PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

LETICIA ESTEVAM ENGEL

AVALIAÇÃO DA REMODELAÇÃO CARDÍACA DE RATOS ESPONTANEAMENTE HIPERTENSOS SUBMETIDOS AO TREINAMENTO INTERVALADO DE ALTA INTENSIDADE

Presidente Prudente – SP
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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal.
Área de Concentração: Fisiopatologia Animal.

Orientadora:
Prof.ª Drª. Francis Lopes Pacagnelli

Presidente Prudente – SP
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Presidente Prudente, 27 de setembro de 2021.

BANCA EXAMINADORA

_____
Profª. Drª. Francis Lopes Pacagnelli
Universidade do Oeste Paulista – Unoeste
Presidente Prudente-SP

_____
Prof. Dr. Ivan Vechetti Junior
University of Nebraska- Lincoln
Lincoln- Estados Unidos

_____
Prof. Dr. Leonardo de Oliveira Mendes
Universidade do Oeste Paulista – Unoeste
Presidente Prudente-SP

DEDICATÓRIA

A minha rainha, luz da minha vida, que virou uma estrelinha, mas que deixou todos os ensinamentos com muito carinho e simplicidade me ensinou como ter determinação e integridade, e que cada momento difícil que passei sempre me incentivava e acendia uma vela para Nossa Senhora. Mãe eu consegui e a pessoa que me tornei foi por você, obrigada Cleide.

Ao meu pai Ismael, que sempre não mediu esforços para incentivar aos estudos e ensinar que ele é prioridade para crescer, exemplo de empenho e bondade. A pessoa que mais se alegra com cada conquista, e que me ajuda a subir cada degrau, sem ele nada disso seria possível.

A minha querida e linda irmã que desde pequena sempre foi um exemplo de guerreira, esforço e inteligência, me ensinou a buscar meus sonhos, acreditou e cuidou de mim sempre que precisei, está sempre ao meu lado me apoiando nas incertezas e desesperos. A minha trajetória teria sido muito difícil, ruim e tortuoso sem você.

Ao meu amado Cristiano que sempre me incentivou e ajudou a estudar, que nos momentos de desânimos, estava comigo com paciência e muito carinho, acreditando e torcendo sempre por mim.

À minha querida orientadora, Profª. Drª. Francis Lopes Pacagnelli que a 7 anos me deu uma oportunidade que mudou toda minha trajetória, não estaria aqui sem o apoio e ensinamentos, minha referência de profissionalismo e exemplo de pessoa.

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Agradeço a todos que de alguma forma contribuíram para o fechamento deste ciclo.

Obrigada!

O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis.” (José de Alencar)

RESUMO

Avaliação da remodelação cardíaca de ratos espontaneamente hipertensos submetidos ao treinamento intervalado de alta intensidade

Introdução: Hipertensão é um grave problema de saúde pública, pode levar à hipertrofia concêntrica – um importante fator de risco para insuficiência cardíaca, que é considerada um preditor de maior morbimortalidade cardiovascular. O Treinamento Intervalado de Alta Intensidade (HIIT) pode ser indicado para hipertensos. Entretanto, o efeito potencial do HIIT tem mostrado resultados controversos e os aspectos moleculares remodelação cardíaca em hipertensos não foram totalmente elucidado. **Objetivo:** analisar os efeitos do HIIT durante a fase compensada da hipertensão arterial sobre os parâmetros estruturais, funcionais e moleculares da remodelação miocárdica em ratos espontaneamente hipertensos (SHR). **Métodos**: Ratos machos (12 meses de idade) foram divididos em três grupos: 1) Ratos Wistar Kyoto (WKY, n=8); 2) ratos sedentários espontaneamente hipertensos (SHR-SED, n =10) e 3) ratos espontaneamente hipertensos treinados (SHR-HIIT, n=10). Os parâmetros de pressão arterial média, capacidade máxima de exercício e perfil ecocardiográfico foram avaliados antes e após o HIIT; a remodelação cardíaca foi avaliada por meio de ecocardiografia, músculo papilar isolado e a expressão gênica em relação à via MAPK: Elk1, cJun, ATF2, MEF2. Para comparação entre os grupos foi utilizado ANOVA seguido de Tukey ou Kruskal-Wallis e Dunn ($p<0.05$). **Resultados**: HIIT diminuiu a PAS (SED-SHR: 229 ± 5.93 mmHg vs HIIT-SHR: 198.6 ± 18.3 mmHg; p = 0.001), aumentou a distância percorrida, sendo 82,7% maior no grupo SHR-HIIT (SHR-SED=183.0±88.08m vs. SHR-HIIT=1126.0±187.1m; $p<0.0001$) e reduziu a tensão de repouso do músculo papilar (WKY=0.77 ± 0.216; SHR-SED=1.26 ± 0.20; SHR-HITT=0.67 ± 0.23; p=0.0001). Na expressão gênica houve uma diminuição da expressão do gene ATF2 nos grupos hipertensos em relação ao grupo controle (WKY: 1.14 ± 0.62; SHR-SED: 0.39 ± 0.11; SHR-HIIT: 0.62 ± 0.21 p = 0.03) sem alterações nos demais genes. **Conclusão**: O HIIT aplicado ratos SHR na fase de hipertensão compensada demonstrou uma técnica adequada para diminuir a pressão arterial, melhorar a capacidade funcional, gerou um aumento da hipertrofia cardíaca, mas atenuou a disfunção diastólica miocárdica, sem prejuízos funcionais ou alteração gênica.

Palavras-chave: exercício físico, músculos papilares, sistema de sinalização da MAP quinase, coração.

ABSTRACT

Evaluation of cardiac remodeling in spontaneously hypertensive rats submitted to high-intensity interval training

Introduction: Hypertension, is a serious public health problem, can lead to concentric hypertrophy - an important risk factor for heart failure, which is considered a predictor of greater cardiovascular morbidity and mortality. High Intensity Interval Training (HIIT) can be indicated for hypertensive patients. However, the potential effect of HIIT has controversial results and the molecular aspects of cardiac remodeling in hypertensive patients have not been fully elucidated. **Objective:** to analyze the effects of HIIT during the compensated phase of arterial hypertension on the possible parameters, give and myocardial remodeling molecules in spontaneously hypertensive rats (SHR). **Methods:** we evaluated the influence of HIIT on blood pressure, exercise tolerance and cardiac remodeling in spontaneously hypertensive rats (SHR). Methods: Male rats (12 months old) were divided into three groups: 1) Wistar Kyoto rats (WKY, n = 8); 2) spontaneously hypertensive sedentary rats (SED-SHR, n = 10) and 3) spontaneously hypertensive trained rats (HIIT-SHR, n = 10). The parameters of mean arterial pressure, maximum exercise capacity and echocardiographic profile were taken before and after HIIT; cardiac remodeling was assessed using echocardiography, isolated papillary muscle and gene expression quantified in relation to the MAPK pathway: Elk1, cJun, ATF2, MEF2. For comparison between the groups, ANOVA was used followed by Tukey or Kruskal-Wallis and Dunn's ($p < 0.05$). **Results**: HIIT decreased SBP (SED-SHR: 229 ± 5,93 mmHg vs HIIT-SHR: 198,6 ± 18,3 mmHg; p = 0,001), increased the distance covered, being 82.7% greater in the SHR- HIIT (SED-SHR = 183.0 ± 88.08m vs. HIIT-SHR = 1126.0 ± 187.1m; $p < 0.0001$) and reduced the resting period of the papillary muscle (WKY = 0.77 ± 0.216 ; SED-SHR = 1.26 ± 0.20; HIIT-SHR = 0.67 ± 0.23; p = 0.0001) and in gene expression there was a decrease in the gene encoding ATF2 protein in hypertensive groups in relation to control group (WKY: 1.14 ± 0.62; SHR-SED: 0.39 ± 0.11; HIIT-SHR: 0.62 ± 0.21 p = 0.03) **Conclusion:** The HIIT applied in SHR models in the compensated hypertension phase, a technique to decrease blood pressure, functional capacity, generated an increase in cardiac hypertrophy, but attenuated myocardial diastolic dysfunction, without dissipating damage or altering the gene.

