UNIVERSITY OF PORTO

FACULTY OF SPORT

LONG-TERM ENDURANCE EXERCISE TRAINING PROVIDES A CARDIOPROTECTIVE PHENOTYPE AGAINST AGE-INDUCED CARDIAC DYSFUNCTION

Academic dissertation submitted with the purpose of obtaining a master degree in Physical Activity for the Elderly, under the decree-law 74/2006 from March 24, supervised by Professor Doctor José Alberto Ramos Duarte and Doctor Daniel Moreira Gonçalves.

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Key words: Regular physical activity; Sedentary behaviour; Cardiac dysfunction; Mitochondrial dysfunction; Cardioprotection.

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To my family

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Honestly, this journey was a concretization of a desire and a realization of a dream. Not many people have the opportunity to experience some dreams in their lifetime, fortunately life bought me this present and now that is ending, I can say that was unforgettable and a life changing experience.

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…and to all of you I genuinely wish… Love and Peace.

TABLE OF CONTENTS

RESUMO

O envelhecimento é um processo complexo e os seus efeitos culminam numa perda progressiva de funcionalidade do organismo. É comummente aceite que o sistema cardiovascular, mesmo com ausência de doença, sofre profundas alterações com o envelhecimento, levando à disfunção cardíaca, sendo esta com mais incidência na funcionalidade diastólica em contraste com uma função sistólica que está relativamente bem preservada. A pratica regular de exercício físico confere um conjunto de benefícios ao sistema cardiovascular, levando a um fenótipo cardíaco melhorado que se define por uma capacidade funcional aumentada. E como forma de realçar esta influencia do exercício físico no sistema cardiovascular, o comportamento sedentário característico da população idosa, leva a uma diminuição da capacidade funcional, induzindo um envelhecimento cardíaco prematuro. Com base neste contexto de benefícios do exercício físico na disfunção cardíaca associada ao envelhecimento e os efeitos deletérios do sedentarismo na função cardíaca, este estudo foi planeado para investigar se as alterações da função cardíaca normalmente associadas ao envelhecimento são consequência do próprio efeito do envelhecimento ou derivado do declínio progressivo da actividade física. O estudo foi realizado com 16 ratos Wistar fêmeas, que foram colocadas aleatoriamente em dois grupos: sedentários (SED; *n*=8; com movimento restrito ao espaço da caixa por 52 semanas) e exercitados (EX; *n*=8; com exercício de passadeira por 52 semanas, 5 dias/semana, 60 min/dia a 20m/min de velocidade). Vinte e quatro horas após o termino do protocolo de exercício, todos os animais foram submetidos a uma avaliação hemodinâmica no ventrículo esquerdo em condições basais e isovolumétricas. Amostras do ventrículo esquerdo (VE) e ventrículo direito (VD) foram colhidas para analises bioquímicas e para análise histológica da área de secção transversal (AST) dos cardiomiócitos, foram também colhidas amostras do VE, VD e septo (SPT). Descobrimos que em condições basais, o grupo EX apresentou uma diminuição da frequência cardíaca (FC) e da pressão tele-diastólica (PTD), enquanto que a pressão sistólica máxima (Pmax) e a pressão tele-sistólica (PTS) aumentaram em relação ao grupo SED. ($P<0.05$) Em condições isovolumétricas, o grupo EX exibiu uma melhoria de resposta ($P<0.05$)

expressa pelo aumento do dP/dt max, pela diminuição pressão mínima (Pmin), PTD e um Tau mais rápido. Ao nível mitocondrial, os animais EX mostram um aumento do nível de expressão da SIRT-3 e também um aumento de actividade do complexo V. ($P<0.05$) O exercício físico induziu um aumento significativo na área de secção transversa (AST) do VE, no entanto o VD e o SPT mostram um aumento significativo da AST no grupo SED. ($P<0.05$) Os dados deste estudo sugerem que o exercício físico de longa duração confere um fenótipo cardioprotetor contra o envelhecimento biológico, manifestado por uma melhoria na função cardíaca a nível basal e uma resposta melhorada contra um aumento brusco da pós-carga. Este aumento da performance cardíaca parece estar relacionado com melhorias a nível mitocondrial.

Palavras chave: Exercício; Sedentarismo; Disfunção cardíaca; Disfunção mitocondrial; Cardioproteção.

