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Exercise volume modulates cardiac protection in a type 2 diabetic rat model: differential effects of high- and low-volume moderate-intensity endurance exercise training on diabetic cardiomyopathy

Rob Simons[1†], Ileana Bonilauri[2†], Lotte Vastmans[1], Lisa Steegen[1], Ellen Heeren[1], Vincent Vandenboer[1], Eline Verghote[1], Sarah D'Haese[1], Guido Claessen[1,3], Ivo Lambrichts[1], Beatriz Martín-Fernández[2], Pieter-Jan Volders[4], Dominique Hansen[1,5], Dorien Deluyker[1†] and Virginie Bito[1*†]

Abstract

Background Cardiomyopathy is a major complication of type 2 diabetes, whose prevalence continues to rise globally. Although major cardiology and endocrinology societies endorse exercise training to reduce cardiovascular risk, the optimal exercise training modality, and specifically the role of exercise volume, in preventing diabetic cardiomyopathy remains unclear.

Methods Male Sprague Dawley rats were fed a high-sugar diet to induce type 2 diabetes. At diet onset, animals were assigned to high-volume (HVE, $N=7$) or low-volume (LVE, $N=8$) moderate-intensity treadmill training, performed five days per week for 18 weeks. Sedentary rats served as controls ($N=6$). Cardiac function was assessed using conventional echocardiography, strain imaging, and invasive hemodynamics. Plasma analyses were used to identify systemic metabolic status, and ex vivo techniques quantified left ventricular cardiac fibrosis, oxidative stress, hypertrophy, inflammation, and metabolism.

Results After 18 weeks of diet, sedentary rats developed characteristic features of early-stage diabetic cardiomyopathy, accompanied by impaired systolic function and increased interstitial myocardial fibrosis. Conversely, high volumes of moderate-intensity exercise training partially prevented pathological cardiac remodelling by improving cardiac metabolic regulation, reducing oxidative stress, and enhancing both cardiac stress-responses and systemic metabolic control. Lower volumes of exercise training primarily influence oxidative stress and inflammatory pathways, resulting in modest cardioprotective effects and preservation of cardiac function. By contrast, high-volume

[†]Dorien Deluyker and Virginie Bito are shared last authors.

[†]Rob Simons and Ileana Bonilauri contributed equally to this work.

*Correspondence:
Virginie Bito
virginie.bito@uhasselt.be

Full list of author information is available at the end of the article

exercise training elicited more pronounced cardioprotective effects, reflected by a significantly higher ejection fraction, cardiac output, stroke volume index, and global longitudinal strain relative to sedentary controls.

Conclusion The progression of diabetic cardiomyopathy appears to be modulated by exercise training volume. Although both volumes preserve cardiac performance, high-volume exercise training elicited more pronounced cardioprotective effects than low-volume exercise training in T2DM rats. These findings emphasise the importance of further considering exercise volume as a critical variable in the optimisation of evidence-based exercise prescriptions for individuals with type 2 diabetes.

Keywords Diabetes, Cardiomyopathy, Aerobic training, Exercise volume, Preclinical study

Background

Diabetes mellitus is a growing global health concern, with approximately 536.6 million individuals affected in 2021 [1]. Despite extensive efforts to mitigate its prevalence, the incidence of this metabolic disorder is still rising at an alarming rate [1]. Individuals with type 2 diabetes mellitus (T2DM) are at a significantly higher risk of developing heart failure with either a preserved ejection fraction (HFpEF; 23% of patients) or reduced ejection fraction (HFrEF; 13% of patients) [2, 3]. Consequently, diabetic cardiomyopathy (DCM) is a major complication of T2DM, characterised by structural and functional myocardial changes independent of coronary artery disease, other cardiac pathologies (e.g., valvular dysfunction), or hypertension [4–6].

Current management of DCM primarily relies on pharmacological interventions aiming to control systemic metabolic derangements (e.g., dyslipidemia, dysglycemia) and cardiovascular function (e.g., hypertension). However, these therapeutic options do not address the underlying metabolic dysfunction seen in DCM. Furthermore, clinical care remains suboptimal, underscoring the need for new preventive, non-pharmacological strategies to improve metabolic control and preserve cardiac function in T2DM [7–9]. In line with this, the European Society of Cardiology and the American Heart Association advocate for structured lifestyle interventions, emphasising regular physical activity, dietary improvements, and weight management as primary strategies to mitigate metabolic dysfunction and reduce cardiovascular risk in T2DM [10, 11]. However, the optimal exercise modality (i.e., intensity, volume, type) for preventing DCM remains unclear. Previous work from our research group demonstrated that moderate-intensity exercise training (40–69% of VO_{2max}) and high-intensity interval training (70–95% of VO_{2max}) exert cardioprotective benefits in a T2DM rodent model [10]. Emerging evidence indicates that prioritising total exercise volume, defined as the product of intensity and duration of each training session, may be more effective than focusing solely on exercise intensity [12, 13]. The current study aims to provide more insights into the cardioprotective effects of exercise training volume on the prevention of DCM in a translatable T2DM rat model. This novel approach represents a promising and cost-effective strategy for mitigating cardiovascular risk in T2DM, with potential implications for the development of evidence-based exercise prescription guidelines in T2DM management.

Methods

Animal experiments

Six-week-old male Sprague Dawley rats (Janvier Labs, France) were housed in a temperature-controlled environment (21 °C) with a 12-hour light/dark cycle. Food and water were provided *ad libitum*. To induce T2DM with DCM, rats were fed a high-sugar Western diet (21% starch, 48% sugars, 16% fat, 15% protein) for 18 weeks [14]. Rats were randomly assigned to a sedentary (SED, $N = 6$), low-volume exercise (LVE, $N = 8$), or high-volume exercise (HVE, $N = 7$) group. Food intake and body weight were monitored weekly. Oral glucose tolerance tests (OGTTs), echocardiography, and blood sampling were performed at baseline and weeks 6, 12, and 18, as shown in Supplemental Figure S1. Invasive left ventricle (LV) hemodynamics were assessed pre-sacrifice via carotid catheterisation (Millar Inc., The Hague, The Netherlands). Rats were anaesthetised with 2% isoflurane and euthanised with sodium pentobarbital (200 mg/kg IP) and heparin (1000 U/kg IP). Organs were weighed and normalised to tibia length. Mid-LV transversal sections were fixed in 4% paraformaldehyde for paraffin embedding, and 8 µm sections were prepared for histological analysis. For transmission electron microscopy (TEM), ultra-thin LV sections were mounted on formvar-coated grids and fixed in 2% glutaraldehyde in 0.05 M cacodylate buffer [15]. Residual LV tissue was crushed in liquid nitrogen for gene expression analysis. All procedures followed the 2010/63/EU directive and were approved by the Local Ethical Committee at Hasselt University (Diepenbeek, Belgium; matrix ID 202102A1).

Exercise training protocol

Rats underwent 2 weeks of acclimatisation to the treadmill (IITC Life Science, Woodland Hills, CA, USA) with progressively increased speed and duration of running (5° inclination, 3 days/week). Starting at the onset of the

diet, exercise training was conducted for 18 weeks. Exercise training consisted of continuous moderate-intensity running (18 m/min, 5° inclination, 5 days/week) for 20 min/day in the LVE group or 60 min/day in the HVE group. Exercise durations were based on the guidelines from the American Heart Association and results from D'Haese S, et al. [10, 16]. Animals in the SED group were not exposed to structured exercise. The total mechanical workload was calculated as an index of performance, taking into account body weight, and calculated by the following formula: Total workload = mean body weight (g) × running distance (km/min) × frequency.

Echocardiographic measurements

Transthoracic LV echocardiography was performed on all rats using a 21 MHz MX250 transducer and a Vevo® 3100 imaging system (v5.6.1; FUJIFILM VisualSonics, Inc., Toronto, Canada), as described by Haesen S, et al. [17]. Heart rate and ECG signals were continuously monitored non-invasively during scanning. LV wall thickness was measured from M-mode parasternal short-axis views and averaged over three heartbeats. End-systolic volumes, end-diastolic volumes and ejection fraction were obtained from B-mode parasternal long-axis views. Mitral flow profiles were assessed using B-mode apical four-chamber views, with pulsed-wave Doppler to determine the E/A ratio and Tissue Doppler to measure peak septal mitral annulus velocity (E') during early filling. Doppler measurements were averaged over three cardiac cycles. Echocardiographic images were analysed using Vevo® LAB software (v5.6.1; FUJIFILM VisualSonics, Inc., Toronto, Canada). LV strain was assessed using Vevo® Strain software (v5.6.1; FUJIFILM VisualSonics, Inc., Toronto, Canada) on imported mid-ventricular parasternal short- and long-axis B-mode cine loops. Optimal cardiac cycles were selected to measure global circumferential strain from short-axis views and peak radial strain plus global longitudinal strain from long-axis views. The endocardial border was manually traced, and the software generated the epicardial border to define 48 equidistant sampling points, dividing the LV into six segments per slice for peak strain quantification.