Keywords: Physical exercise, papillary muscles, MAP kinase signaling system, heart.

LISTA DE ABREVIATURAS

+dT/dt, g/mm^2	- Velocidade máxima de elevação da tensão desenvolvida
µm²	- Micrômetro quadrado
A'	- Onda Diastolica tardia do anel mitral por Doppler tissular
AE	- Átrio esquerdo
ANOVA	- Análise de variância
AO	- Diâmetro da aorta
ATF2	- Ativador o Fator de Transcrição 2
AU	- unidade arbitrária
$CaCl_2$	- Cloreto de cálcio
c-Jun	- Jun Proto-Oncogene, AP-1 Transcription Factor Subunit)
CO_2	- Dióxido de carbono
DDVE	- Diâmetro diastólico do ventíiculo esquerdo
DSVE	- Diâmetro sistólico do ventrículo esquerdo
-dT/dt, g/mm^2	- Velocidade máxima de decréscimo da tensão desenvolvida
E'	- Onda Diastólica precoce do anel mitral por Doppler tissular.
E/A'	- Razão entre picos de velocidade de fluxo de enchimento
E/TDI-E’	- Onda E/Velocidade inicial anular mitral po Doppler tissular
EDPP	- Espessura diastólica da parede posterior
EDSIV	- Espessura diastólica da parede septal ventricular
EFE	- Percentual de encurtamento do endocárdio
EFM	- Percentual de encurtamento do mesocárdio
ELK1	- Fator de Transcrição ETS
ERK1/2, ERK5	- quinase regulada por sinais extracelulares
g	- Grama
h	- Hora
HAS	- Hipertensão Arterial Sistêmica
HIIT	- Treinamento Intervalado de Alta Intensidade
ICC	- Insuficiência cardíaca congestiva
IMVE	- Índice de massa do ventrículo esquerdo
Índice Tei	- Índice de desempenho miocárdico inicial
JNK	- c-Jun N-terminal quinase
KCl	- Cloreto de potássio

km/h - Quilômetro por hora

MAPK - Proteínas quinases ativadas por mitógenos

MEF2 - fator de aumento de miócitos 2

mg/kg - Miligrama por quilo

$MgSO_4$ - Sulfato de magnésio

MHZ - Megahertz

min - Minuto

mmol/L - Milimol por litro

ms - Milissegundo

NaCl - Cloreto de sódio

$NaCO_2$ - Bicarbonato de sódio

O_2 - Oxigênio

onda A - Velocidades de entrada mitral diastólica tardia

onda E - Velocidades de entrada mitral diastólica precoce

p38 proteínas quinases ativadas por mitogênio p38

PAS - Pressão arterial sistólica

PCF - Peso corporal final

PCI - Peso corporal inicial

PEE - Porcentagem de encurtamento do endocárdio

PEM - Porcentagem de encurtamento do mesocárdio

RNA - ácido ribonucléico

RT- qPCR - Reação em cadeia da polimerase em tempo real após a transcrição reversa

S ' - Imagem de Doppler tissular da velocidade sistólica

SHR - Ratos espontaneamente hipertensos

TD - Tensão desenvolvida

TDI-A’ - Velocidade tardia anular mitral por Doppler tissular

TDI-E’ - Velocidade inicial anular mitral por Doppler tissular

TDIS’: - avaliação por imagem de Doppler tissular da velocidade sistólica

TR - Tensão de repouso

TRIV - Tempo de relaxamento isovolumétrico

VE - Ventrículo esquerdo

VEPP - Velocidade de encurtamento da parede posterior

VO_2max - Consumo máximo de oxigenio corporal

WKY - Ratos Wistar-Kyoto

SUMÁRIO

1 ARTIGO CIENTÍFICO

ARTIGO ORIGINAL

The high-intensity interval training mitigates the cardiac remodeling in spontaneously hypertensive rats

Letícia Estevam Engel, Francilene Lima Agostinho de Souza[1], Dijon Henrique Salome Campos[2], Ines Cristina Giometti[1], Katashi Okoshi[2], Thaoan Bruno Mariano[1], Natalia Zamberlan Ferreira[1], Dyovana Gomes Pinheiro[1], Rafael S. Floriano[1], Andreo Fernando Aguiar[4], Antônio Carlos Cicogna[2], Francis Lopes Pacagnelli[1*]

[1] University of Western São Paulo (UNOESTE), Presidente Prudente, SP, 19.067-175, Brazil
[2] São Paulo State University (UNESP), Botucatu, SP, 18618-687, Brazil
[3] São Paulo State University (UNESP), Presidente Prudente, SP, 19060-900, Brazil
[4] University of Northern Paraná (UNOPAR), Londrina, PR, 86047-500, Brazil

Short title: Spontaneously hypertensive rats exposed to HIIT

*Correspondence to: F.L. Pacagnelli (francispacagnelli@unoeste.br), Tel.: +55 18 3229 3264, Fax: +55 18 3229 2080

ABSTRACT

Aim: To evaluate the influence of high-intensity interval training (HIIT) on cardiac structural and functional characteristics and myocardial mitogen-activated protein kinase (MAPK) signaling in hypertensive rats. **Methods:** Male rats (12 months old) were divided into three groups: Wistar Kyoto rats (WKY, n=8); sedentary spontaneously hypertensive rats (SED-SHR, n=10), and trained spontaneously hypertensive rats (HIIT-SHR, n=10). Systolic blood pressure (SBP), functional capacity, echocardiography, isolated papillary muscle, and gene expression of MAPK gene-encoding proteins associated with Elk1, cJun, ATF2, MEF2 were analyzed. **Key findings:** HIIT decreased SBP and increased functional capacity, left ventricular diastolic diameter, posterior wall thickness-left ventricle, relative wall thickness-left ventricle, and resting tension of the papillary muscle. In hypertensive rats, we observed a decrease in the gene-encoding ATF2 protein; this decrease was reversed by HIIT. **Significance:** The influence of HIIT in the SHR model in the compensated hypertension phase generated an increase in cardiac hypertrophy, attenuated myocardial diastolic dysfunction, lowered blood pressure, improved functional capacity, and reversed the alteration in gene-encoding ATF2 protein.

Key words: Physical exercise, papillary muscles, MAP kinase signaling system, hypertension.

