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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Moderate- and high-intensity endurance training as preventive strategies for adverse cardiac remodeling and dysfunction in prediabetes

Iris de Laat

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

SUPERVISOR :
Prof. dr. Virginie BITO

MENTOR :
Mevrouw Sarah D'HAESE

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*Running title: *Exercise prevents diabetes-induced cardiac harm*

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ABSTRACT

Background: Type 2 Diabetes Mellitus is a major risk factor for the development of cardiovascular diseases. Exercise training has been suggested as potential preventive strategy. We aim to investigate whether and how moderate- and high-intensity endurance training can prevent prediabetes-related adverse cardiac remodeling and dysfunction.

Methods: Rats received a chow or Western diet to induce prediabetes with adverse cardiac effects. At onset of diet, WD fed rats were subjected to sedentary lifestyle, moderate-intensity training (MIT), or high-intensity interval training (HIIT). Diverse experiments were performed to assess prediabetes (glucose tolerance test) and cardiac function and structure (echocardiography, hemodynamic measurements, immunohistochemical staining, qPCR).

Results: MIT and HIIT trained rats displayed lower plasma glucose (p=0.06) and plasma insulin concentrations (p<0.05). In the heart, both interventions inhibited the increase in end-systolic pressure (p<0.05), wall thickness (p<0.01), and interstitial fibrosis (p<0.05). Cardiac CD68-positive pan-macrophages were elevated after MIT and HIIT. Gene expression of proinflammatory cytokines and M1 macrophage markers were increased following both exercise modalities (p<0.001), while only HIIT increased gene expression of a ROS-generating enzyme (p=0.08). Anti-inflammatory M2 macrophage markers were increasingly expressed after MIT (p<0.01), whereas anti-oxidative (p<0.05) and dicarbonyl (p<0.01) defense mechanisms were favored by HIIT on gene level. Lastly, advanced glycation end-products deposition in the heart was decreased on protein level following HIIT (p=0.01).

Conclusion: Our findings demonstrate that MIT and HIIT limit prediabetic severity, hypertension, and cardiac hypertrophy. Besides these benefits, long-term endurance training induces cardiac inflammation and oxidative stress, which seem to be counteracted by upregulating intrinsic defense systems.

INTRODUCTION

Diabetes-induced adverse cardiac remodeling and dysfunction

Type 2 Diabetes Mellitus - To date, 537 million individuals suffer from Diabetes Mellitus (DM), corresponding with a global prevalence of 10.5% (1). This prevalence is expected to increase at an alarming rate, as it is estimated that 783 million people will have DM by 2045.

DM is a chronic, metabolic disease featured by persistent hyperglycemia, meaning that fasting blood glucose levels are elevated above 126 mg/dl (2). Two pancreatic hormones play a major role in blood glucose homeostasis, namely glucagon and insulin (3,4). In physiological circumstances, the glucose-mobilizing hormone glucagon is produced by pancreatic α-cells and stimulates blood glucose levels to rise (3). For example, it favors hepatic glucose production; it converts glycogen into glucose (i.e. glycogenolysis), stimulates *de novo* glucose synthesis (i.e. gluconeogenesis), inhibits glucose breakdown (i.e. glycolysis), and inhibits glycogen formation (i.e. glycogenesis) (5). Insulin is produced by the pancreatic β-cells in response to an increase in blood glucose levels (e.g. after food intake) (5,6). Insulin binds to its membrane-bound insulin receptor located on somatic cells of peripheral tissues such as liver, fat, and muscle cells to stimulate glucose uptake from the blood via a glucose transporter, thereby lowering blood glucose levels (6).

DM is conventionally classified into Type 1 DM (T1DM) and Type 2 DM (T2DM) (7). In T1DM, an autoimmune reaction against the pancreatic β-cells causes cellular destruction and, thus, absolute insulin deficiency. T2DM, which accounts for 90% of all diabetic individuals, is a progressive condition and is characterized by relative insulin deficiency.

In the prediabetic phase of T2DM, hyperglycemia occurs because the peripheral insulin receptors become resistant (8). To compensate for the lower uptake of glucose in hepato-, adipo-, and myocytes, pancreatic β-cells secrete more insulin, which results in a hyperinsulinemic state. Despite the increased insulin demand, the β-cells can no longer ensure adequate insulin production and secretion over time, leading to significant hyperglycemia and development of T2DM.

T2DM represents a multifactorial disease caused by genetic and lifestyle factors, including insufficient physical activity (PA) and unhealthy diet (9). Especially, the current epidemic of obesity and metabolic syndrome (MetS), major risk factors for T2DM, is thought to be attributable to the increased consumption of high-caloric diets, including a high-fat diet (HFD), food containing refined sugar, and sweetened beverages (10,11). Besides having an increased risk to develop complications such as nephro-, retino-, and neuropathy, T2DM individuals are highly likely to develop cardiovascular diseases (CVD) (12).

Type 2 Diabetes Mellitus as risk factor for cardiovascular diseases - According to the American Heart Association, T2DM should be designated as an independent risk factor for CVD (13,14). CVD are the cause of death in about 65% of all diabetic individuals and the prognosis of diabetic patients is worse compared to non-diabetic individuals suffering from CVD.

Traditionally, it was thought that diabetes-related cardiac dysfunction and remodeling develops in several (sub)clinical phases (15,16). In early stages of T2DM, abnormalities in the heart manifest as an increased filling pressure, concentric left ventricle (LV) hypertrophy, a reduced LV cavity size, and cardiac fibrosis, leading to diastolic dysfunction and heart failure (HF) with preserved ejection fraction (HFpEF). In a later phase, diastolic dysfunction can progressively change towards systolic dysfunction, due to a cardiac compensation mechanism, which is featured by a dilated LV cavity and cardiomyocyte apoptosis (15,17). This systolic dysfunction can develop into HF with reduced ejection fraction (HFrEF) (15,16). Rather than being consecutive stages of cardiac dysfunction in T2DM, it is currently also hypothesized that HFpEF and HFrEF both develop as separate phenotypes, namely the so-called restrictive and dilated phenotype, respectively (18).

Different molecular mechanisms are responsible for the development of adverse cardiac remodeling and dysfunction in T2DM (16,18,19). As such, systemic factors (e.g. hyperglycemia, hyperinsulinemia, and lipotoxicity) can cause increased oxidative stress, inflammation, and advanced glycation end-products (AGEs)

deposition in the heart. Cardiac oxidative stress is caused by an imbalanced state in generation and degradation of reactive oxygen species (ROS). In physiological circumstances, excessive ROS is removed by endogenous antioxidant mechanisms, which are impaired in DM (20). The disturbed ROS balance also contributes to induction of cardiac inflammation upon infiltration of proinflammatory cytokines and immune cells (i.e. M1-phenotype macrophages). In response to the myocardial inflammatory processes, anti-inflammatory defense can become upregulated in the T2DM heart, for example by macrophage polarization towards the M2-like phenotype. Moreover, ROS can trigger AGEs formation in cardiomyocytes. AGEs are complex compounds formed by the irreversible glycation of amino acids, peptides or proteins, representing posttranslational protein modifications (21,22). Our Western diet (WD), characterized by excess of sugars, is an exogenous source of these molecules, but AGEs also accumulate with aging in the blood and tissues (23-25). AGEs induce detrimental cardiac effects by two different mechanisms (26). In particular, they crosslink extracellular matrix (ECM) and intracellular proteins, thereby directly affecting their structure and function, and interact with the receptor for advanced glycation end-products (RAGE) (26,27). The latter initiates signaling pathways that promote disturbed calcium (Ca^{2+}) handling, inflammation and oxidative stress in the heart (21,28).

Western diet-induced diabetic rat model with adverse cardiac remodeling and dysfunction - To investigate the pathophysiology of cardiac remodeling and dysfunction in DM, a broad spectrum of *in vivo* models is currently used (15). Rodents, such as db/db mice and Zucker diabetic fatty rats, are genetically modified to become resistant to or deficient in the receptor for leptin, which is an adipocyte-originating hormone that regulates food intake and energy consumption. In addition, high concentrations of toxins which damage the insulin-producing β-cells, like streptozotocin (STZ), can be used. However, these genetic and chemically-induced models represent the human phenotype of T2DM insufficiently (29).

The past few years, diet-induced models are increasingly applied to investigate T2DM because these models mimic the human pathogenesis more closely (15,29). The HFD-induced obesity model, in the absence of a high-sugar component, is one of the most frequently used models to investigate T2DM (Figure 1). As such, this model shows insulin-resistance in a prediabetic phase. Nevertheless, the intake of a HFD does not consistently induce cardiac dysfunction in rodents (15). With increasing popularity, low-dose STZ injections are provided on top of HFD intake to reinforce the T2DM phenotype and generate a worse cardiac outcome. However, the additional injection with STZ lowers the translational relevance of the model due to undesired, cytotoxic side effects (30,31).

Interestingly, it has been shown that excessive sugar intake rather than an increased fat intake is associated with the current T2DM pandemic (32,33). However, high-sugar diet-induced models are less investigated than other models regarding cardiac abnormalities in T2DM (34). Overconsumption of fructose seems to induce a mild diabetic phenotype accompanied by notable cardiac detrimental effects (15) (Figure 1). Previously, our research group has developed a diabetic rat model fed a diet rich in dietary sugar sucrose, a disaccharide consisting of glucose and fructose, or so-called WD (34,35). Indeed, rats receiving a WD for 18 weeks displayed features of T2DM and cardiac dysfunction (34). In detail, these WD fed rats presented increased body weight, hyperglycemia, hyperinsulinemia, end-diastolic pressure and anterior wall thickness. At the cellular and molecular level, elevated cardiac interstitial fibrosis and plasma AGEs levels were observed in rats fed a WD. Thus, the high-sucrose diet-induced rat model is clinically relevant because sucrose is abundantly present in the WD and its fructose component possibly exerts the adverse cardiometabolic response (15,36). Therefore, dietary interventions characterized by high fructose concentrations seem useful experimental tools to generate a preclinical rodent model to study cardiac pathology associated with insulin resistance and (pre-)diabetes (15).

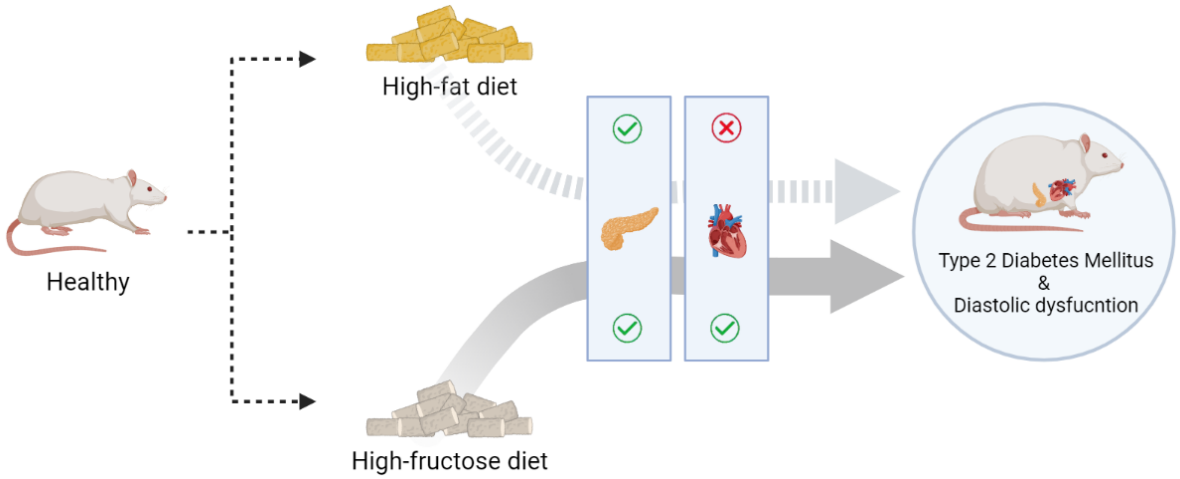

Figure 1: The potential of high-fat diet and high-fructose diet to induce a Type 2 Diabetes Mellitus phenotype presenting cardiovascular traits. Healthy rats fed a high-fat diet demonstrate a prediabetic phenotype of Type 2 Diabetes Mellitus, but inconsistently induce cardiac dysfunction. Although not many is known, healthy rats fed a fructose-rich diet seem to present both a prediabetic phenotype and diastolic dysfunction. Figure modified from Heather *et al*. (2022) (15).

Exercise training as therapeutic strategy

Conventional treatments for Type 2 Diabetes Mellitus with heart failure - Antihyperglycemic agents are frequently used as T2DM treatment and present as a broad spectrum of drugs acting via various pathways (37,38). The European Society of Cardiology (ESC) recommends metformin and sodium-glucose cotransporters 2 (SGLT2) inhibitors as first line treatment in T2DM individuals to reduce HF risk (39). Metformin (i.e. dimethylbiguanide) is a hypoglycemic drug which inhibits hepatic gluconeogenesis (40). The glucose-lowering effects of SGLT2 inhibitors are mediated by inhibiting renal glucose reabsorption and stimulating its excretion via the urinary tract (37,38). Besides metformin and SGLT2 inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists are also able to decrease blood glucose levels safely in patients with HF. GLP-1 receptor agonists induce glucose-dependent insulin release.

Next to treatment of the diabetic state, additional therapies to tackle HF should be considered in diabetic individuals suffering from CVD. To treat HFrEF and hypertension in diabetic patients, pharmacological interventions (i.e. renin-angiotensin-aldosterone system (RAAS)-inhibitors, and beta-blockers) and device therapies (i.e. implantable cardioverter defibrillator, and cardiac resynchronization therapy) are recommended (39,41). However, these therapies are not recognized as effective treatments for HFpEF, indicating the need for new approaches to treat this subgroup of patients (42,43). In addition, as vulnerable patients with T2DM and HF might encounter different comorbidities and use multiple drugs, there is a risk for drug-related side effects (i.e. polypharmacy) (39). Therefore, a multifactorial approach should be implemented in which lifestyle modifications, such as a healthy diet and PA, are included.