OGTT and biochemical assays

Glucose tolerance was measured with a one-hour OGTT as described previously [16]. At baseline and 18 weeks, plasma insulin concentrations were measured using the Mouse/Rat Insulin Kit (K152BZC; Museo Scale, Gaithersburg, MD, USA) electrochemiluminescence assay, following the manufacturer's instructions. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ratio was used to assess insulin resistance and calculated via the following formula: HOMA-IR = (fasting insulin [μIU/mL] × fasting glucose [mmol/L])/22.5.

Histology and transmission electron microscopy

Paraffin-embedded LV transverse sections were deparaffinised in ethanol and stained with haematoxylin and eosin. Images were captured with the Zeiss Axioscan Z.1 (Zeiss, Jena, Germany), and cardiomyocyte cross-sectional area (CSA) was assessed by addressing elliptical cross-sectional shapes for 12 cells per animal with Fiji v1.53c software from ImageJ. Additionally, LV cardiac sections were stained using a Sirius Red/Fast Green Collagen Staining kit (9046, Chondrex Inc., Woodville, TX, USA) according to the manufacturer's protocol. Detection of nitrotyrosine residues was assessed by 3'-nitrotyrosine (3-NT) immunohistochemistry, as described elsewhere [18]. Images were captured with a Leica MC170 camera connected to a Leica DM2000 LED microscope (Leica Microsystems, Diegem, Belgium). LV collagen deposition and 3-NT derivatives were quantified in eight randomly selected fields per section using the colour deconvolution plugin in Fiji v1.53c software. The area of collagen or 3-NT deposition was normalised to the total cardiac area and expressed as a percentage. For TEM analysis, five random pictures of cardiomyocytes were taken per animal at 1000x and 4000x magnification using a JEM-1400Flash transmission electron microscope (JEOL Ltd, Tokyo, Japan) coupled to an Xarosa camera (EMSIS GmbH, Münster, Germany). The mitochondrial density was calculated as the number of mitochondria normalised to the total cell area, using the cell counter tool in Fiji Software.

Gene expression

Total RNA isolation was performed using an RNeasy Fibrous Tissue Kit (Qiagen Benelux B.V., Antwerp, Belgium), following the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Isogen Life Science B.V., Utrecht, Netherlands). cDNA synthesis was performed using a qScript cDNA SuperMix (QuantaBio, Leuven, Belgium). Primers were designed based on the coding sequence of the mRNA for the target and reference genes. RT-qPCR was performed using a MicroAmp™ Fast Optical 96-well reaction plate (Thermo Fisher Scientific, Geel, Belgium) with SYBR Green (Thermo Fisher Scientific, Geel, Belgium) on the QuantStudio 3 PCR system (Thermo Fisher Scientific, Geel, Belgium). Gene expression analysis was performed using the delta CT method. GeNorm analysis identified ribosomal protein L13a (Rpl13a) and cyclin A (Ccna) as the most stably expressed reference genes. Furthermore, pure mRNA samples were sent to Novogene Co., Ltd. (Beijing, China) for whole-transcriptome analysis. Briefly, sample quality control was performed as per standard procedure. Messenger RNA was purified from total RNA via poly-T oligo-attached magnetic beads. Using random

hexamer primers, first- and second-strand cDNA were synthesised. Non-stranded cDNA libraries were quantified using Qubit fluorometry and sequenced on an Illumina NovaSeq X Plus instrument to generate paired-end 150 bp reads. Raw FASTQ files were processed with the nf-core/rnaseq pipeline (version 3.14.0), executed in a Linux high-performance computing environment (Flemish Supercomputer Centre (VSC)), using the Nextflow version 23.04.2. The Rattus norvegicus reference genome GRCr8 and reference transcriptome were obtained from Ensembl version 114. Sample quality was evaluated via the MultiQC reports obtained from the pipeline, and low-quality samples were excluded for further analysis. Gene-level counts quantified with Salmon were used for differential expression analysis with the DESeq2 package in RStudio (version 4.2.2). Differentially expressed genes were defined as those with an adjusted p-value < 0.05. Gene set enrichment analysis for the MSigDB hallmark gene sets was performed with the R packages MSigDBR and fgsea. The top 20 significant hallmark gene sets were plotted in a two-sided bar plot to visualise enriched or depleted pathways.

Statistical analysis

Statistical analyses were performed with GraphPad Prism (GraphPad Software, v10.1.1, San Diego, CA, USA). Outliers were identified using the robust regression and outlier removal (ROUT) method with a false discovery rate set at 1%. Bland–Altman analysis of echocardiographic measurements demonstrated good measurement reproducibility with minimal intra (– 0.04%; [– 2.67; 2.59%]) and inter-observer (– 2.41%; [-12.05; 7.23%]) variability. Due to the limited sample size, a formal assessment of data normality was not performed. Consequently, all statistical analyses, including group comparisons and correlation assessments, were conducted using non-parametric analyses to avoid assumptions regarding the underlying data distribution. Comparisons for multiple groups were performed using a Kruskal–Wallis test followed by Dunn's post hoc test (uncorrected). The Wilcoxon signed-rank test was performed for comparisons against a theoretical mean. Data for different groups measured at multiple time points were evaluated using a mixed-effect model analysis. Associations between cardiac functional parameters and metabolic variables, as well as exercise workload, were assessed using Spearman's rank correlation. Multiple linear regression analyses were conducted to assess the relative contribution of each independent variable to cardiac outcomes, controlling for potential confounders. Model assumptions, including linearity, independence of residuals, homoscedasticity, and normality of residuals, were evaluated using the according diagnostic plots. To evaluate the adequacy of the sample size, post hoc statistical power analysis was performed with G*Power (version 3.1, Germany), via a one-way ANOVA. An achieved statistical power of 0.93 was obtained assuming an effect size of f = 1.06, based on the primary outcome, cardiac output index to body surface area, with a total sample size of $N = 21$. Results are expressed as median with interquartile range. Statistical significance is defined as $p < 0.05$ (2-tailed). Sample size is indicated as 'n'.

Results

Exercise volume training alters metabolic dysfunction in T2DM

Our results demonstrated a progressive weight gain in SED rats, reaching significantly higher final body weights than the HVE and LVE groups after 18 weeks (Fig. 1a and b). These differences occurred in the absence of significant variations in food intake among groups (Fig. 1c). Surprisingly, no differences in heart or lung weight were observed between exercise trained groups and SED controls (Fig. 1d-f). Still, HVE significantly reduced liver weight, whereas LVE training showed a trend toward reduced liver weight compared to SED rats, suggesting a potential effect of both exercise training volumes on hepatic metabolism (Fig. 1g). Further plasma analysis revealed that 18 weeks of HVE training significantly reduced fasting insulin levels compared to SED rats (Fig. 1h). Interestingly, HVE preserved plasma insulin levels, whereas the SED group showed a significant increase of approximately fivefold from baseline (Fig. 1i). LVE-trained rats showed a non-significant tenfold increase in fasting plasma insulin levels compared to baseline values (Fig. 1i).

Subsequent analysis of glycaemic control revealed that HVE-training displayed a downward trend in fasting blood glucose levels compared to SED controls (Fig. 1h). However, no significant improvements in glucose tolerance were observed as all groups showed a twofold increase in fasting glucose compared to baseline (Fig. 1j). Nevertheless, HVE-trained animals had a reduced HOMA-IR index relative to SED controls, indicating a marked improvement in insulin sensitivity (Fig. 1k). A downward trend in insulin resistance was observed for the LVE group; however, this change did not reach statistical significance (Fig. 1k). Overall, HVE training and, to a lesser degree, LVE training were associated with improvements in indices related to insulin sensitivity in T2DM rats, although these interventions confer only partial improvements in glycaemic control.