1. INTRODUCTION

Hypertension is considered a serious public health problem and affects more than a billion people worldwide. An estimated 45% of coronary artery disease cases and 51% of vascular accident cases have a direct relationship with hypertension (Mills & Stefanescu, 2020). Hypertension is also involved in the more than 9.4 million deaths annually from cardiovascular diseases, costing more than €20 billion per year (World Health Organization, 2015). Hypertension is characterized by a chronic pressure overload elevation in the left ventricle (LV) that causes molecular, cellular, and interstitial changes, resulting in alterations to size, mass, geometry, and function. These effects contribute to LV delay, which is a poor prognosis due to its association with pathological hypertrophy, ventricular dysfunction, malignant arrhythmias, and heart failure (Azevedo, Polegato, Minicucci, Paiva, & Zornoff, 2016). Pathological LV hypertrophy is consistently associated with cardiovascular morbidity and mortality, and it is recognized as a marker and mediator of cardiovascular events (Nadruz, 2015).

Mitogen-activated protein kinases (MAPKs) are among the molecular mechanisms that govern cardiac hypertrophy and have been the focus of extensive investigations in recent decades (Rose, Force, & Wang, 2010). The four bestcharacterized MAPK subfamilies, ERK1/2, JNK, p38, and ERK5, are the pharmacological and genetic targets involved in the regulation of aspects of cardiac remodeling, such as reactivation of transcription factors (e.g., Elk1, cJun, ATF2 and MEF2c) (Mutlak & Kehat, 2015).

Research on non-pharmacological alternatives for hypertension and cardioprotection are associated with inhibition of the MAPK signaling pathway as in continuous low- and medium-intensity aerobic training. Miyachi et al., demonstrated that exercise (swimming) reduced the phosphorylation and activity of P38 and ERK1/2, leading to preservation of cardiac function and improved survival rate. (Miyachi et al., 2009). In addition, Pagan et al., showed that aerobic exercise improves physical capacity, myocardial function, reduces the frequency of heart failure features and ERK phosphorylation, and normalizes energy metabolism in SHR (Pagan et al., 2021). Although these studies have demonstrated the benefits induced by aerobic training, different training modalities have also been used in an attempt to mitigate the deleterious effect promoted by hypertension.

High-intensity training (HIIT) may be beneficial to hypertensive individuals by improving cardiometabolic risk factors, cardiovascular performance, adherence to exercise programs (more time efficient) and various metabolic responses in skeletal muscle. Studies have shown that HIIT is more effective than continuous moderate aerobic exercise in improving peak aerobic fitness (VO2peak) in patients with cardiovascular disease ((Krzesiak et al., 2019; Leal, Galliano, & Del Vecchio, 2020; Batacan, Duncan, Dalbo, Connolly, & Fenning, 2016; Gibala, Little, Macdonald, & Hawley, 2012; Gomes-Neto et al., 2017). Despite these benefits, the effects of HIIT in the MAPK signaling during hypertension is not well characterized.

As an initial effort to test the hypothesis that HIIT is a viable nonpharmacological alternative for cardioprotection, we analyzed the effects of HIIT during the compensated phase of hypertension on the structural, functional, and MAPK signaling of myocardial remodeling in SHRs. In support of our hypothesis, we observed that HIIT contributes to the decreased of blood pressure, as a way to improve physical performance, increases exercise tolerance, myocardial function and attenuates transcription changes in myocardial mitogen-activated protein kinase (MAPK) signaling.

2. MATERIALS AND METHODS

2.1. Ethical approval and animals

All experiments involving animals were approved by the Committee for Ethics in Animal Use, São Paulo State University (UNESP) (protocol No. 018/11), in accordance with the Brazilian College of Animal Experimentation (COBEA) and the US National Institute of Health “Guide for the Care and Use of Laboratory Animals.” Male spontaneously hypertensive rats (400-450 g; 12 months old) obtained from the Federal University of São Paulo (UNIFESP, São Paulo, SP, Brazil) were maintained in the Laboratory of Animal Experimentation of the São Paulo State University (UNESP, Botucatu, SP, Brazil), in a controlled environment, and housed (3 or 4 rats per cage) in a temperature-controlled environment (21 ± 1 °C) with 12-hour light/dark cycles (light from 7 am to 7 pm). They had ad libitum access to food (Supralab, Alisul R, Brazil) and water.

2.2. Experimental design

The rats were then divided into three experimental groups: 1) sedentary spontaneously hypertensive rats (SED-SHR, n = 10), 2) trained spontaneously hypertensive rats (HIIT-SHR,

n = 10), and 3) Wistar Kyoto rats (WKY, n = 8). HIITSHR group was submitted to the training protocol for eight consecutive weeks as described below. The parameters for systolic blood pressure (SBP), maximal exercise capacity, and echocardiographic profiles were assessed before and after HIIT; a complementary maximal exercise capacity test was performed at the end of the fourth week to adjust the training load. The rats were euthanized after the period of experimental protocols, and the functional analyses of the isolated papillary muscle and gene expression were then performed (**Figure 1**).

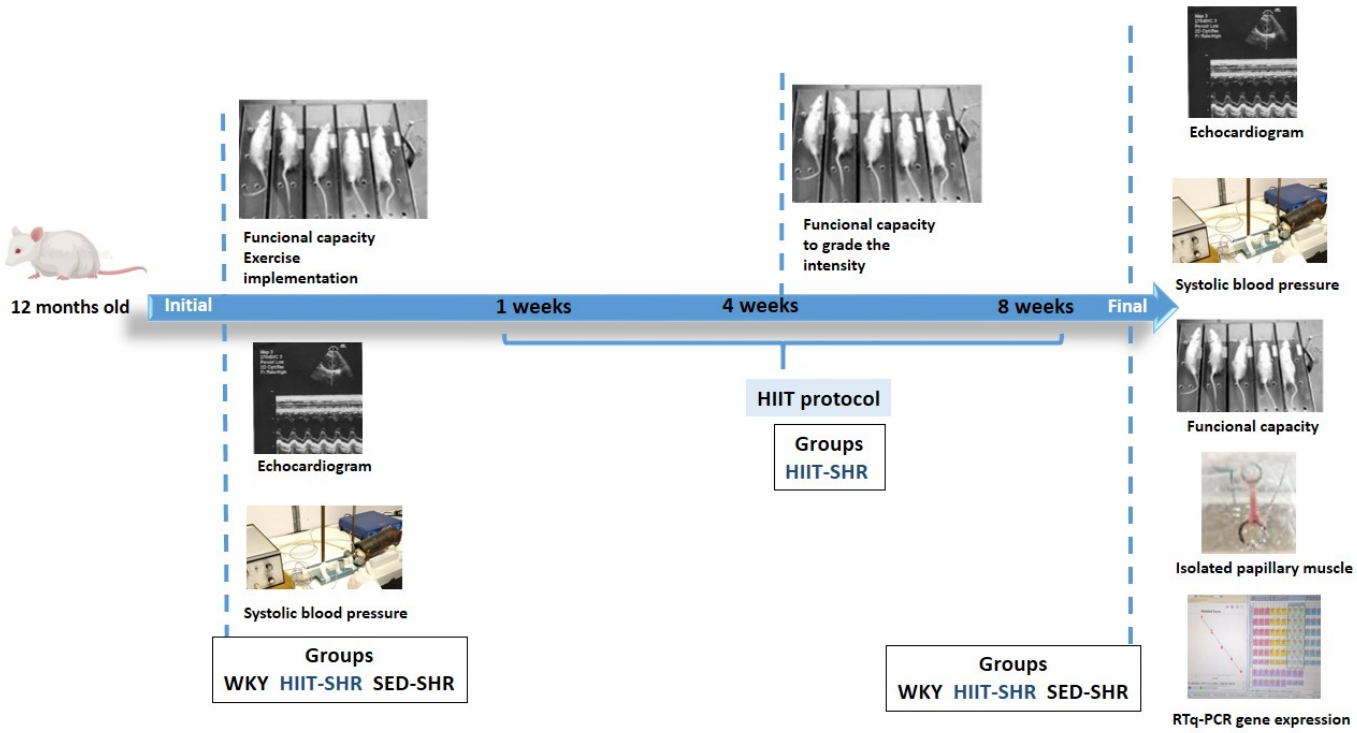

Fig. 1. Schematic figure summarizing the experimental design.

