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Mestrado Integrado

Medicina

Chronic sildenafil therapy in experimental hypertension and metabolic syndrome associated heart failure with preserved ejection fraction rat model

Sara Leite

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2019

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M.ICBAS **2019**

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Mestrado Integrado em Medicina

Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Maio de 2019

Trabalho organizado sob a forma de artigo original de acordo com as normas da revista:

Journal of Cardiovascular Pharmacology and Therapeutics
https://journals.sagepub.com/home/cpt

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Agradecimentos

A realização desta tese de mestrado contou com importantes apoios e incentivos sem os quais a sua realização não teria sido possível. Por essa razão, desejo expressar os meus sinceros agradecimentos:

Ao Professor André Lourenço, meu orientador pela competência científica e acompanhamento do trabalho, pela disponibilidade e paciência.

Ao Professor Adelino Leite Moreira, diretor do departamento de cirurgia e fisiologia da FMUP por me ter cedido todas as condições necessárias à conclusão deste percurso.

À minha família, em especial aos meus pais, irmãos e avó, pela compreensão e por acreditarem sempre em mim e naquilo que faço e por todos os ensinamentos de vida.

Ao Daniel Cruz, fiel companheiro, um agradecimento especial pelo apoio sempre presente e também pela compreensão. Por toda a motivação transmitida e ajuda na concretização dos meus objetivos pessoais.

Financiamento

Este estudo foi financiado por fundos da União Europeia (FP7-HEALTH-2010-MEDIA; ERA-CVD/0001/2016-MINOTAUR) e por fundos da Fundação para a Ciência e a Tecnologia (FCT) Portuguesa (PTDC/DTP-PIC/4104/2014, NORTE-01-0145-FEDER-000003-DocNET, e SAICT-PAC/0047/2015; 01/12/2016-NETDIAMOND, SFRH/BD/110404/2015).

Este estudo recebeu ainda uma menção honrosa nas Jornadas de Terapêutica de 2019.

Resumo

Apesar da diminuição da atividade da proteína quinase G ter sido proposta como um potencial alvo terapêutico na insuficiência cardíaca com fração de ejeção preservada (ICFEP), os resultados dos ensaios clínicos com inibidores da fosfodiesterase tipo 5 (iFDE5) foram neutros. No entanto, permanece a dúvida se estes iFDE5 seriam benéficos em subgrupos de doentes com ICFEP. O nosso objetivo, com este trabalho foi assim, testar uma terapêutica crónica com sildenafil em ratos jovens de ZSF1 obesos machos, que representam um modelo animal de ICFEP associada a hipertensão severa e síndrome metabólico. Para este efeito, ratos machos ZSF1 obesos com dezasseis semanas de idade, foram aleatoriamente distribuídos de forma a receberem SIL 100mg.Kg^{-1}.d^{-1} dissolvido na água que bebiam (ZSF1 Ob SIL, n=8), ou foram integrados no grupo placebo (ZSF1 Ob PL, n=8). Um grupo de ratos Wistar-Kyoto, foi usado como controlo (WKY, n=8). Quatro semanas depois os animais foram submetidos a testes de esforço e resistência, avaliação hemodinâmica, foram feitas preparações com anéis aórticos e foi feita uma quantificação dos níveis de adenosina trifosfato (ATP) no miocárdio por cromatografia líquida de alta eficiência. Os ratos ZSF1 Ob PL exibiram hipertensão sistémica, rigidez aórtica, distúrbios do relaxamento e um aumento da rigidez diastólica do ventrículo esquerdo (VE), associado a uma fração de ejeção e índice cardíaco preservado. Estes ratos, também apresentaram uma resistência ao esforço diminuída e um pico de consumo de oxigénio e quociente respiratório aumentados, sugerindo uma maior dependência pelo metabolismo anaeróbio. Adicionalmente a quantificação dos níveis de ATP apresentou-se diminuída. O tratamento crónico com sildenafil foi capaz de atenuar a hipertensão e diminuir a rigidez do VE, aumentar a tolerância ao esforço com um aumento simultâneo do pico de oxigénio consumido e aumentar os níveis de ATP no miocárdio do VE. Assim concluímos, que o tratamento crónico com sildenafil neste modelo animal de jovens machos com ICFEP associada a várias comorbilidades mal controladas, apresenta efeitos benéficos cardiovasculares que suportam a realização de ensaios clínicos randomizados em subgrupos de doentes com ICFEP associada a características similares.

Palavras-chave: hipertensão; síndrome metabólico; insuficiência cardíaca com fração de ejeção preservada; inibidores da fosfodiesterase tipo-5.