HVE training exhibits partial cardioprotective effects and preserves contractility

Conventional echocardiography revealed a significantly higher ejection fraction (EF) in HVE (70 vs. 58%; $p = 0.0033$) and LVE-trained rats (66 vs. 58%; $p = 0.0402$),

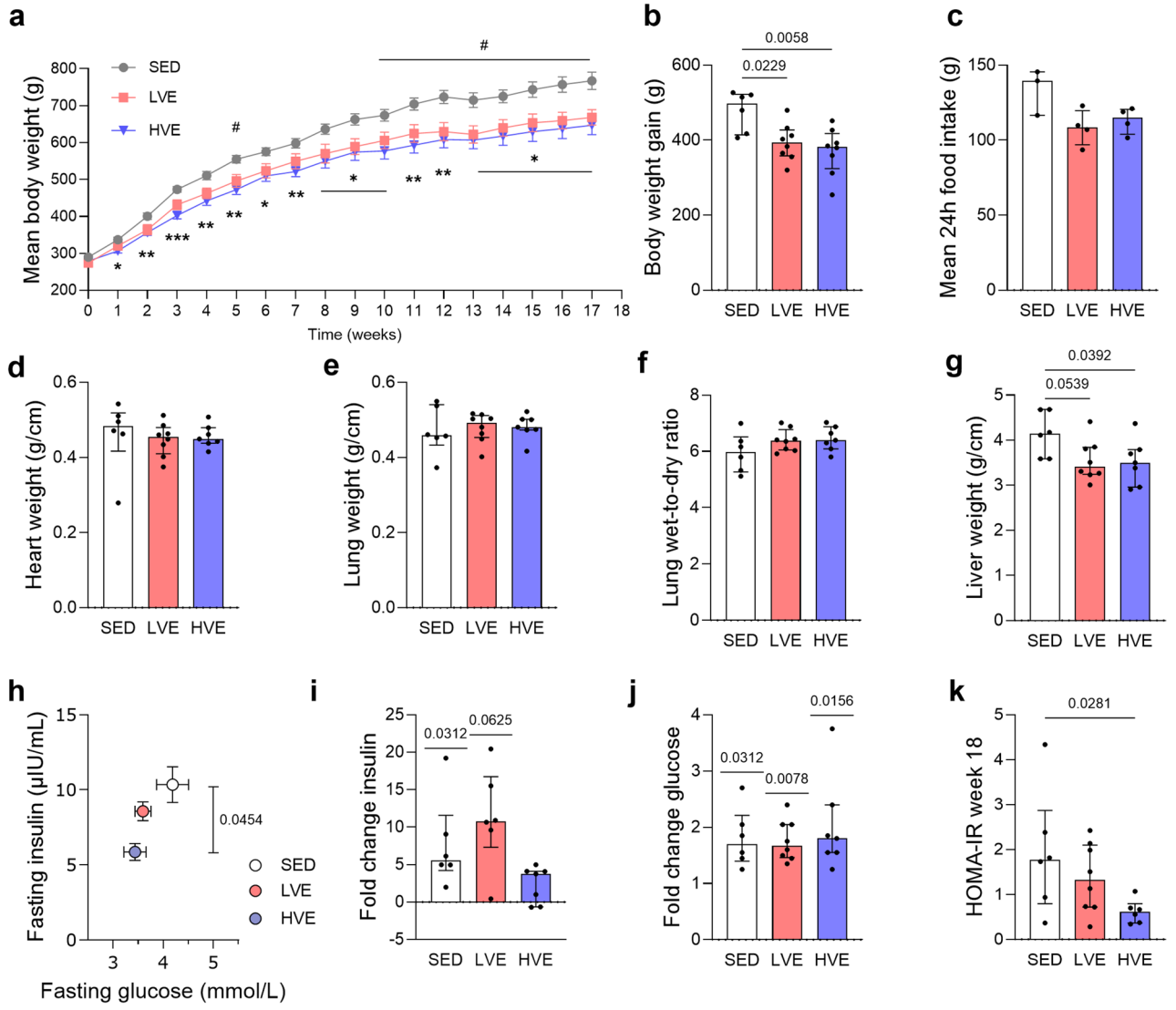

Fig. 1 Metabolic alterations induced by exercise volume training in T2DM rats. (**a**) Mean body weight over 18 weeks. (**b**) Mean body weight gain per group after 18 weeks. (**c**) The average 24-hour food intake per cage. Each data point (cage) represents the housing of two rats from the same group, with $n = 3$–4 per group. (**d**) Heart weight corrected by tibia length at week 18. (**e**) Lung weight corrected by tibia length at week 18. (**f**) Lung weight wet-to-dry ratio as an indicator for pulmonary oedema at week 18. (**g**) Liver weight corrected by tibia length at week 18. (**h**) Fasting insulin and glucose levels at week 18. (**i**) Fold change in fasting insulin from baseline (value = 1). (**j**) Fold change in fasting glucose from baseline (value = 1). (**k**) HOMA-IR ratio. $n = 6$–8 per group. Data are presented as median [Q1, Q3]. * denotes $p < 0.05$; ** denotes $p < 0.01$; *** denotes $p < 0.001$ SED vs. HVE; # denotes $p < 0.05$ SED vs. LVE. HOMA-IR, Homeostatic Model Assessment for Insulin Resistance

compared to SED rats after 18 weeks (Table 1). Consequently, rats exposed to HVE-training exhibited a significantly higher cardiac output (0.27 vs. 0.19 mL/min/cm^2; $p = 0.0009$) and stroke volume index (SV; 0.83 vs. 0.57 µl/cm^2; $p = 0.0029$; Table 1). Similarly, LVE-training resulted in an increased cardiac output (0.24 vs. 0.19 mL/min/cm^2; $p = 0.0367$) and stroke volume index (SV; 0.74 vs. 0.57 µl/cm^2; $p = 0.0440$) compared to SED controls (Table 1). Overall, SED rats developed marginal reductions in EF and cardiac output, whereas HVE-trained rats showed a significant increase in SV index compared to baseline (Fig. 2a-c).

To assess the presence of cardiac hypertrophy, anterior and posterior LV wall thicknesses were evaluated during systole and diastole. Unexpectedly, no significant differences were observed for LVE and HVE-exposed rats compared to SED animals (Table 1). Still, the SED and LVE group showed a significant increase (> 10%) in systolic and diastolic posterior, but not anterior, wall thickness compared to baseline, indicative of LV cardiac remodelling (Supplemental Figure S2). In contrast, HVE-trained animals only showed a significant increase in systolic anterior LV wall thickness over time (Supplemental Figure S2).

Table 1 Conventional LV echocardiographic parameters at week 18

	Week 18		
	SED	**LVE**	**HVE**
EF (%)	58 [56, 59]	66 [59, 70]*	70 [60, 72]**
FS (%)	18 [15, 21]	17 [15, 26]	22 [18, 24]
Cardiac output (mL/min/cm^2)	0.19 [0.16, 0,20]	0.24 [0.20, 0.28]*	0.27 [0.26, 0.29]***
SV index (µl/cm^2)	0.57 [0.51, 0.61]	0.74 [0.61, 0.84]*	0.83 [0.72, 0.91]**
EDV/BSA (µl/ cm^2)	0.97 [0.86, 1.06]	1.19 [0.98, 1,21]	1.25 [1.03, 1.38]*
ESV/BSA (µl/ cm^2)	0.41 [0.33, 0.46]	0.39 [0.31, 0.44]	0.35 [0.31, 0.55]
PWTd (mm)	2.23 [2.11, 2.53]	2.53 [2.05, 2.71]	2.25 [2.04, 2.31]
AWTd (mm)	1.94 [1.93, 2.08]	1.90 [1.79, 2.07]	1.70 [1.58, 1.96]
E/A	1.14 [1.00. 1.66]	1.34 [1.81, 1.06]	1.18 [0.87, 1.62]
E/E'	-24.86 [-27.95, -22.27]	-19.84 [-28.72, -15.81]	-23.47 [-28.23, -21.70]
HR (bpm)	319 [296, 340]	336 [302, 362]	342 [310, 355]
BSA (cm^2)	822 [755, 843]	720 [690, 761]**	723 [690, 755]**

Results from conventional echocardiography from SED (n = 6), LVE (n = 8), and HVE (n = 7) groups at study termination. Data are presented as median [Q1, Q3]. * denotes p < 0.05; ** denotes p < 0.01; *** denotes p < 0.001 vs SED. EF, ejection fraction; FS, fractional shortening; SV; stroke volume; EDV, end-diastolic volume; ESV, end-systolic volume; PWTd, diastolic posterior wall thickness; AWTd, diastolic anterior wall thickness; E/A, ratio of early (E) to late atrial (A) mitral inflow velocities; E/E', ratio of early mitral inflow velocity (E) to early diastolic mitral annular velocity (E'); HR, heart rate; BSA, body surface area

Strain analysis with non-conventional echocardiography revealed that LV global longitudinal strain (GLS) in the HVE group was nearly two-fold higher than in SED rats, reaching a statistically significant difference (Fig. 2d). Additionally, it was shown that high and low-volume training resulted in an upward trend in global radial strain (GRS), but not circumferential strain (GCS), compared to the SED group (Fig. 2e and f). Furthermore, SED rats revealed a significant decline in GLS and a declining trend in GRS and GCS from baseline, highlighting possible progressive functional impairments (Fig. 2g and i). A stabilising trend in GLS, GRS, and GCS was observed in both exercise-trained groups, suggesting preservation of LV contractile function over time (Fig. 2g-i).