Exercise training in the healthy and diabetic heart - Exercise training is generally known to benefit cardiovascular and metabolic outcomes, thereby reducing risk for morbidity and all-cause mortality (44). When prescribing PA, an individual training plan should be assessed in terms of type of exercise, volume (i.e. frequency and duration), and intensity. As such, PA can be divided into several training modes, including resistance training (e.g. lifting weights), flexibility training (e.g. yoga, pilates), and endurance training (e.g. running, cycling, walking) (45,46). Resistance and flexibility training are performed to increase muscle strength and joint motion, respectively (45). Endurance training on regular basis induces a stronger and fitter cardiorespiratory system and mainly relies on the aerobic metabolic system. Furthermore, PA can be performed at light,

moderate or high-to-vigorous intensity, expressed in either absolute or relative terms (Table 1) (44). Interestingly, research over the past years showed that the intensity rather than the duration of exercise is correlated with decreasing all-cause mortality (45). The ESC recommendations regarding PA strive for at least 150 - 300 min per week of moderate intensity or 75 - 150 min per week of high or vigorous-intensity aerobic training, or an equivalent combination of these exercise modalities, for all healthy adults to reduce cardiovascular risk (44).

Lifestyle interventions are not only essential in healthy individuals, but are also important in controlling DM and its cardiovascular complications (39). Therefore, the ESC recommends PA of moderate-to-high intensity for at least 150 min a week for adults with T2DM. Previously, endurance exercise prescriptions favored traditional moderate-intensity training (MIT) to treat T2DM individuals (45). MIT represents aerobic, continuous exercise training at moderate intensity, which is corresponding with approximately 50% of the maximal oxygen consumption ($VO_{2\ max}$) (47). Nevertheless, training at high intensity is proposed as valuable alternative for MIT as potentially being more effective and time-efficient (45). High-intensity interval training (HIIT) is defined as an anaerobic exercise modality containing repeated bouts of vigorous-to-maximal-intensity exercise, exceeding $VO_{2\ max}$ of 60%, separated by periods of low-intensity exercise to ensure recovery (45,47). Indeed, randomized controlled trials suggested that HIIT is a safe, effective intervention for T2DM patients, decreasing total body fat and blood glucose concentrations, and slightly decreasing plasma insulin levels (48-50). Furthermore, HIIT has beneficial effects on cardiorespiratory fitness by showing reduced heart rate in rest and increased peak VO_2 capacity (51,52).

In a previous preclinical study, our research group investigated the therapeutic effects of PA on the diabetic heart in a WD fed rat model (manuscript in preparation). Verboven *et al.* showed that 12 weeks of MIT and HIIT were equally effective to treat T2DM, demonstrated by lower blood glucose and homeostatic model assessment of insulin resistance (HOMA-IR) values than sedentary T2DM rats. Both exercise interventions alleviated diastolic dysfunction and pathological cardiac remodeling, by reducing fibrosis and increasing mitochondrial capacity, while only MIT rescued systolic function in T2DM.

Table 1: Overview of exercise intensities and its absolute and relative measurables.

ABSOLUTE INTENSITY		RELATIVE INTENSITY		
Intensity	**MET**	**% HR**	**% $VO_{2\ max}$**	**Examples**
Light	1.1-2.9	57-63	20-39	Walking (<4 km/h), light household work.
Moderate	3.0-5.9	64-76	40-59	Walking (4.1-6.5 km/h), cycling (<15 km/h)
High-to-vigorous	>6.0	77-95	>60	Running, cycling (>15 km/h)

HR: Heart rate, MET: Metabolic equivalent of task, $VO_{2\ max}$: Maximal oxygen consumption. Table modified from Visseren et al. (2021) (44).

Exercise as preventive approach: the unknown

Taken together, an unhealthy lifestyle (e.g. WD consumption and physical inactivity) is a corner stone in the development of T2DM and its adverse cardiac phenotype. Endurance exercise training, especially MIT, is considered as a non-pharmacological, safe approach to treat T2DM with cardiac dysfunction. The last few years, progress has been made in the understanding of the therapeutic potential of HIIT in T2DM with HF as well. Given the rising prevalence, there is a tremendous need for multifactorial, effective approaches which do not only treat but also prevent the development of cardiac abnormalities in T2DM. In this regard, it remains to be elucidated whether exercise training, especially different exercise intensities, can be suitable herein. In addition, the molecular mechanisms by which exercise training

exerts its potential cardioprotective effects are not fully understood.

In this research, we aim to investigate whether moderate- and high-intensity endurance training prevent the development of T2DM with adverse cardiac remodeling and dysfunction in rats. We also identified whether these training modalities affect inflammatory and oxidative signaling pathways in the heart. We hypothesize that both MIT and HIIT offer cardio-glycemic protection in a WD-induced T2DM rat model with adverse cardiac remodeling and dysfunction, thus being considerable preventive approaches to halt T2DM development.

EXPERIMENTAL PROCEDURES

Ethical approval - All animal experiments were approved by the local ethical committee for animal experimentation (UHasselt, Diepenbeek, Belgium, ID 202102) and have been performed in accordance with the EU Directive 2010/63/EU for animal testing. The animals were housed two per cage and had water and food provided ad libitum. A controlled environment was preserved during the study.

Experimental set-up - Twenty-seven male Sprague-Dawley rats (Charles River Laboratories, L'Arbresle, France), weighing 250 grams, were randomly assigned into four experimental groups. The control group received a control chow rodent diet (CD, n=5), consisting of 24% kcal proteins, 18% kcal fat, and 58% kcal carbohydrates from grains without added sugars (Teklad Global Rodent Diet, ENVIGO, Horst, The Netherlands) and remained sedentary throughout the full study period of 18 weeks. All other animals were fed a sugar-rich or so-called WD, consisting of 15% kcal proteins, 16% kcal total fat, and 69% kcal carbohydrates, of which 48% sugars from sweetened condensed milk and added D-saccharose, as previously described (34,53). At the onset of diet, WD fed rats were subjected to sedentary lifestyle (WD SED, n=7), moderate-intensity training (WD MIT, n=7), or high-intensity interval training (WD HIIT, n=8) for 18 weeks.

Animal body weight and 24-hour food intake were measured weekly (Supplementary Figure S1). Blood sampling, echocardiographic imaging and oral glucose tolerance test (OGTT) were performed at baseline, 6, 12, and 18 weeks after the start of the diet. Hemodynamic measurements are performed at sacrifice. All rats were euthanized using an overdose of sodium pentobarbital (Dolethal, Val d'hony Verdifarm, Beringen, Belgium; 150 mg/kg; i.p.) preceded by an injection with heparine (1000 u/kg; i.p.). The hearts were excised and transversal sections were fixed in 4% paraformaldehyde for 24h. Residual LV tissue was crushed, snap-frozen in liquid nitrogen, and stored at -80°C for further analysis.

Exercise protocol - Treadmill running (Expendable Treadmill Model 805, IITC Life Science, California, USA) was performed for five days a week during 18 weeks, according to the exercise protocols as previously described (54). MIT consists of continuous moderate-intensity running on a treadmill at 18 m/min for 45 min/day at 5° inclination. HIIT consisted of 10 bouts of 2 min high-intensity running at 18 m/min at 30° inclination, separated by 1 min of active rest at 12 m/min at 30° inclination.

Echocardiographic and hemodynamic measurements - Both echocardiographic and hemodynamic procedures are performed under 2% isoflurane anesthesia supplemented with oxygen. Transthoracic echocardiographic parameters were measured with a Vevo 3100 system and a 21 MHz linear probe MX250 (FUJIFILM VisualSonics Inc., Amsterdam, The Netherlands), as described previously (55). Echocardiographic images were analyzed using the Vevo Lab 3.2.6 Software (FUJIFILM VisualSonics Inc.). Standard measures of LV structure, and systolic and diastolic function were analyzed. Analysis of the echocardiographic data was blinded to reduce bias. Details regarding echocardiography are described in Supplementary Experimental Procedures.

Invasive hemodynamic measurements were performed using an SPR-320 MikroTip high-fidelity pressure catheter (Millar Inc, The Hague, The Netherlands) that was inserted into the LV via the right carotid artery, as described previously (55). A quad-bridge amplifier was connected to the pressure catheter and the PowerLab 26 T module (AD Instruments, Oxford, UK) was used to transfer all data to LabChart v7.3.7 software (AD Instruments). Hemodynamic parameters, such as LV end-systolic pressure (LVESP), were obtained from this software.

Oral glucose tolerance test and insulin resistance assessment - By performing a 1h oral glucose tolerance test, as previously described, glucose tolerance was assessed (34,56). After an overnight fasting period, D-glucose (2 g/kg) was administered orally. Glucose concentration was determined from capillary tail blood collection prior to glucose administration (i.e. fasting glucose levels) using the Analox GM7 (Analis SA, Namur, Belgium) and repeated 15, 30 and 60 min after glucose administration. Glucose response was expressed as total area under the curve (AUC).

Plasma insulin concentrations were determined at 0 and 60 min using an electrochemiluminsescent sandwich immunoassay (K152BZC, Meso Scale, Gaithersburg, MD, USA). The HOMA-IR was used to determine insulin resistance and was calculated by the following formula: HOMA-IR=(fasting insulin [µIU/mL] ∗ fasting glucose [mmol/L])/22.5 (57).

Histological staining - 8 µm thick transversal sections were obtained from paraffin-embedded heart tissue and stained using the Sirius Red/Fast Green Collagen Staining kit (Chondrex Inc., Washington, USA), according to the manufacturer's protocol. After staining, sections were mounted with DPX mounting medium. Interstitial fibrosis was assessed in the LV of all animals in four randomly chosen 20x zoomed-in images per section using the Leica MC170 camera connected to a Leica DM2000 LED microscope (Leica Microscystems, Diegem, Belgium). The area of collagen deposition, indicated by red staining, is quantified using the color deconvolution plugin in FIJI/ImageJ software. Total collagen deposition to global cardiac area was calculated, normalized to the total surface area, and expressed as percentage. Two independent researches performed the analysis blinded for group allocation.

Immunohistochemistry – Deparaffinized 8 µm thick heart tissue sections were used for immunohistochemical staining against cluster of differentiation 68 (CD68), AGEs, and lysyl oxidase (LOX). Sections were incubated with a primary antibody against CD68 (1:100), AGEs (1:250), and LOX (1:200) diluted in 1X PBS for 1h at room temperature (RT) for CD86 and AGEs, or overnight at 4°C for LOX, followed by five washes with PBS. For CD68 and AGEs, EnVision™ with Dual Link System-horse reddish peroxidase (HRP) was applied for 30 min at RT. For LOX, a HRP-conjugated secondary antibody (1:400) was incubated at RT for 30 min. The presence of CD68, AGEs and LOX was visualized using 3,3′-diaminobenzidine (DAB). Next, all sections were counterstained with hematoxylin to stain nuclei and mounted using DPX mounting medium. Images were obtained using a Leica MC170 camera which is connected to a Leica DM2000 LED microscope. AGEs and LOX staining was quantitively analyzed at 20x magnification in four random fields per section by use of the color deconvolution plugin in FIJI/ImageJ software (Maryland, USA). The level of AGEs and LOX staining was expressed as percentage of the total surface area of interest. The CD68 staining was assessed semi-quantitatively at 40x magnification. Five scores were provided to evaluate CD68-positive cells in the LV, namely absent or limited staining (0), minor staining (1), moderate staining (2), high staining with presence of small aggregates (3), and high staining with presence of large aggregates (4). The analyses were performed blinded for group allocation by two independent researchers. Additional details are described in Supplementary Experimental Procedures and Supplementary Table S1.

Real-time quantitative polymerase chain reaction - By following the manufacturer's guidelines of the RNeasy fibrous tissue kit (Qiagen, Antwerp, Belgium), total RNA was extracted from 30 mg snap-frozen LV tissue. The concentration and purity of the RNA was assessed using the NanoDrop 2000 spectrophotometer (Isogen Life Science, Tense, Belgium). cDNA synthesis was performed using qScript cDNA Supermix (Quanta Biosciences, Beverly, USA). Primers were designed in the coding sequence of the mRNA (Integrated DNA Technologies, Leuven, Belgium; Table S2). A real-time quantitative polymerase chain reaction (RT-qPCR) experiment was performed using the QuantStudio 3 PCR system (Thermofisher Scientific, Merelbeke, Belgium). Analysis of gene expression data was performed via the ΔΔCT method following the MIQE guidelines (58). The most stable reference genes were assessed with GeNorm (i.e. ribosomal protein L13a (RPL13a) and hydroxymethylbilane synthase (HMBS)).

Statistical analysis - Statistical analysis was performed using GraphPad Prism 9.0.0 software (California, USA). Outliers were identified by the ROUT outlier test. Normal distribution of data was evaluated by the Shapiro-Wilk test. Accordingly, a parametric one-way ANOVA followed by Tukey's multiple comparisons test or non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test was performed. If comparing one measurable variable with two nominal variables, a two-way ANOVA followed by Tukey's multiple comparisons test was applied. All data are expressed as mean ± standard error of the mean (SEM). $P < 0.05$ was considered statistically significant.

RESULTS

Exercise training limits body and heart weight gain in Western diet fed rats - 18 weeks of WD and a sedentary lifestyle caused a significantly increased body weight when compared to CD fed rats (Table 2 and Supplementary Figure S1). Both MIT and HIIT prevented this body weight gain in WD fed rats. Food intake was significantly increased in all rats undergoing WD, regardless of sedentary or physically active lifestyle. In addition, WD fed rats had heavier hearts and livers. Both MIT and HIIT significantly prevented an increase in these organ weights.

Table 2: Effect of exercise training on general animal characteristics of Western diet fed rats.