2.3. Systolic blood pressure (SBP)

Blood pressure was determined through the tail-cuff occlusion method (Narco Bio-System®, model 709-0610, International Biomedical, Inc.) before and after training sessions. The procedure was performed two days before starting the experiment, and the average values for SBP were measured from six consecutive cycles per day

2.4. Maximum exercise capacity evaluation

Prior to assessing maximum exercise capacity, the rats (SED-SHR, HIIT-SHR, WKY) performed adaptation training sessions on a treadmill (model TK 1, Imbramed, São Paulo, SP, Brazil) for 10 min for one week (every day) at a rate of 6 m/min with zero % inclination.

(Pagan et al., 2015); (Haram et al., 2009). The rats were considered exhausted when they refused to run or were unable to coordinate steps. Functional capacity was evaluated by the total distance travelled, which was calculated with consideration to the speed and maximum duration of the test. We evaluated functional capacity before the start of the first training sessions, four weeks after the start of the training to grade the effort intensity, and 24 hours after the final training session (Haram et al., 2009). The percentage of variation Δ (%) of distance and time was calculated.

2.5. High-intensity interval training (HIIT)

Training was performed for approximately 50 min/day, five days a week, for eight weeks, in an inverted cycle. The training sessions occurred from 2 pm to 2:50 pm. Each session consisted of three phases: warm-up, HIIT, and recovery. The warm-up phase included 5 minutes at 60% of the exhaustion speed. HIIT started at 95% of the speed achieved in the exhaustion test for four minutes, interspersed with 65% of the maximum speed for three minutes. The protocol was repeated five times in the first and second weeks. (**Figure 2)** The same HIIT velocities of the first week were used in the third and fourth weeks, but the velocities were repeated six and seven times consecutively. Before the start of the fifth week, a second test was performed to reevaluate the maximum rate of exhaustion; and the training load was reset. In the fifth and sixth weeks, HIIT was performed with an adapted protocol at a speed of 23m/min for four minutes, interspersed at 12m/min for three minutes, with seven repetitions. Speed was increased 15% in the seventh week and 18% in the eighth week, interspersed with 65% of the maximum speed for three minutes, with seven repetitions (Aleixo et al., 2021).

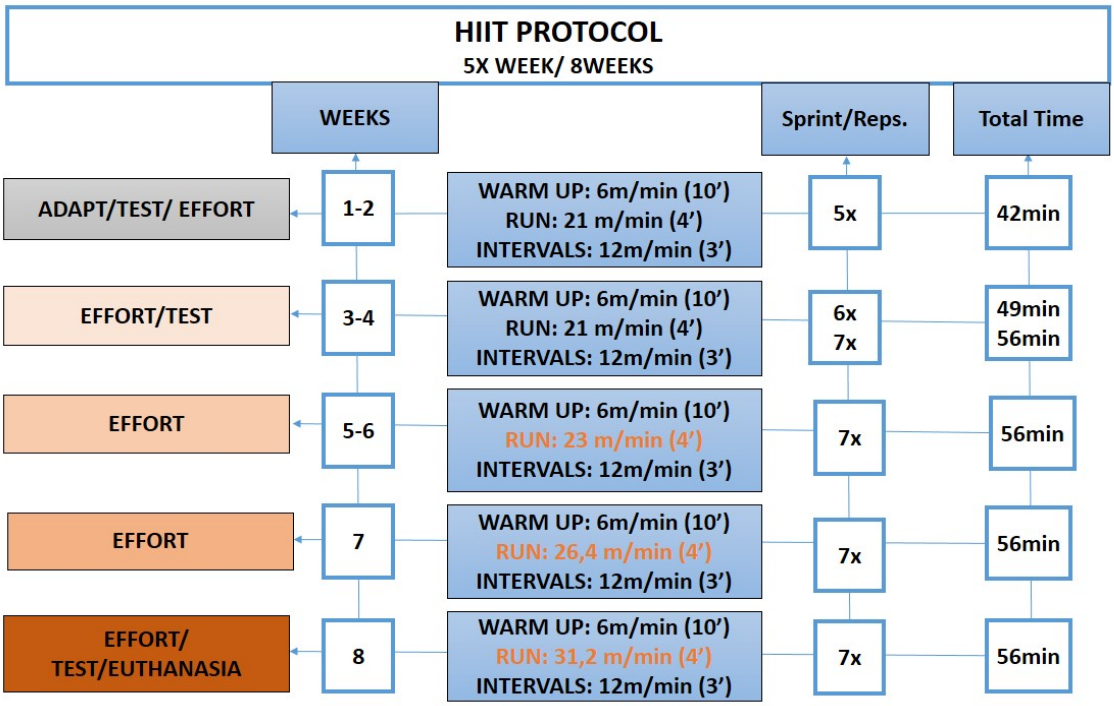

Fig. 2. Schematic figure summarizing HIIT protocol.

2.6. *In vivo* functional and structural studies: echocardiographic analysis

Echocardiographic analysis was performed before and after the training phase (one day after final session) using a cardiovascular ultrasound model Vivid S6+ (General Electric Medical Systems, Tirat Carmel, Israel) equipped with a multifrequency probe (5–11.5 MHz). The rats were anesthetized by an intramuscular injection of a mixture of ketamine (50 mg/kg-1 , Dopalen®) and xylazine (0.5 mg/kg-1 , Anasedan®). Two dimensionally-guided M-mode images were obtained from parasternal short-axis views of the LV just below the tip of the mitral valve leaflets, at the level of the aortic valve, and at the left atrium (Okoshi et al., 2004). Images were acquired at a frame rate (FPS) of 124/sec and at a depth of 2.5 cm. The LV M-mode images were printed (Sony UP-890MD) at a scanning speed of 200 mm/sec. All LV structures and diameters of the aorta and left atrium were manually measured in the images above by the same observer (KO). We used the method of the American Society of Echocardiography (Cicogna, Padovani, Okoshi, Aragon, & Okoshi, 2000; Pagan et al., 2015; Haram et al., 2009; Wisløff et al., 2007; Damatto et al., 2013; Okamoto & Aoki, 1963). Measurements were taken from the mean of at least five cardiac cycles in the M-mode tracings.

To evaluate structural remodeling, the measured variables included the left atrium diameter (LA), LV diastolic diameter (LVDD), posterior wall thickness-left ventricle (PWT),

LV relative wall thickness (RWT), and LV mass. The RWT was calculated using the formula 2 × PWT/LVDD. LV mass was calculated using the formula [(LVDD + PWT + SWT)3 − (LVDD)3] × 1.04. LV systolic function was assessed by mid-wall fractional shortening (MFS). LV diastolic function was assessed from apical four and five chamber views by parameters that included early and late diastolic mitral inflow velocities (E and A waves), E/A ratio, and the isovolumetric relaxation time (IVRT).