Further hemodynamic assessment did not reveal significant differences in LV systolic or diastolic function among the experimental groups (Table 2). Still, LVE-trained rats exhibited a prolonged trend in Tau relative to the SED group, suggesting slower ventricular relaxation. Similarly, isovolumetric contraction was not significantly altered by LVE or HVE compared to SED controls, including intrinsic contractile function and maximal rate of pressure gain during systole (dP/dt_{max}). Again, HVE-trained rats exhibited an upward trend in end-systolic pressure, accompanied by an elevated trend in systolic and diastolic dP/dt_{max} compared with SED rats, indicative of increased preload and contractile function (Table 2).

To investigate whether variations in cardiac function were associated with systemic metabolic status or exercise stimulus, correlation analyses, using cardiac output indexed to body surface area (CO/BSA) as a global marker of cardiac performance, were evaluated. Fasting glucose levels exhibited a modest negative correlation with CO/BSA ($r = -0.463$, $p = 0.035$), whereas fasting insulin and HOMA-IR index were not significantly associated with cardiac function after 18 weeks (Supplemental Figure S3a-c). Mean body weight and body weight gain showed borderline significant negative correlations with CO/BSA, suggesting a partial contribution of body weight to functional differences (Supplemental Figures S3d and 3e). In contrast, mechanical exercise workload demonstrated a strong positive correlation with CO/BSA ($r = 0.743$, $p = 0.0001$, Figure S3f). Multiple linear regression analyses, including mechanical workload and HOMA-IR index, incorporating glucose and insulin levels, as independent variables, indicated that mechanical workload ($p = 0.0019$) was significantly associated with CO/BSA, whereas HOMA-IR ($p = 0.6268$) was not (Table S1). However, when SED animals were excluded from the analysis to reduce group-driven effects, the association between workload and CO/BSA was no longer statistically significant (Table S1).

Together, these findings suggest that exercise exposure is a primary determinant of cardiac performance, with the largest differences observed between SED and exercised animals, rather than reflecting a clear dose-response relationship between low and high exercise workloads.

Molecular alterations in DCM pathology prevention are dependent on exercise volume

Histological analysis of LV cardiac tissue demonstrated significant reductions in cardiac interstitial fibrosis in rats exposed to HVE training compared to SED controls (Fig. 3a and b). Although not significant, rats from the LVE group also showed a downward trend in LV interstitial fibrosis in comparison with SED rats. A similar trend was observed for 3-NT derivatives, an indirect marker

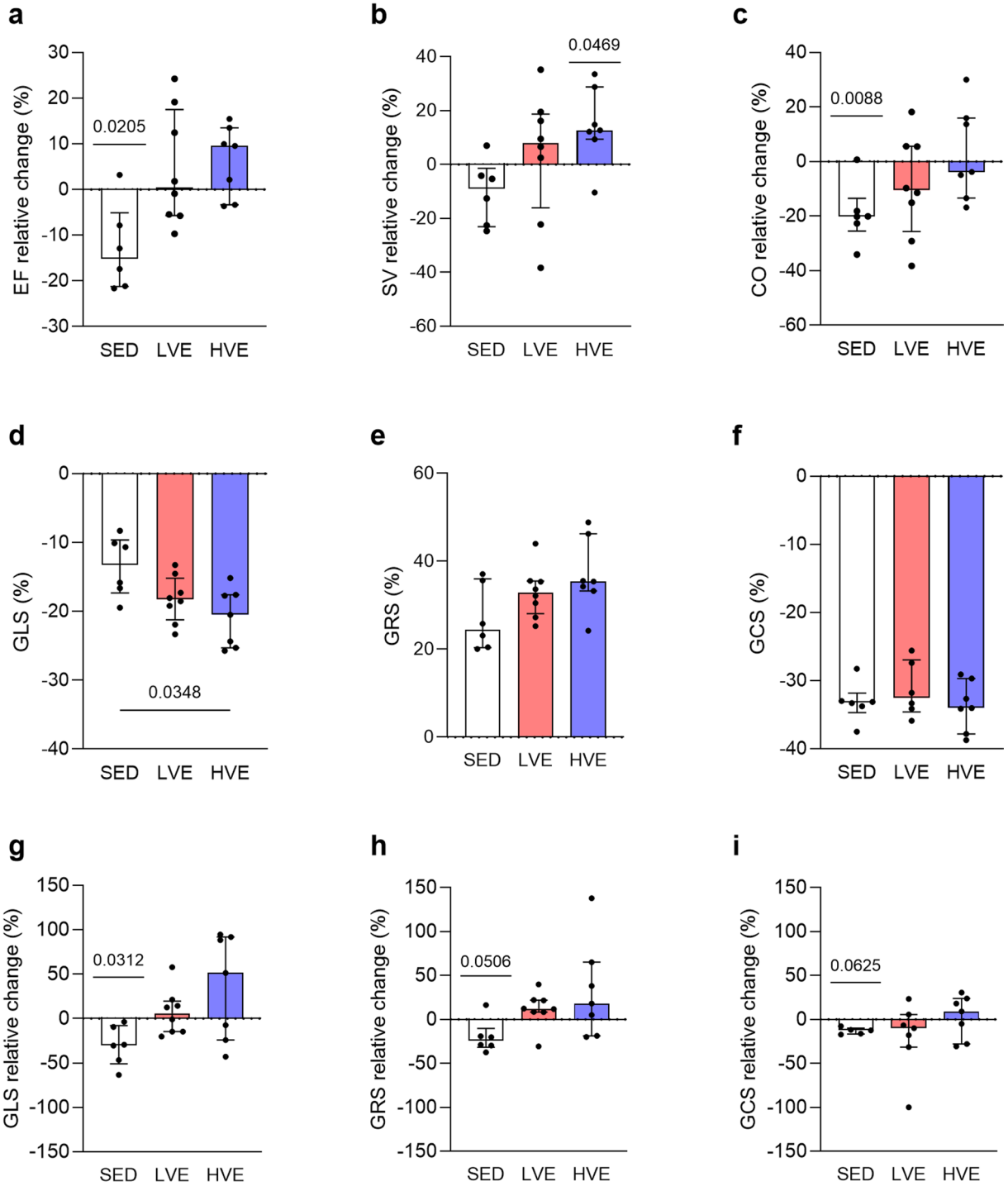

Fig. 2 HVE training alters LV cardiac function. Analysis of LV echocardiography shows relative change in (**a**) EF, (**b**) cardiac index, and (**c**) SV index between baseline (value = 0) and week 18. Left ventricular strain analysis with non-conventional echocardiography shows LV (**d**) GLS (**e**) GRS, and (**f**) GCS at week 18. The relative change in (**g**) GLS, (**h**) GRS, and (**i**) GCS, between baseline (value = 0) and week 18. (n = 6–8 per group). Data are presented as median [Q1, Q3]. LV, left ventricular; EF, ejection fraction; SV, stroke volume; GLS, global longitudinal strain; GRS, global radial strain; GCS, global circumferential strain

for reactive oxygen species (ROS), in IHC-stained LV sections for the HVE and LVE groups, indicating a likely influence of exercise volume on handling oxidative stress in cardiac tissue (Fig. 3c and d).