	CD	WD SED	WD MIT	WD HIIT
Body weight (g)	642 ± 5	777 ± 16****	617 ± 19^{XXXX}	624 ± 26$^{####}$
Body weight increase (%)	238 ± 7	291 ± 12***	240 ± 4^{XXX}	242 ± 7$^{####}$
Food intake (g/day)	30.0 ± 0.2	48.7 ± 1.0****	44.5 ± 0.5	42.0 ± 1.5
Heart weight (g)	1.76 ± 0.04	2.21 ± 0.07**	1.91 ± 0.09^{X}	1.90 ± 0.04$^{#}$
Heart weight/tibia length (g/cm)	0.40 ± 0.01	0.50 ± 0.02**	0.42 ± 0.02^{X}	0.44 ± 0.02 (p=0.06)
Liver weight (g)	17.34 ± 0.83	20.76 ± 0.52*	15.51 ± 0.63^{XXXX}	16.99 ± 0.68$^{##}$
Liver weight/tibia length (g/cm)	3.96 ± 0.18	4.68 ± 0.13*	3.52 ± 0.15^{XXXX}	3.85 ± 0.14$^{###}$

*Biometric measurements and 24h food intake of rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). CD (n=5), WD SED (n=7), WD MIT (n=7) and WD HIIT (n=8). Data are presented as mean ± SEM. * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$, and **** denotes $p < 0.0001$ WD SED vs. CD. ^{X} denotes $p < 0.05$, ^{XXX} denotes $p < 0.001$, and ^{XXXX} denotes $p < 0.0001$ WD SED vs. WD MIT. $^{#}$ denotes $p < 0.05$, $^{##}$ denotes $p < 0.01$, $^{###}$ denotes $p < 0.001$, and $^{####}$ denotes $p < 0.0001$ WD SED vs. WD HIIT.*

Exercise training prevents glucose intolerance and insulin resistance in Western diet fed rats - We examined the effect of MIT and HIIT on glucose tolerance, via an OGTT, and on insulin resistance in WD fed rats. Total blood glucose levels, measured as AUC, tended to be increased in sedentary WD fed rats when compared to the CD fed animals over time (6 weeks: p=0.07, 18 weeks: p=0.18; Figure 2A). MIT limited the increase in glucose concentration present in the WD group (18 weeks: p=0.06; Figure 2A). HIIT significantly prevented a rise in glucose levels after 6 and 12 weeks of training when compared to the WD group. Furthermore, fasting insulin levels rose with time in all groups, but this increase was more pronounced in WD fed animals (Figure 2B). Both exercise modalities significantly decreased fasting plasma insulin concentrations after 18 weeks when compared to rats fed a WD.

Moreover, the fasting and 60 min post-oral glucose administration levels of insulin and glucose were measured after 18 weeks of diet (Figure 2C-E). No differences in fasting glucose and insulin concentrations were observed between the CD and WD group (Figure 2C). While only MIT tended to keep fasting glucose levels low (p=0.06), both exercise modalities significantly prevented a rise in

fasting insulin levels (Figure 2C). The calculated HOMA-IR values, indicators for insulin resistance, confirmed these findings (Figure 2D). 60 min after oral glucose administration, no differences in glucose levels were observed between all groups (Figure 2E). The WD group displayed increased insulin levels compared to CD fed animals following 60 min post-glucose administration (p=0.06), whereas both exercise modalities lost their ability to reduce insulin (Figure 2E).

Exercise training prevents the development of cardiac hypertrophy and diastolic dysfunction in Western diet fed rats - Echocardiographic and hemodynamic measurements were performed to examine cardiac function (Table 3). After 18 weeks, the LVESP, an indicator for hypertension, was increased in the sedentary WD group when compared to the CD group. Both MIT and HIIT prevented the increase in LVESP. In addition, animals fed a WD displayed cardiac hypertrophy, demonstrated by a significantly increased diastolic LV posterior wall thickness (LVPWT; Table 3 and Figure 3). Both exercise modalities limited the rise in LVPWT. Furthermore, the LV ejection fraction (LVEF) remained comparable between all the groups. Interestingly, the LV end-diastolic volume (LVEDV) tended to be higher, whereas stroke volume (SV) and cardiac output (CO) were significantly increased in the WD group. HIIT, but not MIT, tended to impede the rise in SV and CO (p=0.09 and p=0.12, respectively; Table 3).

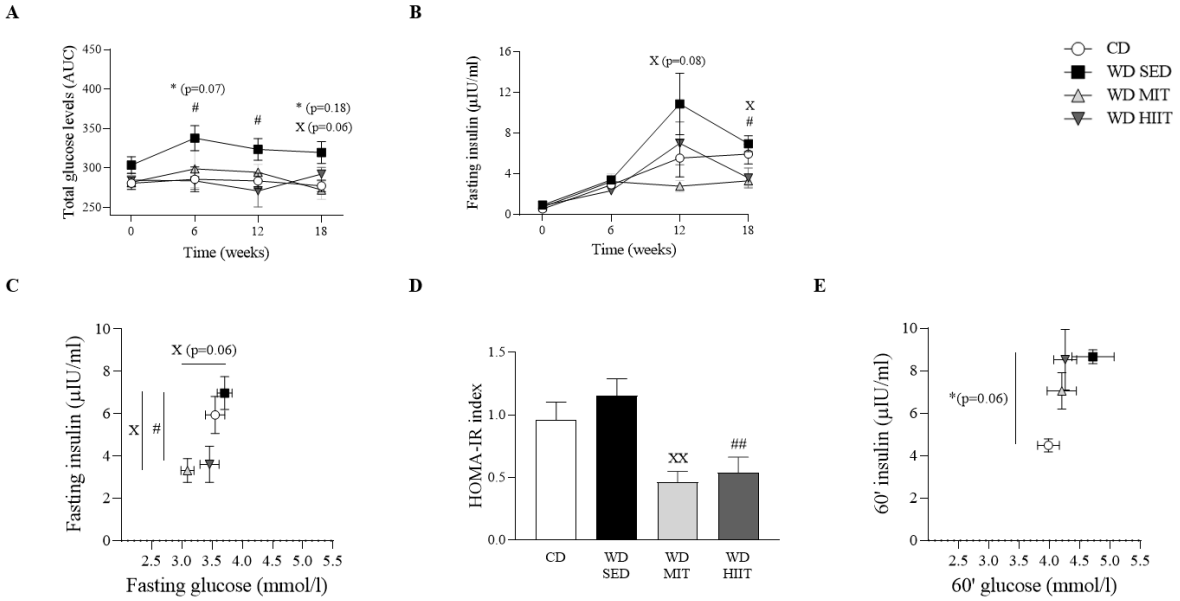

Figure 2: Effect of exercise training on glucose tolerance and insulin sensitivity in Western diet fed rats. Glucose tolerance and insulin sensitivity were assessed in rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). An oral glucose tolerance tests (OGTT) was performed at baseline, 6 weeks, 12 weeks, and 18 weeks. **A**: Total blood glucose levels, expressed as area under the curve (AUC), measured during OGTT over time. **B**: Fasting plasma insulin levels measured during OGTT over time. **C**: Fasting plasma insulin concentrations are plotted against blood glucose concentrations obtained during OGTT at 18 weeks. **D**: Insulin resistance index (homeostatic model assessment of insulin resistance, HOMA-IR) at 18 weeks. **E**: Plasma insulin concentrations are plotted against blood glucose concentrations 60 min post-glucose administration during OGTT at 18 weeks. CD (n=5), WD SED (n=7), WD MIT (n=7) and WD HIIT (n=8). Data are presented as mean ± SEM. * denotes $p < 0.05$ WD SED vs CD. ^{X} denotes $p < 0.05$, and ^{XX} denotes $p < 0.01$ WD SED vs. WD MIT. $^{\#}$ denotes $p < 0.05$, and $^{\#\#}$ denotes $p < 0.01$ WD SED vs. WD HIIT.

Table 3: Effect of exercise training on echocardiographic and hemodynamic measurements in Western diet fed rats.

ECHOCARDIOGRAPHIC PARAMETERS				
	CD	**WD SED**	**WD MIT**	**WD HIIT**
Heart rate (bpm)	302 ± 14	342 ± 12	331 ± 10	321 ± 14
LVPWT, diastole (mm)	2.05 ± 0.12	2.78 ± 0.23*	2.08 ± 0.08^{XX}	1.98 ± 0.06$^{\#\#}$
LVPWT, systole (mm)	3.24 ± 0.21	3.93 ± 0.18	2.89 ± 0.06^{XX}	3.08 ± 0.15$^{\#}$
LVEF (%)	65 ± 6	75 ± 1	70 ± 3	68 ± 2
LVEDV (µl)	570 ± 14	685 ± 40	674 ± 49	616 ± 43
LVESV (µl)	197 ± 38	167 ± 12	201 ± 25	211 ± 26
Cardiac output (ml/min)	114 ± 14	167 ± 14*	141 ± 5	130 ± 11 (p=0.12)
Stroke volume (µl)	373 ± 28	487 ± 29*	434 ± 11	405 ± 25 (p=0.09)
HEMODYNAMIC PARAMETERS				
	CD	**WD SED**	**WD MIT**	**WD HIIT**
LVESP (mmHg)	91 ± 3	109 ± 3**	96 ± 2^{X}	96 ±2$^{\#\#}$
Mean LV pressure (mmHg)	38 ± 0.5	51 ± 2**	39 ± 3^{XX}	43 ±2$^{\#}$

*Hemodynamic and echocardiographic parameters of rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). CD (n=5), WD SED (n=7), WD MIT (n=7) and WD HIIT (n=8). Data are presented as mean ± SEM. * denotes p < 0.05, and ** denotes p < 0.01 WD SED vs. CD. ^{X} denotes p < 0.05, and ^{XX} denotes p < 0.01 WD SED vs. WD MIT. $^{\#}$ denotes p < 0.05, and $^{\#\#}$ denotes p < 0.01 WD SED vs. WD HIIT. LV: Left ventricle, LVEDV: Left ventricular end-diastolic volume, LVESP: Left ventricular end-systolic pressure, LVESV: Left ventricular end-systolic volume, LVPWT: Left ventricular posterior wall thickness.*

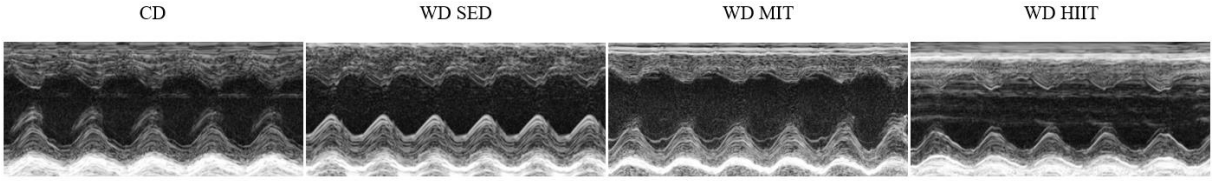

Figure 3: Representative images of echocardiography. Representative echocardiographic images of M-mode measurements obtained at parasternal short-axis of rats receiving control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). CD (n=5), WD SED (n=7), WD MIT (n=7) and WD HIIT (n=8).

Exercise training limits interstitial collagen deposition in the heart of Western diet fed rats - Representative images of interstitial collagen deposition in cardiac sections of all groups, obtained with a Sirius Red/Fast Green staining, are demonstrated in Figure 4A. Interstitial collagen deposition was significantly increased in the LV of WD fed animals compared to CD fed animals, indicating LV fibrosis (Figure 4C). MIT and HIIT prevented this increase in myocardial collagen deposition. To further investigate the involvement of collagen crosslinking, an immunohistochemical staining was performed against LOX in cardiac sections (Figure 4B). Although no statistical difference for LOX was observed between rats fed

a CD or WD, MIT and HIIT significantly lowered cardiac LOX levels (Figure 4D).

Next, expression of fibrosis-related genes was assessed in LV tissue of all rats, and increased in exercised rats (Supplementary Figure S3 C-I). Both exercise interventions increased the gene expression of cardiac collagen types (collagen type I, and collagen type III; Figure S3 C-D). HIIT significantly decreased collagen type I to type III ratio (collagen type I:III; Figure S3 E). Expression of profibrogenic genes in the LV was increased in both exercise groups, however more pronounced following HIIT, compared to sedentary WD fed animals (LOX, transforming growth factor beta (TGF-β); Figure S3 F-G). Furthermore, expression of genes regulating ECM turnover and remodeling seemed to be upregulated in trained animals as well (matrix metalloproteinase 2 (Mmp2), tissue inhibitor of metalloproteinase 1 (Timp1); Figure S3 H-I). In accordance with these findings, MIT and HIIT significantly increased perivascular collagen deposition in the heart, assessed by Sirius Red/Fast Green staining, compared to WD rats subjected to sedentary lifestyle (Figure S3 A-B).