Abstract

Although decreased protein kinase G (PKG) activity was proposed as potential therapeutic target in heart failure with preserved ejection fraction (HFpEF), randomized clinical trials (RCTs) with type-5 phosphodiesterase inhibitors (PDE5i) showed neutral results. Whether specific subgroups of HFpEF patients may benefit from PDE5i remains to be defined. Our aim was to test chronic sildenafil (SIL) therapy in the young male ZSF1 obese rat model of HFpEF with severe hypertension and metabolic syndrome. Sixteen-week-old male ZSF1 obese rats were randomly assigned to receive SIL 100mg.Kg^{-1}.d^{-1} dissolved in drinking water (ZSF1 Ob SIL, n=8), or placebo (ZSF1 Ob PL, n=8). A group of Wistar-Kyoto rats served as control (WKY, n=8). Four weeks later animals underwent peak and endurance effort tests, hemodynamic evaluation, aortic ring preparation, and myocardial adenosine triphosphate (ATP) quantification by ultra-high-performance liquid chromatography. ZSF1 Ob PL rats showed systemic hypertension, aortic stiffening, impaired left ventricular relaxation and increased left ventricle (LV) diastolic stiffness, with preserved ejection fraction and cardiac index. Their endurance capacity was decreased as assessed by maximum workload and peak oxygen consumption and respiratory quotient were increased, denoting more reliance on anaerobic metabolism. Additionally, myocardial ATP levels were decreased. Chronic SIL treatment attenuated hypertension and decreased LV stiffness, modestly enhancing effort tolerance with a concomitant increase in peak oxygen consumption and LV myocardial ATP levels. Chronic SIL therapy in this model of HFpEF of the young male with extensive and poorly controlled comorbidities has beneficial cardiovascular effects which support RCTs in HFpEF patient subgroups with similar features.

Keywords: hypertension; metabolic syndrome; heart failure with preserved ejection fraction; type-5 phosphodiesterase inhibitors.

Lista de Abreviaturas

$\dot{V}O_{2^{max}}$	*maximum O_2 consumption*
$\dot{V}O_2$	*O_2 consumption*
2-CA	*2-Chloro-Adenosine*
ATP	*Adenosine Triphosphate*
BSA	*Body Surface Area*
CO	*Cardiac Output*
HFpEF	*Heart Failure with preserved Ejection Fraction*
L_0	*Slack Length*
LV	*Left Ventricle*
NO	*Nitric Oxide*
Ob	*Obese*
PDE5i	*Type 5 phosphodiesterase inhibitors*
PKG	*Protein kinase G*
PL	*Placebo*
RCTs	*Randomized Clinical Trials*
RQ	*Respiratory Quotient*
SIL	*Sildenafil*
uHPLC	*ultra-High-Performance Liquid Chromatography*
WKY	*Wistar-Kyoto*
ε	*Strain*

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Chronic sildenafil therapy in experimental hypertension and metabolic syndrome associated heart failure with preserved ejection fraction rat model

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Short Title: Chronic sildenafil in experimental heart failure with preserved ejection fraction

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Introduction

Population ageing, caused an increase of comorbidities such as the metabolic syndrome, diabetes mellitus and hypertension and improved care and outcomes of ischemic heart disease have jointly contributed to a change in heart failure epidemiology, since most of heart failure patients now present with preserved ejection fraction (HFpEF)[1]. HFpEF is a syndrome of complex pathophysiology and considerable heterogeneity in which the unifying feature seems to be impaired cardiovascular reserve to any form of hemodynamic stress including exercise which heralds effort intolerance and acute lung edema[2, 3]. Some authors have proposed chronic pro-inflammatory state, endothelial dysfunction and decreased protein kinase G (PKG) activity as unifying pathways that lead to increased cardiovascular stiffness, the hallmark of HFpEF[4]. Type 5 phosphodiesterase inhibitors (PDE5i) that potentiate cyclic guanosine monophosphate effects and PKG activity were consequently investigated as therapeutic targets. Off-label administration of PDE5i attenuated pulmonary hypertension secondary to HFpEF[5], chronic and acute experimental therapy in animal models also reduced cardiovascular remodeling[6] and improved diastolic function[7], respectively. However, the results obtained in a larger heterogeneous population of HFpEF patients were discouraging in the RELAX randomized clinical trial[8]. From many potential explanations for neutral outcomes, the fact that HFpEF remains a syndrome that encompasses a largely heterogeneous set of patient phenotypes that warrant clustering and tailored therapeutic strategies remains the most consensual hypothesis. Still, steps to patient cluster tailored therapy are yet to be taken[9, 10].

Recently, we characterized an experimental model of HFpEF characterized by left ventricular and aortic stiffening with effort intolerance associated with severe metabolic syndrome and systemic arterial hypertension in young ZSF1 obese rats[11-13]. This model mimics a subset of young male HFpEF patients in whom the toll of poorly controlled or previously untreated comorbidities is high. This seems to be a growing group amongst HFpEF patients[14]. The aim of the present work is to evaluate the effect of chronic PDE5i therapy on exercise capacity, vascular function, and left ventricle (LV) hemodynamics and bioenergetics in such an experimental model.

Methods

Animal model

Nine-week-old male Wistar Kyoto (WKY, n=8) and ZSF1 obese rats (ZSF1 Ob, n=16; Charles River, Barcelona, Spain) were kept in individually ventilated chambers and fed *ad libitum* with recommended diet (LabDiet® 5008, International Product Supplies Ltd.), in a controlled environment with a 12:12h light-dark cycle at 22ºC room temperature. At their 16th week of life, ZSF1 Ob were randomized to receive either sildenafil 100 mg.Kg^{-1}.d^{-1} (Sildenafil citrate, PF-01000000-10, Pfizer, Inc.) dissolved in the drinking water (ZSF1 Ob SIL) or placebo (ZSF1 Ob PL). On the 20th week, all animals underwent peak effort and endurance test, hemodynamic evaluation, and samples were collected for aortic ring preparation and LV myocardial adenosine triphosphate (ATP) level quantification. Sildenafil administration was interrupted 24h prior to hemodynamic evaluation and sample collection. All animals received humane care. Experimental procedures complied with the Faculty of Medicine of Porto guidelines, Portuguese law on animal welfare, EU Directive 2010/63/EU for animal experiments and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 2011).

