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RESEARCH ARTICLE

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Myocardial proteome changes in aortic stenosis rats subjected to long-term aerobic exercise

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Abstract

The effects of exercise training (ET) on the heart of aortic stenosis (AS) rats are controversial and the mechanisms involved in alterations induced by ET have been poorly clarified. In this study, we analyzed the myocardial proteome to identify proteins modulated by moderate-intensity aerobic ET in rats with chronic supravalvular AS. Wistar rats were divided into four groups: sedentary control (C-Sed), exercised control (C-Ex), sedentary aortic stenosis (AS-Sed), and exercised AS (AS-Ex). ET consisted of five treadmill running sessions per week for 16 weeks. Statistical analysis was performed by ANOVA or Kruskal–Wallis and Goodman tests. Results were discussed at a significance level of 5%. At the end of the experiment, AS-Ex rats had higher functional capacity, lower blood lactate concentration, and better cardiac structural and left ventricular (LV) functional parameters than the AS-Sed. Myocardial proteome analysis showed that AS-Sed had higher relative protein abundance related to the glycolytic pathway, oxidative stress, and inflammation, and lower relative protein abundance related to beta-oxidation than C-Sed. AS-Ex had higher abundance of one protein related to mitochondrial biogenesis and lower relative protein abundance associated with oxidative stress and inflammation than AS-Sed. Proteomic data were validated for proteins related to lipid and glycolytic metabolism. Chronic pressure overload changes the abundance of myocardial proteins that are mainly involved in lipid and glycolytic energy metabolism in rats. Moderate-intensity aerobic training attenuates changes in proteins related to oxidative stress and inflammation and increases the COX4I1 protein, related to mitochondrial biogenesis. Protein changes are combined with improved functional capacity, cardiac remodeling, and LV function in AS rats.

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KEYWORDS
aerobic exercise training, myocardium, proteomics, rat, supravalvular aortic stenosis, ventricular dysfunction

1 | BACKGROUND

Heart failure (HF) is the final route for several cardiovascular diseases and represents one of the most relevant public health issues, with high morbidity, mortality, and health costs (Tsao et al., 2023).

The heart, in response to several stimuli, undergoes cardiac remodeling, defined as alterations in genome expression, which results in molecular, cellular, and interstitial changes, and clinically manifests as changes in heart size, shape, and function (Cohn et al., 2000). The rodent aortic stenosis (AS) model has been extensively used to induce cardiac remodeling, characterized by left ventricular (LV) hypertrophy, diastolic and systolic dysfunction, and HF (Mazeto et al., 2021; Sant'Ana et al., 2018, 2023; Silva et al., 2022). The mechanisms related to functional impairment in the AS heart are complex and not fully elucidated; alterations in cardiomyocytes, myocardium, and cardiac chambers are involved (Hartupee & Mann, 2017).

Physical exercise has been recommended as a nonpharmacological therapy to attenuate cardiac remodeling in different injury cardiac models (Lin et al., 2020). Although exercise training (ET) induces cardioprotection in AS rats (Mota et al., 2020; de Souza et al., 2015, 2020; Souza et al., 2014), the mechanisms responsible for improving cardiac performance have not been fully elucidated. We have previously shown that the β-adrenergic system (de Souza et al., 2020), renin–angiotensin system (Mota et al., 2020), and cardiomyocyte cell cycle (Mota et al., 2020) are not involved in the functional benefit of exercise in AS rats.

Technological advances have contributed to a better understanding of cardiac remodeling (Pandey & Mann, 2000). Proteomics aims to assess changes in relative protein abundance to elucidate the pathophysiology of cardiovascular disease development and progression (McGregor & Dunn, 2006). Studies with this approach have been conducted in clinical cardiology research (Beck et al., 2018) and experimental models such as myocardial infarction (Mitra et al., 2015), systemic arterial hypertension (Manakov et al., 2016), diabetes mellitus (Ma et al., 2016), obesity (Vileigas, Harman, et al., 2019), and aortic constriction (Bugger et al., 2010; Chang et al., 2013; Dai et al., 2012; Guan et al., 2016; Hollander et al., 2011; Kararigas et al., 2014; Li et al., 2016). Mitochondria (Bugger et al., 2010; Dai et al., 2012; Hollander et al., 2011), lipid droplets (Li et al., 2016), myocardial tissue (Guan et al., 2016; Kararigas et al., 2014), and protein phosphorylation (Chang et al., 2013) have been evaluated in AS models. The effects of ET have also been analyzed by myocardial proteome in healthy rodents (Boluyt et al., 2006; Burniston, 2009; Dantas et al., 2016; Ferreira et al., 2014; Kavazis et al., 2009), obesity cardiomyopathy (Petriz et al., 2013), myocardial infarction (Bansal et al., 2010), arterial hypertension (Petriz et al., 2015) and diabetes mellitus (Cox & Marsh, 2013). The effects of aerobic ET in AS rats using the proteomic approach had not been explored.

In this study, we evaluated myocardial relative protein abundance in sedentary and exercised rats with supravalvular AS-induced cardiac dysfunction by the shotgun proteomics approach followed by marker-free quantification.

2 | METHODS

2.1 | Animals and experimental design

Male Wistar rats (70–90 g, 3 weeks old) from the Central Laboratory of Botucatu Medical School were kept in collective cages in a climate-controlled environment with food and water supplied ad libitum. Two experimental groups were formed initially: sham-operated and supravalvular AS. After 2 weeks, the rats were subjected to echocardiogram and randomized into the following groups: sedentary control (C-Sed; $n = 15$); exercised control (C-Ex; $n = 15$); sedentary AS (AS-Sed; $n = 15$); and exercised AS (AS-Ex; $n = 14$, Figure 1).

AS was surgically induced as previously described (Mazeto et al., 2021; Sant'Ana et al., 2018; Silva et al., 2022). Briefly, rats were anesthetized with a mixture of ketamine hydrochloride (50 mg/kg; i.p.) and xylazine hydrochloride (10 mg/kg; i.p.), and the hearts were exposed through median thoracotomy. A stainless-steel clip (0.6 mm internal diameter) was placed in the ascending aorta, approximately 3 mm from its root. During surgery, the rats received 1 mL of warm saline intraperitoneally and were manually ventilated with positive pressure with 100% oxygen. After the procedure, rats were kept warm until full consciousness was regained. Analgesia was performed with intraperitoneal administration of carprofen (5 mg/kg). Control animals were submitted to the same procedure but without the constriction of the aorta.

At the end of the experiment, rats were anesthetized with a mixture of ketamine hydrochloride (60 mg/kg; i.p.) and xylazine hydrochloride (10 mg/kg; i.p.) and euthanized by decapitation. The heart was dissected, and the left ventricle (LV) was removed and immediately frozen in liquid nitrogen. Blood samples were collected, and serum was separated by centrifugation at 1620*g* for 10 min at 4°C.

The study was approved by the Animal Research Ethics Committee of Botucatu Medical School, Sao Paulo State University (1324/2020), and all protocols were conducted following the Guide for the Care and Use of Laboratory Animals published by the National Academies Collection (2011).

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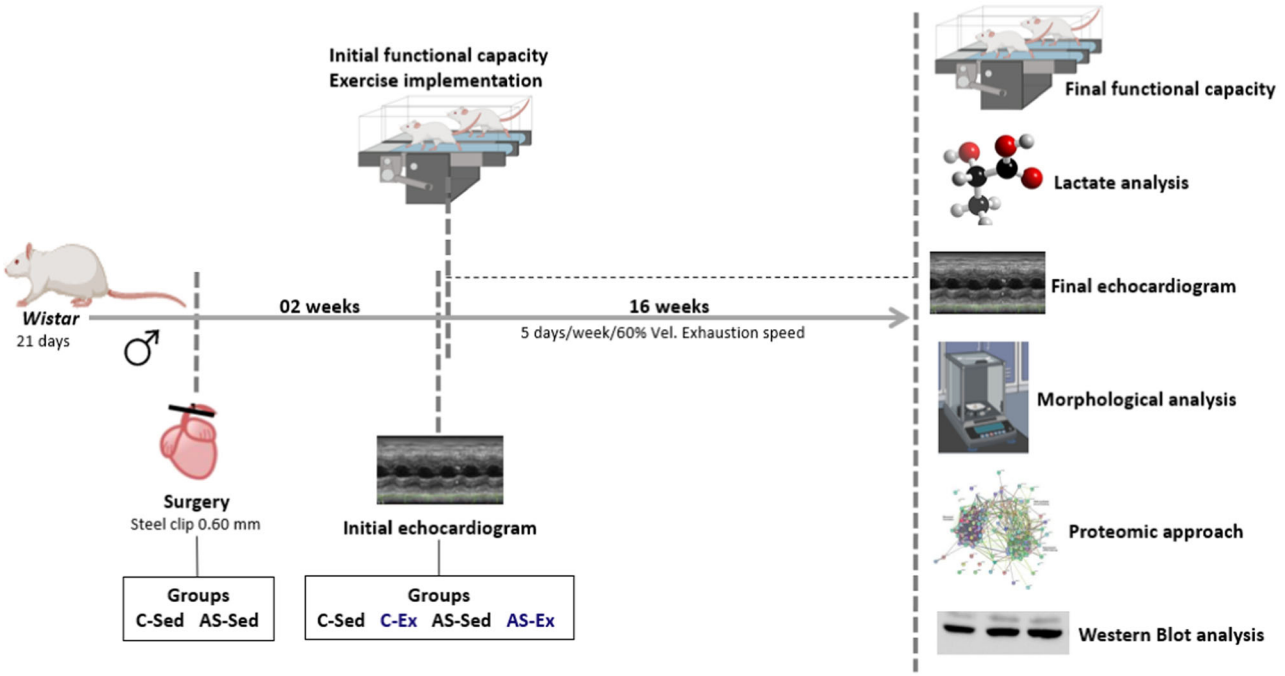

FIGURE 1 Schematic representation of the experimental design. AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis; C-Ex, exercised control; C-Sed, sedentary control.

2.2 | Treadmill exercise testing

Functional capacity was assessed by graded treadmill exercise testing (TET). Maximal speed, total time, and distance run were evaluated as previously described (Mota et al., 2020). TET was performed on a motorized treadmill for rats (AVS Projects) after 5 days of adaptation to the treadmill environment. The rats exercised at a speed of 5 m/min/day with increasing duration from 10 min on the first day to 30 min on the fifth day.

During TET, treadmill speed was initiated at 6 m/min and increased by 3 m/min every 3 min until exhaustion. Exhaustion was defined as the inability to maintain the proposed speed. TET was performed before training to guide running speed, at the end of the third and seventh weeks to adjust running speed, and at the end of the experiment.

2.3 | ET protocol

The ET protocol was modified from previously published protocols (Mota et al., 2020; Wisløff et al., 2007). The rats exercised 5 days/week for 16 weeks, at 60% of the maximal speed achieved during the TET. Exercise duration from the first to the sixth week was progressively increased by 2 min/week until 20 min/day and then remained constant. Animals received low-voltage electrical stimulation during the training.

2.4 | Echocardiographic study

Echocardiograms were performed 2 and 18 weeks after surgery using a commercially available echocardiogram (General Electric Medical Systems, Vivid S6) equipped with a 5–11.5 MHz multifrequency probe as previously described (Gregolin et al., 2021; da Silva et al., 2023; de Souza et al., 2023; Vileigas, Marciano, et al., 2019). All examinations were performed blind by a cardiologist and specialist in echocardiography. Rats were anesthetized by intraperitoneal injection with a mixture of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (1 mg/kg) (Mota et al., 2020). A two-dimensional parasternal short-axis view of the LV was obtained at the level of the papillary muscles. M-mode tracings were obtained from short-axis views of the LV at or just below the tip of the mitral valve leaflets, and at the level of the aortic valve and left atrium. M-mode images of the LV were printed on a black-and-white thermal printer (Sony UP-890MD) at a sweep speed of 100 mm/s. Measurements are the average of at least five cardiac cycles from M-mode tracings. Cardiac structure was evaluated by LV diastolic diameter (LVDD), LV diastolic posterior wall thickness (DPWT), left atrium (LA) and aorta (AO) diameters, and LA/AO ratio. LV function was assessed based on posterior wall shortening velocity (PWSV), ejection fraction (EF), mesocardial shortening fraction (MFS), early and late diastolic mitral inflow velocities (E and A waves), E/A ratio, E-wave deceleration time (EDT), and isovolumetric relaxation time (IVRT). Additionally, LV function was evaluated by tissue Doppler imaging (TDI) of systolic (S′), early (E′), and late (A′) diastolic velocity of the mitral annulus (arithmetic average of the lateral and septal walls), and E/E′ ratio. Heart rate (HR) was also evaluated.

2.5 | HF characterization

During euthanasia, in-vivo (tachypnea) and post-mortem (ascites, pleural effusion, atrial thrombi, and liver congestion) HF features were assessed by two blind observers.

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2.6 | Blood lactate concentration

Lactate concentration, a biomarker of physical fitness, was assessed immediately after the final TET to determine ET protocol effectiveness. After alcohol sterilization, the distal portion of the animal's tail was sectioned with a little prick and 25 μL of blood was collected. Samples were deposited in Eppendorf tubes containing 50 μL of 1% sodium fluoride (Quimesp Quimica). Blood lactate concentration was measured by the electroenzymatic method using a blood lactate analyzer YSI 2300 (Stat Plus Glucose Lactate Analyzer). Collected blood was maintained on ice during the procedures and then stored in a freezer for later analysis.