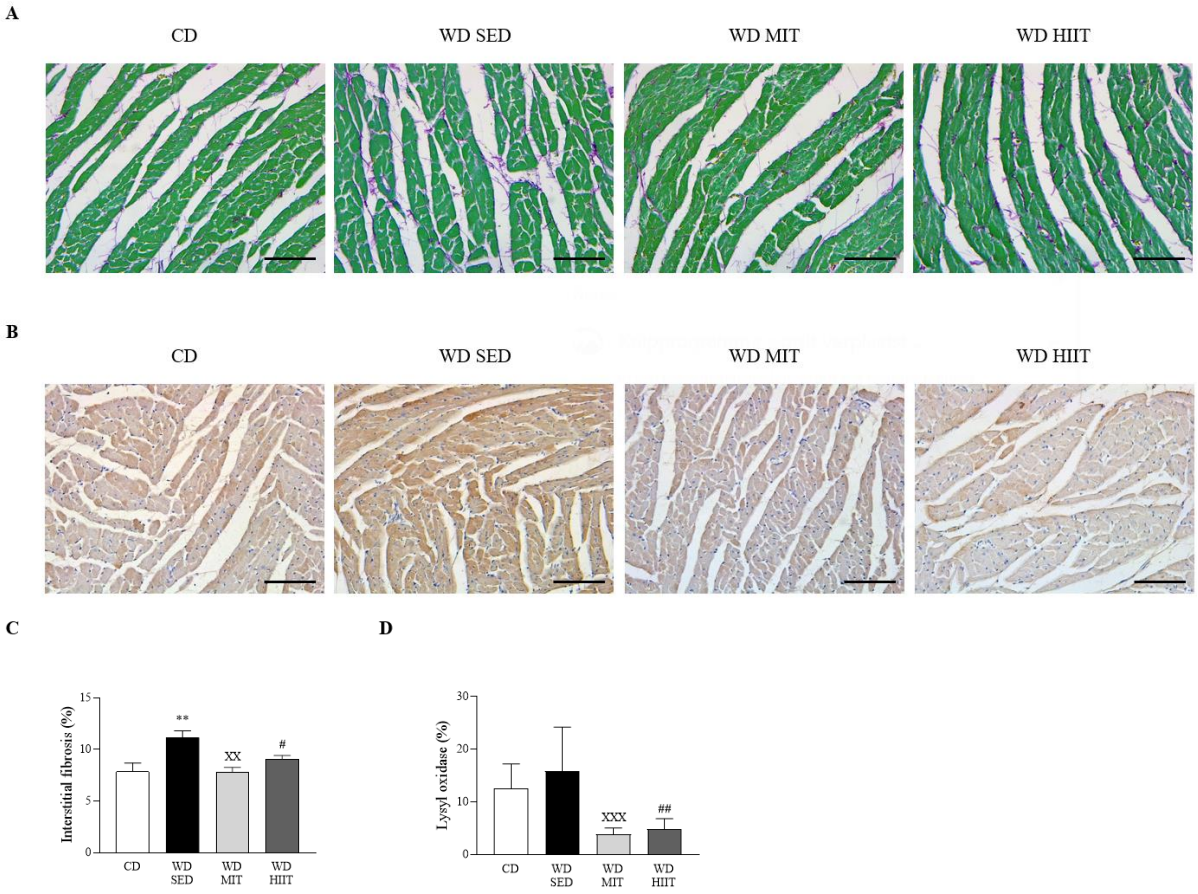

Figure 4: Effect of exercise training on left ventricular interstitial fibrosis and lysyl oxidase in Western diet fed rats. Interstitial collagen deposition and lysyl oxidase were stained in the left ventricle (LV) of rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). **A:** Representative pictures from the Sirius Red/Fast Green staining in the LV of all group. Red-purple staining indicates interstitial collagen deposition. Scale bare represent 100 µm. **B:** Representative pictures from lysyl oxidase staining in the LV of all groups. Scale bare represent 100 µm. **C:** Quantification of interstitial collagen content in LV sections. **D:** Quantification of lysyl oxidase in LV sections. CD (n=5), WD SED (n=7), WD MIT (n=7) and WD HIIT (n=8). Data are presented as mean ± SEM. ** denotes $p < 0.01$ WD SED vs CD. ^{XX} denotes $p < 0.01$, and ^{XXX} denotes $p < 0.001$ WD SED vs. WD MIT. $^{\#}$ denotes $p < 0.05$, and $^{\#\#}$ denotes $p < 0.01$ WD SED vs. WD HIIT.

Exercise training, especially HIIT, upregulates defense mechanisms for advanced glycation end-products and oxidative stress in the heart of Western diet fed rats - AGEs deposition is visualized in LV sections of all groups (Figure 5A). HIIT, but not MIT, induced a significant decrease in the deposition of AGEs in the myocardium compared to other WD fed animals (Figure 5B).

To further examine AGEs pathology, expression of AGEs-related genes was examined via RT-qPCR. RAGE expression was decreased in WD fed sedentary rats compared to rats fed a CD (Figure 5C). RAGE was upregulated in trained animals, especially following MIT, compared to the WD SED group. Furthermore, the expression of glyoxalase 1 (GLO1), a dicarbonyl defense mechanism, is increased in the WD HIIT group compared to other WD fed animals (Figure 5D).

In addition, the expression of genes playing a role in redox homeostasis was evaluated. The expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4), an enzyme which generates ROS, was upregulated following HIIT compared to the WD SED group (Figure 5E). Superoxide dismutase 2 (SOD2) expression, an antioxidant enzyme, was increased following both exercise modalities compared to sedentary animals fed a WD (Figure 5F).

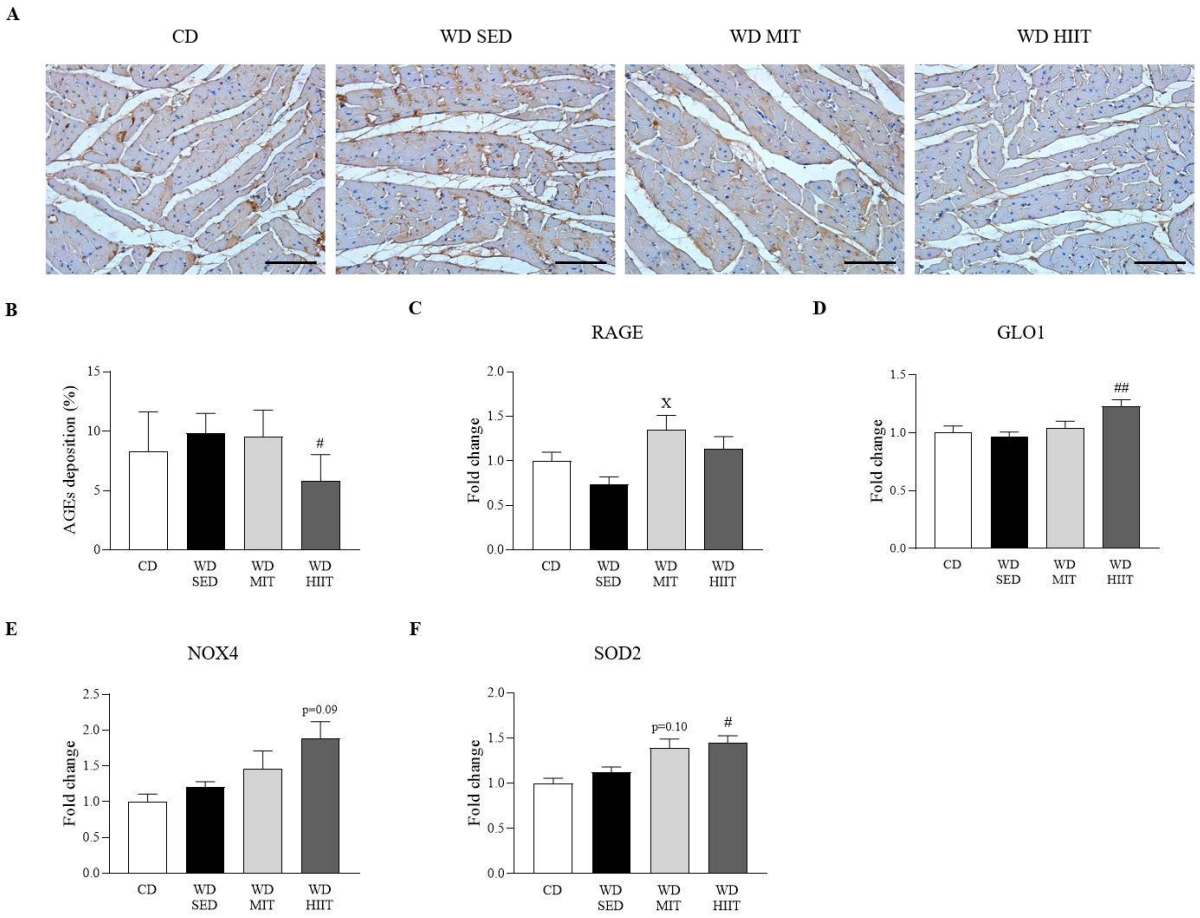

Figure 5: Effect of exercise training on advanced glycation and oxidative stress in the heart of Western diet fed rats. Advanced glycation and oxidative balance is examined in the left ventricle (LV) of rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). **A:** Representative pictures from the advanced glycation end products (AGEs) staining in the LV of all groups. Scale bare represent 100 µm. **B:** Quantification of AGEs deposition in LV sections. Quantification of gene expression of **(C)** receptor for AGEs (RAGE), **(D)** glyoxalase 1 (GLO1), **(E)** NADPH oxidase 4 (NOX4), and **(F)** superoxide dismutase 2 (SOD2). CD (n=5), WD SED (n=7), WD MIT (n=7), and WD HIIT (n=8). Data are presented as mean ± SEM. ^{X} denotes $p < 0.05$ WD SED vs. WD MIT. $^{\#}$ denotes $p < 0.05$, and $^{\#\#}$ denotes $p < 0.01$ WD SED vs. WD HIIT.

Exercise training, especially MIT, induces proinflammatory responses, but also anti-inflammatory responses, in the heart of Western diet fed rats - The presence of pan-macrophages was evaluated via an immunohistochemical staining against CD68 in LV sections of all groups (Figure 6A). CD68-positive macrophage and aggregate content seems to be increased in the LV of all WD fed animals, especially following MIT, compared to CD fed rats (Figure 6B).

To distinguish macrophage phenotypes, gene expression of different macrophage markers was examined in the LV of the four groups. Both MIT and HIIT caused a rise in the gene expression of pan-macrophage marker CD68 (Figure 6C). Both exercise interventions significantly increased the gene expression of proinflammatory macrophage markers (cluster of differentiation 86 (CD86); Figure 6D) and cytokines (interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α); Figure 6E-F). Interestingly, MIT also significantly elevated gene expression of anti-inflammatory macrophage markers (mannose receptor C type 1 (Mrc1), cluster of differentiation 163 (CD163); Figure 6G-H).

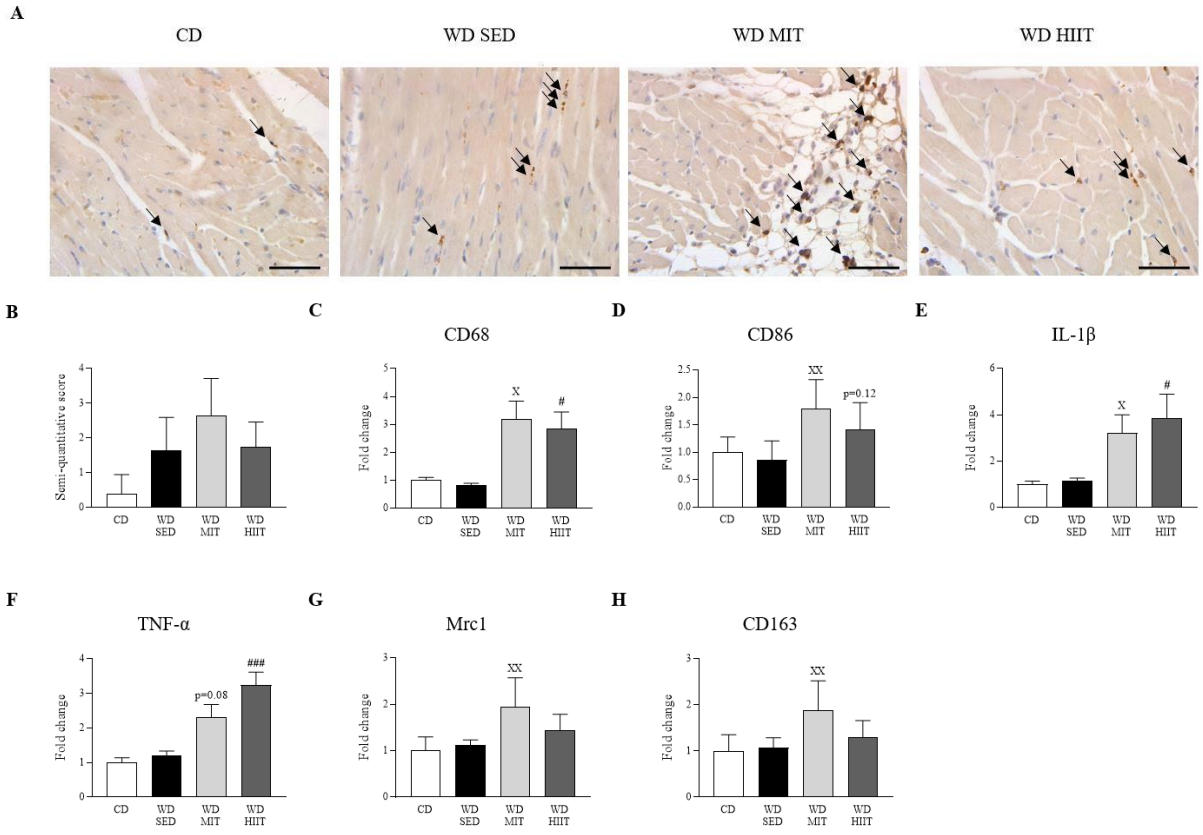

Figure 6: Effect of exercise training on pro- and anti-inflammatory macrophage markers and cytokines in the heart of Western diet fed rats. Pro- and anti-inflammatory macrophage markers and cytokines were assessed in the left ventricle (LV) of rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). **A:** Representative pictures from the cluster of differentiation 68 (CD68) staining in the LV of all groups. CD68-positive macrophages are indicated (arrows) on the pictures. Scale bare represent 50 µm. **B:** Semi-quantitative quantification of the CD68 staining. Quantification of gene expression of **(C)** CD68, **(D)** cluster of differentiation 86 (CD86), **(E)** interleukin 1 beta (IL-1β), **(F)** tumor necrosis factor alpha (TNF-α), **(G)** mannose receptor C type 1 (Mrc1), and **(H)** cluster of differentiation 163 (CD163). CD (n=5), WD SED (n=7), WD MIT (n=7), and WD HIIT (n=8). Data are presented as mean ± SEM. ^{X} denotes $p < 0.05$, and ^{XX} denotes $p < 0.01$ WD SED vs. WD MIT. $^{\#}$ denotes $p < 0.05$, and $^{\#\#\#}$ denotes $p < 0.001$ WD SED vs. WD HIIT.