Hemodynamic evaluation

Animals were sedated and received analgesia by intraperitoneal injection of midazolam 5 mg.kg^{-1} and fentanyl 100 µg.kg^{-1}, respectively, and were then anesthetized with sevoflurane (8% for induction and 2,5-3% for maintenance; Penlon Sigma Delta), orotracheally intubated and mechanically ventilated to achieve normocapnia (TOPO, Kent scientific). Fluid replacement with warm Ringer's lactate 32 mL.kg^{-1}.h^{-1} (NE-1000, New Era Pump Systems) was instituted through a dorsal foot vein, rectal temperature was held at 38 ºC on a heating pad and electrocardiogram was continuously recorded. Surgical preparation consisted of a left thoracotomy, apical insertion of a pressure-volume catheter in the LV (SPR-847, Millar Instruments) and placement of an ascending aorta flow probe (2.5PS, Transonic) for continuous real-time cardiac output (CO) measurement. Data was continuously acquired (MPVS 300, Millar Instruments), recorded at 2000 Hz (ML880 PowerLab 16/30, AD instruments) and analysed offline (PVAN 3.5, Millar Instruments). Hemodynamic recordings were made after a stabilization period of 15 min, with ventilation held at end-expiration. Transient inferior vena cava occlusions were obtained to derive end-systolic and end-diastolic pressure-volume relationships. Systemic arterial pressure was determined by advancing the LV catheter into the aorta. Volume signal was calibrated for parallel conductance by 40-60 µL 10% saline injection and for slope factor α by simultaneous measurement of CO by the flowmeter. Upon experiment completion, animals were euthanized

by exsanguination under anesthesia. Blood samples were collected, organs were excised and weighed, and tibia length was measured. Tissue samples were snap frozen in liquid nitrogen and stored at -80˚C. Organ weights were normalized to tibial length, considering the differences observed in body weight between groups. Time constant of isovolumic relaxation was computed by the monoexponential method. Systemic vascular resistance was computed neglecting central venous pressure. To account for large differences in body weight between groups, volumes were indexed for body surface area (BSA) as estimated by 9.1*body weight in grams$^{2/3}$.

Aortic ring preparation

Two 1.5 mm rings were isolated from ascending aorta samples and mounted between metal pins in an organ bath system (770MO, Danish Myo Technology). After stabilization, a passive-length tension relationship was obtained by progressive monoaxial stretching of the vascular rings, at intervals of 20%, from slack length (L_0) up to 200% of L_0. Strain (ε) was defined as percent increase in length from L_0. Exponential fitting of the curve enabled derivation of vascular stiffness constant. By dose-response curves we assessed vascular reactivity to phenylephrine (10^{-9} to 10^{-5}, at 0.5 log unit intervals) and then endothelial function by responsiveness to acetylcholine and direct vasodilator response to nitric oxide (NO) donor sodium nitroprusside (both, 10^{-9} to 10^{-4}, at 0.5 log unit intervals) with relaxation assessed as percentual decrease of tension after pre-contraction with phenylephrine.

Peak effort test and endurance capacity

Effort testing with maximum O_2 consumption ($\dot{V}O_{2^{max}}$) determination was performed on a closed-chamber treadmill with a stimulation grid coupled to a gas analyser (LE8700C and LE405, Panlab ®, Harvard Apparatus). The fresh air flow was 700 mL.min^{-1} and treadmill inclination was 10̊. Animals were weighted at the beginning and end of experiments while O_2 and CO_2 concentrations, velocity, distance and number of shocks were continuously recorded. Tests were interrupted whenever animals became incapable to maintain the rhythm and stayed on the stimulation grid longer than 3s. Each animal underwent a peak effort test and an endurance test separated by 24h. After an initial adaption period at 15 cm/s, peak effort testing to assess $\dot{V}O_{2^{max}}$ consisted of changing velocity to 30 cm/s and then stepping up 5 cm/s every 60s, whereas endurance testing consisted of stepping up velocity by 5 cm/s every 15 min to assess fatigue. Data analysis was performed with Metabolism software (version 2.2.01, Panlab ®, Harvard Apparatus).

Myocardial ATP levels

LV myocardium (25-60 mg) was homogenized and proteins precipitated in 10% methanol. An additional volume of ultrapure water with 5% 2-chloro-adenosine (2-CA) was added. The sample was then left on ice for 10 min and vortexed every 2 min in order to solubilize adenine nucleotides after which it was centrifuged at 16000g (-5 °C) for 20 min (Mikro 200R, Zentrifugen). The precipitate was stored at -80 ºC for total protein quantification by Bradford's method while the supernatant was dehydrated in a liquid nitrogen concentrator for 2 to 4 hours and then rehydrated on ice with 700 µL of mobile phase (100 mM KH_2PO_4, 1,5 mM TBA, 40 % methanol, pH=6,5 adjusted with KOH). Samples (20 µL) then underwent ultra-high-performance liquid chromatography at 4 ºC (uHPLC, Flexar FX-10 Ultra High Performance LC 10,000 PSI, PerkinElmer) with a 3 µm particle specially treated octadecyl-coupled silica surface column (SupelcosilTM LC18-T column, 15 cm x 4.6 mm, Sigma-Aldrich). Separation was carried out at 1 mL/min and 2800-4200 psi by reversed-phase ion pairing. Optical density of ATP was recorded at 256 nm. Quantification was performed based on standard curves for ATP and 2-CA as internal control for extraction efficiency (Chromera® software, version 3.2.0, Perkin Elmer). All chemical products were obtained of Sigma-Aldrich (Sintra, Portugal).