2.7 | Shotgun proteomics followed by label-free quantification

For full experimental details of this analysis, see supplementary materials. LV non-pooled samples from 10 rats in each group were used in the proteomics analysis. LV protein was extracted by homogenization in 500 μL of 50 mmol/L ammonium bicarbonate containing protease inhibitors (Sigma Fast Protease Inhibitor Cocktail Tablets EDTA-Free), using a bead beater homogenizer (Bullet Blender®, Next Advance, Inc.), followed by centrifugation at 5000 rpm for three cycles (5 min with a 1-min interval at 2–8°C). Total protein quantification was determined by a Pierce BCA Protein Assay Kit (Thermo Scientific) according to manufacturer instructions.

For in-solution digestion, 50 μg of protein was incubated at 80°C with RapiGest (0.05% w/v final concentration, Waters), digested with trypsin (Trypsin Gold Mass Spectrometry, Promega) and incubated at 37°C. The samples containing peptides were analyzed by liquid chromatography coupled with mass spectrometry (LC-MS/MS). Peptide identification was performed using Xevo QTof mass spectrometer (Waters).

The resulting raw data were processed using Progenesis QI for Proteomics v.4.0 (Nonlinear Dynamics, Waters) for quantitative assay. The peak list was exploited by the UniProt database of *Rattus norvegicus* using Mascot v.2.6 (Matrix Science, UL; 7,989 sequences; 4,044,314 residues, June 2022). Trypsin was the specified enzyme, and a missed cleavage was allowed. Cysteine carbamidomethylation was defined as a fixed modification and methionine oxidation as a variable modification. A precursor mass tolerance of 10 ppm and a fragment ion tolerance of 0.01 Da were applied. A false discovery rate (FDR) of 1% was considered. A relative quantification, using nonconflicting peptides, was selected to quantify proteins. The criteria used to consider proteins differentially up or downregulated were: identification and quantification using at least two unique peptides and a q value < 0.05.

2.8 | Proteomic bioinformatics

The proteins differentially identified by label-free shotgun were subjected to enrichment analysis for the gene ontology (GO) terms "molecular function," "biological process," and "cellular component" using the "KEGG Orthology-Based Annotation System" tool (KOBAS 3.0; http://kobas.cbi.pku.edu.cn/). Protein–protein interaction networks were constructed using the online database Search Tool for the Retrieval of Interacting Genes (STRING, v.11.0; https://string-db.org). All STRING network analysis was performed with a medium confidence level of 0.4.

2.9 | Western blot analysis

After identifying the proteins differentially abundant between the groups, the expression of four proteins was validated by Western blot analysis (Deus et al., 2019). As most modulated proteins are involved in metabolic processes, proteins linked to lipid metabolism (fatty acid-binding protein, Fabp3, and medium-chain acyl-CoA dehydrogenase, Mcad) and the glycolytic pathway (lactate dehydrogenase, Ldh, and pyruvate kinase, Pk) were selected.

LV protein was extracted by homogenization in RIPA buffer containing protease (Sigma-Aldrich) and phosphatase (Roche Diagnostics) inhibitors using a bead beater homogenizer (Bullet Blender®, Next Advance, Inc.), followed by centrifugation (12,000*g*, 4°C, 20 min). Proteins were quantified, separated on a polyacrylamide gel, and transferred to a nitrocellulose membrane. The membranes were incubated with primary antibodies overnight at 4°C and then with the secondary antibodies. Immunodetection was performed using the chemiluminescence method according to the manufacturer's instructions (Enhancer Chemi-Luminescence, Amersham Biosciences). The nitrocellulose membranes were analyzed in an ImageQuant™ LAS 4000 photodocumenter (GE Healthcare Life Sciences) and quantitative analyses of the blots were performed using ImageJ software (National Institutes of Health). The primary antibodies were: anti-*LDHA* (2012S), PK (3186S) (Cell Signaling), *Fabp3* (ab133585), and *MCAD* (ab110296) (Abcam). Protein levels were normalized to glyceraldehyde-3-phosphate dehydrogenase—GAPDH (sc-32233, Santa Cruz Biotechnology).

2.10 | Statistical analysis

Data were tested for normality using the Shapiro–Wilk test and were expressed using descriptive measures. Comparisons between groups were performed by two-way ANOVA followed by Bonferroni for parametric data or Kruskal–Wallis, followed by Dunn for non-parametric data. The Goodman test was used to compare the frequency of HF features. Statistical conclusions were discussed at the 5% significance level. Statistical analyses were performed using Sigma Plot 12.0 (Microsoft) and graphs were generated using GraphPad Prisma 9 (GraphPad Software).

3 | RESULTS

The C-Ex and AS-Ex groups had higher exhaustion speed, total time, and run distance in the treadmill than their respective controls, showing that ET improved functional capacity (Figure 2). The trained

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groups presented lower lactate levels than their controls (Figure 2). The AS-Ex had attenuation of clinical and pathological HF features compared to AS-Sed, with a lower frequency of tachypnea, pleural effusion, atrial thrombi, hepatic congestion, and ascites (Figure 3). Survival rates did not differ between AS-Ex and AS-Sed groups (data not shown).

We performed echocardiographic evaluation before ET to assure homogeneity between controls and AS groups (Supporting

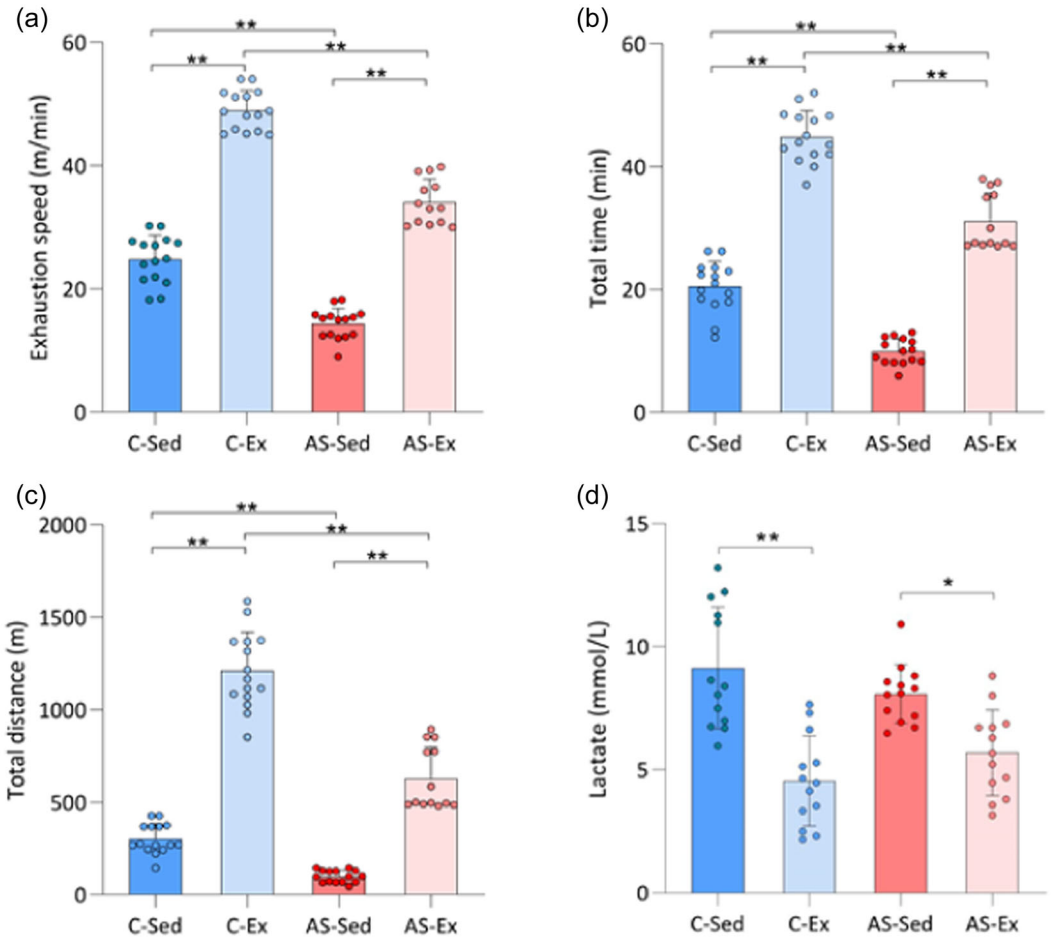

FIGURE 2 Final treadmill exercise testing and lactate concentration. Exhaustion speed (a), total time in treadmill (b), total run distance (c), and blood lactate concentration (d). AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis; C-Ex, exercised control; C-Sed, sedentary control; $n = 13–15$ per group. Data are mean ± SD and individual values; two-way ANOVA and Bonferroni; * $p < 0.05$; ** $p < 0.001$.

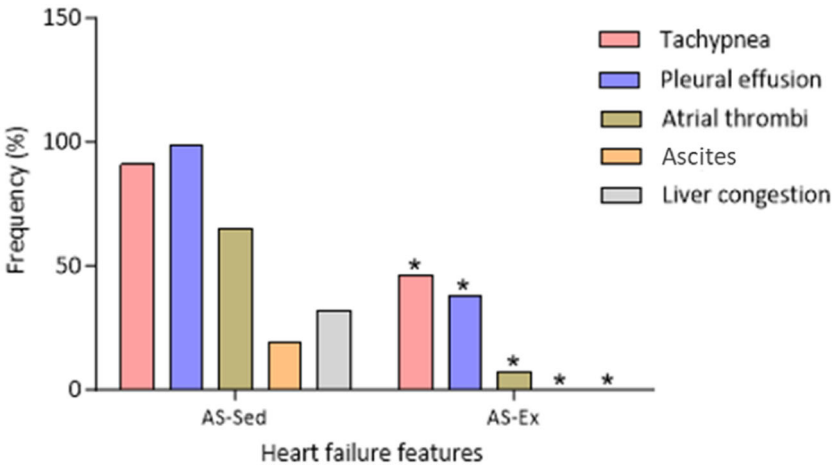

FIGURE 3 Frequency of heart failure features. AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis; $n = 13–15$ per group. Data are expressed as percentages. Goodman test; * $p < 0.05$ versus AS-Sed.

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TABLE 1 Final echocardiographic data.

	C-Sed	C-Ex	AS-Sed	AS-Ex
BW (g)	491 ± 66.9	430 ± 54.4§	422 ± 48.6*	364 ± 49.1#&
HR (bpm)	289 ± 25	323 ± 38	316 ± 53	321 ± 55
LVDD (mm)	7.48 ± 0.50	7.47 ± 0.45	8.42 ± 0.61*	7.47 ± 0.93#
DPWT (mm)	1.53 (1.53–1.53)	1.53 (1.50–1.53)	2.81 (2.65–2.81)*	2.55 (2.30–2.68)&
RWT	0.42 ± 0.04	0.41 ± 0.03	0.67 ± 0.06*	0.68 ± 0.10&
LA (mm)	4.85 (4.60–5.11)	4.85 (4.60–5.11)	7.92 (7.66–8.43)*	7.15 (6.39–8.17)&
LA/AO	1.24 ± 0.10	1.23 ± 0.08	1.99 ± 0.19*	1.78 ± 0.34#&
EF (%)	93.4 ± 2.00	93.5 ± 2.00	85.0 ± 5.00*	90.0 ± 3.00#&
MFS (%)	27.2 ± 3.89	27.9 ± 3.00	20.6 ± 3.78*	23.7 ± 2.52#&
PWSV (mm/s)	72.5 ± 3.80	72.8 ± 7.26	44.6 ± 6.68*	56.1 ± 11.8#&
S′ (cm/s)	5.80 (5.70–5.90)	5.70 (5.60–5.80)	4.20 (3.80–4.40)*	4.70 (4.40–5.10)&
IRVT (ms)	24.0 (22.0–25.0)	22.0 (22.0–25.0)	14.0 (12.0–15.0)*	15.0 (13.5–22.0)&
EDT (ms)	48.6 ± 2.80	48.3 ± 2.99	26.1 ± 6.44*	36.4 ± 10.3#&
Mitral E (cm/s)	83.8 (82.0–87.5)	82.0 (79.0–86.3)	132 (116–143)*	105 (95–121)&
Mitral A (cm/s)	51.2 (48.2–53.6)	55.4 (49.3–62.7)	22.8 (20.3–25.5)*	23.9 (19.7–66.1)&
E/A (cm/s)	1.61 ± 0.17	1.50 ± 0.19	5.86 ± 0.96*	4.07 ± 2.48#&
TDI E′ (cm/s)	5.65 ± 0.69	6.14 ± 0.73	5.08 ± 0.70*	5.52 ± 0.74&
TDI A′ (cm/s)	3.83 ± 0.70	4.00 ± 0.38	2.77 ± 0.84*	3.52 ± 1.00#
E/E′ (cm/s)	15.3 ± 1.08	13.7 ± 1.36	26.2 ± 4.98*	20.1 ± 3.95#&

Note: Data are mean ± SD or median and percentiles. Two-way ANOVA and Bonferroni or Kruskal–Wallis and Dunn; $n = 13–15$ per group; $p < 0.05$: * vs C-Sed; # vs AS-Sed; & vs C-Ex.