2.7. Euthanasia and anatomical data

The rats were euthanized using methods approved for their specific species, developmental stage, and size. Two days after the last HIIT session, the rats were anesthetized by an overdose of a mixture of ketamine and xylazine administered intraperitoneally and decapitated (Aleixo et al., 2021). The rats were then submitted to median thoracotomy, the heart was removed, and the atria and ventricles were separated and weighed. The left ventricular + septum (LV) and atrial weights were normalized by final body weight (FBW) (Pagan et al., 2015; Haram et al., 2009).

2.8. *In vitro* functional study: isolated papillary muscle

The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. Papillary muscle was dissected carefully from the left ventricle, clipped at its edges, placed vertically in a chamber containing Krebs-Henseleit solution at 28°C, oxygenated with a mixture of 0.95 O2 and 0.5 CO2 (pH 7.38), and stimulated with two electrodes in the solution at a rate of 0.2 Hz (Cicogna et al., 1997). The following basal parameters were measured from isometric contraction: peak developed tension (DT, g/mm2), resting tension (RT, g/mm2), time to peak tension (TPT, ms), maximum rate of tension development (+dT/dt, g/mm2 per s), and maximum rate of tension decline (−dT/dt, g/mm2 per s). To evaluate the myocardial contractile reserve, mechanical performance was analyzed at the basal condition and after the following positive inotropic stimulations: post-rest contractions (10, 30, and 60 s), increased extracellular Ca2+ concentration (external calcium concentrations of 0.5, 1.5, and 2.5 mM), and the addition of β-adrenergic agonist isoproterenol (10-8 , 10-7 , and 10-6 M) to the nutrient solution (Cicogna, Padovani, Okoshi, Aragon, & Okoshi, 2000). Papillary muscle cross-sectional areas (CSA, mm2) were calculated from muscle weight and length by assuming cylindrical uniformity and a specific

gravity of 1.0. All force data were normalized for muscle CSA. Papillary muscles with CSA 1.5 mm2 were excluded from analysis (Mazeto & Okoshi, 2021).

2.9 Gene expression

Total RNA was extracted from LV tissue using TRIzol (ThermoScientific, Waltham, United States) and then treated with DNAse deoxyribonuclease I (ThermoScientific), in accordance with the manufacturer's instructions. The HighCapacity Reverse Transcriptional Kit (ThermoScientific) was used for the synthesis of complementary RNA (cDNA) from 1000 ng of total RNA from each sample. Aliquots of cDNA were then submitted to real-time PCR reaction using a customized assay containing the following Taqman (Applied Biosystems, Foster City, United States) probes: ETS Transcription Factor (elk1, Rn01756649_g1), Jun Proto-Oncogene, AP-1 Transcription Factor Subunit (c-Jun, Rn99999045_s1), Activating Transcription Factor 2 (atf2, Rn00578832_m1), Myocyte Enhancer Factor 2A (mef2a, Rn01478096_m1). The Taqman™ Universal Master Mix II (AppliedBiosystems) and the StepOne Plus system (ThermoScientific) were used for qPCR. All samples were analyzed in duplicates. The cycling conditions were at 50°C for 2 minutes and 95°C for 10 minutes. This was followed by 40 cycles of denaturation at 95°C for 15 seconds and the final extension at 60°C for 1 minute. After normalization by expression levels of the betaactin reference gene (Actb, Rn00667869_m1) using the 2 -ΔΔCt method (Livak & Schmittgen, 2001)

2.10. Data analysis

The data were expressed as mean ± SD. The normality and homogeneity for outcome measurements were performed using the Shapiro-Wilk and Levene's tests, respectively. Variables (SBP, functional capacity, anatomical parameters, echocardiographic analysis, isolated papillary muscle) were evaluated by analysis of variance One-Way ANOVA. When significant differences were found ($p< 0.05$), the post hoc Tukey's multiple comparisons test was performed. Variables (gene expression) were evaluated by the Kruskal Wallis test, the post hoc Dunn's multiple comparisons test was performed. All data analyses were performed using SPSS software (v. 20.0; Chicago, USA). Significance level was set at 5%.

3. RESULTS

Initial readings of SBP were greater in SHR group rats (SED-SHR and HIITSHR) when compared to normotensive rats (WKY: 131.1 ± 6.95 mmHg; SED-SHR: 201.5 ± 24.08

mmHg; HIIT-SHR: 210.8 ± 18.9 mmHg; p = 0.001). HIIT-SHR group rats showed a significant decrease of final SBP when compared to the SED-SHR group (WKY: 133.6 ± 9.63 mmHg; SED-SHR: 229 ± 5.93 mmHg; HIIT-SHR: 198.6 ± 18.3 mmHg; p = 0.001) (**Figure 3**).

The HIIT protocol induced an increase in the distance traveled when compared to the SED-SHR group; after training, distances were higher for the HIIT-SHR group (1198.0 ± 227.2 m) when compared to the WKY group (440.0 ± 131.7 m, p < 0.0001) and SED-SHR group (275.3 ± 51.0 m; p < 0.0001).

HIIT also contributed to body weight (BW) loss; rats in the HIIT-SHR group weighed less than the SED-SHR group (SED-SHR: 420.1 ± 27.72; HIIT-SHR: 352.9 ± 33.26; p < 0.05). The LV/FBW were higher in SED-SHR and HIIT-SHR rats when compared to the WKY group, after training, were LV/BWF higher for the HIIT-SHR group when compared to the SED-SHR (WKY: 1.67 ± 0.09 g/kg; SED-SHR: 2.65 ± 0.27 g/kg; HIIT-SHR: 3.02 ± 0.33 g/kg; p < 0.001). The atria weight normalized by FBW were higher in SED-SHR and HIIT-SHR groups when compared to the WKY group (WKY: 0.70 ± 0.07 g/kg; SED-SHR: 0.86 ± 0.06 g/kg; HIIT-SHR: 0.92 ± 0.2 g/kg). The rats from all groups showed no signs of heart failure, such as pleural effusion, ascites, tachypnea, or atrial thrombus.

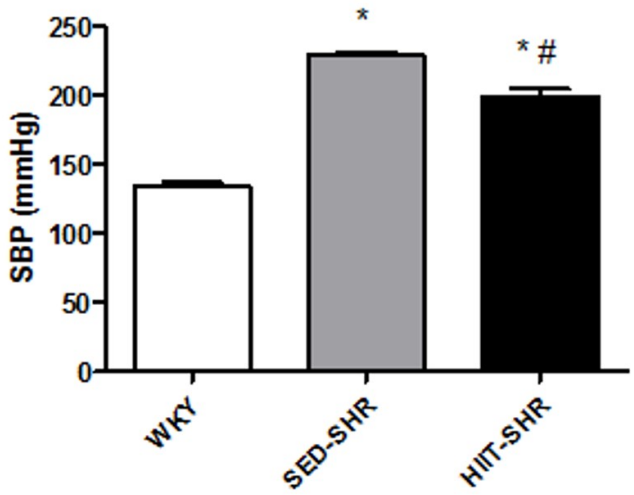

Fig. 2. Systolic blood pressure (SBP) measured by the tail cuff occlusion method in Wistar Kyoto rats (WKY), sedentary spontaneously hypertensive rats (SED-SHR), and trained spontaneously hypertensive rats (HIIT-SHR). Data is presented as mean ± SD. One-way ANOVA and Tukey's test. *p

Initial echocardiographic readings showed that SHR group rats had already demonstrated increases in LVDD/BW, PWT, LA/BW, and LVM/BW when compared to the control (WKY). At the end of the experimental protocol, echocardiographic analysis revealed that SED-SHR and HIIT-SHR group rats showed increased LVDD/BW, PWT, RWT, LA/BW and LVM/BW; these increases led to considerably higher measurements when compared to the control (WKY). LVDD/BW, PWT, RWT, and LA/BW increased in HIIT-SHR group rats when compared to SED-SHR. All other measured parameters did not differ among groups (**Table 1**).