Surprisingly, 18 weeks of low-volume training did not result in significant differences in CSA compared to SED rats, but a downward trend was seen for animals exposed to HVE training (Fig. 3e and f). Furthermore, profound

Table 2 Hemodynamic parameters at week 18

	Week 18		
	SED	**LVE**	**HVE**
ESP (mmHg)	85.96 [84.69, 91.48]	88.19 [85.46, 91.35]	93.15 [91.45, 94.42]
EDP (mmHg)	8.19 [7.26, 8.96]	7.05 [6.16, 9.50]	7.99 [7.71, 10.52]
Tau (s)	0.011 [0.009, 0.012]	0.015 [0.012, 0.019]	0.011 [0.010, 0.012]
SPTI (mmHg*S)	6.44 [6.21, 7.08]	6.90 [6.53, 7.05]	6.84 [6.64, 7.17]
Diastolic Duration (s)	0.109 [0.098, 0.113]	0.104 [0.091, 0.118]	0.097 [0.095; 0.109]
Systolic Duration (s)	0.204 [0.193, 0.212]	0.194 [0.183, 0.211]	0.187 [0.175; 0.195]
dP/dt_{max} (mmHg/s)	6013.66 [5659.61, 6251.22]	5597.07 [5399.49, 6095.62]	6022.14 [5868.31, 6357.91]
dP/dt_{min} (mmHg/s)	-5128.91 [-5557.89, -4568.51]	-4879.44 [-6110.59, -4639.21]	-6012.81 [-6102.86, -5790.58]
Contractility index (1/s)	115.88 [108.27, 125.94]	114.59 [111.47, 117.48]	107.94 [102.93, 112.33]

Hemodynamic measurements of western diet-fed rats exposed to a SED lifestyle ($n=6$), LVE training ($n=8$), and HVE training ($n=5$) after 18 weeks. Data represent median [Q1, Q3]. EDP, end-diastolic pressure; ESP, end-systolic pressure; SPTI, systolic pressure–time index; Tau, time constant for isovolumetric relaxation; dP/dt, rate of change in pressure over time

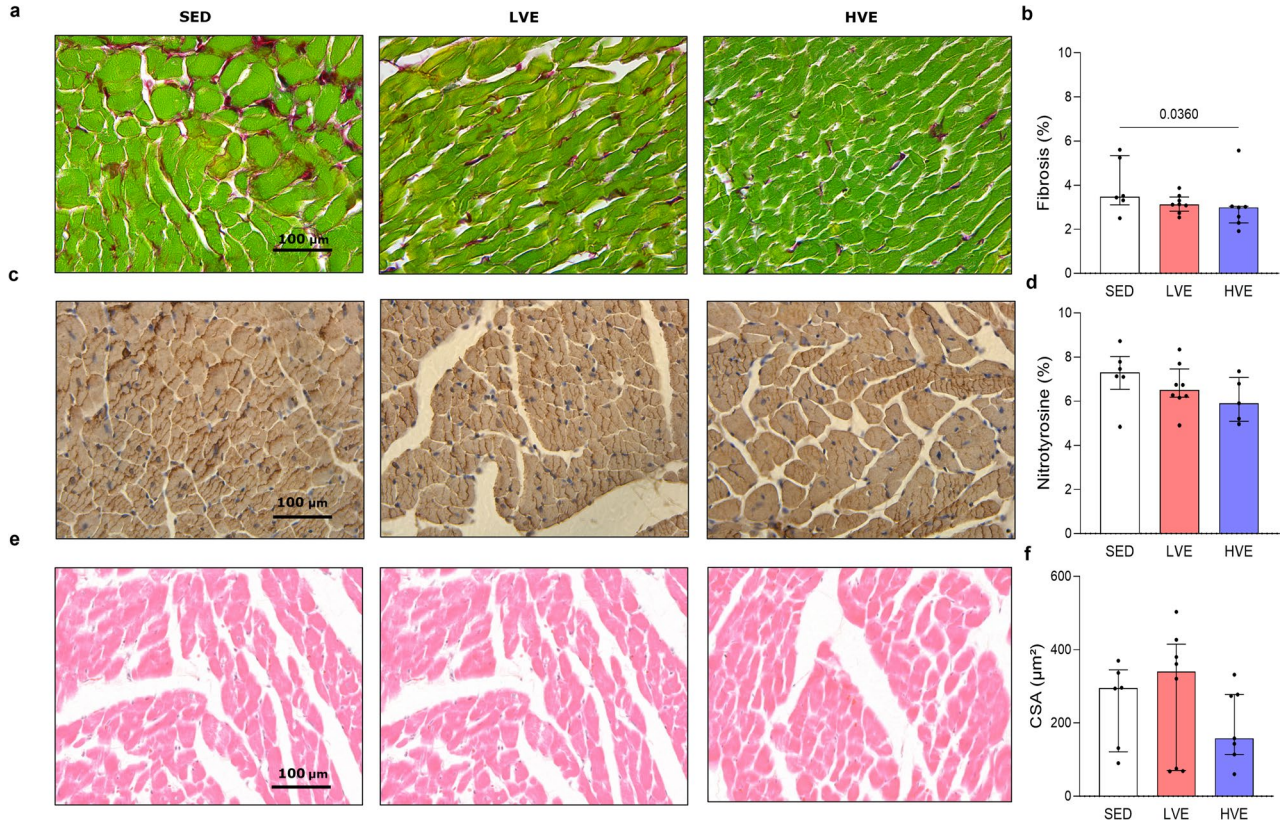

Fig. 3 HVE training alters the progression of DCM. (**a**) Representative images of Sirius Red/Fast Green staining showing interstitial fibrosis (purple/red) in cardiac tissue of SED, LVE and HVE animals. (**b**) Representative images of nitrotyrosine-IHC-stained LV cardiac sections illustrating 3'-nitrotyrosine derivatives (dark brown). (**c**) H&E staining of LV cardiac tissue showing cardiomyocytes (pink). (**d**) Percentage of interstitial fibrosis to total surface area. (**e**) Quantification of the DAB-positive area to total surface area. (**f**) Quantification of the cross-sectional area. ($n=5$–8 per group). Images captured at 40x magnification. Data are presented as median [Q1, Q3]. LV, left ventricle; H&E, haematoxylin and eosin; IHC, Immunohistochemical; DAB, 3,3'-Diaminobenzidine

analysis of cardiomyocytes revealed no influence of exercise training volume on mitochondrial density, despite systemic metabolic alterations and improvement in DCM (Fig. 4a and b).

LV gene expression analysis showed no significant changes in fibrotic, redox, or inflammatory markers following LVE or HVE training, despite minor non-significant downward trends in selected genes (Fig. 5a-f). Still, a significantly reduced expression of the oxidative enzyme, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase 2 (Nox2), was noticed in exercised trained animals when compared to SED rats (Fig. 5c).

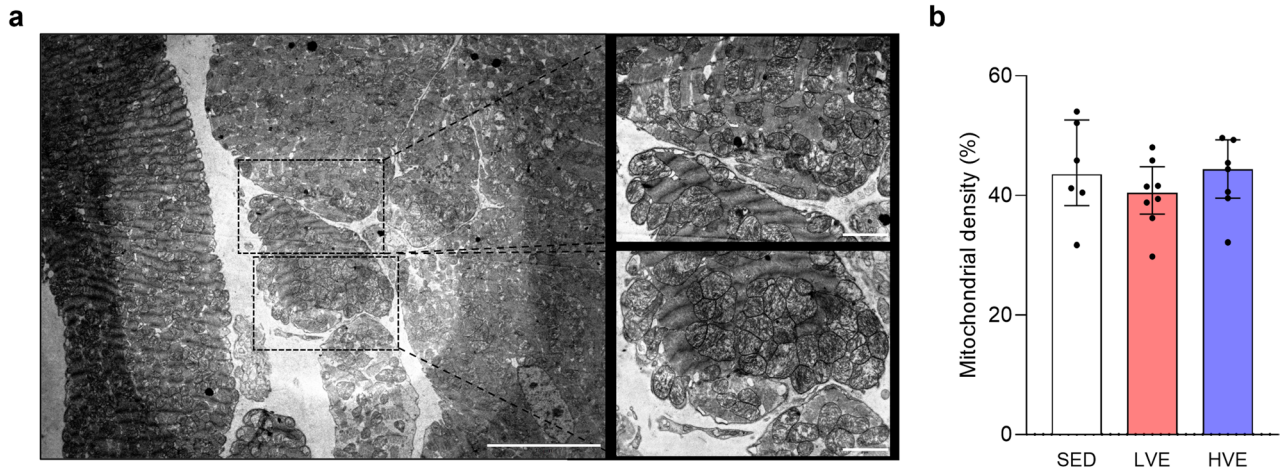

Fig. 4 Exercise training volume does not affect mitochondrial density in cardiomyocytes. (**a**) Representative TEM images of cardiomyocytes with mitochondria. Images were captured at 1000x (left) and 4000x (right) magnification. Scale bars are 10 µm (left) and 2 µm (right). (**b**) Quantification of mitochondrial density ($n = 6–8$ per group). Data are presented as median [Q1, Q3]. TEM, transmission electron microscopy

