reported significant improvements in diastolic and endothelial functions in 20-month-old rats following a 10-12 week exercise program, supporting the notion that physical activity can enhance myocardial relaxation capacity and coronary blood flow in the aging heart.

During the investigation of molecular mechanisms, we focused particularly on the analysis of the PKG–STAT3–Opa1 signaling pathway, which Chang and colleagues described as an inducer of mitochondrial fusion in a diabetic cardiomyopathy mouse model. Considering that the imbalance between mitochondrial fusion and fission is a key factor in the aging process of cardiac muscle, we performed Western blot analysis to examine the expression of proteins within this pathway in the left ventricle. In the myocardium of the aged, physically inactive animals we detected decreased PKG expression, further supporting the diagnosis of diastolic dysfunction and consistent with findings from previous studies using different model systems. The reduction of PKG levels impairs myocardial relaxation by increasing the stiffness of the giant titin molecule and disturbing calcium homeostasis, as well as promoting fibrosis and cardiomyocyte hypertrophy. These combined effects contribute to left ventricular diastolic dysfunction and structural remodeling, especially in metabolic and age-related pathological conditions. The moderate expression of the STAT3 transcription factor in the physically inactive group indicates a decline in cardioprotective mechanisms associated with aging. This finding is supported by evidence that decreased STAT3 expression and activation significantly contribute to the progression of heart failure, given its key role in myocardial stress adaptation and maintenance of cardiac homeostasis. The reduced expression of Opa1 observed in the aged, sedentary group suggests the presence of impaired mitochondrial fusion, which may lead to

dysfunctional mitochondrial dynamics, thereby directly contributing to the pathomechanism of heart failure. This result aligns with previous studies where heterozygous $Opa1^{+/-}$ mice exhibited mitochondrial dysfunction, ATP depletion and increased reactive oxygen species production. Our molecular analyses from the second experiment clearly confirmed the age-related downregulation of the PKG–STAT3–Opa1 signaling pathway.

Further analysis of our Western blot results revealed - to our knowledge for the first time - that physical exercise activates the cardioprotective PKG–STAT3–Opa1 axis. Previous studies have already demonstrated that PKG induces protective signaling pathways in the heart under various pathological conditions. In the present study, the exercise-induced increase in PKG expression and its presumed beneficial effect are consistent with the findings of Heerebeek and colleagues, who emphasized that restoring myocardial PKG level is crucial in the treatment of diastolic heart failure. The elevated STAT3 levels observed in physically active animals are also supported by recent research showing that, unlike in oncogenesis, STAT3 activation is necessary to mediate cardioprotective processes. Moreover, accumulating evidence suggests that restoration of the Opa1-mediated mitochondrial fusion represents one of the most promising strategies for improving tissue function in the context of disrupted mitochondrial dynamics. For example, in a diabetic cardiomyopathy model, punicalagin and paeonol were shown to enhance mitochondrial fusion through regulation of STAT3 and Opa1. Additionally, activation of the κ-opioid receptor promotes mitochondrial fusion, which increases myocardial resilience against ischemia/reperfusion injury via the STAT3–Opa1 pathway.

In our experiment using the aging rat model, to further elucidate the molecular mechanisms, we also investigated the expression and activity of ATP synthase (ATPS). Our results showed that ATPS activity was significantly decreased in the aged animals modeling a physically inactive lifestyle. This indicates impaired ATP production as well as increased ROS generation, which leads to oxidative stress and may indirectly contribute to age-related cardiac dysfunction. This hypothesis is supported by literature data showing that mitochondrial dysfunction results in an energy-deficient state in the heart and exacerbates the development of heart failure. Further analysis of activity measurements suggests that the increase in ATPS activity, detectable exclusively in response to forced exercise, follows Opa1-dependent mitochondrial fusion. This is corroborated by studies demonstrating that Opa1-mediated fusion enhances oxidative phosphorylation, increases ATP production and reduces ROS accumulation. Ultimately, our results indicate that forced physical exercise, through activation of the PKG–

STAT3–Opa1 axis and presumably by promoting mitochondrial fusion, enhances energy production in the myocardium of aged rats.

It is also important to mention several limitations of this study. Although rats are widely used as an animal model for investigating human aging and exercise adaptation processes, they cannot fully represent the complex changes occurring in the human body. Secondly, the study was conducted exclusively on male animals; however, including both sexes would have provided more generalizable results from a translational perspective.

In conclusion of our second experiment, this study represents the first experimental approach to investigate the effects of voluntary and forced physical activity on diastolic function in the context of aging through the myocardial PKG–STAT3–Opa1 signaling pathway and ATP synthase (ATPS) activity. Our results indicate that forced exercise was significantly more effective and also promoted the improvement of mitochondrial function. Although further research is required to elucidate the precise molecular mechanisms, this experiment provides new insights into the mechanisms of exercise effects on the aging heart and potentially identifies novel therapeutic targets for the treatment of cardiac dysfunction.