Table 1. Structural and functional echocardiographic final data from the experimental groups: Wistar-Kyoto rats (WKY), sedentary spontaneously hypertensive rats (SEDSHR), and trained spontaneously hypertensive rats (HIIT-SHR).

Variables	**Experimental groups**		
	WKY	**SED-SHR**	**HIIT-SHR**
LVDD/BW (mm/kg)			
Initial	13.78 ± 1.57	16.90 ± 0.74*	17.90 ± 2.32*
Final	12.4 ± 0.94	17.40 ± 0.63*	20.35 ± 1.64*#
PWT (mm)			
Initial	1.36 ± 0.10	1.54 ± 0.13*	1.60 ± 0.09*
Final	1.45 ± 0.04	1.52 ± 0.07*	1.62 ± 0.10*#
RWT (mm)			
Initial	0.35 ± 0.02	0.37 ± 0.02	0.36 ± 0.03
Final	0.35 ± 0.02	0.39 ± 0.02*	0.44 ± 0.03*#
LA/BW (mm/kg)			
Initial	10.26 ± 0.88	11.08 ± 1.19*	14.15 ± 1.90*
Final	9.31 ± 0.73	13.93 ± 1.27*	16.49 ± 1.33*#
LVM/BW (g/kg)			
Initial	0.78 ± 0.06	0.83 ± 0.06	0.80 ± 0.08
Final	0.78 ± 0.09	0.84 ± 0.04*	0.90 ± 0.16*
MFS (%)			
Initial	34.45 ± 3.19	32.49±3.23	31.75 ± 2.77
Final	32.91 ± 5.34	31.52 ± 3.02	28.99 ± 4.69

E/A			
Initial	1.81 ± 0.52	1.84 ± 0.58	2.1 ± 0.50
Final	1.99 ± 0.37	1.63 ± 0.56	1.66 ± 0.14
IVRT (ms)			
Initial	32.67 ± 6.86	30.00 ± 3.80	29.82 ± 5.60
Final	32.67 ± 6.26	30.00 ± 3.24	30.63 ± 2.38

The absolute values are the mean ± SD. BW "body weight"; LVDD "left ventricular diastolic diameter"; PWT-LV "posterior wall thickness-left ventricle"; RWT-LV "relative wall thickness-left ventricle"; LA "left atrial diameter"; LVM "left ventricular mass"; MFS "mid-wall fractional shortening"; E/A "ratio between early (E) to late (A) diastolic mitral inflow"; IVRT "isovolumic relaxation time". One-way ANOVA and Tukey's test. * $p< 0.05$ vs WKY. # $p< 0.05$ vs HIIT-SHR.

The data related to LV papillary muscle function are summarized in **Figure 4**; only resting tension (RT) was significantly different among groups. Our results suggested that HIIT protocol recovered the resting tension (RT) when compared to the SED-SHR group.

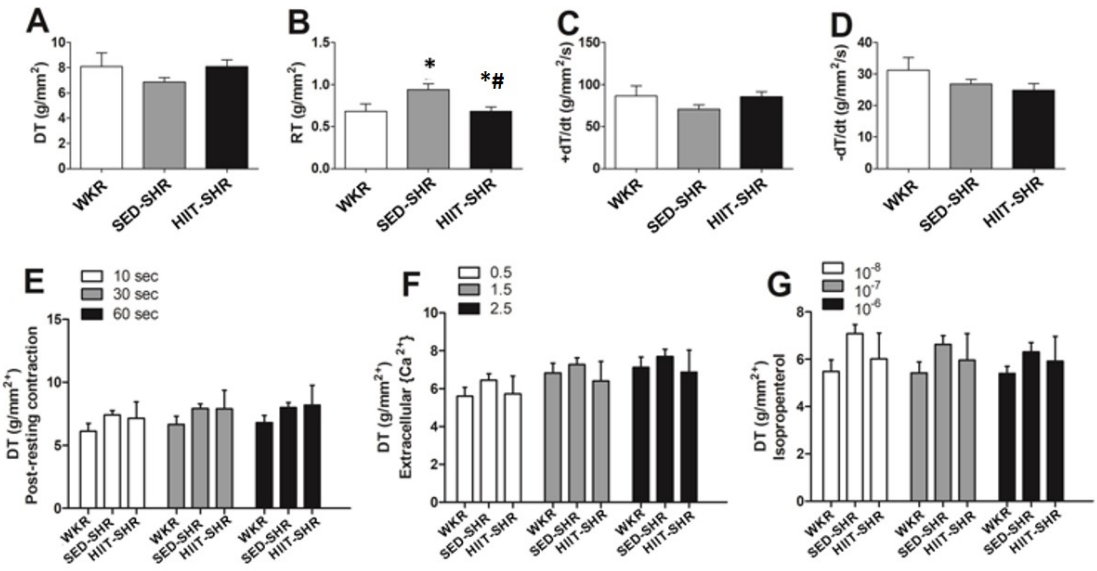

Fig. 4. Analysis of functional data of isolated papillary muscle from Wistar Kyoto rats (WKY), sedentary spontaneously hypertensive rats (SED-SHR), and spontaneously trained hypertensive rats (HIIT-SHR). A. Developed tension (DT); B. Resting tension (RT); C. Maximum development voltage rate (+ dT/dt); D. Maximum rate of decay voltage (−dT / dt); E. Post-contraction developed tension (DT) at rest at 10, 30, and 60 seconds; F. Developed

tension (DT) after positive inotropic stimulation: extracellular calcium concentration increased to 0.5, 1.5, and 2.5 mm; G. Developed tension (DT) after positive inotropic stimulation: Iso 10−6 M β-adrenergic agonist isoproterenol (10−8 , 10−7 , and 10−6 M) added to saline. Data are expressed as mean ± SD. One-way ANOVA and Tukey's test. *$p < 0.05$ compared to WKY; # $p < 0.05$ compared to SED-SHR

The analysis of MAPK pathway revealed that the HIIT protocol restored the expression of the gene-encoding ATF2 protein (WKY: 0.99 (0.57-1.84); SED-SHR: 0.41 (0.23-0.44); HIIT-SHR: 0.81 (0.76-0.91); $p = 0.0002$). All other measured parameters did not differ between groups (**Figure 5)**