ABSTRACT

Aging is a complex process and its effects in the organism culminate in a progressive functional decline. It is known that with aging and even in the absence of underlying diseases, the cardiovascular system suffers profound alterations, leading to the development of cardiac dysfunction, with a more expressive incidence of diastolic dysfunction in contrast to a relatively well-preserved systolic function. Regular physical exercise has a myriad of benefits to the cardiovascular system, leading to an improved cardiac phenotype that expresses itself by a better functional capacity. Importantly, sedentary behavior has deleterious effects in the cardiovascular system and has been shown to decrease functional capacity and induce premature cardiac aging. So with this context of exercise beneficial alteration in age-associated cardiac dysfunction and the deleterious effects of sedentary behavior, this experiment was planned to investigate if the cardiac features normally associated with aging are a consequence of the aging process or from the progressive decline in physical activity. The study was designed with female Wistar rats (*n* = 16; weight=130±2.4g). The animals were randomly displaced into two groups: sedentary (SED; *n*=8; restricted movement to the cage space for 52 weeks) and exercise (EX; *n*=8; treadmill exercise training for 52 weeks, 5 days/week, 60 min/day at 20 m/min). Twenty-four hours after ending the training protocol, all the animals were submitted to left ventricle (LV) hemodynamic evaluation with a conductance catheter in baseline conditions and under acute occlusion of the ascending aorta (ISO). Samples from LV and RV were collected for biochemical analysis and samples from LV, RV and SPT were also collected for histological CSA evaluation. We found that in baseline conditions EX animals presented a lower HR and a decreased EDP, while MaxP and ESP were increased in comparison to the SED group ($P<0.05$). Under isovolumetric conditions the EX group exhibited an improved response ($P<0.05$) as shown by the increased dP/dt max, decreased MinP, EDP and faster Tau. At the mitochondrial level EX animals show increased expression levels of SIRT-3 and also increased activity of ATP-synthase. ($P<0.05$) Exercise training also induced significant increase in the LV cardiomyocyte CSA, although the SED animals show significantly increased RV and SPT cardiomyocyte CSA. ($P<0.05$) Our data suggest that long-term endurance exercise training of moderate intensity provides a cardioprotective phenotype against biological aging, manifested by improved cardiac function in baseline and against acute afterload elevations. This improved cardiac performance may be related to improvements at the mitochondrial level.

Key words: Regular physical activity; Sedentary behaviour; Cardiac dysfunction; Mitochondrial dysfunction; Cardioprotection.