Gene set enrichment analysis (GSEA) was performed for the hallmark gene sets. Both LVE- and HVE-trained groups showed significantly enhanced gene sets for coagulation, fatty acid metabolism, complement, and xenobiotic metabolism compared to SED controls (Fig. 5g and h). Moreover, HVE-trained rats showed a significantly elevated expression for hypoxia, estrogen response late, and oxidative phosphorylation gene sets (Fig. 5g). In contrast, LVE-trained rats had a marked decline in normalised enrichment score for the inflammatory response, interferon alpha and gamma response, bile acid metabolism, G2M checkpoint, and E2F target gene sets (Fig. 5h). Interestingly, HVE-trained rats exhibited significantly higher gene enrichment scores for metabolic, inflammatory and cellular stress-related gene panels, including oxidative phosphorylation, fatty acid metabolism, adipogenesis, cholesterol homeostasis, interferon alpha and gamma response, TNF alpha and IL6–JAK–STAT3 signalling, p53 pathway, MYC targets V1 and V2, apoptosis, DNA repair, allograft rejection, xenobiotic metabolism, UV response up, and hypoxia, compared to the LVE group. In contrast, animals exposed to LVE training exhibited enhanced gene enrichment scores involved in cell-cycle-related signalling and tissue maintenance, including hedgehog signalling, mitotic spindle, and UV response down, compared to HVE-trained animals (Fig. 5i). Overall, both LVE and HVE training appear to engage overlapping as well as distinct molecular pathways that possibly contribute to the attenuation of pathological cardiac remodelling.

Discussion

This study demonstrates that exercise volume plays a potential role in modulating the progression of early DCM. Accordingly, selected exercise volumes were designed to model two clinically relevant levels of exposure: a low-volume intervention approximating the minimal recommended physical activity for individuals at increased risk of cardiovascular disease, and a high-volume intervention reflecting sustained aerobic training commonly required to elicit robust cardiac remodelling. The most prominent findings are that high-volume moderate-intensity endurance exercise (HVE) training more effectively preserves cardiac systolic function, reduces interstitial fibrosis, lowers oxidative stress markers, and induces stronger systemic metabolic reprogramming than low-volume endurance exercise (LVE) training. While LVE training partially preserved cardiac function and attenuated cardiac inflammatory and cell-responsive stress pathways on the transcriptional level, HVE induced more pronounced alterations in cardiac metabolic pathways and produced profound improvements in cardiac contractile performance. These results suggest that exercise volume is an important determinant of cardioprotection in this T2DM model.

Exercise volume determines systemic metabolic control in rats fed a Western diet

A notable finding is that exercise volume significantly influences the regulation of circulating insulin in the T2DM rat model. Our results indicate that HVE training, and to a lesser extent LVE training, improve parameters related to insulin sensitivity and reduce hyperinsulinemia. Although the underlying mechanisms were not directly assessed in this study, previous work indicates that prolonged moderate-intensity exercise training likely increases the activation of AMP-activated protein kinase (AMPK), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and Rho guanosine triphosphate hydrolases (Rho-GTPases) in skeletal muscle, likely

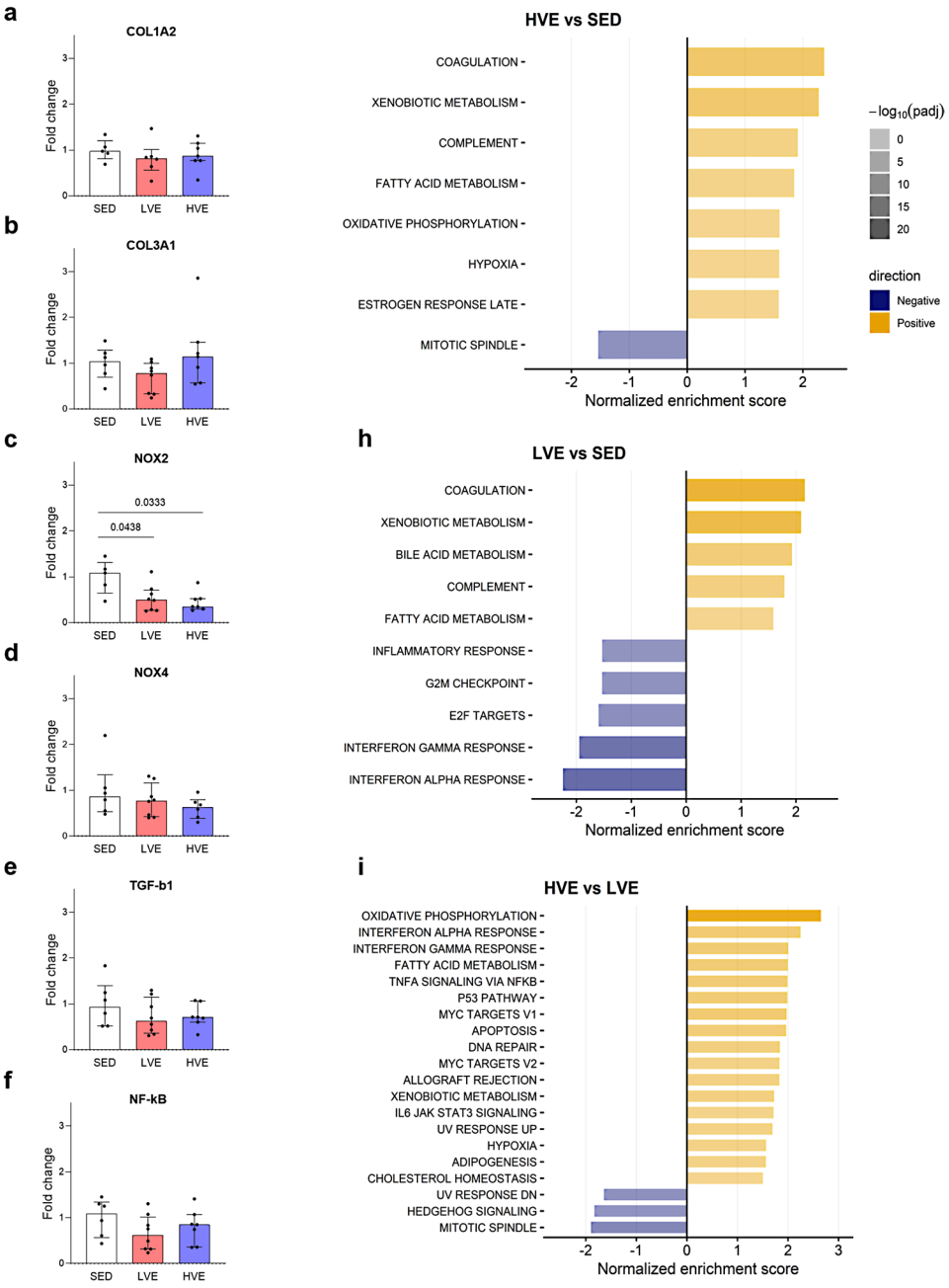

Fig. 5 Influence of exercise training volume on LV cardiac gene expression. RT-qPCR analysis was performed for (**a**, **b**) fibrosis-related genes, (**c**, **d**) redox genes, and (**e**, **f**) inflammatory-related genes. Data are presented as median [Q1, Q3]. * denotes $p < 0.05$ vs. SED. Normalised gene enrichment scores of hallmark gene sets with a significantly altered expression for HVE (**g**) and LVE (**h**) trained rats, compared to SED controls, and (**i**) HVE-trained animals compared to the LVE group. ($n = 5$–8 per group). COL, collagen; NOX, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase; Nf-κB, nuclear factor kappa B; TGF-β, transforming growth factor-β

through elevated intracellular AMP levels, increased calcium influx, and muscle contractions [19, 20]. Activation of these signalling pathways enhances peripheral insulin sensitivity and promotes the translocation of glucose transporter 4 (GLUT4)-containing vesicles to the sarcolemma, thereby enhancing skeletal muscle glucose uptake during and after exercise in an insulin-independent manner [21].