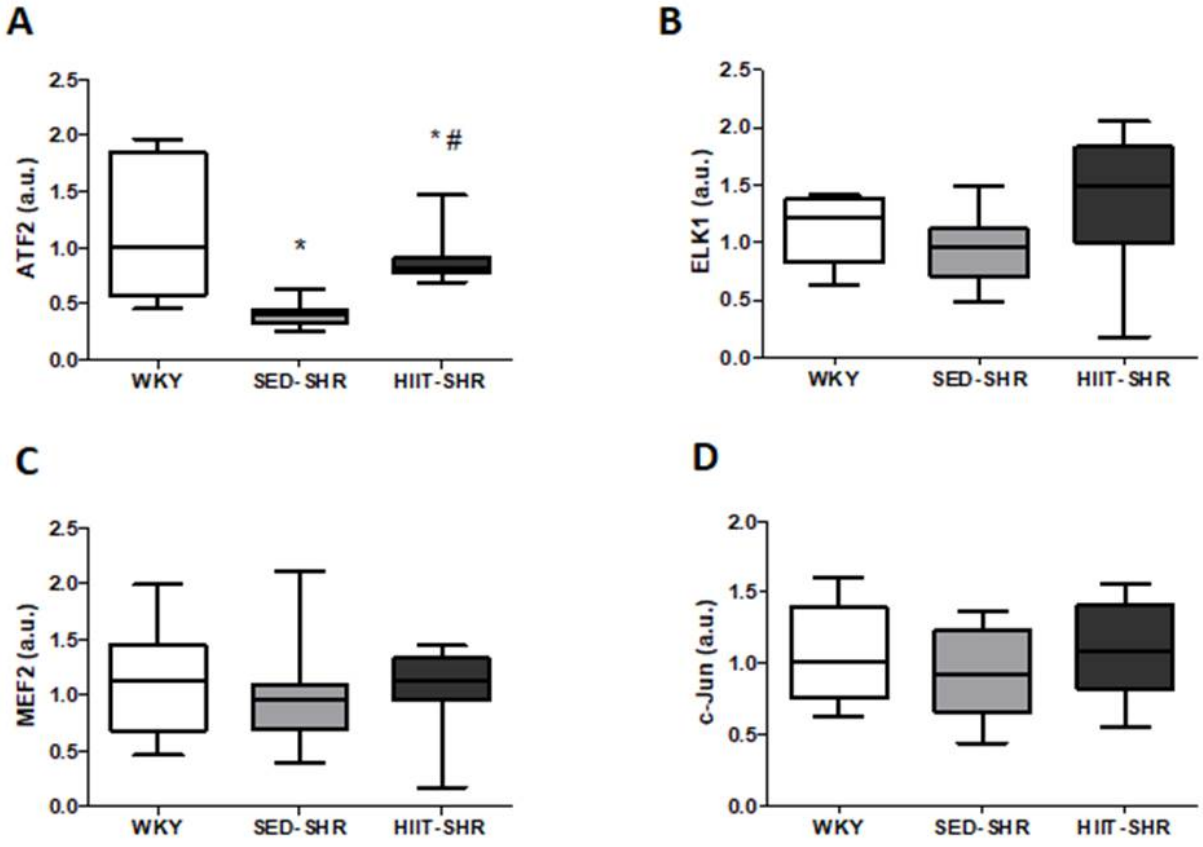

Fig. 5. Gene expression of transcription factors via MAPKs: A) ATF2, B) ELK1, C) MEF2, D) c-Jun. Sedentary hypertensive (SED-SHR, n=8), trained hypertensive (HIITSHR, n=10), and sedentary normotensive Wistar Kyoto (WKY, n=6); The box plot shows the median (line), interquartile range (box), and the maximum and minimum values (whiskers).

au=arbitrary unit. Kruskal-Wallis and Dunn's test. *$p < 0.05$ compared to WKY; # $p < 0.05$ compared to SED-SHR

4. DISCUSSION

In this study, we have demonstrated in spontaneously hypertensive rats that HIIT reduced blood pressure, increased exercise tolerance, and attenuated myocardial diastolic dysfunction. HIIT increased cardiac hypertrophy but did not lead to functional worsening. In addition, the HIIT protocol restored the expression levels of the ATF2 transcript. The main findings of this study are summarized in Fig. 6.

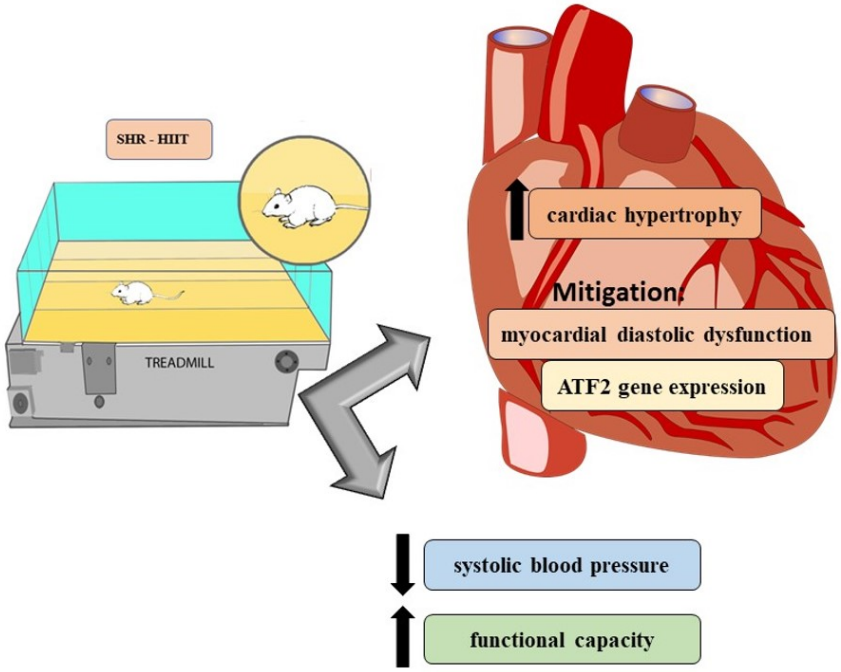

Fig. 6. Schematic figure summarizing main findings.

The SHR is an experimental model of genetic hypertension. The SHR presents early systemic arterial hypertension and, as an adaptive response, pathological LV hypertrophy (<18 months) which progresses to heart failure during maturity and senescence. (Pagan et al., 2019; Damatto et al., 2016). In the present study, HIIT training was performed in the compensated hypertension phase (12 months old) that precedes the eventual occurrence of heart failure.

Increasing evidence suggests that exercise training is beneficial in the treatment of hypertension (Cornelissen & Smart, 2013). In our study, 8-weeks of HIIT induced a

significant decrease in SBP, highlighting its benefits for reduction of cardiovascular disease risks. In contrast to our results, 4-weeks of HIIT did not reduce blood pressure in hypertensive rats (Holloway et al., 2015). These different results suggest that a higher length of HIIT is necessary to induce hypotensive effects in hypertensive rats (Shenasa & Shenasa, 2017). The progressive HIIT protocol used in our study promoted a significant improvement in functional capacity, which increases effort tolerance, a fundamental aspect and a predictor of increased survival (Li, Chen, & Zhu, 2021).