DISCUSSION

Currently, CVD are the leading cause of death among individuals with T2DM (14). The T2DM epidemic is associated with overconsumption of sugar, abundantly present in our WD and sugar-sweetened beverages (10,15). Endurance exercise training, at moderate- and high-intensity, has been found to be an effective treatment for adverse cardiac remodeling and dysfunction associated with T2DM (Verboven *et al.*; manuscript in preparation) (59,60). However, the potential, preventive effect of different exercise training intensities on cardiac function and structure in T2DM is not understood. In this study, we investigated whether two exercise training modalities, namely MIT and HIIT, could limit the development of adverse cardiac remodeling and dysfunction in a WD-induced prediabetic rat model. Furthermore, we evaluated which underlying pathways are activated by these interventions.

Rat model for prediabetes-induced adverse cardiac remodeling and dysfunction – This study used a prediabetic rat model with cardiac dysfunction induced by a diet high in sucrose or so-called WD, as previously described (34). After 18 weeks of diet, we evaluated systemic and cardiac changes to validate the phenotype.

Our results show that WD (48% kcal added sugars, 16% kcal fat) intake, caused a substantial gain in body and organ weights (i.e. liver, heart) in sedentary animals. This was also confirmed by a review of Suleiman *et al.*, which states that high-sucrose diet-induced obese rat models can show, but not necessarily, substantial body weight gain and increased adipose tissue (61). Being overweight or obesity are known to be correlated with higher liver fat accumulation, which suggests metabolic dysfunction (62). Liver steatosis can be a sign of non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) from which its progression is related with T2DM (62-65). Interestingly, both obesity and NASH are associated with cardiometabolic HFpEF (65,66). In addition, we assessed the presence of hyperglycemia and hyperinsulinemia, two risk factors for the development of T2DM (67). Although HOMA-IR values of the CD and WD group remained comparable, we found that fasting insulin levels over time and 60' post-glucose administration insulin levels increased, indicating an early stage of insulin resistance. The impaired insulin sensitivity led to elevated total blood glucose levels over time, altogether confirming a mild prediabetic phenotype in sedentary WD fed rats. In accordance with our results, a review from Heather *et al.* stated that a high-fructose (60-72% kcal) dietary intervention for 4-12 weeks induces a mild prediabetic phenotype in rodents showing insulin resistance and slightly-elevated blood glucose levels (15). Elevated blood glucose concentrations and HOMA-IR values were also seen in rabbits undergoing a moderate-fructose (30% kcal) low-fat diet (10% kcal) for 12 weeks (68). Furthermore, rats fed a moderate-fructose (20% kcal) and -fat (30% kcal) diet showed significantly elevated fasting blood glucose levels and HOMA-IR values (69).

Metabolic impairments including obesity, NAFLD, hyperglycemia and hyperinsulinemia are known to promote aortic stiffness (70,71). This contributes to the development of a hypertensive state, which occurs in 73% of all T2DM patients (72,73). If left untreated, pathological cardiac remodeling occurs as myocardial compensatory mechanism against the chronically elevated workload on the heart muscle (74). Therefore, T2DM patients initially display a concentric hypertrophic heart (i.e. reduced or preserved LV cavity size, increased wall thickness) (75). At this early stage, diastolic dysfunction or even HFpEF can be present, characterized by an elevated LVESP (i.e. afterload) and decreased CO and SV(76). In the current study, WD fed sedentary rats developed diastolic dysfunction, confirmed by a preserved LVEF, and increased LVESP and LVPWT. The observed increased heart weight can be a result of the myocardial concentric hypertrophy or myocardial fat deposition (15,77). It has also been demonstrated by others that high-fructose dietary interventions cause a notable cardiac phenotype displaying features of diastolic dysfunction or HFpEF (15). Remarkably, we also observed a slightly higher LVEDV, a sign of starting LV dilation, and significantly higher SV and CO in rats subjected to WD and sedentary lifestyle. Interestingly, an increased circulating blood volume (i.e. preload) is seen in T2DM and obese patients, resulting in a higher SV and CO (78,79). The existing insulin resistance in T2DM promotes sympathetic hyperactivity which in its turn

stimulates the RAAS pathway. This pathway favors sodium reabsorption, retention of water, and thereby increasing circulating blood volume, SV, and peripheral resistance. Additionally, obesity-related excessive adipose tissue accumulation also causes this hypervolemic state, to meet the increased metabolic demand, by releasing endocrine molecules (62,78-80). Eventually, the volume overload can induce a dilated LV cavity via eccentric hypertrophic remodeling (i.e. increased LV cavity size, preserved wall thickness) leading to systolic dysfunction (74,75,81). Thus, literature suggests that the HFpEF-like phenotype in T2DM or obesity can eventually evolve towards a HFrEF-like phenotype over time. The shift from diastolic towards systolic dysfunction has been shown by Maurya *et al.* in mice after 24 weeks of WD (21% kcal sucrose, 45% kcal fat) consumption (9). Nevertheless, another theory suggests that both HFpEF and HFrEF develop as separate cardiac phenotypes, rather than being successive stages (15,18). The underlying factor which determines whether a diabetic individual develops HFpEF or HFrEF is likely multifactorial, consisting of genetic and environmental influences. Although the exact determinants are not elucidated yet, it is known that only a small subset of patients suffering from diabetic cardiomyopathy presents with HFrEF whereas the majority suffers from HFpEF (82).

In brief, our prediabetic rat model presents with diastolic dysfunction and developing systolic impairment after 18 weeks of WD consumption. As this animal model recapitulates early stages of T2DM with cardiac impairments, it is suitable to study the impact of preventive measures on the disease progression.

Exercise training during the Western diet regimen prevents the development of the prediabetic phenotype – To date, exercise training is considered an indispensable tool in the management of obesity (83). In general, both MIT and HIIT seem effective methods to reduce total body weight and fat deposition in obese individuals. Furthermore, weight loss in individuals with overweight ameliorates or resolves the metabolic risk factors for T2DM and CVD (84). Our findings show that MIT and HIIT are equally effective in preventing body and organ weights to rise in WD fed rats. We also proved that both exercise modalities prevent the development of prediabetes in rats by limiting the rise in fasting insulin levels, HOMA-IR values and total blood glucose levels. MIT also halted fasting glucose levels to increase during the WD regimen. In accordance with our findings, Chengji *et al.* stated that HFD/STZ-induced (% kcal unknown) diabetic rats displayed declined fasting blood glucose levels and improved insulin resistance after 6 weeks of low- and moderate-intensity treadmill exercise (85). In addition, Kesherwani *et al.* discovered that 8 weeks of swimming exercise is able to prevent weight gain in HFD (46% kcal) fed mice (86). Moreover, Khakdan *et al* investigated the effects of 8 weeks MIT or HIIT running in moderate-fructose (20% kcal) and moderate-fat (30% kcal) diet-induced diabetic rats (69). Significantly lower blood glucose levels and HOMA-IR indexes were seen following HIIT, however no significant reduction in total body weight could be observed. Li *et al*. validated these results in HFD/STZ-induced (% kcal unknown) diabetic rats following 8 weeks of aerobic treadmill running or resistance training on a ladder (87). A review from Myers *et al*. stated that marked improvements are seen in fasting plasma glucose, cardiorespiratory fitness, and metabolic risk profile of patients with MetS are associated with higher levels of PA (88). Interestingly, the enhanced metabolic profile following aerobic exercise also translates into improvements of cardiovascular risk factors in adults with MetS (89).

Exercise training prevents the development of Western diet-induced adverse left ventricle remodeling and dysfunction – Next to offering metabolic protection, MIT and HIIT showed comparable preventive effects against the development of diastolic dysfunction (90). As such, both modalities limited the increase in ESP and wall thickness, thereby preventing concentric hypertrophy in WD fed rats. Accordingly, Kar *et al.* observed normalized values of ventricular pressure during relaxation and hypertrophy following 20 weeks of MIT running in HFD (45% kcal) fed mice (91). Additionally, Epp *et al.* stated that 12 weeks of voluntary treadmill running seems to prevent impaired diastolic parameters in HFD/STZ-induced (40% kcal) diabetic rats (92). The clinical trial of Hollekim-Strand *et al*. showed that both MIT and HIIT induce diastolic function improvements at rest in individuals suffering from T2DM, however HIIT seems to be more beneficial than MIT (93). In

addition, Schmidt *et al*. observed improved diastolic parameters after 24 weeks of soccer training in a T2DM population (94).

Besides preventing diastolic dysfunction, we found that moderate- and high-intensity endurance exercise also seems to have beneficial effects in preventing systolic dysfunction (90). Kar *et al.* stated that systolic contractility is normalized following 20 weeks of MIT in a HFD (45% kcal) mouse model (91). A randomized controlled trial of Cassidy *et al*. showed that HIIT was able to improve systolic parameters such as SV and LVEF after 12 weeks of HIIT in participants suffering from T2DM (95).

In brief, literature elucidated on the importance of exercise training in the management of diabetic cardiomyopathy because of its cardioprotective effects and its ability to reduce cardiac pathological hypertrophic remodeling (96,97).

Exercise training prevented the development of interstitial fibrosis in the heart – Cardiac hypertrophy encompasses both physiological and pathological remodeling (97). Literature states that pathological cardiac hypertrophy is associated with increased interstitial fibrosis, which is one of the hallmarks of a (pre-)diabetic heart, and can eventually lead to HF (97-100). This type is commonly associated with diseases such as hypertension and cardiomyopathy (97). Indeed, our prediabetic rat model showed an increased cardiac wall thickness and ESP after 18 weeks of WD consumption. Staining of the heart revealed that the pathological concentric hypertrophy was accompanied by significantly increased levels of interstitial fibrosis and, although not significant, slightly elevated LOX protein expression. LOX favors enzymatically-induced ECM crosslinking by catalyzing oxidative deamination of lysine side chains on collagen and elastin (101,102). In accordance with our results, Zibadi *et al*. showed the coincidence between the LOX-induced fibrotic response, myocardial stiffness and diastolic dysfunction in a HFD-induced (35% kcal) mouse model of MetS (103). Of note, interstitial fibrosis levels are often increased in the presence of AGEs, oxidative stress and inflammation, as explained later. As such, AGEs are known to contribute to ECM crosslinking by inducing AGEs-AGEs intermolecular covalent bonds between collagen fibrils (101,104). Furthermore, inflammatory processes are known to induce upregulation of LOX in the myocardial ECM via the NF-κβ and smad2/3 pathway (101,105).

Although a WD and sedentary lifestyle induced pathological remodeling of the heart, exercise training is known to exert cardioprotective effects via physiological cardiac remodeling (97). The latter induces adaptive, reversible growth of the cardiac muscle and allows increased cardiomyocyte contractility. In the current study, both MIT and HIIT prevented the development of interstitial fibrosis in the heart of WD fed rats. Analogously, both exercise modalities limited a rise in myocardial LOX levels. As the trained rats show non-significantly, slightly dilated LV cavities but not cardiac wall thickening, our findings may suggest that MIT and HIIT induced starting physiological, eccentric hypertrophy. Indeed, Boraita *et al.* showed that eccentric hypertrophy may occur following long-term exercise in athletes (106). Furthermore, Yazdani *et al*. and Lund *et al.* discovered that increased myocardial collagen content could be prevented by, respectively, 8 weeks of MIT in HFD/STZ (58% kcal) rats, and 10 weeks of high-intensity treadmill running in HFD (46% kcal) fed mice (107,108). Wang *et al*. showed that 12 weeks of moderate-intensity treadmill exercise efficiently prevented myocardial fibrosis in HFD/STZ (% kcal unknown) mice (109).

Opposite to these findings, we observed that MIT and HIIT induced more perivascular fibrosis and increased profibrogenic gene expression, including LOX, TGF-β, Mmp2, Timp1, collagen I and III, in hearts of WD fed rats. LOX increases insoluble cardiac collagen deposition (i.e. fibrosis), thereby contributing to ECM remodeling (101,110). By upregulating the PI3K/Akt/mTOR and TGF-β pathway, LOX induces cardiac fibroblast transformation into collagen-producing myofibroblasts (101,105). Besides LOX-mediated upregulation of TGF-β, this profibrogenic growth factor is also induced by excess ROS due to the involvement of the NF-κβ, MAPK, or JNK signaling pathway (111). On its turn, TGF-β induces LOX mRNA synthesis via the smad2/3 signaling pathway (112). Moreover, the smad2/3 signaling pathway leads to TGF-β-induced fibrosis (111). TGF-β activation occurs via Mmp2, which is also upregulated via the NF-κβ, MAPK, or JNK signaling pathway (111,113). Nevertheless, it

should be noted that long-term strenuous exercise is known to promote vascular remodeling (e.g. tunica media fibrosis) through RAAS-activated Mmp2-involving processes (114). This might explain the elevated profibrogenic gene expression in the LV following MIT and HIIT in WD fed rats. Besides, the gene expression of antifibrogenic gene Timp1 was also increased in trained animals fed a WD. If Timp1 is present in sufficient amounts, it is capable to inhibit Mmp2-mediated TGF-β activation (115). The upregulation of Timp1 gene expression possibly suggests a cardiac defense mechanism to regain collagen synthesis/breakdown homeostasis.