LIST OF ABREVIATIONS

CVD: cardiovascular diseases

LV: left ventricle

EDV: end-diastolic volume

EDP: end-diastolic pressure

EF: ejection fraction

ESV: end-systolic volume

HR: heart rate

β-AR: beta adrenergic receptor

Ca2+: calcium

PKA: protein kinase A

cAMP: cyclic adenosine monophosphate

E-C: excitation-contraction coupling

SERCA: sarcoplasmic reticulum Ca2+ ATPase

PLB: phospholamban

CaMK: calcium/calmodulin-dependent protein kinase

LCC: L-type Ca2+ channels

NFAT: nuclear factor of activated T cells

K+: potassium

AP: action potential

RYR: ryanodine-sensitive Ca2+ release channel

Ca2+ Sparks: sarcoplasmic reticulum Ca2+ release events

NCX: sodium-calcium exchanger

Na+: sodium

CSQ: calsequestrin

TRD: triadin

JNC: junctin

SR: sarcoplasmic reticulum

ATP: adenosine-5’-triphosphate

FA: fatty acids

FAT/CD36: fatty acid translocase CD36

CPT: carnitine palmitoytransferase

ROS: reactive oxygen species

PPAR-α: peroxisome proliferator-activated receptor-α

NAD+: nicotinamide adenine dinucleotide

SIRT-3: sirtuin-3

ETC: electron transfer chain

ACC : acid citric cycle

SDH : succinate dehydrogenase

SSM : mitochondrial subsarcolemmal type

IFM: mitochondrial interfibrilhar type

mitDNA : mitochondrial DNA

O2.- : superoxide

O2 : oxygen

mitCAT : mitochondrial catalase

HNE : 4-hydroxynonenal

MDA: melondialdehyde

mPTP: mitochondrial permeability transition pore

N.O: nitric oxide

ONOO-: peroxynitrite

eNOS: endothelial nitric oxide synthase

iNOS: inducible nitric oxide synthase

AGEP: advanced glycation end products

ECM: extracellular matrix

vSMC: vascular smooth muscle cells

ANGII: angiotensin II

TGF-β1: transforming growth factor beta

MMPII: matrix metalloproteinase II

ET-1: endothelin-1

NF-KB: nuclear factor kappa light-chain enhancer of activated B cells

MCP-1: monocyte chemoattractant protein-1

ACE: angiotensin converting enzyme

H_2O_2 : hydrogen peroxide

Mn-SOD: manganese superoxide dismutase

VCAM: vascular cell adhesion molecule

TNF-α: tumor necrosis factor alpha

MHC: myosin heavy chain

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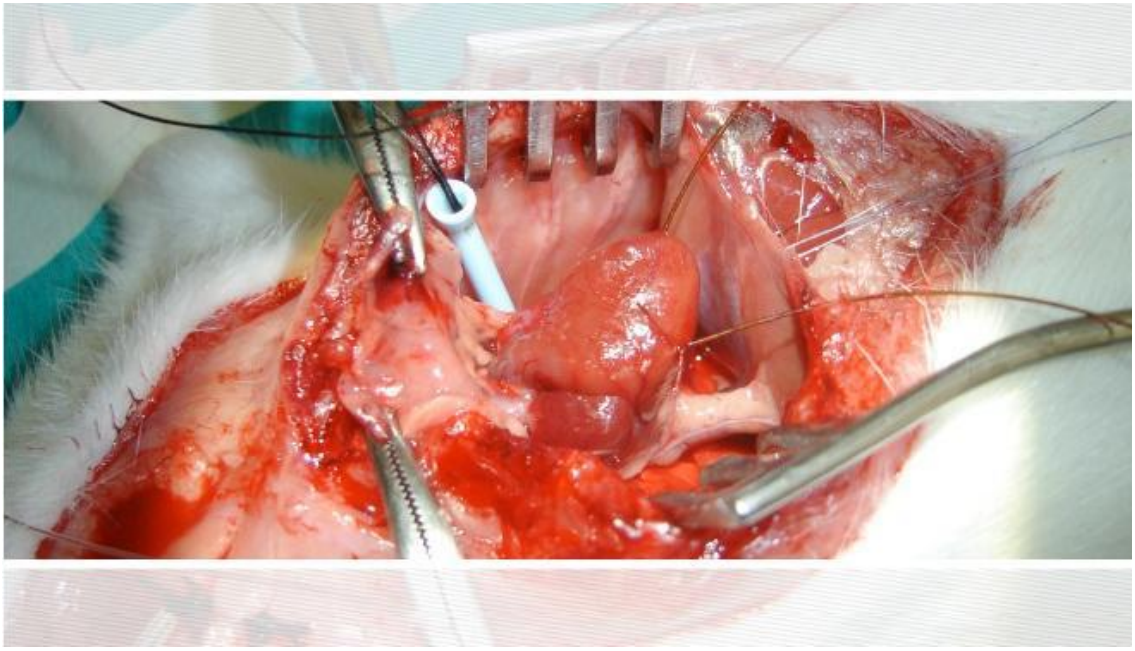

1 THE PROCESS OF AGING AND MAJOR CARDIOVASCULAR ALTERATIONS

1.1 *The Aging process and epidemiology of CVD*

The overall aging process relies in a wide compound of factors, therefore its genesis and development is extremely complex to determine. Numerous theories have been elaborated in an attempt to explain the aging phenomenon. There seems to be two main opposite fields of viewpoints in the theories foundations. One is mainly marked by the particular influence of the genes in the longevity, while the other states that the exposure to the environmental properties dictates the dynamics of the aging process (for further information please read [1, 2]). Mainly, aging results from the interaction between genomic and stochastic factors that put the organism in a constant imbalance. Over time the severity of this state has repercussions in the progressive development of this phenomenon, resulting in a gradual loss of function and a decreased ability to maintain homeostasis. Therefore, an enhanced susceptibility to lesion and deficits accumulation will take place, leaving the organism with a progressive reduction of its redundancy, which in turn will increase the risk for disease development and ultimately death. [1-3] A related concept is that as we age, the time exposure to different risk factors also increases. So, time *per se* indirectly increases the susceptibility for the development and manifestation of diseases. [4]

According to the Portuguese national institute of statistics, the elderly population is increasing in relation to the overall population. In fact, 18.2% of Portuguese people are over 65 years old and the median lifespan is more favorable to woman than man, with 82.1 and 76.1 years old, respectively. [5] Advanced age is associated with a high incidence of metabolic and degenerative diseases such as diabetes, Parkinson's disease, Alzheimer's disease and dementia. [3] Aging is also associated with the development of cardiovascular diseases (CVD) which are in fact considered the leading cause of death, disability and mortality in the elderly population and account for 31.9% of Portuguese deaths. [5, 6] Indeed, 70% of patients with arterial fibrillation, 75% of patients with congestive heart failure and more than 80% of patients with coronary heart disease are above 60 years hold. According to the heart

and stroke's statistics from the American Heart Association, the susceptibility to the occurrence of the first cardiovascular event exhibits a progressive rise in both men and woman. For instance, in men, there is an incidence of 3 per 1000 events from 35 to 44 years old, increasing to 74 per 1000 at age of 85 to 94 years old. [6]