Abbreviations: A′, TDI of end-diastolic velocity of mitral annulus; AO, aorta diameter; AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis; BW: body weight; C-Ex, exercised control; C-Sed, sedentary control; DPWT, diastolic posterior wall thickness; E′, TDI of early diastolic velocity of mitral annulus; E/A, ratio of early (E)-to-late (A) diastolic mitral inflow; EDT, E-wave deceleration time; EF, ejection fraction; HR, heart rate; IVRT, isovolumetric relaxation time; LA, left atrium diameter; LVDD, left ventricular diastolic diameter; MSF, myocardial shortening fraction; PWSV, posterior wall shortening velocity; RWT, relative wall thickness; S′, tissue Doppler imaging (TDI) of systolic shortening velocity.

TABLE 2 Anatomical data.

	C-Sed	C-Ex	AS-Sed	AS-Ex
Tibia (cm)	4.36 ± 0.07	4.30 ± 0.06	4.25 ± 0.12	4.24 ± 0.10
Heart (g)	1.21 ± 0.13	1.10 ± 0.18	2.45 ± 0.32*	1.86 ± 0.30,#&
LV (g)	0.85 ± 0.09	0.77 ± 0.13	1.49 ± 0.19*	1.27 ± 0.15,#&
RV (g)	0.25 ± 0.02	0.22 ± 0.03	0.52 ± 0.08*	0.32 ± 0.08,#&
AT (g)	0.11 ± 0.01	0.11 ± 0.02	0.44 ± 0.09*	0.27 ± 0.09,#&
Heart/Tibia (g/cm)	0.28 ± 0.03	0.25 ± 0.04	0.58 ± 0.07*	0.44 ± 0.07,#&
LV/Tibia (g/cm)	0.19 ± 0.02	0.18 ± 0.03	0.35 ± 0.04*	0.30 ± 0.04,#&
RV/Tibia (g/cm)	0.057 ± 0.006	0.052 ± 0.008	0.123 ± 0.019*	0.075 ± 0.019,#&
AT/Tibia (g/cm)	0.026 ± 0.003	0.025 ± 0.004	0.102 ± 0.021*	0.063 ± 0.021,#&

Note: Data are mean ± SD. $n = 13–15$ per group. Two-way ANOVA and Bonferroni; $p < 0.05$: *vs C-Sed; #vs AS-Sed; &vs C-Ex.

Abbreviations: AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis; AT, atria weight; C-Ex, exercised control; C-Sed, sedentary control; LV, left ventricle weight; RV, right ventricle weight; Tibia, tibia length.

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Information: Table S1). Table 1 shows the final echocardiogram data after ET protocol. AS-Sed had higher LVDD, DPWT, RWT, LA, LA/AO ratio, E wave, and E/A and E/E′ ratios, and lower BW, EF, MFS, PWSV, S′, IRVT, EDT, Mitral A, and TDI E′ and A′ than C-Sed. AS-Ex had lower BW, LVDD, LA/AO, E/A, and E/E′, and higher EF, MFS, PWSV, EDT, and TDI A′ than AS-Sed. These results show that ET attenuates structural changes and systolic and diastolic dysfunction in AS rats (Table 1).

The post mortem cardiac macroscopic data are presented in Table 2. For better understanding, we have also added Supporting Information: Table S2, with the actual numbers used to generate the percentages (Supporting Information: Table S2). Except for the tibia, all parameters were higher in AS-Sed than C-Sed and AS-Ex groups.

3.1 | Identification of differentially abundant proteins

Analysis by Progenesis software identified 404 myocardial proteins; 165 proteins were considered for the comparative study, as they met the criterion of two or more unique peptides. ANOVA was used to

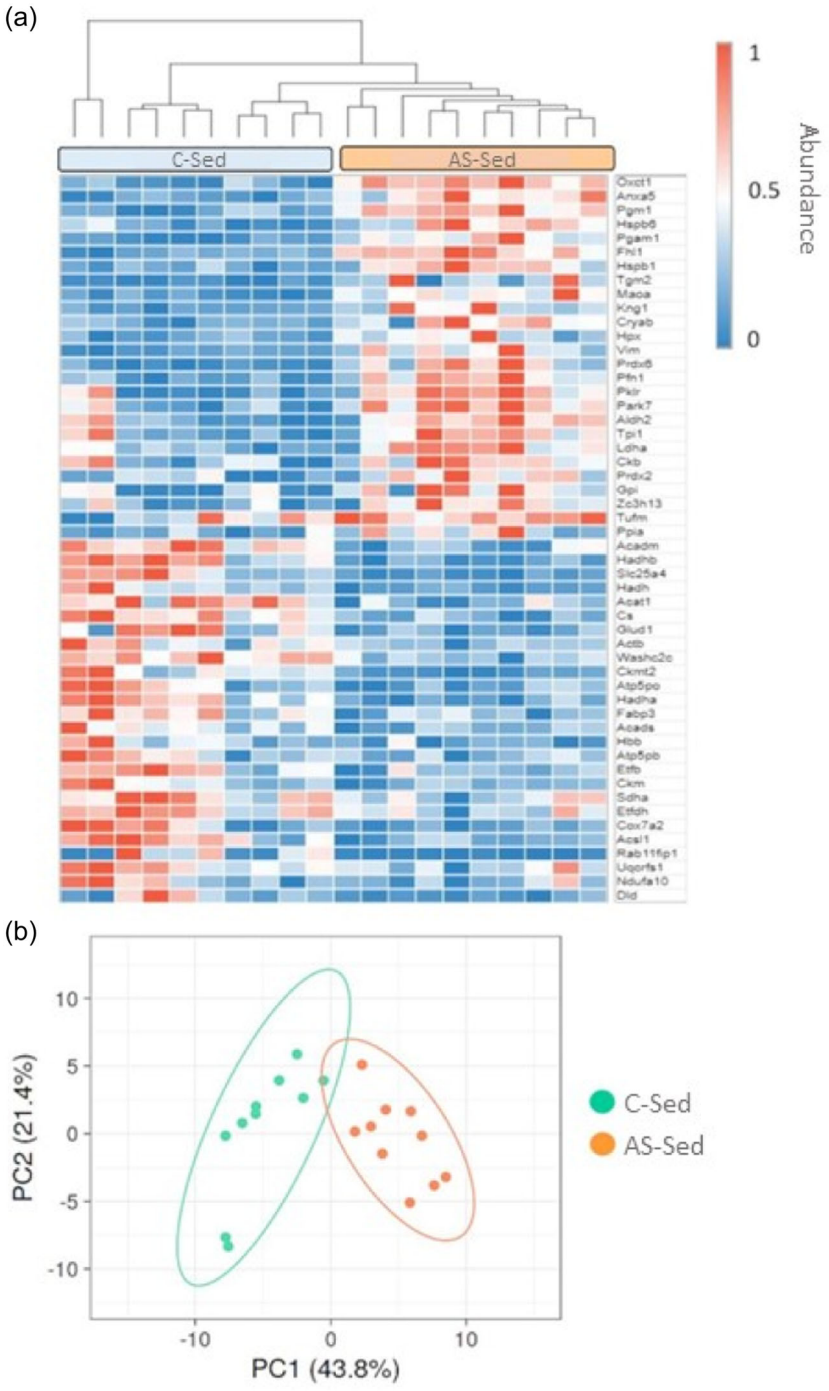

FIGURE 4 Label-free myocardial proteomic data of AS-Sed versus C-Sed groups. (a) Hierarchical clustering analyses (heatmap) using unsupervised Euclidean distance of all differentially abundant proteins between groups. A detailed description of protein names is shown in Supporting Information: Table S3. (b) Unsupervised multivariate principal component analysis (PCA). AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis.

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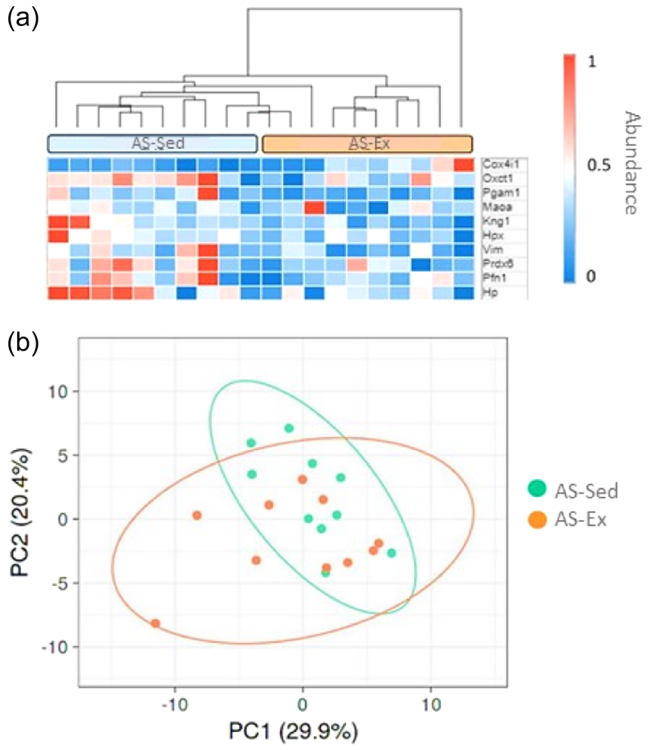

FIGURE 5 Label-free myocardial proteomic data of AS-Ex versus AS-Sed groups. (A) Hierarchical clustering analyses (heatmap) using unsupervised Euclidean distance of all differentially abundant proteins between groups. A detailed description of protein names is shown in Supporting Information: Table S3. (B) Unsupervised multivariate principal component analysis (PCA). AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis.

identify comparisons of interest. The abundance of 58 proteins significantly differed between groups; AS-Sed had 26 proteins increased and 26 proteins decreased in relation to C-Sed. AS-Ex had the abundance of one protein increased and nine proteins decreased compared to the AS-Sed. This information is detailed in Supporting Information: Table S3. To graphically represent the myocardial proteome results and the level of significance and magnitude of the changes, we performed a hierarchical cluster analysis of all proteins differentially abundant between AS-Sed versus C-Sed and AS-Ex versus AS-Sed; this revealed evident separation between groups (Figures 4a and 5a, respectively). We also performed multivariate principal component analysis (PCA), showing differences according to treatment (Figures 4b and 5b).

3.2 | Bioinformatics analysis

Proteomic results were acquired by label-free quantitative analysis (shotgun label-free) and were submitted to a functional enrichment analysis using GO terms and the KOBAS software. The procedure allowed the identification of biological processes linked to the differentially abundant proteins with both increased and decreased abundance, focusing on two comparisons, AS-Sed versus C-Sed and AS-Ex versus AS-Sed. The list of proteins with their related biological process is presented in Tables 3 and 4, and the gene names are detailed in Supporting Information: Table S3.

A protein–protein interaction network was constructed using the STRING database to visualize the possible relationship between proteins differentially observed. A network of interactions was observed between most of the 52 proteins that were differentially abundant. Most changed proteins from AS-Sed versus C-Sed rats were related to metabolic processes; these are represented by red circles (FDR: 6.49e−18). The network analysis also highlighted changes in lipid metabolism (gray circle); this functional category had the highest number of changed proteins (Figure 6). AS-Ex had alterations in 10 proteins compared to AS-Sed; the interaction network showed that 50% of these are related to biological regulation. Network analysis highlighted strong evidence for changes in cellular oxidant detoxification (gray circle) and acute inflammatory response (Figure 7).

GO enrichment analysis showed changes in molecular function, such as acetyl-CoAtransferase activity, fatty acyl-CoA binding, and electron transfer activity, with most proteins downregulated in AS-Sed compared to C-Sed (Figure 8). Most of the enriched pathways for the GO biological process showed a decrease in proteins involved in lipid metabolism, highlighting fatty acid beta-oxidation, electron transport chain, and tricarboxylic acid cycle. On the other hand, upregulated proteins were related to the glycolytic process, carbohydrate metabolism, response to oxidative stress, and hypoxia. Other altered proteins were involved in cell death regulation, stress-activated MAPK cascade, and intermediate filament-based process. Finally, GO cellular component analysis suggested that most of the changed proteins were related to mitochondria and mitochondrial matrix.

Analysis of the GO molecular functions for AS-Ex versus AS-Sed (Figure 9) showed that exercise had beneficial effects on oxidant and cytochrome c-oxidase activities, and cytoskeleton constituents. Evaluation of GO biological processes shows alterations in cellular oxidant detoxification, vasodilation, regulation of the glycolytic process, and immune system. Interestingly, all these pathways were reduced by ET. As for the GO cellular component, most of the enriched pathways were related to mitochondria, cytoskeleton, contractile machinery, and the haptoglobin-hemoglobin complex, which were decreased by ET.

3.3 | Validation of differentially expressed proteins by Western blot analysis

To validate differentially abundant proteins, Western blot analysis was performed to probe four proteins involved in cardiac energy metabolism, which were selected based on confidence in protein identification, magnitude of change by proteomics, and biological role

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TABLE 3 Biological process of AS-Sed versus C-Sed groups.