As a result of this increased interstitial fibrosis, LV stiffness can occur which is indicated by the collagen type I:III ratio (98,116). Collagen type I and III are the principal collagen types in the myocardium (117). Collagen type I is more rigid, while collagen type III is more elastic and provides structural maintenance in case of expansion. For this reason, higher type I:III ratios implicate a more rigid LV. A stiffer LV is less resistant to LV diastolic expansion, inducing impaired filling and eventually diastolic dysfunction (118). This impaired filling is mediated by myocardial stiffness-induced concentric hypertrophy (119). In the current study, WD fed sedentary male rats show no changes in gene expression of collagen type I:III ratio compared to the control group. Although Manrique *et al*. showed significant elevated collagen type I/III ratio in WD fed female mice, they did not found significant increase in WD fed male mice (116). The current data suggests that HIIT is effectively preventing the development of cardiac stiffness, as it lowers the collagen I:III ratio. Indeed, a review of Lindgren *et al*. mentioned that regular aerobic exercise is able to prevent cardiac stiffness in HFpEF patients (120). In addition, a randomized controlled trial of Howden *et al*. stated that cardiac stiffness is decreased following 2 years of intensive exercise training in healthy, middle-aged sedentary participants (121).

Exercise training upregulates anti-oxidative and dicarbonyl defense mechanisms in the heart – Endogenous AGEs formation *in vivo* mainly occurs following the Maillard reaction, which is accelerated in hyperglycemic conditions (122). Through the Maillard reaction, instable Schiff bases are formed following the non-enzymatical reaction of reducing sugars with an amino group of proteins, lipids, and nucleic acids. These Schiff bases become rearranged into Amadori products, which become oxidized in the presence of oxidative stress, eventually forming stable AGEs (104,122). Next to completing the Maillard reaction, instable Schiff bases can also enter the Namiki pathway to form AGEs (122). In the latter, methylglyoxal (MG, i.e. a highly reactive dicarbonyl) is formed as reactive intermediate following auto-oxidative cleavage. Besides, MG also originates as inevitable glycolytic by-product since MG is mainly formed by the fragmentation of the glycolytic intermediates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (123). Once they are formed, AGEs can bind to their full-length membrane bound receptor (RAGE), thereby activating oxidative stress and inflammatory pathways, or soluble receptors (sRAGE) present in the serum (122). As sRAGE scavenges AGEs, it is responsible for AGEs clearance and thus preventing AGEs bioavailability. In literature, it is described that diabetic patients experiencing complications have lower levels of serum sRAGE in comparison with healthy controls (124). Besides the protective sRAGE, growing evidence suggests that the glyoxalase system can prevent endogenous AGEs formation by detoxifying AGEs precursors (123,125). GLO1 is the rate-limiting enzyme of the glyoxalase system, serving as a reactive dicarbonyl defense mechanism as it detoxifies MG into D-lactate (125). Due to the hyperglycemic state, GLO1 expression has been found to be decreased while the AGEs precursor MG has been found to be increased in T2DM patients (126). In this study, we found that WD fed rats showed slightly increased AGEs protein deposition in the LV compared to CD rats. In addition, gene expression of RAGE was slightly lower and gene expression of GLO1 remained comparable in WD fed rats, although not significant. Although data are sparse, exercise training seems to exert beneficial effects on the AGEs-RAGE pathway in the heart (98,127). Wright *et al*. notified that moderate-intensity interval training evoked a remarkable decline in AGEs deposition in the heart of senescent rats (128). Additionally, this result is also seen in rats suffering from diabetic cardiomyopathy which are subjected to aerobic interval training and pharmacologic Liraglutide (i.e. GLP-1 analog) administration for 8 weeks (129). Notably, different

clinical studies did examine the effect of exercise training on sRAGE in the blood of diabetic or obese individuals. For example, a recent clinical trial of Legaard *et al*. demonstrated that sRAGE levels are increased in the blood upon aerobic exercise training in diabetic patients (130). This result was confirmed by Choi *et al*. in diabetic individuals subjected to 12 weeks of MIT (131). In the current study, we found that HIIT significantly decreased AGEs deposition in the LV of WD fed rats. In addition, we found that WD fed animals subjected to HIIT training showed an upregulated gene expression of GLO1. Although no literature is available which describes the effects of exercise on myocardial GLO1 expression, aerobic exercise has been demonstrated to increase GLO1 expression in skeletal muscle in T2DM (132). Thus, anaerobic HIIT might possibly lower myocardial AGEs or their precursor MG by upregulation of GLO1 in the heart of WD fed rats. Remarkably, we also found that MIT induced higher RAGE gene expression in WD fed rats. In accordance, RAGE has been found to be a key mediator in the lung response after long-term voluntary wheel running because RAGE mediates a lower lung compliance (i.e. stiffer lung), which is disadvantageous in exercise context (133). Additionally, literature states that RAGE is expressed on multiple cell types implicated in CVD, including monocytes and macrophages (134,135). As explained later, our data suggests that macrophage content is increased in rats subjected to MIT, which can explain why RAGE is upregulated in these animals.

Furthermore, literature states that oxidative stress is abundantly present in a T2DM and HFpEF phenotype (20,136). In particular, NOX4 is the most common cardiac NADPH oxidase subtype and the major source of oxidative stress in the failing heart (137). NOX4 is known to produce hydrogen peroxidase, and to a lesser extend superoxide radicals, by transferring electrons from NADPH (138). Interestingly, it is known that AGEs-RAGE interaction also contributes to ROS formation by activation of NADPH oxidases (i.e. NOX4) (98,111,139,140). In its turn, ROS signaling also favors RAGE upregulation (139,141). The positive feedback loop between oxidative stress and AGEs-RAGE signaling causes consistent worsening of the disease progression. Additionally, T2DM-associeted dyslipidemia, present in our rat model as previously described, are known to directly induce ROS generation through NOX activation (34,142). Since ROS is upregulated, the anti-oxidative defense system can be activated. An important anti-oxidative enzyme is SOD2, which is able to scavenge superoxide anion radicals (143). Here, we found that the gene expression of NOX4 and SOD2 were slightly upregulated, although not significant, in WD fed animals.

Different studies examining the effect of exercise on the heart of diet-induced T2DM models generally state that PA reduces oxidative stress by impeding ROS production and promoting antioxidant mechanisms (144). For example, Wang *et al.* stated that 16 weeks of treadmill running ameliorates cardiac function by decreasing ROS production (e.g. NOX4 expression) and improving mitochondrial function in a HFD/STZ (45% kcal) mouse model (143). Likewise, Lund *et al.* showed that in HFD fed (46% kcal) mice, high-intensity treadmill running resolves myocardial ROS content (108). A randomized controlled trial of Legaard *et al*. showed that circulating oxidative stress markers decreased following a 12 months exercise-based lifestyle intervention in T2DM individuals (130). Nevertheless, we observed increased gene expression of NOX4 in HIIT trained, WD fed rats in the current study. In accordance with our findings, a review of Kawamura *et al*. summarizes that oxidative stress in the blood and heart is generated upon acute exercise in healthy individuals (145). Additionally, a review of Lu *et al.* confirmed these findings solely in the blood of healthy individuals following long-term HIIT (146). To maintain the redox balance, the antioxidant system can be stimulated upon increased oxidative stress levels. In this study, we observed that HIIT, and to a lesser extend MIT, induces increased SOD2 gene expression, potentially as intrinsic defense mechanism against this impaired oxidative balance. Indeed, Wang *et al*. observed that HFD/STZ (45% kcal) mice showed significantly decreased levels of SOD2, while 16 weeks of treadmill running was able to normalize SOD2 levels (143). In addition, total SOD levels show the same expressional changes in HFD-induced (40% kcal) T2DM rats and T2DM rats subjected to 8 weeks of MIT (147). Also Ghorbanzadeh *et al*. suggested that moderate-fat diet (22% kcal) fed rats subjected to 8 weeks of voluntary running showed significant upregulated

SOD expression compared to sedentary T2DM rats (148). Moreover, Hancock *et al*. showed that NOX4, which was upregulated in cardiomyocytes isolated from mice following acute exercise training, is responsible for the beneficial effect of PA on NOX4/Nrf2 axis-mediated cardiomyocyte antioxidant defense (149,150).

Exercise training induces a pro-and anti-inflammatory response in the heart – Different studies have shown the contributing role of different inflammatory cells, especially macrophages, and their secreted cytokines in the development of diabetic heart diseases (151). Macrophages are classified into proinflammatory M1-like (i.e. classically-activated macrophages) and anti-inflammatory M2-like macrophages (i.e. alternatively-activated macrophages) (152). Kaur *et al*. highlighted the importance of the predominant M1 macrophage and M1 polarization (i.e. M2 macrophage differentiation) in the progression of diabetic cardiomyopathy (153). Moreover, *in vitro* experimentation of Pavillard *et al*. showed that monocytes which are incubated with serum originating from high-sucrose (32% kcal) diet fed mice significantly upregulate proinflammatory gene expression (154). Ngcobo *et al*. mentioned that monocyte (i.e. precursor of infiltrating, mature macrophages) function is altered in T2DM patients favoring inflammation by producing proinflammatory cytokines (155). In addition, a clinical study of Pierzynová *et al*. showed an elevated presence of CD68-positive macrophages in the atria of obese T2DM patients (156). Lastly, it is also known that T2DM is associated with chronic systemic low-grade inflammation in which predominantly proinflammatory monocyte polarization plays an important role (157). Notably, the role of inflammation in diabetic heart diseases is also linked to other underlying pathways. As such, ROS-induced inflammation occurs upon activation of the NF-κβ, MAPK and JNK signaling pathways in the diabetic heart (111). Oxidative stress-related AGEs formation on its turn is predominantly able induce macrophages to release inflammatory cytokines, but also induce M1 polarization (158). Moreover, this inflammatory response is known to upregulate RAGE in response to ROS via NF-κβ signaling, providing a positive feedback loop (139). In the current study, WD fed rats presented with slightly elevated pan-macrophages content compared to CD rats, although gene expression of pro-and anti-inflammatory markers remained unchanged.

To date, literature shows no consensus on the effect of exercise training, especially its intensity and duration, on systemic and cardiac inflammation. A systematic review of Cerqueira *et al*. revealed that moderate exercise with appropriate resting periods achieves maximal benefit, whereas intense long exercise can lead to higher proinflammatory systemic responses (i.e. IL-1β, TNF-α) in healthy adults (159). The latter was confirmed here in cardiac samples of our prediabetes rat model. Indeed, we observed that long-term exercise intervention at moderate and high intensity increases myocardial pan-macrophage content, accompanied by an increased gene expression in M1 macrophage markers and proinflammatory cytokines. This was contrary to findings of other research groups working on the effect of exercise training on systemic and cardiac inflammation in preclinical models for T2DM. For example, Kesherwani *et al*. showed decreased cardiac TNF-α levels following swimming exercise in HFD (46% kcal) fed mice (86). Additionally, Kar *et al*. described a normalization in IL-1β levels in the LV of HFD (45% kcal) fed mice following 20 weeks of MIT (91). In this study, we also found that long-term exercise intervention at moderate and high intensity increases gene expression in M2 macrophage markers and anti-inflammatory cytokines, indicating a physiological protective mechanism. This is in accordance with findings of Wang *et al*., as HIIT also induces an increase in anti-inflammatory polarization of macrophages in T2DM mice (160). Likewise, Kesherwani *et al*. demonstrated an increased cardiac anti-inflammatory IL-10 protein expression in HFD (46% kcal) fed mice following 8 weeks of swimming exercise (86). In addition, Botta *et al*. demonstrated that 22 weeks of MIT limited cardiac-specific macrophage infiltration in a db/db mouse model (161). In clinical settings, PA has been suggested as a strong systemic anti-inflammatory strategy in T2DM patients (162). Furthermore, Pedersen *et al*. showed that PA exerts anti-inflammatory effects involving IL-10 upregulation in the context of T2DM and CVD (163). Lastly, Noz *et al*. showed that 16 weeks of low-intensity PA is able to shift the innate immune system towards a less proinflammatory state due to a

reduced capacity to produce proinflammatory cytokines in non-diabetic individuals with an increased cardiometabolic risk (164).

Limitations of this study and future prospects – Due to practical considerations regarding animal treadmill experiments, the sample size of rats within each group is limited. Accordingly, within-group variations become larger and statistical power is low, however sufficient. To boost statistical power, this *in vivo* study is currently repeated and the results will be pooled accordingly.

Our WD-induced rodent model is highly translational to the overweighted T2DM patient pathophysiology (i.e. 90% of the T2DM population) (165). However, the concurrent development of obesity doesn't allow researchers to investigate solely the effect of T2DM on the heart. Because of existing associations between components of the MetS, it is interesting to further investigate T2DM comorbidities such as obesity (i.e. determine blood lipids/cholesterols, body composition) or liver disease (i.e. NAFLD and NASH) in future studies (68).

As data is sparse, more research is needed to provide a general, reliable understanding of sugar-mediated effects in T2DM with adverse cardiac remodeling and dysfunction. Since sucrose is present in our WD in tremendous amounts, the use of a high-sucrose diet fed model is highly relevant. Considering other diet-induced diabetic cardiomyopathy rodent studies, the diet composition is highly variable (i.e. fat and sugar composition) and not always properly showed. The same accounts for describing the exercise protocols (i.e. exercise mode, duration, intensity, frequency). The indistinct description and high variation in diet composition and exercise protocols makes data comparison complicated. So, standardization and transparency should be implemented in future research in this specific research field.