Cardiac structures and LV function were analyzed by anatomical analysis and conventional thoracic echocardiography. We observed cardiac remodeling in SHR rats that was characterized as hypertrophy (LV/tibia length, LVDD/BW, PWT, RWT, and LA/BW), which corroborates with previous animal studies that show detrimental effects of chronic pressure on hearts overloaded with hypertension (da Costa Rebelo, Schreckenberg, & Schlüter, 2012; Holloway et al., 2015). These hypertrophic indicators were also increased after HIIT. Our results are in agreement with previous studies that demonstrated HIIT-induced left ventricular hypertrophy in rodents (Kemi, Haram, Wisløff, & Ellingsen, 2004; Kemi, Loennechen, Wisløff, & Ellingsen, 2002). Although the exercise protocol used in our study induced a hypertrophic response in the LV of hypertensive rats, this structural alteration was not harmful, as the functional data of the LV did not suggest impairment or interference with systolic and diastolic functions (MFS, E/A, IVRT) (Table 1). Increased cardiac output due to aerobic training induces cardiac overload and physiological left ventricular hypertrophy, which are considered adaptive responses to maintain high cardiac performance during training (Carvalho et al., 2021). Exercise-induced cardiac hypertrophy is usually a physiological process associated with preserved or enhanced cardiac function without myocardial fibrosis, cardiomyocyte apoptosis, or altered gene expression (Oláh et al., 2016; Oláh et al., 2019).

Next, we evaluated myocardial function in isolated LV papillary muscle preparations, which allows for the measurement of myocardial contractility regardless of the alterations to cardiac load, heart rate, and ventricular chamber geometry. In our study, the HIIT protocol was able to restore the levels of RT when compared with SEDSHR group (Figure 4). Increase in RT has been demonstrated to increase myocardial collagen content in hypertensive rats (Matsubara, Matsubara, Okoshi, Franco, & Cicogna, 1997; Cezar et al., 2015). Pagan et al., suggest that exercise reduce the amount of collagen and attenuated changes in the extracellular matrix in SHR-SED, which may affect myocardial stiffness and contribute to LV dysfunction (Pagan et al., 2019). The fact that SHR-HIIT did not present these changes may

have contributed to functional variables improvement, suggesting that the training volume did not negatively affect the myocardial function.

Cellular mechanisms that contribute to the transition of increased hemodynamic loads to altered gene expression and ventricular dysfunction remain incomplete. We investigated the activation of the MAPK phosphorylation cascade leading to the translocation of the main protein kinases of this pathway: ERK, JNK and p38 (Rose et al., 2010). ERK catalyzes the phosphorylation of transcription factors (eg, Elk1 and MEF2) that stimulate protein synthesis and cell growth. JNK and p38 catalyze transcription factors (c-Jun and ATF2) that modulate myocardial apoptosis, inflammatory cytokine synthesis and fibrosis (Mutlak & Kehat, 2015). In our study, we did not observed changes in the main genes associated with MAPK signaling pathway (Figure 5); however, HIIT prevented a decrease in the gene expression of the ATF2 transcription factor observed in SHR rats (Figure 5). Interestingly, ATF-2 plays a critical role in cardiac development and is involved in cardiac pathological hypertrophy triggered by TGF-β in vitro (Monzen et al., 2001; Lim et al., 2005). A previous experimental study found that suppression of ATF2 attenuated the hypertrophic response of LV (Lim et al., 2005). Li and colleagues observed that the transverse aortic constriction (TAC) mouse model showed an increase in phosphorylation of MAPK pathway signaling in the first phase of pressure overload stimulation, whereas a decreased at the time of functional decompensation was observed (Gallo et al., 2019). Together with our results, these observations suggest that MAPK signaling might be associated with the transition from compensated hypertrophy to maladaptive hypertrophic heart failure. While speculative, our results suggest that HIIT delayed the progression between hypertension to heart failure.

The changes observed in our study, increase in cardiac hypertrophy without alteration of the LV function and MAPK pathway, is in agreement with the idea that physical training leads to the conversion of pathological hypertrophy into physiological hypertrophy (Garciarena et al., 2009). Physical training causes physiological cardiac hypertrophy, considered a favorable adaptation to the cardiovascular system, as exercise can induce mechanical stretching of the cardiac muscle in vivo (Gleeson & Baldwin, 1981). Interestingly, chronic exercise training for 4–12 weeks attenuates the activation of cardiac MAPK signaling pathways following a single exercise session. After 12 weeks of exercise training, the MAPK activation response to a single acute bout of exercise disappears, although significant cardiac hypertrophy is evident (Iemitsu et al., 2006). Thus, we proposed that 8-weeks of HIIT induced activation of multiple cardiac MAPK pathways, which was later gradually decreased with the development of exercise-induced cardiac hypertrophy. Our results suggest that chronic

exercise possibly helps to prevent an increase in myocardial p38 that is related to the development of pathological hypertrophy. (Miyachi et al., 2009; Reyes et al., 2019). Similar data in middle-aged rats showed that moderate-intensity exercise reduced ERK1/2, p38, and fibrosis fibrosis (Baghaiee, Karimi, Siahkouhian, & Pescatello, 2018).

The current study is not without limitations. The results rely exclusively on gene expression data to infer changes in the MAPK pathways. In view of this, future studies need to be conducted to better understand the mechanisms involved with the effects of hypertension and HIIT on cardiac hypertrophy related to MAPK pathways at the protein levels. Although limiting, previous studies have shown that signal transduction mediated by extracellular factors such as pressure overload results in signaling cascades, protein activation, and nuclear changes in cardiomyocytes. These results give us confidence that the changes observed here also have physiological significance. (Iemitsu et al., 2006; Liu et al., 2019). Our findings demonstrate a relevant clinical implication that HIIT may contribute to an alternative therapeutic method for patients in the early stages of hypertension by preventing blood pressure increases and cardiac dysfunction. HIIT has been widely practiced by healthy individuals and athletes as an intense exercise modality to improve physical performance and exercise tolerance.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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ANEXOS

ANEXO A - APROVAÇÃO ÉTICA

UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PEIC - Programa Especial de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado "**EFEITOS DO TREINAMENTO INTERVALADO DE ALTA INTENSIDADE NA VIA MAP-K NO CORAÇÃO DE RATOS ESPONTANEAMENTE HPERTENSOS**", cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **6589** e tendo como participante(s) **LETICIA ESTEVAM ENGEL (discente), ESTER GARCIA SANTOS (discente), INES CRISTINA GIOMETTI CEDA (docente), FRANCIS LOPES PACAGNELLI (orientador responsável)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e COMISSÃO DE ÉTICA USO DE ANIMAIS (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Este Projeto de Pesquisa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de Julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido APROVADO em reunião realizada em 09/12/2020.

MATERIAL ARMAZENADO/DOADO

Protocolo(s)	Data Aprovação	Armazenado (local)	É doação	Detalhes armazenamento
1167-2016	25/02/2016	UNOESTE	NÃO	Biofreezer na Unesp de Botucatu

Presidente Prudente, 19 de Abril de 2021.

Prof. Dr. Luiz Rodrigues Garcia Jr.
Coordenador Científico da CPDI

Profª Dra. Adriana Falco de Brito
Coordenadora da CEUA - UNOESTE

ANEXO B - NORMAS DA REVISTA

PREPARATION

NEW SUBMISSIONS

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2b. Affiliations of all authors. If necessary, use superscripted lowercase letters after the author's name to distinguish affiliations.

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Acknowledgements

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