Biological process	Proteins	
	Up	Down
Lipid metabolism		
Receptor of fatty acids	—	FABP3
Mitochondrial β-Oxidation	—	ACADM; HADHB; HADH; ACAT1; HADHA; ACADS; ETFB; ACSL1
Glucose metabolism		
Glycolysis	PGM1; PGAM1; PKLR; TPI1; LDHA; GPI	—
Ketone body metabolism		
Catabolism	OXCT1	—
Tricarboxylic acid cycle	—	CS; GLUD1
Oxidative phosphorylation		
Electron transport chain	—	ETFDH; SDHA; COX7A2; UQCRFS1; NDUFA10; DLD
ATP-synthase complex	—	ATP5PB
Energy transfer	CKB	CKM; CKMT2
Oxidative stress and inflammation	KNG1; PPIA; HPX; ALDH2	—
Antioxidant defense	PRDX6; PRDX2; PARK7	—
Cytoskeleton	PFN1; FHL1; VIM; TGM2	ACTB; WASHC2C
Apoptosis	ANXA5; ZC3H13; MAOA; TUFM	—
Antiapoptosis	HSPB6; HSPB1; CRYAB	—
Transport		
Protons	—	SLC25A4; ATP5PO
Oxygen	—	HBB
Intracellular protein	—	UQCRFS1

Note: Biological process of the main up- and downregulated proteins identified by proteomics. A detailed description of proteins is shown in Supporting Information: Table S3.

Abbreviations: AS-Sed, sedentary aortic stenosis; C-Sed, sedentary control.

in cardiac remodeling (Figure 10 and Supporting Information: Figure S1). The proteomic data were confirmed for proteins MCAD and FABP3 which were lower and LDH which was higher in AS-Sed than C-Sed. PK expression did not differ between the groups, thus not corroborating with proteomic results.

4 | DISCUSSION

The molecular changes related to cardioprotection from ET in AS rodents are still poorly understood. Label-free shotgun proteomics is a robust tool to provide insights into biological mechanisms. The technique identified 58 proteins differentially abundance between the groups, most involved in energy lipid and glycolytic metabolism, oxidative stress, inflammation, and structural architecture.

4.1 | Effects of physical training on functional capacity and the heart

Exercise prevents damage to the heart caused by various types of injury (Pazzianotto-Forti et al., 2020; Saco-Ledo et al., 2020; Sjölin et al., 2020; Suppan et al., 2020). As rats with persistent pressure overload are intolerant to physical effort, we used a moderate-volume, low-intensity training protocol (de Souza et al., 2020; Mota et al., 2020). ET improved functional capacity in both C-Ex and AS-Ex groups, which may be related to improved muscular and cardiocirculatory oxidative efficiency (Souza et al., 2014). Decreased lactate concentration in the trained groups reinforces improved functional capacity. Reduced lactate concentration may be due to higher oxidation of fatty acids, the conversion of lactate into glycogen, and lower anaerobic glycolysis (Brooks, 2018).

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TABLE 4 Biological process of AS-Ex versus AS-Sed groups.

Biological process	Proteins	
	Up	Down
Ketone body metabolism		
Catabolism	—	OXCT1
Glucose metabolism		
Glycolysis	—	PGAM1
Oxidative Phosphorylation		
Electron transport chain	COX4I1	—
Antioxidant defense	—	MAOA; HPX; PRDX6; HP
Oxidative stress andinflammation	—	KNG1
Cytoskeleton	—	VIM; PFN1

Note: Biological process of the main up- and downregulated proteins identified by proteomics. A detailed description of proteins is shown in Supporting Information: Table S3.

Abbreviations: AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis.

AS had LV and atrial hypertrophy, which was attenuated by ET. Studies have suggested that exercise-activated physiological pathways counter-regulate pathological pathways (Bernardo et al., 2018). Long-term AS induces concentric hypertrophy; if this process persists, LV function is impaired (Mota et al., 2020). At the end of the experiment, most AS rats presented HF features, which were attenuated by ET. Physical training attenuated LV and atrial structural changes and improved systolic and diastolic function in AS-Ex.

Research on the influence of exercise in pressure overloaded-induced cardiac remodeling has produced disparate results in rodents. Spontaneously hypertensive rats subjected to low-intensity exercise had improved contractility and cardiac remodeling (Pagan et al., 2015, 2021, 2019). However, when subjected to voluntary wheel running, which is characterized by short periods of intense activity (Gomes et al., 2016), hypertensive rats (Pagan et al., 2019) and AS mice (van Deel et al., 2011) had impaired cardiac remodeling. Furthermore, exercise initiated 18 weeks after AS induction failed to improve ventricular function in rats (Gomes et al., 2016; Reyes et al., 2019). Our data showed that starting training before functional deterioration is useful to reduce pathological remodeling. Thus, both

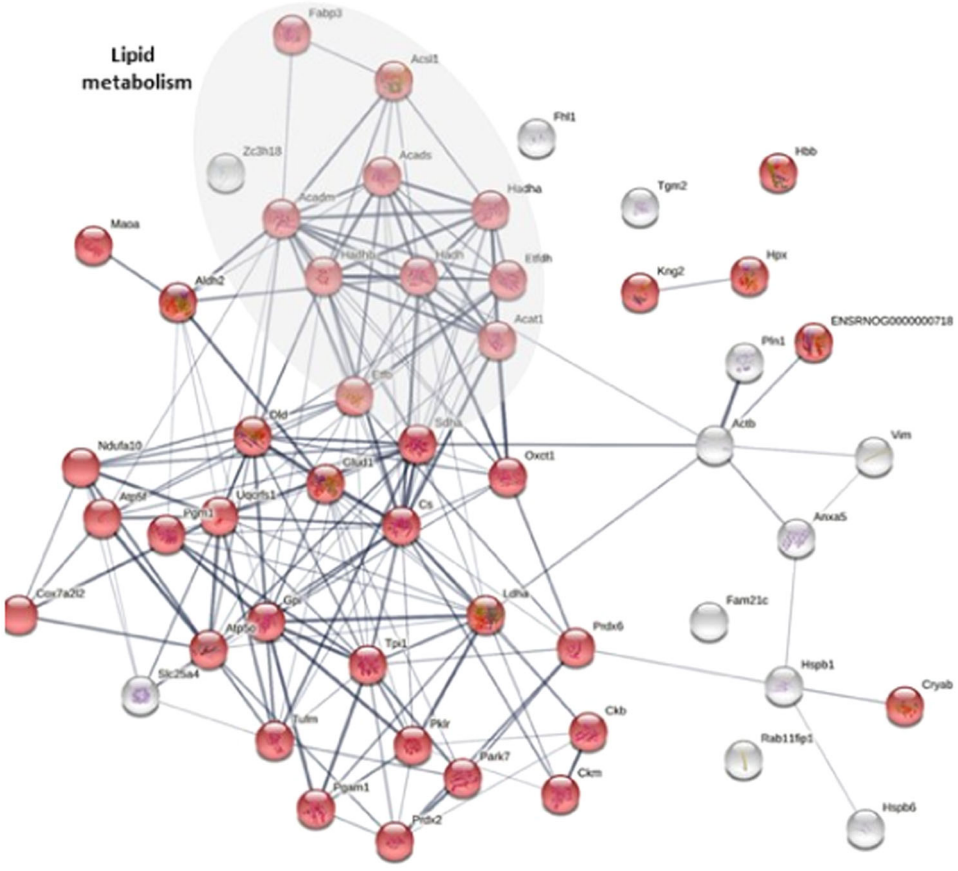

FIGURE 6 Network of protein–protein interactions differentially abundant in the myocardium of AS-Sed versus C-Sed groups. The interaction network analysis was constructed using STRING online software with a medium confidence level of 0.4. Circles represent proteins, while straight lines represent interactions between different proteins. Line thickness indicates strength of evidence, with thicker connections indicating greater interaction robustness. Red circles represent metabolic process proteins and gray circles represent lipid metabolism proteins. AS-Sed, sedentary aortic stenosis; C-Sed, sedentary control.

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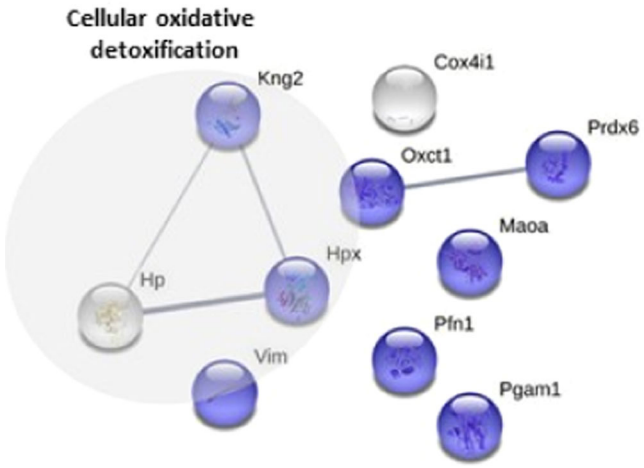

FIGURE 7 Protein–protein interaction network for all differentially abundant proteins in the myocardium of rats from AS-Ex versus AS-Sed groups. Interaction network analyses were constructed using STRING online software with a medium confidence level of 0.4. Circles represent proteins, while straight lines represent interactions between different proteins. Line thickness indicates strength of evidence, with thicker connections indicating greater interaction robustness. Blue circles represent proteins involved in biological regulatory processes and gray circles represent proteins involved in lipid metabolism. AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis.

intensity and timing of physical training are important players in adaptations occurring during pressure overload-induced cardiac remodeling.

4.2 | Effects of AS on the myocardial proteome

The most evident influence of AS on the cardiac proteome was related to the energy metabolism. AS-Sed had lower lipid-related protein abundance and higher glycolytic pathway-related proteins than C-Sed. Energy metabolism changes play a major role in ventricular dysfunction (Nakamura & Sadoshima, 2018). The heart has a high metabolic plasticity; it can use different substrates such as fatty acids (FAs), glucose, lactate, ketone bodies, and amino acids to generate ATP (Kadkhodayan et al., 2013). FA is the main source of energy; under physiological conditions, approximately 50%–70% of heart ATP synthesis comes from FA oxidation; glycolytic pathway contribution is around 30%–40% (Kolwicz et al., 2013). In pathological remodeling, decreased FA metabolism is secondary to lower expression and activity of the proteins involved in FA uptake and oxidation. Glycolysis is increased, although with no proportional increase in glucose mitochondrial oxidation (Nakamura & Sadoshima, 2018). In fact, the abundance of proteins involved in the Krebs cycle, electron transport chain, and ATP-synthase complex was lower in AS-Sed, suggesting an impairment in mitochondrial energy production.

Myocardial changes in ketone body metabolism have received increasing attention (Nakamura & Sadoshima, 2018). OXCT1 protein, an enzyme responsible for ketone body catabolism, was higher in AS-Sed than C-Sed. Serum ketone bodies are increased in HF and may have a cardioprotective role by generating less reactive oxygen species (ROS) (Schugar et al., 2014). In accordance with our results, the proteins involved in FA use were lower and β-hydroxybutyrate dehydrogenase 1, a key enzyme in ketone body oxidation, was higher in AS mice (Aubert et al., 2016). High concentrations of circulating ketones appear to reduce myocardial inflammation and prevent HF development (Byrne et al., 2020).

Metabolic alterations in the failing heart, mainly decreased β-oxidation, mitochondrial dysfunction, and increased glucose oxidation, can increase ROS production. In agreement, our data showed alterations in proteins related to antioxidant defense. Two mitochondrial antioxidant enzymes were increased: PRDX2, responsible for ROS detoxification, and PRDX6, which plays a role in the turnover of cell phospholipids, repairing the peroxidized cell membrane (Fisher, 2017).

Parkinson's protein 7 (PARK7/DJ-1) was higher in AS-Sed than C-Sed. PARK7 reduces the proapoptotic pathway and stimulates the PI3K/Akt pathway (Xin et al., 2020). Higher PARK7 levels are related to reduced cell death, decreased ROS production, and increased antioxidant enzyme synthesis (Lu et al., 2012; Yan et al., 2015). The proteolytic activity of PARK7 against glycative stress protects from ischemia-reperfusion-induced cardiac injury (Lee et al., 2012; Shimizu et al., 2020). On the other hand, myocardial PARK7 deficiency increases vulnerability to oxidative stress (Bonifati et al., 2003) and its absence has been linked to worse post-ischemia-reperfusion injury and pressure overload-induced ventricular dysfunction (Billia et al., 2013; Dongworth et al., 2014). In this study, changed PRDX6, MAOA, PRDX2, and PARK7 abundance may be a compensatory response to increased ROS. Although increased antioxidant defense is observed in HF (Tsutsui et al., 2011), it is not sufficient to prevent a pro-oxidant state. Also, COX-2, an oxidative stress and inflammation marker, was higher in AS-Sed than C-Sed (data not shown). Inflammation-related PPIA, KNG1, HPX, and ALDH2 proteins were upregulated in AS-Sed compared to C-Sed. An increase in these proteins has been associated with impaired cardiomyocyte injury in different experimental models (Alvarado et al., 2020; Belcher et al., 2014; Cheng et al., 2021; Perrucci et al., 2015).

Cytoskeleton-associated proteins PFN1, FHL1, VIM, and TGM2 were higher, and ACTB and WASHC2C were lower in AS-Sed than C-Sed. The cardiac cytoskeleton is important for maintaining cardiomyocyte structural and functional integrity (Sequeira & van der Velden, 2014). An imbalance in structural proteins may explain, at least in part, the cardiac dysfunction seen in AS-Sed. Some proteins that participate in apoptosis (ANXA5, ZC3H13, and MAOA) were higher in AS-Sed as were some antiapoptotic proteins (HSPB6, HSPB1, and CRYAB). The proteome of AS rodents has also shown increased abundance of apoptosis-related proteins (Cheng et al., 2013; Lee et al., 2006; Quindry et al., 2012).