The current research investigated two different exercise modalities, namely MIT and HIIT. Previous research by our research group supposed that both modalities exert their beneficial effects via distinct underlying mechanisms. In a therapeutic setting, it seemed that MIT alters inflammatory response, whereas HIIT improves mitochondrial function (Verboven *et al*.; manuscript in preparation). The current data in a preventive setting suggests that MIT favors M2 macrophage polarization, while HIIT more efficiently activates the dicarbonyl defense mechanism. Future experimental methods will provide us with more information on these underlying mechanisms. We will perform an AGEs enzyme-linked immunosorbent assay to determine total plasma AGEs levels in all groups. More specific results related to MG (i.e. an AGEs precursor) levels will be obtained from tandem mass spectrometry analysis as previously described (166). Furthermore, intracellular oxidative stress and mitochondrial function should be quantitively examined in isolated cardiomyocytes by, respectively, performing a CellROX^{TM} Green Assay and a Seahorse XF extracellular flux analyzer experiment in a new follow-up study if practically feasible (167,168). To investigate M1- and M2-like macrophages in the LV tissue, a multiplex fluorescent staining will be performed and quantified. Furthermore, CD68 analysis should be redone in a quantitative manner using the cell counter plugin of FIJI/ImageJ. Besides more in-depth investigation of the AGEs and inflammation mechanisms, other processes (e.g. Ca^{2+} homeostasis, mitochondrial dysfunction, lipotoxicity, and autophagy) are worth investigating in our high-sucrose fed rat model in the future. Exercise benefits these pathways as shown by Hafstad *et al*. (98).

Future research should be performed focusing on the preventive capacities of exercise training on diabetic cardiomyopathy to gain additional knowledge on the preventive effect of exercise on diabetic cardiomyopathy. The majority of current publications focusses on exercise as therapy of T2DM with cardiac remodeling and dysfunction. However, there is a tremendous need for implementation of preventive strategies, hence it will flatten or even decrease the rising prevalence of T2DM. Prevention of individuals developing T2DM and associated CVD encompasses both positive societal impacts (e.g. lower medical costs, less hospitalizations) and benefit for the individual at risk.

Taken together, further research into exercise training in diabetes-associated cardiac remodeling and dysfunction is necessary to further unravel involved signaling pathways and determine a general optimal training modality (i.e. duration, frequency, intensity) for diabetic patients suffering from diastolic dysfunction.

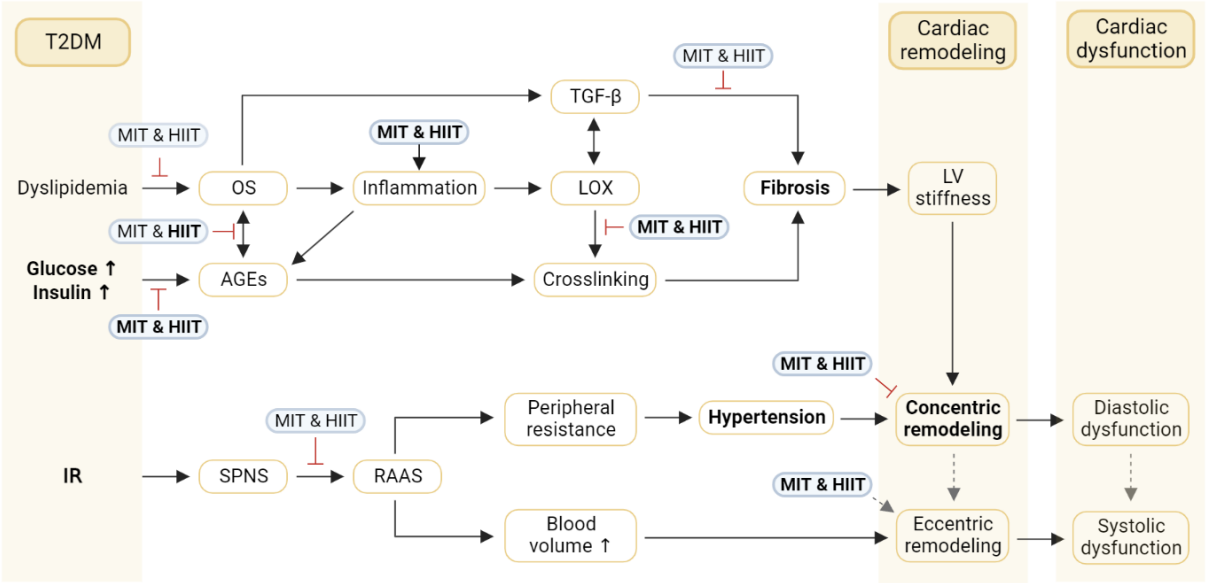

Figure 6: Overview of the molecular mechanisms of T2DM-induced cardiac remodeling and the potential of exercise to interfere upon several stages of this pathway. T2DM hallmarks (i.e. dyslipidemia, dysregulated insulin and glucose concentrations, and insulin resistance) favor distinct detrimental pathways. Advanced glycation end-products, oxidative stress, inflammation, and fibrosis all contribute to the development of cardiac remodeling, which ultimately evolves in cardiac dysfunction. Both MIT and HIIT are able to interfere with several stages in this dysfunction-evoking pathway. This figure is compiled based on existing literature. Both the effects of WD and MIT or HIIT that are confirmed by the current research are indicated in bold. AGEs: Advances glycation end-products, HIIT: High-intensity interval training, IR: Insulin resistance, LOX: Lysyl oxidase, LV: Left ventricle, MIT: Moderate-intensity training, OS: Oxidative stress, RAAS: Renin-angiotensin-aldosterone system, SPNS: Sympathetic autonomous peripheral nervous system, T2DM: Type 2 Diabetes Mellitus, TGF-β: Transforming growth factor beta.

CONCLUSION

In summary, prediabetes and T2DM together represent about 65% of HFpEF patients (83). This pathological phenotype is remarkably related to the consumption of WD and sugar-sweetened beverages, containing tremendous amounts of deleterious fructose. Overconsumption of fructose is suggested to cause metabolic dysregulation (i.e. hyperglycemia, hyperinsulinemia, dyslipidemia, and insulin resistance) causing a hypertensive state, cardiac remodeling (i.e. concentric hypertrophy, collagen deposition, LV stiffness) and eventually diastolic dysfunction (i.e. HFpEF). In this study, we created a rat model presenting with impaired glucose tolerance, minor insulin resistance, hypertrophy, and hypertension.

Although exercise training has been considered as indispensable therapeutic tool in the management of T2DM with cardiac remodeling and dysfunction, we now demonstrated its valuable potential as preventive method as well (Figure 6). Endurance exercise training, both at moderate and high intensity, was able to prevent dysregulated glucose levels, insulin resistance, hypertrophy, and hypertension. Regarding underlying mechanisms, we found that both MIT and HIIT limited oxidative disbalance, LV inflammation and pathological interstitial fibrosis. Although exercise induced a cardiac inflammatory response, it also upregulated defense mechanisms as anti-inflammatory M2-like macrophage polarization. HIIT might be a more effective preventive strategy because it also effectively limits advanced glycation in the LV. By investigating differences in the underlying activated pathways of MIT and HIIT, this research will contribute to future exercise prescription for patients suffering from diabetic cardiomyopathy. Nevertheless, it should be considered that intervention through exercise at moderate or vigorous intensity should be performed with appropriate resting periods to achieve maximum benefit.

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Author contributions – SD, DD and VB conceived and designed the research. SD and IDL performed experiments and data analysis. DD provided assistance with the OGTT and echocardiographic measurements. IDL wrote the paper. SD critically reviewed and carefully edited the manuscript. SD approved the final version of the manuscript.

SUPPLEMENTARY PROCEDURES, TABLES, AND FIGURES

Supplementary Experimental Procedures

Echocardiographic measurements - Transthoracic echocardiographic parameters were measured with a Vevo 3100 system and a 21 MHz linear probe MX250 (FUJIFILM VisualSonics Inc., Amsterdam, The Netherlands), as described previously (55). These measurements were performed under 2% isoflurane anesthesia supplemented with oxygen at baseline, 6 weeks, 12 weeks, and 18 weeks. Animals were placed in supine position, the thoraxes were shaved, and depilatory cream was applied to prevent hair-based artifacts. Parasternal long-axis images and short-axis views at mid-ventricular level were obtained in both B-mode and M-mode. To estimate the diastolic function, mitral inflow profiles are obtained pulsed wave Doppler in the apical four-chamber view. Additionally, diastolic annular velocities were obtained by tissue Doppler imaging at the septal mitral annulus. Echocardiographic images were analyzed using the Vevo Lab 3.2.6 Software (FUJIFILM VisualSonics Inc.). Standard measures of LV structure, and systolic and diastolic function were analyzed. Analysis of the echocardiographic data was blinded to reduce bias.

Histological staining (perivascular) - 8 µm thick transversal sections were obtained from paraffin-embedded heart tissue and stained using the Sirius Red/Fast Green Collagen Staining kit (Chondrex Inc., Washington, USA), according to the manufacturer's protocol. After staining, sections were mounted with DPX mounting medium. For perivascular fibrosis, six until eight heart blood vessels per section were assessed. Using FIJI/ImageJ software (Maryland, USA), the fibrotic area was determined, normalized to luminal area and expressed as percentage.

Immunohistochemistry - Deparaffinized 8 µm thick heart tissue sections were used for immunohistochemical staining against cluster of differentiation 68 (CD68), AGEs, and lysyl oxidase (LOX). For CD68 and AGEs staining, deparaffinized sections underwent heat-mediated antigen retrieval using citrate buffer (pH=6). Subsequently, sections were washed with 1X phosphate buffered saline (PBS) and endogenous peroxidase was blocked with 30% hydrogen peroxide (1:100). Sections were rewashed and permeabilized with 0.05% Triton X-100 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Then, sections were washed and protein block serum-free (X0909, Dako, Glostrup, Denmark) was applied to minimize background staining. Afterwards, sections were incubated with a primary antibody against CD68 (1:100, MCA341R, mouse anti-rat, Bio-Rad abD Serotec, Oxford, UK), AGEs (1:250, ab23722, rabbit polyclonal, Abcam), and LOX (1:200, ab31238, rabbit polyclonal, Abcam, Cambridge, United Kingdom) diluted in PBS for 1h at room temperature for CD86 and AGEs, or overnight at 4°C for LOX, followed by five washes with PBS. For CD68 and AGEs, EnVision™ with Dual Link System-horse reddish peroxidase (HRP) (K4061, anti-rabbit/anti-mouse, Dako) was applied for 30 min at RT. For LOX, a HRP-conjugated secondary antibody (1:400, P0448, polyclonal goat anti-rabbit, Dako) was incubated at RT for 30 min. The presence of CD68, AGEs and LOX was visualized using 3,3′-diaminobenzidine (DAB). Next, all sections were counterstained with hematoxylin to stain nuclei and mounted using dibutylphthalate polystyrene xylene (DPX) mounting medium. Images were obtained using a Leica MC170 camera which is connected to a Leica DM2000 LED microscope.

AGEs and LOX staining was quantitively analyzed at 20x magnification in four random fields per section by use of the color deconvolution plugin in FIJI/ImageJ software (Maryland, USA). The level of AGEs and LOX staining was expressed as percentage of the total surface area of interest.

The CD68 staining was assessed semi-quantitatively at 40x magnification. Five scores were provided to evaluate CD68-positive cells in the LV, namely absent or limited staining (0), minor staining (1), moderate staining (2), high staining with presence of small aggregates (3), and high staining with presence of large aggregates (4). The analyses were performed blinded for group allocation by two independent researchers.

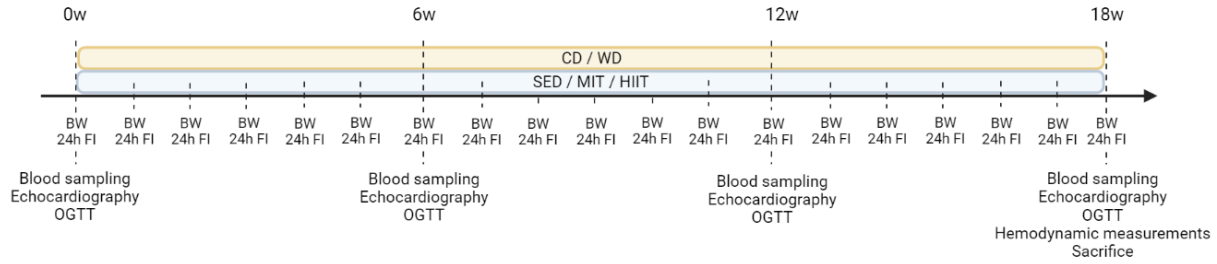

Figure S1: Experimental design of the *in vivo* rodent study. Animals were fed a control diet (CD) or Western diet (WD) for 18 weeks. Concurrently, WD fed rats were subjected to sedentary lifestyle (SED), moderate-intensity training (MIT), or high-intensity interval training (HIIT). Animal body weight (BW) and 24-hour food intake (24h FI) were measured weekly. Blood sampling, echocardiographic imaging and oral glucose tolerance test (OGTT) were performed at baseline (0w), 6 weeks (6w), 12 weeks (12w), and 18 weeks (18w) after the start of the diet. Hemodynamic measurements are performed at sacrifice. CD (n=5), WD SED (n=7), WD MIT (n=7), and WD HIIT (n=8). 24h FI: 24-hour food intake, BW: Body weight, CD: Control diet, HIIT: High-intensity interval training, MIT: Moderate-intensity training, OGTT: Oral glucose tolerance test, SED: Sedentary lifestyle, W: weeks, WD: Western diet.

Table S1: General information of primary and secondary antibodies used for immunohistochemical staining.

PRIMARY ANTIBODY			
Antigen	**Species**	**Cat. Number**	**Supplier**
CD68	Mouse anti-rat	MCA341R	Bio-Rad abD Serotec, Oxford, UK
AGEs	Rabbit polyclonal	ab23722	Abcam, Cambridge, UK
LOX	Rabbit polyclonal	ab31238	Abcam, Cambridge, UK
SECONDARY ANTIBODY			
Type	**Species**	**Cat. number**	**Supplier**
EnVision™ Dual Link System-HRP	anti-rabbit/anti-mouse	K4061	Dako, Glostrup, Denmark
HRP-conjugated Immunoglobulins	polyclonal goat anti-rabbit	P0448	Dako, Glostrup, Denmark

AGEs: Advanced glycation end-products, CD68: Cluster of differentiation 68, HRP: Horse reddish peroxidase, LOX: Lysyl oxidase.