5. Summary

The aim of our research group was to highlight the potential positive effects of the natural interventions in the therapeutic context of cardiac dysfunction related to hypercholesterolaemia and aging. It is important to emphasize that in both experimental series, the investigations focused on already established pathological conditions; therefore, the applied interventions were evaluated in a therapeutic rather than a preventive context. In our first experiment, we created a rabbit model of atherosclerosis and cardiac dysfunction using atherogenic diet and after 12 weeks of the treatment with anthocyanin-rich sour cherry extract we observed a significant improvement in the diastolic parameters, accompanied by an increase in the myocardial eNOS, PKG and SERCA2a expressions. Our findings also highlight that HO-1 may act as a potential "double-edged sword", indicating the need to re-evaluate its universally protective role. In the second examination, we compared the effects of the voluntary and the forced physical activity on age-associated diastolic dysfunction in Wistar rats. Based on our results, forced physical exercise improved left ventricular relaxation more effectively, a beneficial effect presumably attributable to the activation of the PKG–STAT3–Opa1 signaling pathway and the consequent increase in ATPS activity.

6. Novel findings of the doctoral thesis

- In an atherosclerotic rabbit model, cardiac dysfunction arising from hypercholesterolemia and consequent atherosclerosis is improved by oral administration of a sour cherry extract rich in anthocyanins, likely mediated by increased myocardial expression of eNOS, PKG and SERCA2a proteins; meanwhile, the extract has no effect on the vascular status.
- Paradoxically, PCE treatment leads to a decrease in myocardial HO-1 expression in hypercholesterolemic rabbits, suggesting that the protective role of HO-1 is not universal but may vary depending on the metabolic environment and oxidative status.
- In the aging rat model, diastolic dysfunction associated with old age is more effectively alleviated by forced exercise than by voluntary physical activity.
- In the myocardium of aged rats, both voluntary and forced physical activity reduce the extent of perivascular fibrosis and upregulate the PKG-STAT3-Opa1 signaling pathway, but only forced exercise increases ATP synthase activity.

7. List of publications

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Candidate: Réka Szekeres
Doctoral School: Doctoral School of Nutrition and Food Sciences

List of publications related to the dissertation

1. **Szekeres, R.**, Priksz, D., Bombicz, M., Pelles-Taskó, B., Szilágyi, A., Bernát, B., Pósa, A., Varga, B., Gesztelyi, R., Somodi, S., Szabó, Z., Szilvássy, Z., Juhász, B.: Exercise Types: Physical Activity Mitigates Cardiac Aging and Enhances Mitochondrial Function via PKG-STAT3-Opa1 Axis.
   Aging Dis. [Epub ahead of print], 2024.
   DOI: http://dx.doi.org/10.14336/AD.2024.0959
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2. **Szekeres, R.**, Priksz, D., Kiss, R., Romanescu, D. D., Bombicz, M., Varga, B., Gesztelyi, R., Szilágyi, A., Takács, B., Tarjányi, V., Pelles-Taskó, B., Forgács, I. N., Gálné Remenyik, J., Szilvássy, Z., Juhász, B.: Therapeutic Aspects of Prunus cerasus Extract in a Rabbit Model of Atherosclerosis-Associated Diastolic Dysfunction.
   Int. J. Mol. Sci. 24 (17), 1-19, 2023.
   DOI: http://dx.doi.org/10.3390/ijms241713253
   IF: 4.9

List of other publications

3. Piros, Z., Kispál, K., **Szekeres, R.**, Takács, B., Kiss, R., Szabó, A. M., Ujvárosy, D., Szabó, Z., Szilvássy, Z., Gesztelyi, R., Juhász, B.: Alarming Signal from the Ear: Tinnitus, a Possible Epiphenomenon of Silent Hyperlipidaemia.
   Biomedicines. 12 (12), 1-14, 2024.
   DOI: http://dx.doi.org/10.3390/biomedicines12122837
   IF: 3.9
4. Szilágyi, A., Takács, B., **Szekeres, R.**, Tarjányi, V., Nagy, D., Priksz, D., Bombicz, M., Kiss, R., Szabó, A. M., Lehoczki, A., Gesztelyi, R., Juhász, B., Szilvássy, Z., Varga, B.: Effects of voluntary and forced physical exercise on the retinal health of aging Wistar rats.
   GeroScience. 46 (5), 4707-4728, 2024.
   DOI: http://dx.doi.org/10.1007/s11357-024-01208-x
   IF: 5.4

5. Pelles-Taskó, B., **Szekeres, R.**, Takács, B., Szilágyi, A., Ujvárosy, D., Bombicz, M., Priksz, D., Varga, B., Gesztelyi, R., Szabó, Z., Szilvássy, Z., Juhász, B.: From Nature to Treatment: The Impact of Pterostilbene on Mitigating Retinal Ischemia-Reperfusion Damage by Reducing Oxidative Stress, Inflammation, and Apoptosis.
   Life (Basel). 14 (9), 1-13, 2024.
   DOI: http://dx.doi.org/10.3390/life14091148
   IF: 3.4