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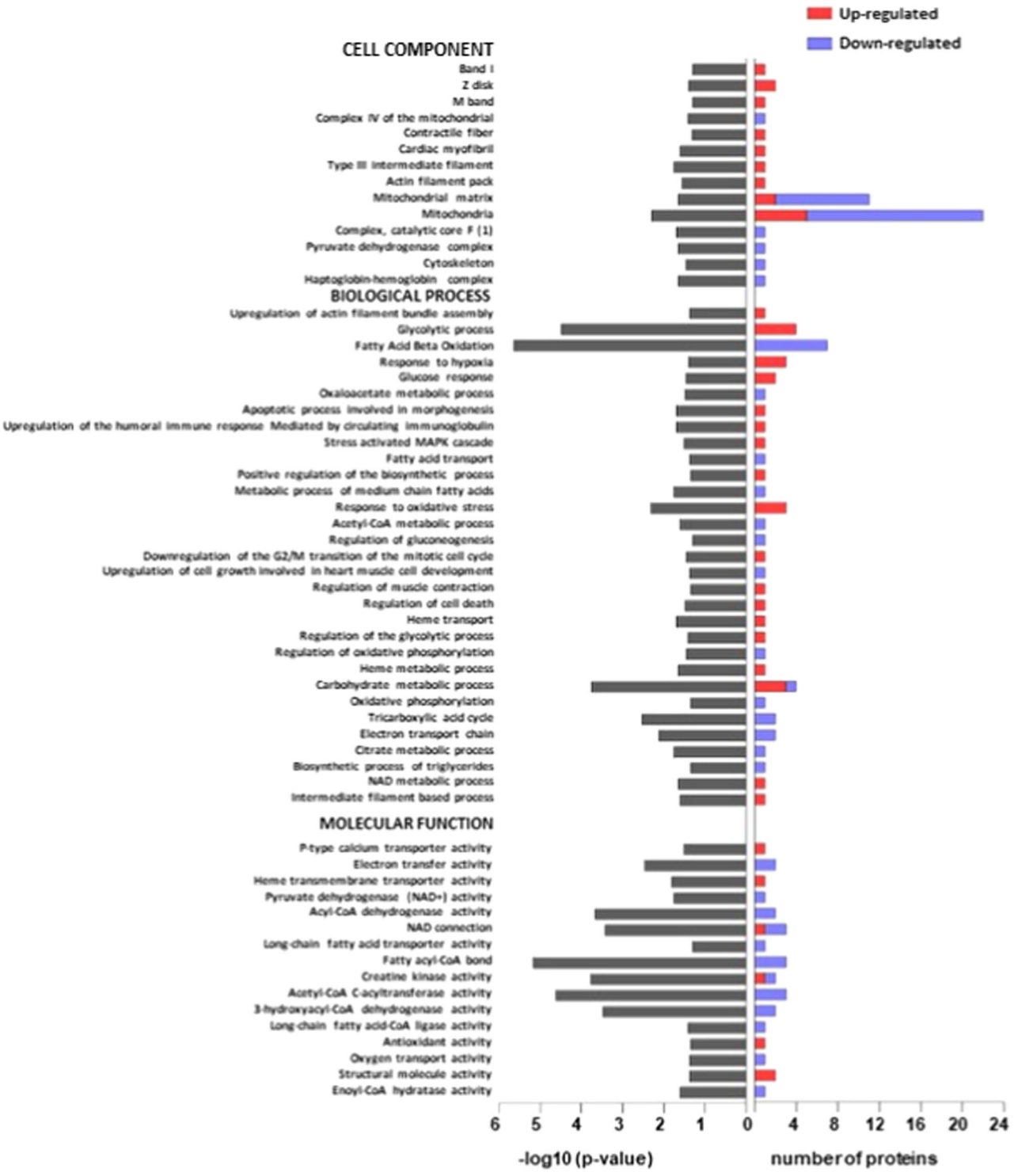

FIGURE 8 Gene ontology enrichment analysis for up- and downregulated myocardial proteins from AS-Sed versus C-Sed group. Analysis was performed using the Kobas tool (http://kobas.cbi.pku.edu.cn/) providing the terms significantly enriched GO, Molecular Function, Biological Process, and Cellular Component. In the left panel, the horizontal axis indicates the significance (−log10 *p* value) of the functional association, which is dependent on the number of proteins altered in each class. In the right panel, changes in the pattern of cellular components, biological process, and molecular function are displayed as the number of proteins with increased or decreased levels (horizontal axis). AS-Sed, sedentary aortic stenosis; C-Sed, sedentary control.

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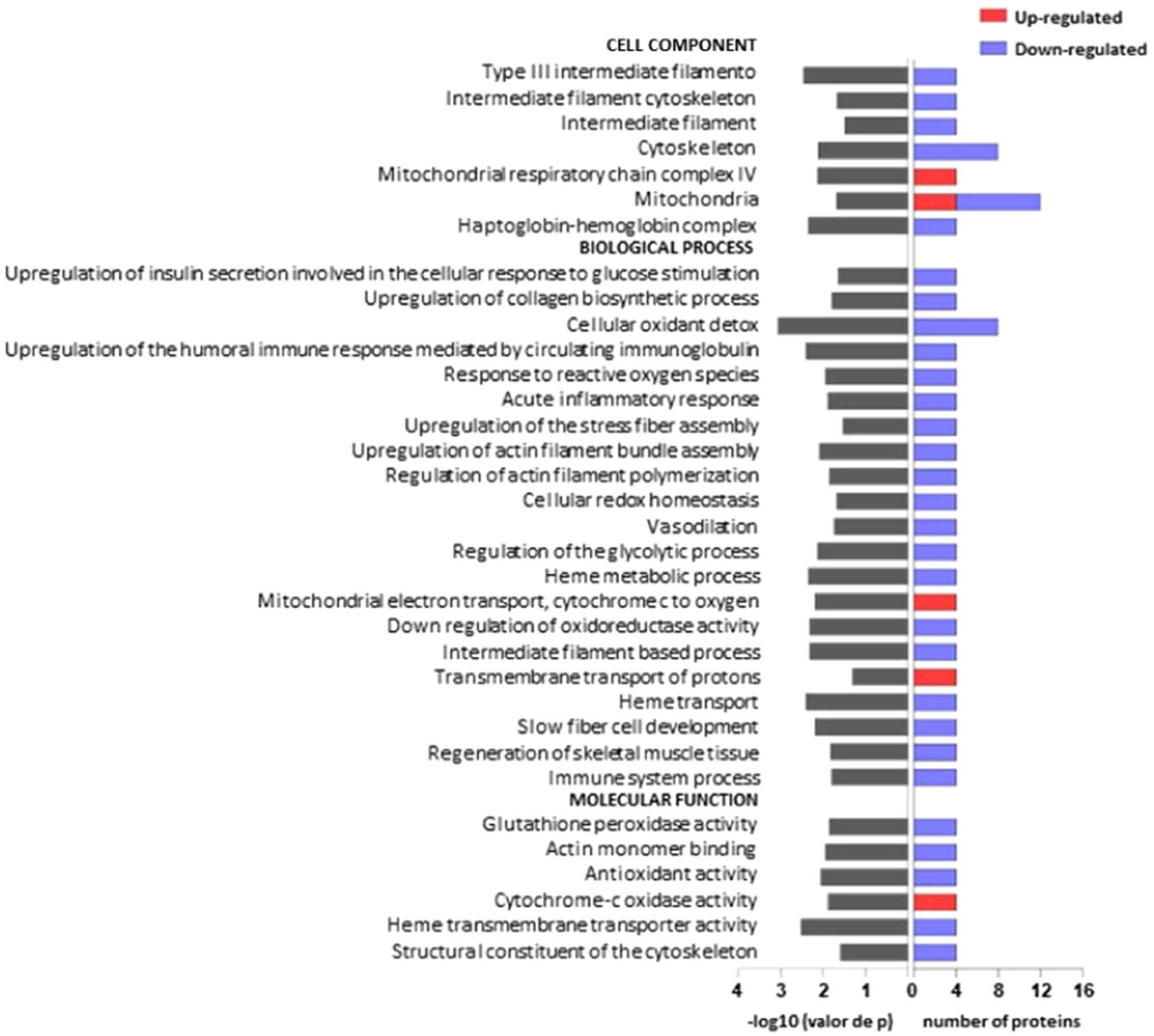

FIGURE 9 Gene ontology enrichment analysis for up- and downregulated myocardial proteins from AS-Ex versus AS-Sed group. Analysis was performed using the Kobas tool (http://kobas.cbi.pku.edu.cn/) providing the terms significantly enriched GO, Molecular Function, Biological Process, and Cellular Component. In the left panel, the horizontal axis indicates the significance (−log10 *p* value) of the functional association, which is dependent on the number of proteins altered in each class. In the right panel, changes in the pattern of cellular components, biological process, and molecular function are displayed as the number of proteins with increased or decreased levels (horizontal axis). AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis.

4.3 | Effects of aerobic physical training on the myocardial proteome

Moderate-intensity aerobic exercise had no effect on the myocardial proteome when applied to healthy rats. ET modulated 10 proteins. Although the number of changed proteins may be modest, the total number is considerable, particularly when taking into account the updated Swiss-Prot database used for identifying rat proteins, which contains less than half the number of proteins reviewed for *Homo sapiens*. Abundance of COX4I1, a protein related to mitochondrial biogenesis, was higher in AS-E than in AS-Sed. Tissue hypoxia stimulates myocardial changes (Semenza, 2014). A master regulator of the hypoxia response, hypoxia-inducible factor (HIF-1α), regulates oxygen-dependent oxidative enzyme cytochrome C-oxidases (COX4) (Fukuda et al., 2007). Under physiological conditions, COX4I1 gene expression is high, and COX4I2 is repressed. Under hypoxia, HIF-1α activity is elevated along with other genes, including COX4I2, necessary for degradation of COX4I1 (Fukuda et al., 2007). In our study, the higher COX4I1 abundance suggests increased tissue oxygen supply. Reinforcing this, myocardial relative protein abundance of HIF-1α was lower in AS-E than AS-Sed (data not shown). Our results therefore suggest that one of the cardioprotection

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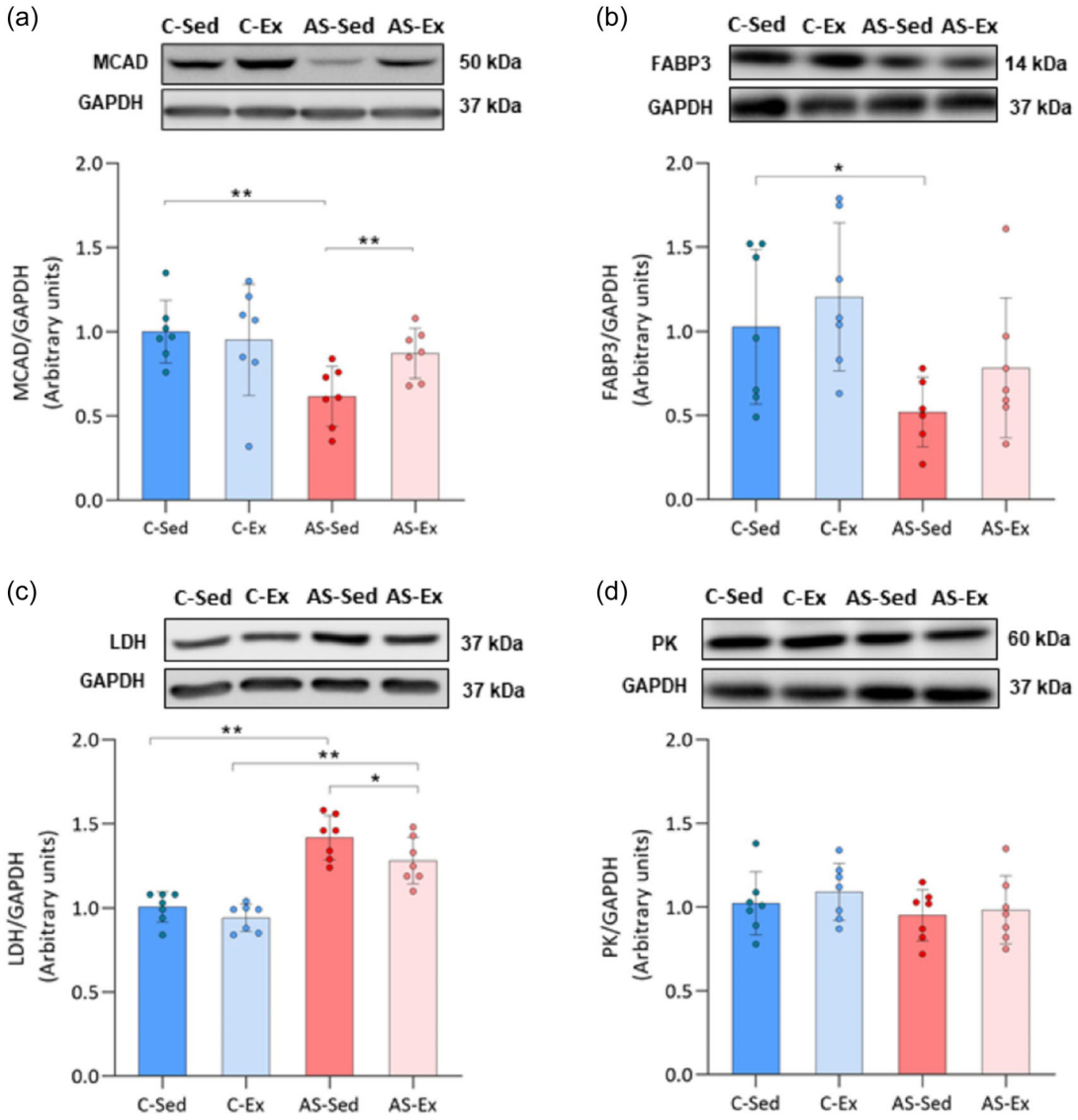

FIGURE 10 Validation of proteomic results by Western blot. Data are mean, SD, and individual values. AS-Ex, exercised aortic stenosis; AS-Sed, sedentary aortic stenosis; C-Ex, exercised control; C-Sed, sedentary control; FABP3, fatty acid-binding protein 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; PK, pyruvate kinase. Two-way ANOVA and Bonferroni; $n = 6–7$ per group; * $p < 0.05$; ** $p < 0.001$.

mechanisms generated by ET is related to mitochondrial biogenesis. Interestingly, OXCT1 abundance decreased with ET. This enzyme regulates ketone body catabolism. We have not found any studies describing how training modulates OXCT1 during HF. By decreasing OXCT1, physical training could be harmful to the heart.