Statistical analysis

Assumption of normality was checked by Shapiro-Wilk's test and of homogeneity of variances by Levene's test. When assumptions were met data were analysed by parametric one-way ANOVA. Otherwise they were analysed by non-parametric Kruskal-Wallis test. Post hoc comparisons were performed with Student-Newman-Keuls method. Coefficients derived by regression in pressure-volume hemodynamic analysis and passive length-tension curves of aortic rings were compared between groups with analysis of covariance. Differences were considered significant at two-tailed $P < 0.05$. Data are mean ± SEM or median (interquartile range) according to distribution.

Results

Hemodynamics and morphometrics

ZSF1 Ob rats showed larger body weights, LV weights and lung weights which were unchanged upon chronic sildenafil therapy (Table 1). ZSF1 Ob rats also showed systemic arterial hypertension, elevated systemic vascular resistance and elevated afterload which were significantly attenuated by sildenafil as assessed by mean arterial pressure, systemic vascular resistance index and effective arterial elastance, respectively. No differences were observed between groups regarding heart rate, cardiac index, end-diastolic volume or ejection fraction. Systolic function indexes such as maximum rate of pressure rise and the slope of the end-systolic pressure-volume relationship (end-systolic elastance) were increased in both ZSF1 Ob groups but preload recruitable stroke work, which is a geometry and chamber size-independent index of contractility was unchanged as well as ventricular-vascular coupling (Table 2). Untreated ZSF1 Ob showed delayed relaxation, elevated end-diastolic pressures and upward-shifted end-diastolic pressure volume relationship which were attenuated by sildenafil (Representative loops in Figure 1).

Vascular Function

ZSF1 Ob presented an upward shifted passive length-tension relationship compared with WKY denoting higher aortic stiffness (Fig. 2, panel A), which was attenuated by sildenafil. Developed tension and receptor sensitivity to phenylephrine was increased while endothelium-dependent vasodilation induced by acetylcholine and response to direct vasodilation by NO donor sodium nitroprusside was impaired in both ZSF1 Ob groups (Table 3 and Figure 2, panels B-D).

Effort tolerance and myocardial ATP levels

ZSF1 Ob showed lower $\dot{V}O_{2}{}^{max}$ and maximum workload compared with WKY without a significant effect of SIL in peak effort testing (28 ± 1, 14 ± 1 and 15 ± 1 mL.min^{-1}.Kg^{-1} for $\dot{V}O_{2}{}^{max}$, and 87 ± 7, 46 ± 3 and 48 ± 4 m.Kg^{-1} for maximum workload, in WKY, ZSF1 Ob PL and ZSF1 Ob SIL, respectively). Contrastingly, in endurance effort testing, sildenafil improved maximum workload, the peak $\dot{V}O_{2}$ achieved at maximum workload and also attenuated the rise in respiratory quotient (RQ) (Figure 3, panels A-C). Myocardial ATP levels were decreased in ZSF1 Ob PL compared with WKY and restored by sildenafil in ZSF1 Ob SIL (Figure 3, panel D). No differences were found in 2-CA concentration and protein quantities between groups (33 ± 5, 45 ± 5 and 36 ± 4 µg.mL, for 2-CA, and 1.6 ± 0.3, 2.2 ± 0.3 and 1.9 ± 0.2 mg, for proteins, in WKY, ZSF1 Ob PL and ZSF1 Ob SIL, respectively).

Discussion

With this work, we described the effects of sildenafil on vascular function, hemodynamic status, effort tolerance and myocardial bioenergetics in the ZSF1 Obese rat model of HFpEF, which mimics a subset of young male HFpEF patients with poorly controlled comorbidities.

RCTs of sildenafil in HFpEF are contradictory. While Guazzi *et al*. showed positive effects in HFpEF patients with pulmonary hypertension[5], the RELAX-trial enrolling 216 patients with HFpEF diagnosis had disappointing results on exercise capacity, clinical status, quality of life, LV remodeling or diastolic function[8]. Author's possible explanations are the higher levels of hypertension and LV mass as well as the concomitant presence of pulmonary hypertension in Guazzi's study[8]. Furthermore, in the RELAX trial the effects on exercise capacity were hampered by an abnormally high proportion of patients with chronotropic incompetence and an inferior dose and duration of therapy. More recently, the potential role of mitochondrial dysfunction and endoplasmic reticulum stress caused by sildenafil[15] and pharmacogenomics of liver metabolizing enzymes[16] were pointed out as other possible explanations. Nevertheless, the lack of therapeutic success in HFpEF has been largely attributed to the fact that this syndrome groups a vast heterogeneity of patient phenotypes that is still far from resolved and therapeutic strategies should be personalized according to an eagerly awaited effective clustering of these patients[9]. Indeed, in more homogeneous patient groups such as severe symptomatic aortic stenosis with preserved ejection fraction and resistant hypertension sildenafil showed favourable hemodynamic effects, such as decreased pulmonary capillary wedge pressure and improved LV stroke volume[17] and reduced blood pressure and peripheral vascular resistance with improved diastolic function[18], respectively, suggesting that in selected HFpEF cases there may be some clinical benefit. The ZSF1 obese rat model is a young male model of severe uncontrolled and untreated systemic arterial hypertension and metabolic syndrome and mimics one of the subgroups of HFpEF patients that is increasing in prevalence[14]. In this model chronic treatment with sildenafil was able to reduce systemic arterial hypertension, ameliorate diastolic LV function and modestly improve peak $\dot{V}O_2$ and maximum workload during endurance effort. Additionally, we observed direct beneficial myocardial bioenergetic effects, such as restored ATP levels.