Accordingly, HVE and LVE-trained animals exhibited a reduced trend of fasting glucose levels at week 18, compared to SED controls. Despite these improvements, fasting glucose levels increased in all groups after 18 weeks, indicating a modest effect of exercise volume on glycaemic control. Still, both LVE and HVE moderate-intensity trained groups showed significant reductions in total body and liver weight, suggesting that moderate-intensity

exercise training improves metabolic control [22, 23]. These findings are consistent with the observations by Chan et al., who reported that low-volume aerobic exercise in sucrose-fed rats reduced body weight and transiently improved insulin resistance, without producing sustained reductions in fasting glucose levels [24]. Thus, while moderate-intensity exercise training improves insulin sensitivity in a volume-dependent manner, its impact on glycemia remains limited in the T2DM rat model.

Exercise training preserves cardiac function, with a tendency toward greater improvements at higher volumes

A central observation of this study is that HVE tends to preserve cardiac function more effectively than LVE. HVE animals exhibited a greater ejection fraction, cardiac output, SV index, and better preserved GLS, indicating that a sufficient exercise volume is required to counteract early systolic impairments in the diabetic myocardium. Still, LVE showed modest, yet significant, improvements, suggesting that a minimal training stimulus can induce measurable enhancements in myocardial performance.

This volume-dependent response aligns with adaptations seen in human endurance athletes, where higher cumulative load is a primary determinant of cardiac functional adaptation [25–27]. In human endurance athletes, higher training volumes promote increased preload, enhanced SV, and improved myocardial efficiency. Although the diabetic rat heart does not remodel structurally to the same extent as the healthy athlete's heart, the functional pattern observed here suggests that HVE may partially recapitulate the performance-related benefits of endurance training, even in the absence of hypertrophy. Notably, cardiac adaptations are not solely driven by cumulative workload per se, but rather by the sustained integration of metabolic and mechanical stressors over time. Our data indicate that estimated mechanical workload was positively associated with cardiac performance, whereas fasting glucose levels show a modest negative correlation with cardiac output index, suggesting that glycaemic status may act as a mild stressor. Importantly, the strength of this association with cardiac performance was considerably greater for exercise workload than for metabolic parameters, highlighting the dominant contribution of exercise exposure.

However, additional analyses indicated that the observed relationship between workload and cardiac performance was largely driven by differences between SED and exercised animals, rather than reflecting a clear dose-response relationship. This finding suggests that the presence of a sustained exercise stimulus, rather than incremental increases in training load, represents the primary determinant of cardiac adaptation in this model. From a physiological perspective, both exercise training regimens are likely to impose sufficient haemodynamic loading, increase metabolic flux, and sustain myocardial stress, directly driving cardiac adaptation [28], and potentially reaching a threshold beyond which larger increments in workload are expected to provide additional functional benefits.

Importantly, the divergence between functional and structural adaptation in this model offers insight into the interaction between volume load and metabolic disease. In healthy athletes, structural eccentric hypertrophy develops because the myocardium is responsive to physiological growth pathways and experiences repetitive volume overload under non-pathological conditions [29]. In contrast, the diabetic myocardium is exposed to oxidative stress, inflammation, and impaired mitochondrial energetics, all of which may inhibit physiological hypertrophic signalling [4, 5]. This could explain why HVE and LVE preserved contractility and strain parameters without inducing chamber enlargement or wall thickening. The heart becomes functionally more efficient but remains structurally constrained by the unfavourable metabolic environment.

Yet, despite the absence of athlete-like hypertrophy, the significant improvement in GLS in the HVE group is particularly noteworthy. GLS is one of the earliest markers of subclinical systolic impairment in cardiomyopathy [30], and its preservation in HVE animals suggests that higher training volumes may prevent or delay the transition from metabolic dysfunction to overt systolic failure. The observed increase in SV index further implies a more favourable interaction between preload, contractility, and myocardial energy handling during HVE.

Exercise volume modulates fibrosis and oxidative stress in the T2DM rat model

Our data further indicate that training volume is an important determinant of cardiac remodelling. Only the HVE group exhibited a statistically significant reduction in LV interstitial fibrosis, whereas the LVE group showed a non-significant downward trend. The absence of major differences in, cardiac weight, hemodynamic parameters, and cardiomyocyte CSA between exercise-trained groups and controls suggest that these effects occurred at an earlier stage of cardiac remodelling, consistent with D'Haese et al. [31]. Although reductions in transforming growth factor β (TGF-β) and collagen gene expression were modest, cardiac fibrosis is also influenced by extracellular matrix turnover and post-transcriptional regulation [32]. Exercise-induced alterations in oxidative stress may attenuate fibroblast activation and matrix remodelling systems, thereby limiting collagen deposition despite minimal changes in fibrotic gene expression [33].

The observed decline in 3-NT derivatives, the significant downregulation of Nox2, and the downward trend

in nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase 4 (Nox4) expression imply that both LVE and HVE mitigate ROS-driven signalling in the diabetic myocardium. Because NOX enzymes are critical generators of ROS that promote fibroblast activation and maladaptive remodelling [34], their suppression likely contributes to the observed cardioprotective effects. Consistent with previous pre-clinical exercise training studies, the downregulation of Nox2 and Nox4 possibly correlates with reduced pathological remodelling [35–37]. Together, these findings suggest that oxidative stress represents an important mechanism through which both exercise training volumes confer intrinsic cardioprotection in a T2DM rat model.

Volume-dependent cardiac mitochondrial and metabolic adaptations

Despite reductions in oxidative stress and fibrosis, the extent to which exercise training volume induces adaptive changes in cardiac mitochondria remains variable. Exercise induces alterations in mitochondrial function and density; however, the extent of these adaptations depends on the specific characteristics of the exercise stimulus, including its type, intensity, and volume [38] . It is possible that neither low-volume nor high-volume moderate-intensity training reached the intensity and duration threshold required to affect cardiac mitochondrial density in T2DM rats. Alternatively, these observations may reflect adaptive changes in mitochondrial function, potentially supported by the intrinsic reserve capacity of existing mitochondria and their ability to accommodate increased energetic demands. Bækkerud FH, et al. reported a preserved cardiac mitochondrial density in male T2DM mice in response to high-intensity exercise training, despite clear improvements in cardiac mitochondrial oxidative phosphorylation capacity [39]. Similarly, Granata C, et al. revealed an intricate and timely remodelling of the mitochondrial transcriptome, proteome, and lipidome, independent of changes in overall mitochondrial content in human skeletal muscle in response to exercise training [40].

Transcriptomic data from LV cardiac tissue revealed a significantly increased enrichment score for the oxidative phosphorylation gene set in HVE-trained animals compared to SED and LVE-trained rats. These results point to volume-dependent adaptations in pathways related to mitochondrial oxidative metabolism and mitochondrial function [41]. Synergistically, both high-volume and low-volume-trained rats showed an enhanced gene enrichment for the fatty acid (FA) metabolism gene sets, suggesting possible cardioprotective metabolic adaptations [42–44]. Nevertheless, HVE-trained rats exhibited a significantly higher gene enrichment score for FA metabolism, adipogenesis and cholesterol homeostasis compared to the LVE group, highlighting volume-dependent alterations at the transcriptional level associated with FA metabolism-related pathways. Alterations in FA metabolism pathways are often accompanied by enhanced FA transport and mitochondrial oxidative capacity, thereby preventing the accumulation of excess fats and lipotoxic intermediates [45]. Likewise, HVE, and to a lesser extent LVE training, induced a significant upregulation of the xenobiotic-metabolism gene sets, suggesting coordinated activation of cardiac detoxification pathways that possibly regulate shifts in circulating lipids and endogenous metabolites [46]. Therefore, moderate-intensity training appears to promote beneficial changes in cardiac metabolic function by altering the expression of oxidative phosphorylation and FA metabolism-related pathways, adaptations that are considered cardioprotective in T2DM and largely dependent on exercise training volume.

Despite the absence of changes in cardiac mitochondrial density, these findings reinforce the concept that exercise-induced improvements in mitochondrial function are likely supported by the intrinsic reserve capacity of existing mitochondria and regulated by exercise volume. Therefore, mitochondrial bioenergetics may be regulated by upstream control points, such as substrate availability, tricarboxylic acid flux, and the modulation of downstream energy demands, independent of changes in cardiac mitochondrial content.

Exercise volume shapes cardiac inflammatory and stress-responsive gene signature

Analysis of normalised gene set enrichment scores further revealed distinct volume-dependent mechanisms. HVE-trained rats showed an elevated normalised enrichment score for hypoxia-related gene sets when compared to SED and LVE-trained rats, suggesting possible cardioprotective adaptations involved in angiogenesis and cell survival [47, 48]. Additionally, HVE training upregulated gene sets associated with late estrogen signalling. The estrogen late response involves the modulation of heat shock factors (HSFs), which in turn transcribe target genes of heat shock proteins (HSPs) [49]. Upregulation of HSPs has been implicated in cardioprotective mechanisms by modulating inflammatory signalling, attenuating oxidative stress, and limiting fibrotic remodelling via MMPs [50, 51]. Although the underlying mechanisms were not evaluated, higher volumes of moderate-intensity exercise appear to be associated with transcriptional changes in these pathways, potentially reflecting repeated physiological stress stimuli [51].