Another protein negatively modulated by exercise was the enzyme PGAM1, which catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate (Tran & Wang, 2019). As the glycolytic pathway is increased in HF, a reduction in PGAM1 suggests decreased myocardial glycolysis. Interestingly, exercise did not change beta-oxidation. Decreased glucose and ketone body metabolism with unchanged beta-oxidation may suggest that ET is not a beneficial therapy in AS. However, echocardiogram did show LV structural and functional improvement after ET.

ET decreased the abundance of proteins related to oxidative stress and inflammation. Our results suggest that counter-regulation of the damage caused by increased ROS may be one of the mechanisms for attenuating cardiac dysfunction by exercise. In fact, regular exercise decreases systemic oxidative stress (Bozi et al., 2016; Cardoso et al., 2013) and attenuates inflammation in rats with ventricular dysfunction (Liao et al., 2019; Reyes et al., 2019). KNG1, a protein with pro-inflammatory, proapoptotic, and pro-oxidant function, was higher in AS-Es=x than AS-Sed; we can therefore infer that reduction in inflammatory, apoptotic, and oxidant processes was involved in the improvement of AS-Ex hearts (Xu et al., 2018). Another protein reduced by exercise was HPX, a plasma protein with the highest binding affinity for heme. In the cardiovascular system, heme modulates antioxidant defense, signal transduction, oxygen

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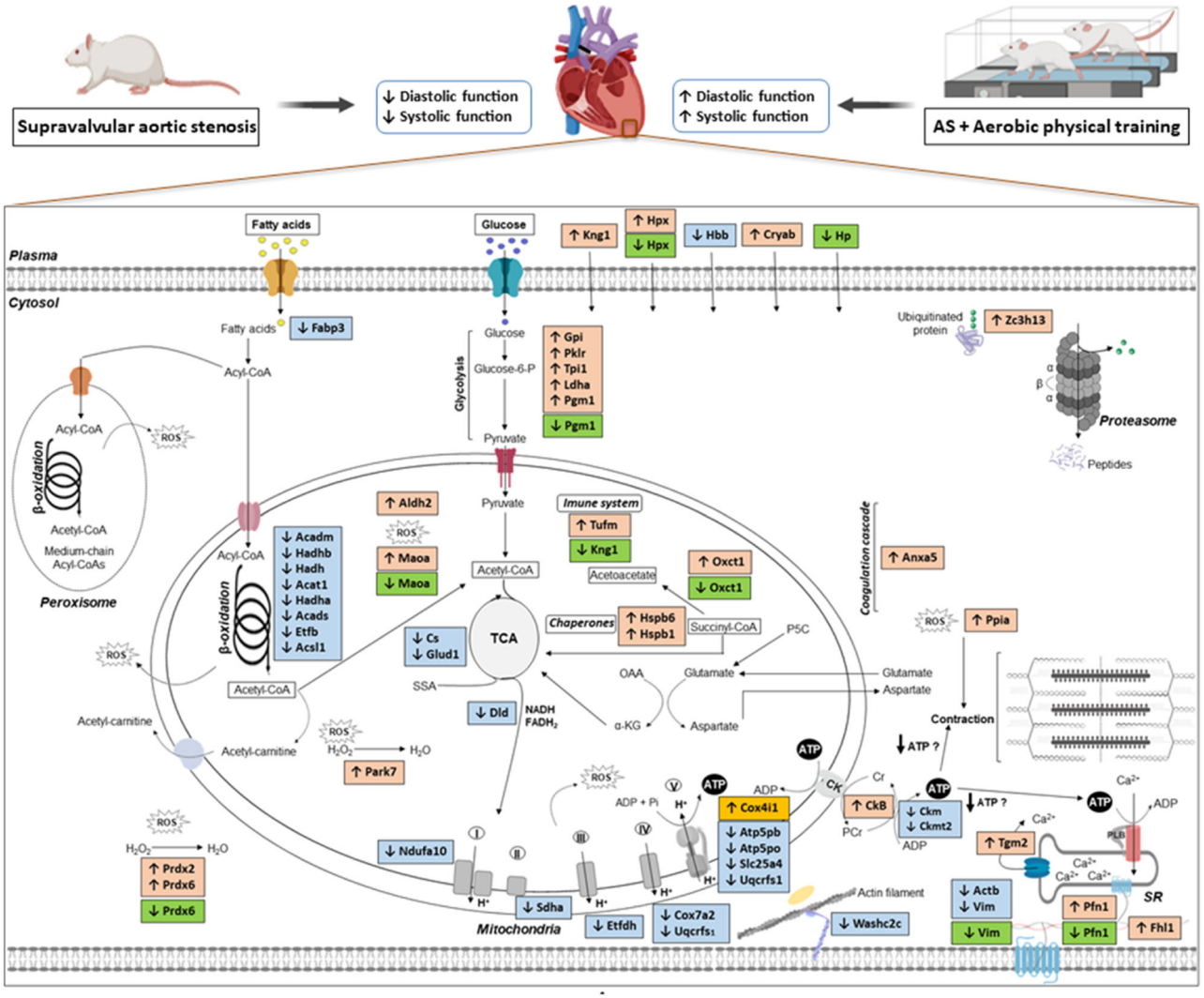

FIGURE 11 Overview of the effects of supravalvular aortic stenosis (AS) and aerobic physical training on the rat myocardial proteome. Identified proteins are shown according to the change: red for upregulated proteins and blue for downregulated proteins in the myocardium of AS-Sed versus C-Sed rats; orange for upregulated proteins and green for downregulated proteins in the myocardium of AS-Ex versus AS-Sed rats. α-KG, α-ketoglutarate; AGE, advanced glycation end product; CK, creatine kinase; Cr, creatine; P5C, pyrroline-5-carboxylate; OAA, oxaloacetate; PCr, phosphocreatine; ROS, reactive oxygen species; SR, sarcoplasmic reticulum; SSA, semialdehyde succinate; TCA, tricarboxylic acid cycle; I, II, III, IV, and V, mitochondrial oxidative phosphorylation complexes (I–IV: respiratory chain complexes; V: ATP synthase complex). Description of protein names is shown in Supporting Information: Table S3.

transport, hemoglobin storage, and mitochondrial electron transport (Yanatori et al., 2019). Increased heme levels are related to increased oxidative stress in cardiomyocytes during HF (Bhoite-Solomon et al., 1993; Khechaduri et al., 2013). Finally, ET decreased PFN1 and vimentin (VIM), proteins related to myocardial architecture. PFN1 modulates cytoskeletal dynamics, promoting actin polymerization, and is linked to endothelial dysfunction and increased inflammation. VIM is a Type III intermediate filament protein that participates in supporting and anchoring cytosolic organelles (Katsumoto et al., 1990). We have not found any studies addressing VIM expression in pressure-overloaded animals subjected to ET. Additional research is needed to validate all proteins with changed relative abundance by more precise methods such as Western blot and ELISA.

Figure 11 shows an overview of the effects of supravalvular AS and ET on the rat myocardial proteome. The proteins encoded by the genes are detailed in Supporting Information: Table S3.

5 | CONCLUSION

Chronic pressure overload changes the abundance of myocardial proteins that are mainly involved in lipidic and glycolytic energy metabolism in rats. Moderate-intensity aerobic training attenuates changes in proteins related to oxidative stress and inflammation and increases the COX4I1 protein related to mitochondrial biogenesis. Protein changes are combined with improved

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functional capacity, cardiac remodeling, and left ventricular function in AS rats.

AUTHOR CONTRIBUTIONS

Gustavo Augusto Ferreira Mota and Antonio Carlos Cicogna conceived and designed the study. Sérgio Luiz Borges de Souza, Danielle Fernandes Vileigas, Vitor Loureiro da Silva, Paula Grippa Sant'Ana, Carlos Roberto Padovani, Licia Carla da Silva Costa, Silméia Garcia Zanatti Bazan, Marília Afonso Rabelo Buzalaf, Lucilene Delazari dos Santos, Marina Politi Okoshi, and Mariana Gatto contributed to the acquisition or interpretation of data. Gustavo Augusto Ferreira Mota, Mariana Gatto, Marina Politi Okoshi, and Antonio Carlos Cicogna drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

All experiments and procedures were approved by the Ethics Committee of Botucatu Medical School, UNESP (1308-2019).

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REFERENCES

Alvarado, G., Tóth, A., Csősz, É., Kalló, G., Dankó, K., Csernátony, Z., Smith, A., Gram, M., Akerström, B., Édes, I., Balla, G., Papp, Z., & Balla, J. (2020). Heme-induced oxidation of cysteine groups of myofilament proteins leads to contractile dysfunction of permeabilized human skeletal muscle fibres. *International Journal of Molecular Sciences*, *21*(21):8172.

Aubert, G., Martin, O. J., Horton, J. L., Lai, L., Vega, R. B., Leone, T. C., Koves, T., Gardell, S. J., Krüger, M., Hoppel, C. L., Lewandowski, E. D., Crawford, P. A., Muoio, D. M., & Kelly, D. P. (2016). The failing heart relies on ketone bodies as a fuel. *Circulation*, *133*(8), 698–705.

Bansal, A., Dai, Q., Chiao, Y. A., Hakala, K. W., Zhang, J. Q., Weintraub, S. T., & Lindsey, M. L. (2010). Proteomic analysis reveals late exercise effects on cardiac remodeling following myocardial infarction. *Journal of Proteomics*, *73*(10), 2041–2049.

Beck, H. C., Jensen, L. O., Gils, C., Ilondo, A. M. M., Frydland, M., Hassager, C., Møller-Helgestad, O. K., Møller, J. E., & Rasmussen, L. M. (2018). Proteomic discovery and validation of the confounding effect of heparin administration on the analysis of candidate cardiovascular biomarkers. *Clinical Chemistry*, *64*(10), 1474–1484.

Belcher, J. D., Chen, C., Nguyen, J., Milbauer, L., Abdulla, F., Alayash, A. I., Smith, A., Nath, K. A., Hebbel, R. P., & Vercellotti, G. M. (2014). Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. *Blood*, *123*(3), 377–390.

Bernardo, B. C., Ooi, J. Y. Y., Weeks, K. L., Patterson, N. L., & McMullen, J. R. (2018). Understanding key mechanisms of exercise-induced cardiac protection to mitigate disease: Current knowledge and emerging concepts. *Physiological Reviews*, *98*(1), 419–475.

Bhoite-Solomon, V., Kessler-Icekson, G., & Shaklai, N. (1993). Myocyte injury by hemin. *In Vitro Cellular & Developmental Biology—Animal*, *29A*(8), 636–642.

Billia, F., Hauck, L., Grothe, D., Konecny, F., Rao, V., Kim, R. H., & Mak, T. W. (2013). Parkinson-susceptibility gene DJ-1/PARK7 protects the murine heart from oxidative damage in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(15), 6085–6090.

Boluyt, M. O., Brevick, J. L., Rogers, D. S., Randall, M. J., Scalia, A. F., & Li, Z. B. (2006). Changes in the rat heart proteome induced by exercise training: Increased abundance of heat shock protein hsp20. *Proteomics*, *6*(10), 3154–3169.

Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J. C., Brice, A., van Duijn, C. M., Oostra, B., Meco, G., & Heutink, P. (2003). DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurological Sciences*, *24*(3), 159–160.

Bozi, L. H. M., Jannig, P. R., Rolim, N., Voltarelli, V. A., Dourado, P. M. M., Wisløff, U., & Brum, P. C. (2016). Aerobic exercise training rescues cardiac protein quality control and blunts endoplasmic reticulum stress in heart failure rats. *Journal of Cellular and Molecular Medicine*, *20*(11), 2208–2212.

Brooks, G. A. (2018). The science and translation of lactate shuttle theory. *Cell Metabolism*, *27*(4), 757–785.

Bugger, H., Schwarzer, M., Chen, D., Schrepper, A., Amorim, P. A., Schoepe, M., Nguyen, T. D., Mohr, F. W., Khalimonchuk, O., Weimer, B. C., & Doenst, T. (2010). Proteomic remodelling of mitochondrial oxidative pathways in pressure overload-induced heart failure. *Cardiovascular Research*, *85*(2), 376–384.

Burniston, J. G. (2009). Adaptation of the rat cardiac proteome in response to intensity-controlled endurance exercise. *Proteomics*, *9*(1), 106–115.