Table S2: Sequences of forward and reverse primers used in RT-qPCR.

REFERENCE GENES		
Gene	**Sequence**	**T_m (°C)**
CycA	Forward: 5' TAT-CTG-CAT-GCC-AAG-ACT-GAG-TG 3' Reverse: 5' CTT-CTT-GCT-GGT-CTT-GCC-ATT-CC 3'	60.7
HMBS	Forward: 5' TCC-TGG-CTT-TAC-CAT-TGG-AG 3' Reverse: 5' TGA-ATT-CCA-GGT-GAG-GGA-AC 3'	56.8
HPRT	Forward: 5' TCC-CAG-CGT-CGT-GAT-TAG-TG 3' Reverse: 5' GCA-AGT-CTT-TCA-GTC-CTG-TCC 3'	59.0
PGK1	Forward: 5' ATG-CAA-AGA-CTG-GCC-AAG-CTA-C 3' Reverse: 5' AGC-CAC-AGC-CTC-AGC-ATA-TTC 3'	60.9
RPL13A	Forward: 5' GGA-TCC-CTC-CAC-CCT-ATG-ACA 3' Reverse: 5' CTG-GTA-CTT-CCA-CCC-GAC-CTC 3'	63.5
YWHAZ	Forward: 5' GAT-GAA-GCC-ATT-GCT-GAA-CTT-G 3' Reverse: 5' GTC-TCC-TTG-GGT-ATC-CGA-TGT-C 3'	59.0
TARGET GENES		
Gene	**Sequence**	**T_m (°C)**
CD163	Forward: 5' ATC-ACA-GCA-TGG-CAC-AGG-T 3' Reverse: 5' TCC-AGA-TCA-TCC-GTC-TTC-G 3'	56.6
CD68	Forward: 5' CAC-TTG-GCT-CTC-TCA-TTC-CCT 3' Reverse: 5' GCT-GAG-AAT-GTC-CAC-TGT-GCT 3'	61.3
CD86	Forward: 5' GTC-AAG-ACA-TGT-GTA-ACC-TGC-ACC 3' Reverse: 5' ACG-AGC-TCA-CTC-GGG-CTT-AT 3'	59.5
Col1A2	Forward: 5' GCC-AAG-AAT-GCA-TAC-AGC-CG 3' Reverse: 5' GAC-ACC-CCT-TCT-GCG-TTG-TA 3'	58.2
Col3A1	Forward: 5' AAC-TGG-AGC-ACG-AGG-TCT-TG 3' Reverse: 5' CGT-TCC-CCA-TTA-TGG-CCA-CT 3'	59.2
GLO1	Forward: 5' GAA-GAT-GAC-GAG-ACG-CAG-AGT-TAC 3' Reverse: 5' CAG-GAT-CTT-GAA-CGA-ACG-CCA-GAC 3'	61.8
IL-1β	Forward: 5' ACC-CAA-GCA-CCT-TCT-TTT-CCT-T 3' Reverse: 5' TGC-AGC-TGT-CTA-ATG-GGA-ACA-T 3'	60.2
LOX	Forward: 5' AGC-TGC-CAC-CAA-CAT-TAC-CA 3' Reverse: 5' GGG-ACT-CAA-CCC-CTG-TGT-G 3'	64.9
Mmp2	Forward: 5' CTG-GGT-TTA-CCC-CCT-GAT-GTC-C 3' Reverse: 5' AAC-CGG-GGT-CCA-TTT-TCT-TCT-TT 3'	62.2
Mrc1	Forward: 5' AAG-GTT-CCG-GTT-TGT-GGA-G 3' Reverse: 5' TGC-ATT-GCC-CAG-TAA-GGA-G 3'	56.6
NOX4	Forward: 5' TCA-TGG-ATC-TTT-GCC-TGG-AGG-GTT 3' Reverse: 5' AGG-TCT-GTG-GGA-AAT-GAG-CTT-GGA 3'	64.8
RAGE	Forward: 5' CAG-GGT-CAC-AGA-AAC-CGG 3' Reverse: 5' ATT-CAG-CTC-TGC-ACG-TTC-CT 3'	57.8
SOD2	Forward: 5' AGC-TGC-ACC-ACA-GCA-AGC-AC 3' Reverse: 5' TCC-ACC-ACC-CTT-AGG-GCT-CA 3'	61.6
TGF-β1	Forward: 5' ACC-GCA-ACA-ACG-CAA-TCT-ATG 3' Reverse: 5' GCA-CTG-CTT-CCC-GAA-TGT-CT 3'	59.4
Timp1	Forward: 5' ATA-GTG-CTG-GCT-GTG-GGG-TGT-G 3' Reverse: 5' TGA-TCG-CTC-TGG-TAG-CCC-TTC-TC 3'	64.9
TNF-α	Forward: 5' CTT-ATC-TAC-TCC-CAG-GTT-CTC-TTC-AA 3' Reverse: 5' GAG-ACT-CCT-CCC-AGG-TAC-ATG-G 3'	60.2

CD163: Cluster of differentiation 163, CD68: Cluster of differentiation 68, CD86: Cluster of differentiation 86, Col1A2: collagen type I alpha 2 chain, Col3A1: collagen type III alpha 1 chain, CycA: Cyclin A, GLO1: Glyoxalase 1, HMBS: Hydroxymethylbilane synthase, HPRT: Hypoxanthine guanine phosphoribosyl, IL-1β: Interleukin 1 beta, LOX: Lysyl oxidase, Mmp2: Matrix metallopeptidase 2, Mrc1: Mannose receptor type 1, NOX4: NADPH oxidase 4, PGK1: Phosphoglycerate kinase 1, RAGE: Receptor for advanced glycation end-products, RPL13A: Ribosomal protein L13a, SOD2: Superoxide dismutase 2, TGF-β1: Transforming growth factor beta 1, Timp1: Metallopeptidase inhibitor 1, T_m: Melt temperature, TNF-α: Tumor necrosis factor alpha, YWHAZ: Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta.

Supplementary Results

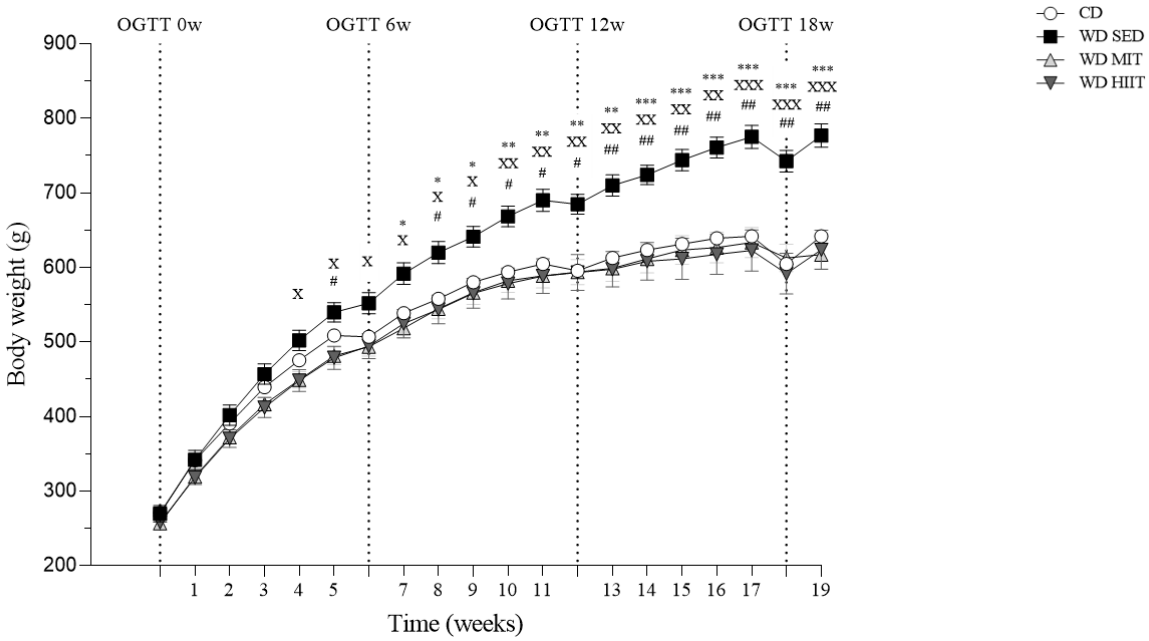

Figure S2: Effect of exercise training on body weight gain of Western diet fed rats. Animals were fed a control diet (CD) or Western diet (WD) for 18 weeks. Concurrently, WD fed rats were subjected to sedentary lifestyle (SED), moderate-intensity training (MIT), or high-intensity interval training (HIIT). Animal body weight was measured weekly. The circles, squares, up-pointing triangles, and down-pointing triangles, respectively, represent the CD, WD SED, WD MIT, and WD HIIT group. Data are presented as mean ± SEM. CD (n=5), WD SED (n=7), WD MIT (n=7), and WD HIIT (n=8). * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ WD SED vs CD. ^{X} denotes $p < 0.05$, ^{XX} denotes $p < 0.01$, ^{XXX} denotes $p < 0.001$ WD SED vs. WD MIT. $^{\#}$ denotes $p < 0.05$, and $^{\#\#}$ denotes $p < 0.01$ WD SED vs. WD HIIT.

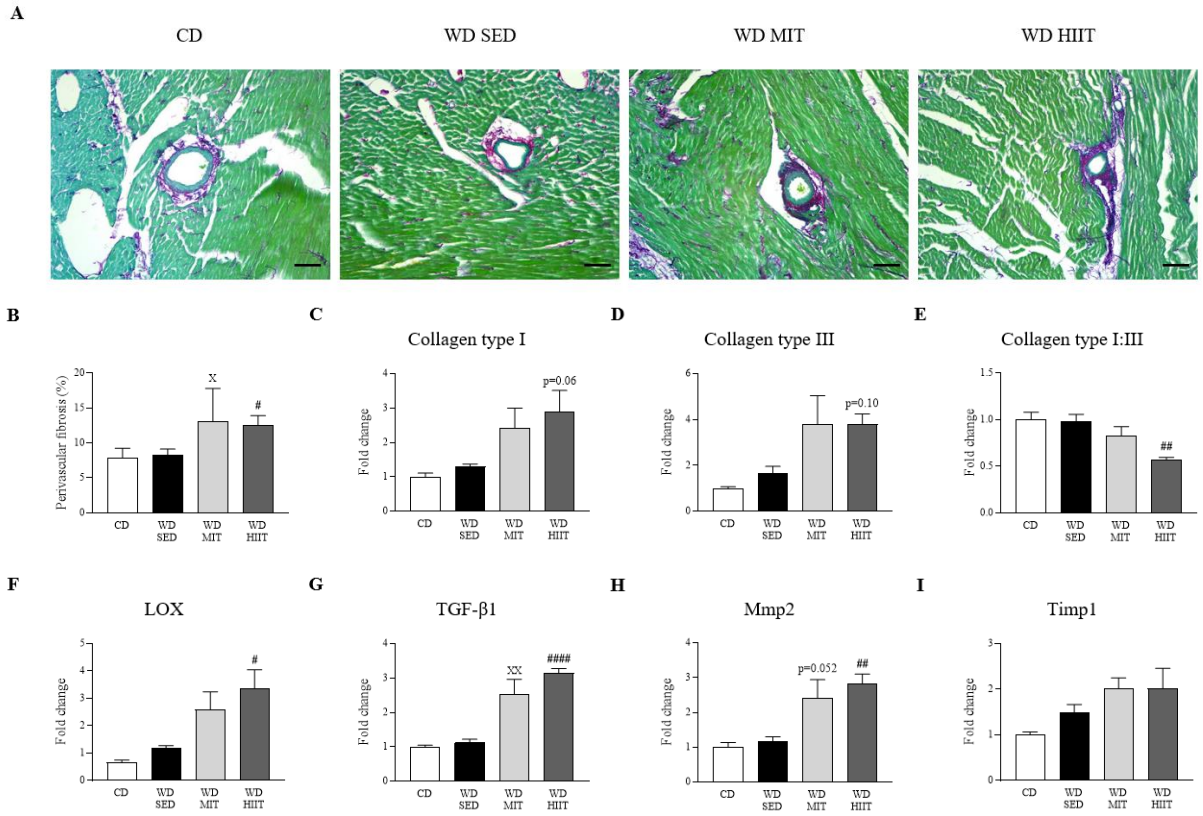

Figure S3: Effect of exercise training on fibrosis-related gene expression and perivascular fibrosis in left ventricular tissue. Perivascular fibrose and expression of fibrosis-related genes are examined in the left ventricle (LV) of rats receiving a control diet (CD), Western diet (WD) and subjected to sedentary lifestyle (SED), or performing either moderate-intensity training (MIT) or high-intensity interval training (HIIT). **A:** Representative pictures from collagen deposition around cardiac blood vessels visualized by the Sirius Red/Fast Green staining in the LV of all groups. Scale bare represent 100 µm. **B:** Quantification of perivascular fibrosis in LV sections. Quantification of gene expression of **(C)** collagen type I, **(D)** collagen type III, **(E)** collagen type I:III ratio, **(F)** lysyl oxidase (LOX), **(G)** transforming growth factor beta 1 (TGF-β1), **(H)** matrix metalloproteinase 2 (Mmp2), and **(I)** tissue inhibitor of metalloproteinase 1 (Timp1). CD (n=5), WD SED (n=7), WD MIT (n=7), and WD HIIT (n=8). Data are presented as mean ± SEM. ^{X} denotes $p < 0.05$, and ^{XX} denotes $p < 0.01$ WD SED vs. WD MIT. $^{\#}$ denotes $p < 0.05$, $^{\#\#}$ denotes $p < 0.01$, and $^{\#\#\#\#}$ denotes $p < 0.0001$ WD SED vs. WD HIIT.