6. Czine, P., Balogh, P., Blága, Z., Szabó, Z., **Szekeres, R.**, Hess, S., Juhász, B.: Is It Sufficient to Select the Optimal Class Number Based Only on Information Criteria in Fixed- and Random-Parameter Latent Class Discrete Choice Modeling Approaches?
   Econometrics. 12 (3), 1-16, 2024.
   DOI: http://dx.doi.org/10.3390/econometrics12030022
   IF: 1.4

7. Bernát, B., Erdélyi, R., Fazekas, L., Garami, G., **Szekeres, R.**, Takács, B., Bombicz, M., Varga, B., Sárkány, F., Ráduly, A. P., Romanescu, D. D., Papp, Z., Tóth, A., Szilvássy, Z., Juhász, B., Priksz, D.: Drug Candidate BGP-15 Prevents Isoproterenol-Induced Arrhythmias and Alters Heart Rate Variability (HRV) in Telemetry-Implanted Rats.
   Pharmaceuticals (Basel). 16 (3), 1-22, 2023.
   DOI: http://dx.doi.org/10.3390/ph16030359
   IF: 4.3

8. Blága, Z., Czine, P., Takács, B., Szilágyi, A., **Szekeres, R.**, Wachal, Z., Hegedűs, C., Buchholcz, G., Varga, B., Priksz, D., Bombicz, M., Szabó, A. M., Kiss, R., Gesztelyi, R., Romanescu, D. D., Szabó, Z., Szűcs, M., Balogh, P., Szilvássy, Z., Juhász, B.: Examination of Preferences for COVID-19 Vaccines in Hungary Based on Their Properties: Examining the Impact of Pandemic Awareness with a Hybrid Choice Approach.
   Int. J. Environ. Res. Public Health. 20 (2), 1-16, 2023.
   DOI: http://dx.doi.org/10.3390/ijerph20021270

9. Kozma, M., Bombicz, M., Varga, B., Priksz, D., Gesztelyi, R., Tarjányi, V., Kiss, R., **Szekeres, R.**, Takács, B., Ménes, Á., Balla, J., Balla, G., Szilvássy, J., Szilvássy, Z., Juhász, B.: Cardioprotective Role of BGP-15 in Ageing Zucker Diabetic Fatty Rat (ZDF) Model: extended Mitochondrial Longevity.
   Pharmaceutics. 14 (2), 1-16, 2022.
   DOI: http://dx.doi.org/10.3390/pharmaceutics14020226
   IF: 5.4

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10. Priksz, D., Lampé, N., Kovács, Á., Herwig, M., Bombicz, M., Varga, B., Wilisicz, T., Szilvássy, J., Pósa, A., Kiss, R., Gesztelyi, R., Ráduly, A. P., **Szekeres, R.**, Sieme, M., Papp, Z., Tóth, A., Hamdani, N., Szilvássy, Z., Juhász, B.: Nicotinic-acid derivative BGP-15 improves diastolic function in a rabbit model of atherosclerotic cardiomyopathy.
Br. J. Pharmacol. 179 (10), 2240-2258, 2022.
DOI: http://dx.doi.org/10.1111/bph.15749
IF: 7.3

11. Viczján, G., Szilágyi, A., Takács, B., Óvári, I., **Szekeres, R.**, Tarjányi, V., Erdei, T. D., Teleki, V., Zsuga, J., Szilvássy, Z., Juhász, B., Varga, B., Gesztelyi, R.: The effect of a long-term treatment with cannabidiol-rich hemp extract oil on the adenosinergic system of the Zucker Diabetic Fatty (ZDF) rat atrium.
Front. Pharmacol. 13, 1-12, 2022.
DOI: http://dx.doi.org/10.3389/fphar.2022.1043275
IF: 5.6

12. Szilágyi, A., Takács, B., **Szekeres, R.**, Tarjányi, V., Bombicz, M., Priksz, D., Kovács, A. I., Juhász, B., Frecska, E., Szilvássy, Z., Varga, B.: Therapeutic Properties of Ayahuasca Components in Ischemia/Reperfusion Injury of the Eye.
Biomedicines. 10 (5), 1-19, 2022.
DOI: http://dx.doi.org/10.3390/biomedicines10050997
IF: 4.7

13. Viczján, G., Erdei, T. D., Óvári, I., Lampé, N., **Szekeres, R.**, Bombicz, M., Takács, B., Szilágyi, A., Zsuga, J., Szilvássy, Z., Juhász, B., Gesztelyi, R.: A Body of Circumstantial Evidence for the Irreversible Ectonucleotidase Inhibitory Action of FSCPX, an Agent Known as a Selective Irreversible A1 Adenosine Receptor Antagonist So Far.
Int. J. Mol. Sci. 22 (18), 1-21, 2021.
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