Byrne, N. J., Soni, S., Takahara, S., Ferdaoussi, M., Al Batran, R., Darwesh, A. M., Levasseur, J. L., Beker, D., Vos, D. Y., Schmidt, M. A., Alam, A. S., Maayah, Z. H., Schertzer, J. D., Seubert, J. M., Ussher, J. R., & Dyck, J. R. B. (2020). Chronically elevating circulating ketones can reduce cardiac inflammation and blunt the development of heart failure. *Circulation: Heart Failure*, *13*(6):e006573.

Cardoso, A. M., Martins, C. C., Fiorin, F. S., Schmatz, R., Abdalla, F. H., Gutierres, J., Zanini, D., Fiorenza, A. M., Stefanello, N., Serres, J. D. S., Carvalho, F., Castro, V. P., Mazzanti, C. M., Royes, L. F. F., Belló-Klein, A., Goularte, J. F., Morsch, V. M., Bagatini, M. D., & Schetinger, M. R. C. (2013). Physical training prevents oxidative stress in L-NAME-induced hypertension rats. *Cell Biochemistry and Function*, *31*(2), 136–151.

Chang, Y.-W., Chang, Y.-T., Wang, Q., Lin, J. J.-C., Chen, Y.-J., & Chen, C.-C. (2013). Quantitative phosphoproteomic study of pressure-overloaded mouse heart reveals dynamin-related protein 1 as a modulator of cardiac hypertrophy. *Molecular & Cellular Proteomics*, *12*(11), 3094–3107.

Cheng, S.-M., Ho, T.-J., Yang, A.-L., Chen, I.-J., Kao, C.-L., Wu, F.-N., Lin, J. A., Kuo, C. H., Ou, H. C., Huang, C. Y., & Lee, S. D. (2013). Exercise training enhances cardiac IGFI-R/PI3K/Akt and Bcl-2 family associated pro-survival pathways in streptozotocin-induced diabetic rats. *International Journal of Cardiology*, *167*(2), 478–485.

Cheng, X., Liu, D., Song, H., Tian, X., Yan, C., & Han, Y. (2021). Overexpression of Kininogen-1 aggravates oxidative stress and

10974652, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.31199 by Univ of Sao Paulo - Brazil, Wiley Online Library on [15/04/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

mitochondrial dysfunction in DOX-induced cardiotoxicity. *Biochemical and Biophysical Research Communications*, *550*, 142–150.

Cohn, J. N., Ferrari, R., & Sharpe, N. (2000). Cardiac remodeling—Concepts and clinical implications: A consensus paper from an international forum on cardiac remodeling. *Journal of the American College of Cardiology*, *35*(3), 569–582.

Cox, E. J., & Marsh, S. A. (2013). Exercise and diabetes have opposite effects on the assembly and O-GlcNAc modification of the mSin3A/HDAC1/2 complex in the heart. *Cardiovascular Diabetology*, *12*, 101.

Dai, D.-F., Hsieh, E. J., Liu, Y., Chen, T., Beyer, R. P., Chin, M. T., MacCoss, M. J., & Rabinovitch, P. S. (2012). Mitochondrial proteome remodelling in pressure overload-induced heart failure: The role of mitochondrial oxidative stress. *Cardiovascular Research*, *93*(1), 79–88.

Dantas, P. S., Sakata, M. M., Perez, J. D., Watanabe, R. L. H., Bizerra, F. C., Neves, V. J., Guzzoni, V., Marcondes, F. K., Casarini, D. E., & Cunha, T. S. (2016). Unraveling the role of high-intensity resistance training on left ventricle proteome: Is there a shift towards maladaptation? *Life Sciences*, *152*, 156–164.

van Deel, E. D., de Boer, M., Kuster, D. W., Boontje, N. M., Holemans, P., Sipido, K. R., van der Velden, J., & Duncker, D. J. (2011). Exercise training does not improve cardiac function in compensated or decompensated left ventricular hypertrophy induced by aortic stenosis. *Journal of Molecular and Cellular Cardiology*, *50*(6), 1017–1025.

Deus, A. F., Silva, V. L., de Souza, S. L. B., Mota, G. A. F., Sant'Ana, P. G., Vileigas, D. F., Lima-Leopoldo, A. P., Leopoldo, A. S., Campos, D. H. S., de Tomasi, L. C., Padovani, C. R., Kolwicz, S. C., & Cicogna, A. C. (2019). Myocardial dysfunction after severe food restriction is linked to changes in the calcium-handling properties in rats. *Nutrients*, *11*(9):1985.

Dongworth, R. K., Mukherjee, U. A., Hall, A. R., Astin, R., Ong, S.-B., Yao, Z., Dyson, A., Szabadkai, G., Davidson, S. M., Yellon, D. M., & Hausenloy, D. J. (2014). DJ-1 protects against cell death following acute cardiac ischemia-reperfusion injury. *Cell Death & Disease*, *5*, e1082.

Ferreira, R., Vitorino, R., Padrão, A. I., Espadas, G., Mancuso, F. M., Moreira-Gonçalves, D., Castro-Sousa, G., Henriques-Coelho, T., Oliveira, P. A., Barros, A. S., Duarte, J. A., Sabidó, E., & Amado, F. (2014). Lifelong exercise training modulates cardiac mitochondrial phosphoproteome in rats. *Journal of Proteome Research*, *13*(4), 2045–2055.

Fisher, A. B. (2017). Peroxiredoxin 6 in the repair of peroxidized cell membranes and cell signaling. *Archives of Biochemistry and Biophysics*, *617*, 68–83.

Fukuda, R., Zhang, H., Kim, J., Shimoda, L., Dang, C. V., & Semenza, G. L. (2007). HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell*, *129*(1), 111–122.

Gomes, M. J., Martinez, P. F., Campos, D. H. S., Pagan, L. U., Bonomo, C., Lima, A. R. R., Damatto, R. L., Cezar, M. D. M., Damatto, F. C., Rosa, C. M., Garcia, C. M., Reyes, D. R. A., Fernandes, A. A. H., Fernandes, D. C., Laurindo, F. R., Okoshi, K., & Okoshi, M. P. (2016). Beneficial effects of physical exercise on functional capacity and skeletal muscle oxidative stress in rats with aortic stenosis-induced heart failure. *Oxidative Medicine and Cellular Longevity*, *2016*, 1–12.

Gregolin, C. S., do Nascimento, M., Borges de Souza, S. L., Ferreira Mota, G. A., Bomfim, G. F., de Azevedo Melo Luvizotto, R., Sugizaki, M. M., Zanati Bazan, S. G., Salomé de Campos, D. H., Dias, M. C., Correa, C. R., Cicogna, A. C., & Ferreira do Nascimento, A. (2021). Myocardial dysfunction in cirrhotic cardiomyopathy is associated with alterations of phospholamban phosphorylation and IL-6 levels. *Archives of Medical Research*, *52*(3), 284–293.

Guan, D., Zhao, Y., Zhang, Y., Tang, D., & Wu, Q. (2016). Proteomics analysis revealed an altered left ventricle protein profile in a mouse model of transverse aortic constriction. *Protein & Peptide Letters*, *23*(2), 125–131.

Hartupee, J., & Mann, D. L. (2017). Neurohormonal activation in heart failure with reduced ejection fraction. *Nature Reviews Cardiology*, *14*(1), 30–38.

Hollander, J. M., Baseler, W. A., & Dabkowski, E. R. (2011). Proteomic remodeling of mitochondria in heart failure. *Congestive Heart Failure*, *17*(6), 262–268.

Kadkhodayan, A., Coggan, A. R., & Peterson, L. R. (2013). A "PET" area of interest: Myocardial metabolism in human systolic heart failure. *Heart Failure Reviews*, *18*(5), 567–574.

Karagas, G., Fliegner, D., Forler, S., Klein, O., Schubert, C., Gustafsson, J.-Å., Klose, J., & Regitz-Zagrosek, V. (2014). Comparative proteomic analysis reveals sex and estrogen receptor β effects in the pressure overloaded heart. *Journal of Proteome Research*, *13*(12), 5829–5836.

Katsumoto, T., Mitsushima, A., & Kurimura, T. (1990). The role of the vimentin intermediate filaments in rat 3Y1 cells elucidated by immunoelectron microscopy and computer-graphic reconstruction. *Biology of the Cell*, *68*(2), 139–146.

Kavazis, A. N., Alvarez, S., Talbert, E., Lee, Y., & Powers, S. K. (2009). Exercise training induces a cardioprotective phenotype and alterations in cardiac subsarcolemmal and intermyofibrillar mitochondrial proteins. *American Journal of Physiology-Heart and Circulatory Physiology*, *297*(1), H144–H152.

Khechaduri, A., Bayeva, M., Chang, H.-C., & Ardehali, H. (2013). Heme levels are increased in human failing hearts. *Journal of the American College of Cardiology*, *61*(18), 1884–1893.

Kolwicz, S. C., Purohit, S., & Tian, R. (2013). Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. *Circulation Research*, *113*(5), 603–616.

Lee, J., Song, J., Kwon, K., Jang, S., Kim, C., Baek, K., Kim, J., & Park, C. (2012). Human DJ-1 and its homologs are novel glyoxalases. *Human Molecular Genetics*, *21*(14), 3215–3225.

Lee, Y. I., Cho, J. Y., Kim, M. H., Kim, K. B., Lee, D. J., & Lee, K. S. (2006). Effects of exercise training on pathological cardiac hypertrophy related gene expression and apoptosis. *European Journal of Applied Physiology*, *97*(2), 216–224.

Li, L., Zhang, H., Wang, W., Hong, Y., Wang, J., Zhang, S., Xu, S., Shu, Q., Li, J., Yang, F., Zheng, M., Qian, Z., & Liu, P. (2016). Comparative proteomics reveals abnormal binding of ATGL and dysferlin on lipid droplets from pressure overload-induced dysfunctional rat hearts. *Scientific Reports*, *6*, 19782.

Liao, Z., Li, D., Chen, Y., Li, Y., Huang, R., Zhu, K., Chen, H., Yuan, Z., Zheng, X., Zhao, H., Pu, Q., Qi, X., & Cai, D. (2019). Early moderate exercise benefits myocardial infarction healing via improvement of inflammation and ventricular remodelling in rats. *Journal of Cellular and Molecular Medicine*, *23*(12), 8328–8342.

Lin, H., Sardana, M., Zhang, Y., Liu, C., Trinquart, L., Benjamin, E. J., Manders, E. S., Fusco, K., Kornej, J., Hammond, M. M., Spartano, N. L., Pathiravasan, C. H., Kheterpal, V., Nowak, C., Borrelli, B., Murabito, J. M., & McManus, D. D. (2020). Association of habitual physical activity with cardiovascular disease risk. *Circulation Research*, *127*(10), 1253–1260.

Lu, H.-S., Chen, H.-P., Wang, S., Yu, H.-H., Huang, X.-S., Huang, Q.-R., & He, M. (2012). Hypoxic preconditioning up-regulates DJ-1 protein expression in rat heart-derived H9c2 cells through the activation of extracellular-regulated kinase 1/2 pathway. *Molecular and Cellular Biochemistry*, *370*(1–2), 231–240.

Ma, J., Banerjee, P., Whelan, S. A., Liu, T., Wei, A.-C., Ramirez-Correa, G., McComb, M. E., Costello, C. E., O'Rourke, B., Murphy, A., & Hart, G. W. (2016). Comparative proteomics reveals dysregulated mitochondrial O-GlcNAcylation in diabetic hearts. *Journal of Proteome Research*, *15*(7), 2254–2264.

Manakov, D., Ujcikova, H., Pravenec, M., & Novotny, J. (2016). Alterations in the cardiac proteome of the spontaneously hypertensive rat induced by transgenic expression of CD36. *Journal of Proteomics*, *145*, 177–186.

10974652, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.31199 by Univ of Sao Paulo - Brazil, Wiley Online Library on [15/04/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Mazeto, I. F. S., Okoshi, K., Silveira, C. F. S. M. P., Sant'Ana, P. G., Silva, V. L., Mota, G. A. F., Mota, G. A. F., Souza, S. L. B., Vileigas, D. F., Padovani, C. R., & Cicogna, A. C. (2021). Calcium homeostasis behavior and cardiac function on left ventricular remodeling by pressure overload. *Brazilian Journal of Medical and Biological Research*, *54*(4), 1–9.

McGregor, E., & Dunn, M. J. (2006). Proteomics of the heart: Unraveling disease. *Circulation Research*, *98*(3), 309–321.

Mitra, A., Basak, T., Ahmad, S., Datta, K., Datta, R., Sengupta, S., & Sarkar, S. (2015). Comparative proteome profiling during cardiac hypertrophy and myocardial infarction reveals altered glucose oxidation by differential activation of pyruvate dehydrogenase E1 component subunit β. *Journal of Molecular Biology*, *427*(11), 2104–2120.

Mota, G. A. F., de Souza, S. L. B., da Silva, V. L., Gatto, M., de Campos, D. H. S., Sant'Ana, P. G., Vileigas, D. F., Padovani, C. R., Casarini, D. E., Oliveira, E. M., Bazan, S. G. Z., Fernandes, T., Sugizaki, M. M., de Morais Gomes, E. R., & Cicogna, A. C. (2020). Cardioprotection generated by aerobic exercise training is not related to the proliferation of cardiomyocytes and angiotensin-(1-7) levels in the hearts of rats with supravalvar aortic stenosis. *Cellular Physiology and Biochemistry*, *54*(4), 719–735.