Attenuation of systemic arterial hypertension, systemic vascular resistance and aortic stiffness by sildenafil were expected while the absence of effect in endothelial dysfunction and response to NO as assessed by vascular response to acetylcholine and nitroprusside, respectively, may be explained by drug withholding 24h before evaluation and are in accordance with the lack of improvement in endothelial function, assessed with the non-invasive flow-mediated dilation

method in brachial artery in hypertensive patients[18]. Chronically decreased afterload and myocardial effects of sildenafil may explain improved relaxation, decreased end-diastolic pressure and improved LV compliance. Of note, no differences were found in LV or lung weights suggesting either a minor role in remodeling or an insufficient duration of therapy to achieve anti-hypertrophic effects. Sildenafil therapy modestly improved peak $\dot{V}O_2$ and effort capacity during endurance suggesting that hemodynamic benefits may partly translate into improved functional performance. Nevertheless, the determinants of exercise capacity in HFpEF are complex and seem to be mainly determined by systemic inflammation and skeletal muscle mass[19] which may partly explain the discreet improvement of only around 10% in both peak $\dot{V}O_2$ and workload. Alternatively, a longer course of therapy might have achieved better results in endurance effort testing. Interestingly, chronic sildenafil therapy also prevented the rise in RQ suggesting enhanced oxidative metabolism of fatty acids and restored myocardial ATP levels. These metabolic and bioenergetic effects may be partly due to direct myocardial effects of sildenafil. Indeed, improved oxidative phosphorylation and mitochondrial function involving multiple subcellular pathways were reported in ischemia and genetic cardiomyopathy models[20, 21] and preconditioning with sildenafil improved functional recovery of the rat heart after cardioplegic arrest which was abolished by ATP-sensitive mitochondrial K^+ channel blockade[22].
Myocardial bioenergetic impairment in HFpEF has been reported with P^{31} cardiac magnetic resonance spectroscopy showing decreased phosphor-creatine content with or without concomitant ATP reduction and proposed by Phan *et al*. to be one of the determinants of effort intolerance and pulmonary congestion in HFpEF[23]. In heart failure progression, cardiomyocytes undergo a series of metabolic changes, which limit the ability to adapt to variations in substrate bioavailability and favour glucose usage by non-oxidative pathways instead of fatty acids [24]. In parallel, cardiomyocyte energy stores become depleted[25]. Although this is far from established, multiple mechanisms link bioenergetic depletion to cardiac dysfunction and it is appealing to assume that impaired bioenergetics partly underlie myocardial dysfunction[26]. In this work we demonstrate that myocardial ATP levels are indeed reduced in the ZSF1 obese rat model of HFpEF through a precise analytical laboratory method. The levels of ATP measured by the uHPLC procedure in healthy control rats conform with previous reports[27]. All care was taken to snap freeze samples in liquid nitrogen as fast as possible upon collection and to carry out all experimental steps at negative temperatures to preserve the stability of adenosine nucleotides. We did not observe differences in the amounts of proteins or 2-CA between groups, which ensures the robustness of the experimental methodology.
In conclusion, chronic treatment with sildenafil reduced blood pressure, systemic vascular resistance and aortic stiffness, it improved myocardial diastolic function and compliance as well

as modestly restored endurance effort capacity and peak $\dot{V}O_2$ in the ZSF1 obese rat model of HFpEF. A model which mimics a subset of younger male patients with HFpEF in whom the role of poorly controlled or untreated comorbidities seems to dominate pathophysiology. The clinical translation to young patients with metabolic syndrome, refractory hypertension, or other similar subgroups of HFpEF patients remains to be established and should be investigated.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This study was supported by grants from the European Union (FP7-HEALTH-2010-MEDIA; ERA-CVD/0001/2016-MINOTAUR), grants from Portuguese Foundation for Science and Technology (PTDC/DTP-PIC/4104/2014, NORTE-01-0145-FEDER-000003-DocNET, and SAICT-PAC/0047/2015; 01/12/2016-NETDIAMOND) and by Portuguese Foundation for Science and Technology Grant SFRH/BD/110404/2015 (to S. Leite).

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Tables

Table 1*- Morphometry.*

	WKY	**ZSF1 Ob PL**	**ZSF1 Ob SIL**
Body weight, *g*	345 ± 8	601 ± 4*	600 ± 5*
TL, *mm*	38 ± 01	40 ± 1*	40 ± 1*
LV weight/TL, *mg.mm^{-1}*	31.9 ± 1.2	37.4 ± 1.0*	36.6 ± 0.8*
Lung weight/TL, *mg.mm^{-1}*	37.5 ± 1.7	59.2 ± 3.9*	62.3 ± 3.1*

TL, tibial length; LV, left ventricle. Values are mean ± SEM, n=8 *per* group. *P < 0,05 *vs* WKY by one-way ANOVA.