Interpreting the inflammatory response remains challenging, as both LVE- and HVE-trained rats exhibited a reduced trend in nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-κB) expression, which

depends on a complex interplay of stimuli [52], compared to SED controls. Nevertheless, our data revealed reduced normalised enrichment scores for inflammation-related gene sets in LV tissue from rats exposed to LVE training, implying inflammatory dampening. In contrast, HVE-trained animals exhibited significantly upregulated pro-inflammatory gene panels compared to the LVE-trained group; however, the functional consequences of these transcriptional adaptations on cardiac performance remain unclear. For instance, acute IL-6 - JAK - STAT3 signalling has been associated with higher exercise workloads conferring cardioprotective effects, whereas chronic activation has been associated with the development of maladaptive remodelling [53]. In that context, our results suggest that adaptations in the expression of genes related to both innate and adaptive inflammatory pathways are influenced by exercise training volume and potentially alter the progression of DCM. Our data also show a significant reduction in gene set enrichment score for cell cycle-related pathways and upregulation of hedgehog signalling in the LVE group, likely reflecting adaptive remodelling of cardiac tissue via non-myocyte populations, such as endothelial cells. Recent studies focusing on cell cycle-related pathways and upregulation of hedgehog signalling in cardiac pathologies indicate that these mechanisms contribute to cardiovascular protection [54–58]. Alternatively, higher gene set enrichment scores of cell-cycle-related pathways in the SED group may reflect transcriptional alterations in fibroblasts or immune cells, rather than endothelial cells themselves. Furthermore, both HVE and LVE training show altered gene set enrichment scores for DNA damage response pathways, suggesting genomic stabilisation against ROS and stress signalling likely induced by metabolic and exercise-induced stress. Collectively, our findings demonstrate that exercise training induces volume-dependent transcriptional adaptations in the heart, encompassing angiogenic, stress-response, inflammatory, and remodelling pathways. LVE and HVE training appear to differentially engage in pathways related to adaptive non-myocyte remodelling, cardioprotective signalling, and inflammatory modulation, whereas excessive pro-inflammatory and cell cycle-related signalling in sedentary or high-volume groups may reflect stress-associated responses.

Study limitations

While our findings provide novel insights into the volume-dependent effects of endurance exercise training on cardiac remodelling in a T2DM rat model, this study also has its limitations. Although the relatively small sample size represents a limitation of the study and may increase susceptibility to inter-biological variability, post-hoc power analyses indicate that the study was sufficiently powered. Nonetheless, the limited sample size possibly constrains multivariable analyses, which may affect the robustness and generalizability of the observed associations. A second limitation of this study is the lack of a comprehensive proteomic analysis. While transcriptomic alterations provide important mechanistic insight and were consistently reflected at the functional and phenotypic levels in the present study, it is acknowledged that post-transcriptional regulation and translational modifications may further modulate protein abundance and activity. Although future studies incorporating proteomic approaches would strengthen mechanistic resolution, the current findings remain robust and provide meaningful insight into the volume-dependent cardioprotective effects of exercise training during early diabetic cardiomyopathy. Furthermore, the absence of temporal resolution in the assessment of oxidative stress and cardiac fibrosis constrains the mechanistic interpretation of the observed alterations in myocardial strain parameters. In addition, while these results demonstrate that low and high exercise training volumes exert protective effects against the development of DCM in a T2DM rat model, the translational relevance to human populations remains to be confirmed. Finally, the exclusive use of male rats, to eliminate confounding cardioprotective hormonal influences, limits the translation of our work to female individuals. Therefore, future studies incorporating female cohorts and hormonal modulation are warranted to improve the translational relevance of this work.

Conclusion

Both low and high-volume exercise training prevent early-stage diabetic cardiomyopathy but engage distinct mechanisms. HVE more effectively preserves systolic function, reduces fibrosis and enhances the cardiometabolic profile, whereas LVE primarily engages in inflammatory and stress signalling pathways. These findings indicate that modest exercise interventions confer cardioprotective effects, while sufficient exercise volume is essential to maximise functional and molecular benefits, providing mechanistic support for volume-dependent training recommendations in populations at risk for diabetic cardiomyopathy.

Abbreviations

3-NT	3′-nitrotyrosine
AMPK	AMP-activated protein kinase
AWTd	Diastolic anterior wall thickness
CaMKII	Ca2+/calmodulin-dependent protein kinase II
CO/BSA	Cardiac output to body surface area
CSA	Cross-sectional area
Ccna	Cyclin A
DAB 3	3′-Diaminobenzidine
DCM	Diabetic cardiomyopathy
dP/dt	Rate of change in pressure over time
E/A	Early to late atrial mitral inflow velocities
EDP	End-diastolic pressure

EDV	End-diastolic volume
E/E	Early mitral inflow velocity to early diastolic mitral annular velocity
EF	Ejection fraction
ESP	End-systolic pressure
ESV	End-systolic volume
FA	Fatty acid
FS	Fractional shortening
HR	Heart rate
GCS	Global circumferential strain
GLS	Global longitudinal strain
GLUT4	Glucose transporter 4
GSEA	Gene set enrichment analysis
GRS	Global radial strain
HFpEF	Preserved ejection fraction
HFreF	Reduced ejection fraction
HIF-1	Hypoxia-inducible factor 1
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HSF	Heat shock factor
HVE	High-volume exercise
IFN-α	Interferon-α
IFN-γ	Interferon-γ
IHC	Immunohistochemical
IQR	Interquartile range
LV	Left ventricle
LVE	Low-volume exercise
MMP	Matrix metalloproteinases
Nf-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NOX	Nicotinamide adenine dinucleotide phosphate hydrogen oxidase
Nox2	Nicotinamide adenine dinucleotide phosphate hydrogen oxidase 2
Nox4	Nicotinamide adenine dinucleotide phosphate hydrogen oxidase 4
OGTT	Oral glucose tolerance test
PWTd	Diastolic posterior wall thickness
Rho-GTPases	Rho guanosine triphosphate hydrolases
ROS	Reactive oxygen species
ROUT	Robust regression outlier removal
Rpl13a	Ribosomal protein L13a
SED	Sedentary
SPTI	Systolic pressure-time index
SV	Stroke volume
T2DM	Type 2 diabetes mellitus
Tau	Time constant for isovolumetric relaxation
TEM	Transmission electron microscopy
TGF-β	Fibrotic signalling factorβ

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40842-026-00288-2.

Supplementary Material 1

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Author contributions

RS wrote the manuscript and performed the ex vivo experiments and data analysis. IB wrote the manuscript and supported exercise interventions and echocardiographic analysis. LV performed exercise interventions and in vivo measurements. LS performed exercise interventions and in vivo measurements. EH performed exercise interventions and in vivo measurements. VV supported with RT-qPCR. EV performed exercise interventions and supported with TEM analysis. SD designed the study and supported the study setup. GC contributed to the clinical translation of echocardiographic data. IL provided access to the transmission electron microscope. BMF conceived and designed the study. PV performed the GSEA analysis. DH conceived and designed the study. DD contributed to the exercise training protocols, in- and ex-vivo experiments, GSEA data analysis and figures. VB conceived and designed the study. All authors have read and approved the final manuscript.

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Data availability

Data will be made available upon request.

Declarations

Ethical approval

All procedures followed the 2010/63/EU directive and were approved by the Local Ethical Committee at Hasselt University (Diepenbeek, Belgium; matrix ID 202102A1).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]BIOMED Research Institute, Department of Cardio & Organsystems, Hasselt University, Agoralaan, Diepenbeek 3590, Belgium
[2]Cell Biology Area, Molecular Biology Department, University of León, León 24007, Spain
[3]Department of Cardiology and Jessa & Science, Jessa Hospital, Stadsomvaart, Hasselt 3500, Belgium
[4]BIOMED Research Institute, Department of Immunology & Infection, Hasselt University, Agoralaan, Diepenbeek 3590, Belgium
[5]Rehabilitation Research Centre (REVAL), Department of Rehabilitation Sciences, Hasselt University, Wetenschapspark 5-7, Diepenbeek 3590, Belgium

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