Nakamura, M., & Sadoshima, J. (2018). Mechanisms of physiological and pathological cardiac hypertrophy. *Nature Reviews Cardiology*, *15*(7), 387–407.

National Academies Collection. (2011). *Guide for the care and use of laboratory animals*. National Academies Press. https://doi.org/10.17226/12910

Pagan, L. U., Damatto, R. L., Gomes, M. J., Lima, A. R. R., Cezar, M. D. M., Damatto, F. C., Reyes, D. R. A., Caldonazo, T. M. M., Polegato, B. F., Okoshi, M. P., & Okoshi, K. (2019). Low-intensity aerobic exercise improves cardiac remodelling of adult spontaneously hypertensive rats. *Journal of Cellular and Molecular Medicine*, *23*(9), 6504–6507.

Pagan, L. U., Damatto, R. L., Cezar, M. D. M., Lima, A. R. R., Bonomo, C., Campos, D. H. S., Gomes, M. J., Martinez, P. F., Oliveira, Jr., S. A., Gimenes, R., Rosa, C. M., Guizoni, D. M., Moukbel, Y. C., Cicogna, A. C., Okoshi, M. P., & Okoshi, K. (2015). Long-term low intensity physical exercise attenuates heart failure development in aging spontaneously hypertensive rats. *Cellular Physiology and Biochemistry*, *36*(1), 61–74.

Pagan, L. U., Gomes, M. J., Damatto, R. L., Lima, A. R. R., Cezar, M. D. M., Damatto, F. C., Reyes, D. R. A., Campos, D. H. S., Caldonazo, T. M. M., Polegato, B. F., Fernandes, D. C., Laurindo, F. R., Fernandes, A. A. H., Lloret, A., Cicogna, A. C., Okoshi, M. P., & Okoshi, K. (2021). Aerobic exercise during advance stage of uncontrolled arterial hypertension. *Frontiers in Physiology*, *12*, 675778.

Pandey, A., & Mann, M. (2000). Proteomics to study genes and genomes. *Nature*, *405*(6788), 837–846.

Pazzianotto-Forti, E. M., Moreno, M. A., Plater, E., Baruki, S. B. S., Rasera-Junior, I., & Reid, W. D. (2020). Impact of physical training programs on physical fitness in people with class II and III obesity: A systematic review and meta-analysis. *Physical Therapy*, *100*(6), 963–978.

Perrucci, G. L., Gowran, A., Zanobini, M., Capogrossi, M. C., Pompilio, G., & Nigro, P. (2015). Peptidyl-prolyl isomerases: A full cast of critical actors in cardiovascular diseases. *Cardiovascular Research*, *106*(3), 353–364.

Petriz, B. A., Almeida, J. A., Gomes, C. P. C., Pereira, R. W., Murad, A. M., & Franco, O. L. (2015). NanoUPLC/MS(E) proteomic analysis reveals modulation on left ventricle proteome from hypertensive rats after exercise training. *Journal of Proteomics*, *113*, 351–365.

Petriz, B. A., Cunha, V. N., Villeth, G. R. C., Mehta, Â., Rocha, L. A. O., Silva, O. N., Almeida, J. A., Morais, P. K., Simões, H. G., & Franco, O. L. (2013). Effects of acute exercise over heart proteome from monogenic obese (ob/ob) mice. *Journal of Cellular Physiology*, *228*(4), 824–834.

Quindry, J. C., Miller, L., McGinnis, G., Kliszczewicz, B., Irwin, J. M., Landram, M., Urbiztondo, Z., Nanayakkara, G., & Amin, R. (2012). Ischemia reperfusion injury, KATP channels, and exercise-induced cardioprotection against apoptosis. *Journal of Applied Physiology*, *113*(3), 498–506.

Reyes, D. R. A., Gomes, M. J., Rosa, C. M., Pagan, L. U., Zanati, S. G., Damatto, R. L., Rodrigues, E. A., Carvalho, R. F., Fernandes, A. A. H., Martinez, P. F., Lima, A. R. R., Cezar, M. D. M., Carvalho, L. E. F. M., Okoshi, K., & Okoshi, M. P. (2019). Exercise during transition from compensated left ventricular hypertrophy to heart failure in aortic stenosis rats. *Journal of Cellular and Molecular Medicine*, *23*(2), 1235–1245.

Saco-Ledo, G., Valenzuela, P. L., Ruiz-Hurtado, G., Ruilope, L. M., & Lucia, A. (2020). Exercise reduces ambulatory blood pressure in patients with hypertension: A systematic review and meta-analysis of randomized controlled trials. *Journal of the American Heart Association*, *9*(24):e018487.

Sant'Ana, P. G., Tomasi, L. C., Murata, G. M., Vileigas, D. F., Mota, G. A. F., Souza, S. L. B., Silva, V. L., Campos, L. P., Okoshi, K., Padovani, C. R., & Cicogna, A. C. (2023). Hypoxia-inducible factor 1-alpha and glucose metabolism during cardiac remodeling progression from hypertrophy to heart failure. *International Journal of Molecular Sciences*, *24*(7):6201.

Sant'Ana, P. G., Batah, S. S., Leão, P. S., Teodoro, W. R., de Souza, S. L. B., Ferreira Mota, G. A., Vileigas, D. F., da Silva, V. L., de Campos, D. H. S., Okoshi, K., Capelozzi, V. L., Cicogna, A. C., & Fabro, A. T. (2018). Heart remodeling produced by aortic stenosis promotes cardiomyocyte apoptosis mediated by collagen V imbalance. *Pathophysiology*, *25*(4), 373–379.

Schugar, R. C., Moll, A. R., André d'Avignon, D., Weinheimer, C. J., Kovacs, A., & Crawford, P. A. (2014). Cardiomyocyte-specific deficiency of ketone body metabolism promotes accelerated pathological remodeling. *Molecular Metabolism*, *3*(7), 754–769.

Semenza, G. L. (2014). Hypoxia-inducible factor 1 and cardiovascular disease. *Annual Review of Physiology*, *76*, 39–56.

Sequeira, V., Nijenkamp, L. L. A. M., Regan, J. A., & van der Velden, J. (2014). The physiological role of cardiac cytoskeleton and its alterations in heart failure. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1838*(2), 700–722.

Shimizu, Y., Nicholson, C. K., Polavarapu, R., Pantner, Y., Husain, A., Naqvi, N., Chin, L. S., Li, L., & Calvert, J. W. (2020). Role of DJ-1 in modulating glycative stress in heart failure. *Journal of the American Heart Association*, *9*(4):e014691.

Silva, V., Souza, S. L. B., Mota, G. A. F., Campos, D. H. S., Melo, A. B., Vileigas, D. F., Sant'Ana, P. G., Coelho, P. M., Bazan, S., Leopoldo, A. S., & Cicogna, A. C. (2022). The dysfunctional scenario of the major components responsible for myocardial calcium balance in heart failure induced by aortic stenosis. *Arquivos Brasileiros de Cardiologia*, *118*(2), 463–475.

da Silva, V. L., Mota, G. A. F., de Souza, S. L. B., de Campos, D. H. S., Melo, A. B., Vileigas, D. F., Coelho, P. M., Sant'Ana, P. G., Padovani, C., Lima-Leopoldo, A. P., Bazan, S. G. Z., Leopoldo, A. S., & Cicogna, A. C. (2023). Aerobic exercise training improves calcium handling and cardiac function in rats with heart failure resulting from aortic stenosis. *International Journal of Molecular Sciences*, *24*(15):12306.

Sjölin, I., Bäck, M., Nilsson, L., Schiopu, A., & Leosdottir, M. (2020). Association between attending exercise-based cardiac rehabilitation and cardiovascular risk factors at one-year post myocardial infarction. *PLoS ONE*, *15*(5):e0232772.

de Souza, P. A., de Souza, R. W., Soares, L. C., Piedade, W. P., Campos, D. H., Carvalho, R. F., Padovani, C. R., Okoshi, K., Cicogna, A. C., Matheus, S. M., & Dal-Pai-Silva, M. (2015). Aerobic training attenuates nicotinic acethylcholine receptor changes in the diaphragm muscle during heart failure. *Histology and Histopathology*, *30*(7), 801–811.

10974652, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.31199 by Univ of Sao Paulo - Brazil, Wiley Online Library on [15/04/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Souza, R. W. A., Piedade, W. P., Soares, L. C., Souza, P. A. T., Aguiar, A. F., Vechetti-Júnior, I. J., Campos, D. H. S., Fernandes, A. A. H., Okoshi, K., Carvalho, R. F., Cicogna, A. C., & Dal-Pai-Silva, M. (2014). Aerobic exercise training prevents heart failure-induced skeletal muscle atrophy by anti-catabolic, but not anabolic actions. *PLoS ONE*, *9*(10):e110020.

de Souza, S. L. B., Ferreira Mota, G. A., da Silva, V. L., Sant'Ana, P. G., Fernandes Vileigas, D., de Campos, D. H. S., Padovani, C. R., Marchesan Rodrigues, M. A., Ferreira do Nascimento, A., Sugizaki, M. M., Garcia Zanati Bazan, S., Chakur Brum, P., & Cicogna, A. C. (2020). Adjustments in β-adrenergic signaling contribute to the amelioration of cardiac dysfunction by exercise training in supravalvular aortic stenosis. *Cellular Physiology and Biochemistry*, *54*(4), 665–681.

de Souza, S. L. B., Mota, G. A. F., da Silva, V. L., Vileigas, D. F., Sant'Ana, P. G., Gregolin, C. S., Figueira, R. L., Batah, S. S., Fabro, A. T., Murata, G. M., Bazan, S. G. Z., Okoshi, M. P., & Cicogna, A. C. (2023). Effects of early exercise on cardiac function and lipid metabolism pathway in heart failure. *Journal of Cellular and Molecular Medicine*, *27*(19), 2956–2969.

Suppan, M., Barcelos, G., Luise, S., Diaper, J., Frei, A., Ellenberger, C., Adamopoulos, D., Noble, S., & Licker, M. (2020). Improved exercise tolerance, oxygen delivery, and oxygen utilization after transcatheter aortic valve implantation for severe aortic stenosis. *CJC open*, *2*(6), 490–496.

Tran, D. H., & Wang, Z. V. (2019). Glucose metabolism in cardiac hypertrophy and heart failure. *Journal of the American Heart Association*, *8*(12):e012673.

Tsao, C. W., Aday, A. W., Almarzooq, Z. I., Anderson, C. A. M., Arora, P., Avery, C. L., Baker-Smith, C. M., Beaton, A. Z., Boehme, A. K., Buxton, A. E., Commodore-Mensah, Y., Elkind, M. S. V., Evenson, K. R., Eze-Nliam, C., Fugar, S., Generoso, G., Heard, D. G., Hiremath, S., Ho, J. E., … Martin, S. S. (2023). Heart Disease and Stroke Statistics-2023 update: A report from the American Heart Association. *Circulation*, *147*(8), e93–e621.

Tsutsui, H., Kinugawa, S., & Matsushima, S. (2011). Oxidative stress and heart failure. *American Journal of Physiology-Heart and Circulatory Physiology*, *301*(6), H2181–H2190.

Vileigas, D. F., Marciano, C. L. C., Mota, G. A. F., Souza, S. L. B., Sant'Ana, P. G., Okoshi, K., Padovani, C. R., & Cicogna, A. C. (2019). Temporal measures in cardiac structure and function during the development of obesity induced by different types of western diet in a rat model. *Nutrients*, *12*(1):68.

Vileigas, D. F., Harman, V. M., Freire, P. P., Marciano, C. L. C., Sant'Ana, P. G., de Souza, S. L. B., Mota, G. A. F., da Silva, V. L., Campos, D. H. S., Padovani, C. R., Okoshi, K., Beynon, R. J., Santos, L. D., & Cicogna, A. C. (2019). Landscape of heart proteome changes in a diet-induced obesity model. *Scientific Reports*, *9*(1):18050.

Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognmo, Ø., Haram, P. M., Tjønna, A. E., Helgerud, J., Slørdahl, S. A., Lee, S. J., Videm, V., Bye, A., Smith, G. L., Najjar, S. M., Ellingsen, Ø., & Skjærpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation*, *115*(24), 3086–3094.

Xin, L.-H., Liu, W.-J., Song, T., & Zhang, L. (2020). Overexpression of DJ-1 expression protects cardiomyocyte apoptosis induced by ischemia reperfusion. *European Review for Medical and Pharmacological Sciences*, *24*(23):11988.

Xu, J., Fang, J., Cheng, Z., Fan, L., Hu, W., Zhou, F., & Shen, H. (2018). Overexpression of the Kininogen-1 inhibits proliferation and induces apoptosis of glioma cells. *Journal of Experimental & Clinical Cancer Research*, *37*(1):180.

Yan, Y.-F., Chen, H.-P., Huang, X.-S., Qiu, L.-Y., Liao, Z.-P., & Huang, Q.-R. (2015). DJ-1 mediates the delayed cardioprotection of hypoxic preconditioning through activation of Nrf2 and subsequent upregulation of antioxidative enzymes. *Journal of Cardiovascular Pharmacology*, *66*(2), 148–158.

Yanatori, I., Richardson, D. R., Toyokuni, S., & Kishi, F. (2019). How iron is handled in the course of heme catabolism: Integration of heme oxygenase with intracellular iron transport mechanisms mediated by poly (rC)-binding protein-2. *Archives of Biochemistry and Biophysics*, *672*, 108071.

SUPPORTING INFORMATION

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