Table 2 - *Hemodynamics.*

	WKY	**ZSF1 Ob PL**	**ZSF1 Ob SIL**
BSA, cm^2	455 ± 5	651 ± 4*	647 ± 4*
HR, min^{-1}	335 (290-367)	317 (291-356)	322 (303-351)
MAP, *mmHg*	107 ± 13	165 ± 4*	150 ± 4*†
SVRI, $mmHg.min^{1}.cm^{2}.\ \mu L^{-1}$	0.78 (0.65-1.11)	1.21 (1.15-1.50)*	1.00 (0.99-1.11)†
EDP, *mmHg*	6 ± 1	13 ± 1*	10 ± 1*†
dP/dt_{max}, $mmHg.s^{-1}$	8790 ± 660	12000 ± 510*	11000 ± 510*
τ, *ms*	8.8 (7.8-9.8)	12.5 (9.6-13.2)*	11.2 (9.8-12.0)
CI, $\mu L.min^{-1}.cm^{-2}$	127 ± 8	133 ± 6	141 ± 4
EF, *%*	60 (49-68)	62 (44-71)	62 (57-71)
EDV_i, $\mu L.cm^{-2}$	0.66 ± 0.04	0.74 ± 0.05	0.72 ± 0.04
E_{ai}, $mmHg.\mu L^{-1}.cm^{-2}$	253 (223-331)	448 (344-517)*	387 (337-411)*†
E_{esi}, $mmHg.\mu L^{-1}.cm^{-2}$	252 ± 33	369 ± 55*	367 ± 19*
VVC	0.96 ± 0.12	0.87 ± 0.13	1.02 ± 0.07
PRSW, *mmHg*	73 ± 6	88 ± 14	58 ± 4
β_i, $\mu L^{-1}.cm^{-2}$	4.4 ± 0.6	7.8 ± 0.9*	5.8 ± 0.4

BSA, body surface area; HR, heart rate; MAP, mean arterial pressure; SVRI, systemic vascular resistance for indexed volumes; EDP, end-diastolic pressure; dP/dt_{max}, maximum rate of pressure rise; *τ,* time-constant of isovolumic relaxation by the monoexponential method ; CI, cardiac index; EF, ejection fraction; EDV_i, end-diastolic volume for indexed volumes; E_{ai}, effective arterial elastance for indexed volumes; E_{esi}, end-systolic elastance for indexed volumes or end-systolic pressure-volume relationship slope; VVC, ventricular-vascular coupling by E_a/E_{es}; PRSW, preload recruitable stroke work; β_i, left ventricular chamber stiffness constant derived from end-diastolic pressure-volume relationships. BSA was estimated as $9.1 \cdot (\text{BW in g})^{2/3}$. Values are mean ± SEM, n=8 *per* group. $^{*}P < 0,05$ *vs* WKY; $^{\dagger}P < 0,05$ *vs* ZSF1 Ob PL, by one-way ANOVA.

Table 3 – *Vascular function*

		WKY	ZSF1 Ob PL	ZSF1 Ob SIL
Aortic rings				
	Phenylephrine			
F_{max}, mN/mm		21 ± 1	25 ± 1*	26 ± 1*
EC_{50}, |log M|		6.7 ± 0.1	7.4 ± 0.1*	7.3 ± 0.1*
	Acetylcholine			
$Relaxation_{max}$, %		75 ± 5	24 ± 3*	21 ± 2*
EC_{50}, |log M|		7.2 ± 0.2	6.8 ± 0.1	6.8 ± 0.1
	Nitroprusside			
$Relaxation_{max}$, %		87 ± 4	58 ± 3*	51 ± 2*
EC_{50}, |log M|		7.4 ± 0.2	7.2 ± 0.4	7.3 ± 0.1*

F_{max}, maximum developed force; EC_{50}, half maximal effective concentration. Values are mean ± SEM, n=8 *per* group. $^{*}P < 0,05$ *vs* WKY by one-way ANOVA.

Figure legends

Figure 1. ***Representative pressure-volume loops of Wistar-Kyoto control animals (WKY, light grey lines), placebo-treated ZSF1 obese (ZSF1 Ob PL, darker grey lines) and sildenafil-treated ZSF1 obese rats (ZSF1 Ob SIL, black lines).*** Transient inferior vena cava occlusions, linear end-systolic pressure-volume relationship and exponential end-diastolic pressure-volume relationship is represented. In the basal loop, effective arterial elastance (E_a) is plotted (dashed lines). In the insert on the lower right, the end-diastolic pressure-volume relationship is shown in detail. $^{*}P < 0.05$ *vs* WKY by one-way ANOVA, n=8 *per* group.

Figure 2. ***Passive-length tension relationship (panel A), contraction with phenylephrine (panel B) and subsequent relaxation with acetylcholine (panel C) and sodium nitroprusside (panel D) in Wistar-Kyoto (WKY, white circles, light grey dashed lines), placebo-treated ZSF1 obese (ZSF1 Ob PL, dark grey squares, dark grey dash-dotted lines) and sildenafil-treated ZSF1 obese rats (ZSF1 Ob SIL, black triangles, solid black line).*** In panel A, vascular stiffness constant derived from exponential fitting is shown as insert in point-plot/box-plot format. Half-maximum effective dose (ED50) is presented as dotted vertical lines on panels B-D. In panels C and D vasodilatory response is reported as percent relaxation from maximum developed force upon phenylephrine. For detailed results please refer to table 3. ε, strain; L, length; L_0, slack length. Values are mean ± SEM, n=8 *per* group. $^{*}P < 0.05$ *vs* WKY; $^{\dagger}P < 0.05$ *vs* ZSF1 Ob PL by Kruskal-Wallis with *post hoc* comparisons by Student-Newman-Keuls.

Figure 3. ***Endurance effort test*** **(panels A-C) and myocardial ATP levels (paned D)** ***in Wistar-Kyoto (WKY, white symbols and bars), placebo-treated ZSF1 obese (ZSF1 Ob PL, grey symbols and bars) and sildenafil-treated ZSF1 obese rats (ZSF1 Ob SIL, black symbols and bars).*** Peak $\dot{V}O_2$ (panel A), maximum workload (panel B), and maximum respiratory quotient achieved during endurance effort testing before fatigue (panel C) are represented alongside myocardial ATP levels (panel D). $^{*}P < 0.05$ *vs* WKY; $^{\dagger}P < 0.05$ *vs* ZSF1 Ob PL by one-way ANOVA or Kruskal-Wallis with *post hoc* comparisons by Student-Newman-Keuls, n=8 *per* group.

Figures

Figure 1

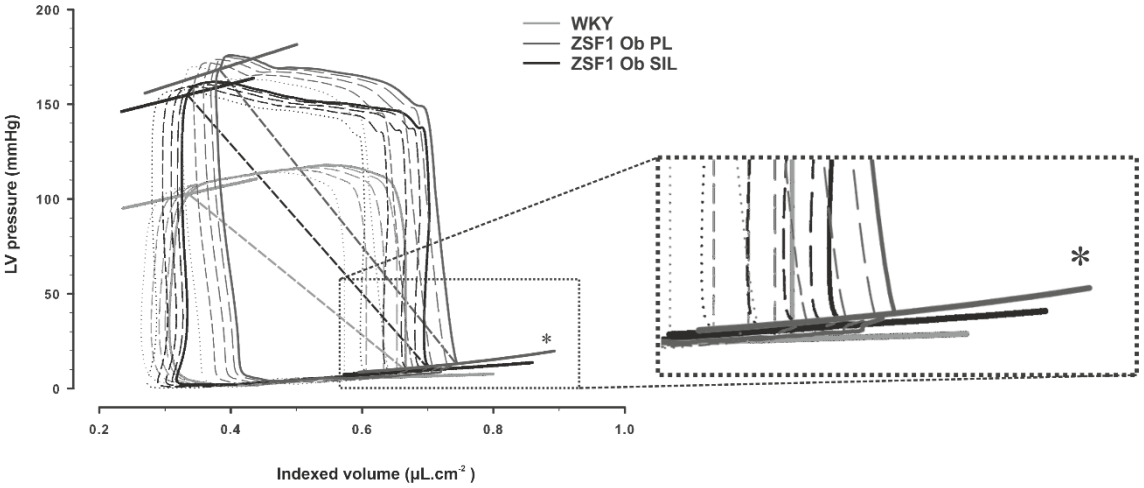


Figure 2

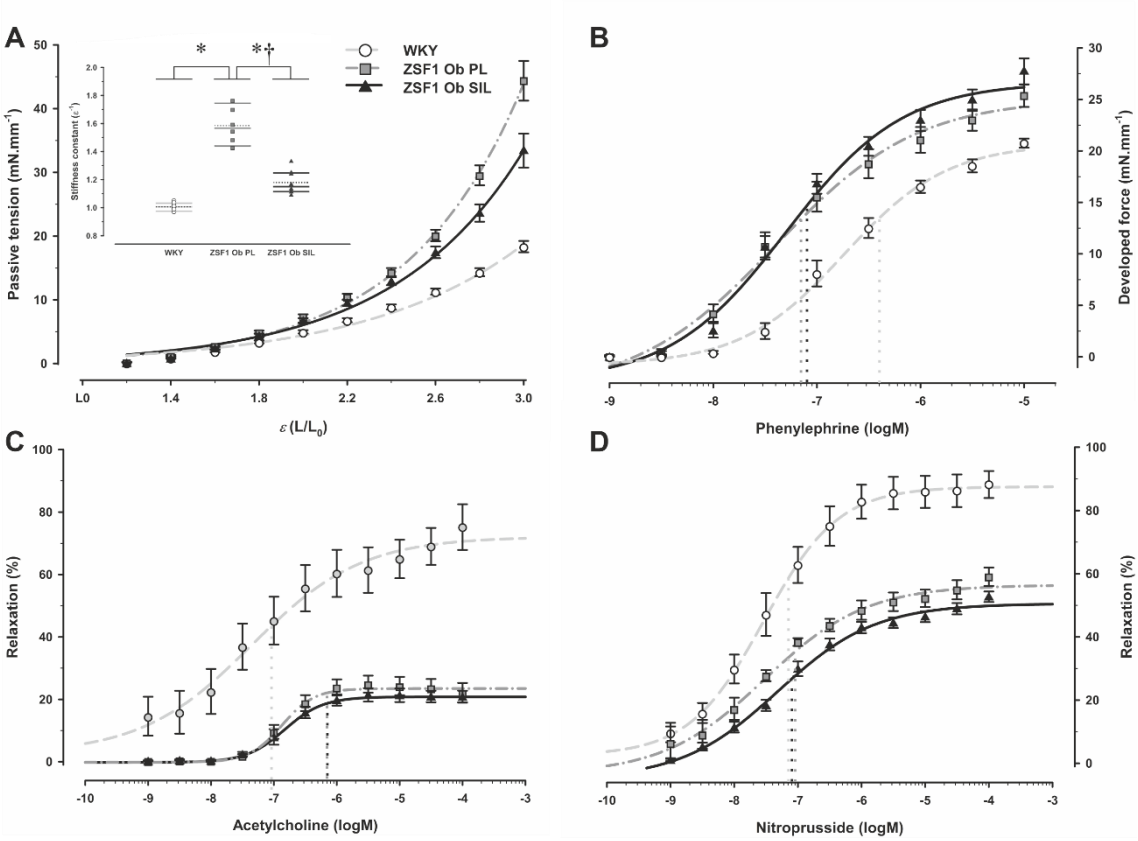
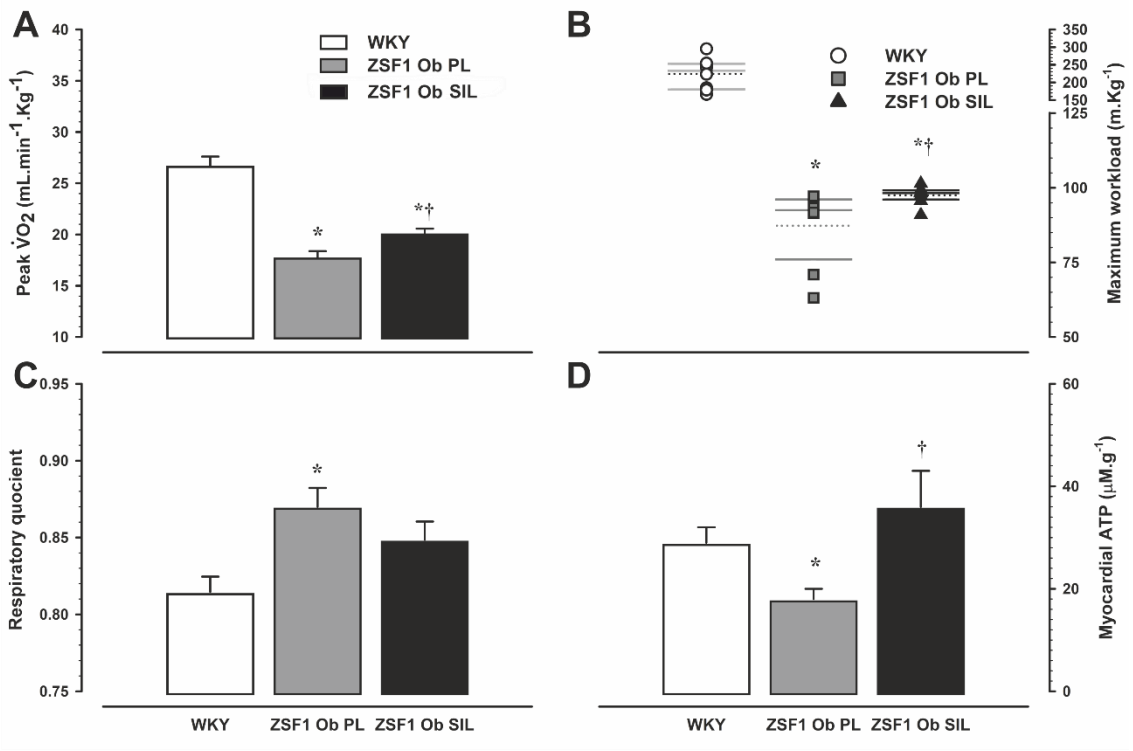

A
B
C
D
WKY
ZSF1 Ob PL
ZSF1 Ob SIL
Passive tension (mN.mm-1)
ε (L/L0)
Stiffness constant (ε-1)
Developed force (mN.mm-1)
Phenylephrine (logM)
Relaxation (%)
Acetylcholine (logM)
Nitroprusside (logM)

Figure 3


A
B
C
D
WKY
ZSF1 Ob PL
ZSF1 Ob SIL
Peak $\dot{V}O_2$ (mL.min^{-1}.Kg^{-1})
Maximum workload (m.Kg^{-1})
Respiratory quocient
Myocardial ATP (µM.g^{-1})
*
*†
†
WKY
ZSF1 Ob PL
ZSF1 Ob SIL

Resumo Biográfico e Curricular

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Bolsas e Prémios

Menção Honrosa nas Jornadas de Terapêutica de 2019, com o trabalho: Terapêutica crónica com Sildenafil na hipertensão arterial e síndrome metabólico experimentais associados a insuficiência cardíaca com fração de ejeção preservada.

Prémio melhor Poster no Congresso Português de Aterosclerose em 2018, com o trabalho: Disfunção e remodelagem arterial num modelo experimental de insuficiência cardíaca com fração de ejeção preservada.

Bolsa de Doutoramento concedida pela FCT em 2015, com o projeto "Modulation of myocardial diastolic stiffness by stretch. New physiological mechanism, diagnostic and therapeutic implications in heart failure with preserved ejection fraction".

Bolsas de Estudo por Mérito, pelo aproveitamento escolar excecional no mestrado em Fisiopatologia Cardiovascular no ano letivo 2013/2014 e 2014/2015.