1.2 *Major structural and functional cardiac alterations with aging*

The aging process is accompanied by several structural and functional alterations of the cardiovascular system, even in the absence of an underlying disease, that may lead to the development of cardiac dysfunction and failure. [3, 7-9] There is an age-dependent hypertrophy of the left ventricle (LV) and a progressive increase in LV wall thickness.[3, 7, 8] This change is thought to be part of an adaptive response to the age-associated increase in mean arterial blood pressure secondary to the loss of arterial compliance. [8] This cardiac phenotype is further characterized by a progressive reduction in the total number of cardiomyocytes due to cellular death from necrosis and apoptosis, in part compensated by the hypertrophy of the surviving cardiomyocytes, as well as by hyperplasia. It has been estimated that from 20 to 100 years of age, the myocyte compartment is replaced 15 times in women and 11 times in men, highlighting that the heart is a dynamic organ [10].

Regarding to the extracellular matrix, it also suffers remodeling, manifested by increased fibrosis and alteration of the collagen properties [11, 12] [13] leading to increased cardiac stiffness. [14, 15] This specific alteration is particularly important to the relaxation and compliance properties of the cardiac muscle, mainly impairing diastolic function. Indeed, aging is intimately associated with disturbed diastolic function and decreased ventricular compliance. [3, 7, 8, 13] The LV early diastolic filling is gradually compromised, starting to decline after the age of 20 and exhibiting a 50% reduction at the age of 80. [16] In order to compensate for this loss, more filling occurs in the late diastolic phase, in part due to a more vigorous atrial contraction. [17] This compensatory mechanism helps to maintain LV filling but contributes to the age-associated atrial hypertrophy and increased risk for atrial fibrillation [7] . So, with aging, the ratio between early and late diastolic filling declines dramatically, which is clinically interpreted as an indication of diastolic dysfunction. [7]

Interestingly, the end-diastolic volume (EDV) does not decrease with age, but end-diastolic pressure (EDP) is typically elevated in old persons. [13]

Regarding to systolic function, it is relatively well preserved at rest [3, 7, 8], with normal resting end-systolic volume (ESV) and ejection fraction (EF) despite the increased systolic pressure [18]. However, when the aged heart is stressed such as with exercise, systolic function becomes impaired. In fact, in response to exhaustive exercise the aged heart exhibits a reduced cardiac output (CO), heart rate (HR) and EF when compared to younger persons. [19] To counteract this age-related depression of systolic performance, older individuals make a greater use of the Frank-Starling mechanism in an attempt to preserve stroke volume over a wide range of cardiac performance demand. Although EDV increases to a greater extent during vigorous exercise, the above-mentioned mechanisms becomes inefficient due to the inability of the aged heart to appropriately reduce the ESV [8, 18, 19]. Aging is associated with a deficit in maximal intrinsic contractility and a decline in contractile reserve. [8] This has been related to the impairment of the β-adrenergic responsiveness and to calcium handling abnormalities that will be explained latter. In addition to the age-induced cardiac alterations, a progressive detrimental change in the function and structure of arteries also takes place. They become thicken, stiffer, develop endothelial dysfunction and display a chronic low-grade inflammatory state. [4, 8, 20-23] These age-induced arterial alterations have an important repercussion in the hemodynamic profile since they contribute to raise the pulse pressure and systolic blood pressure which alters the afterload the heart has to cope with [8, 21]. So the interaction between the LV and the arterial system, which is known as arterial-ventricular coupling, is a central determinant of the cardiovascular performance, that becomes significantly impaired during vigorous exercise with age, and contribute to the reduced cardiovascular functional capacity. [9]

1.3 *Molecular alterations in Aging Heart*

There are many biological processes that underlie the aging process that remain poorly defined and unclear. For the purpose of this work, we will summarize the main molecular alterations that have been related to the age-associated cardiac and vascular alterations.

1.3.1β-adrenergic receptor signaling stimulation

β-adrenergic receptor (β-AR)-mediated modulation of cardiac function is a key component of cardiovascular reserve function. [24] Cardiac aging is characterized by a depression in the efficacy of the β-AR stimulation signaling to modulate contraction, resulting in a decreased contractile response in both β1-AR and β2-AR stimulation. [25, 26] This depression is frequently attributed to an age-associated reduction in postsynaptic response to β-AR stimulation whish seems to be due an apparent receptor desensitization caused by a chronic state of elevated plasma levels of norepinephrine and epinephrine. [27] Also, there is a failure of the β-AR stimulation in cells of aged hearts to augment intracellular calcium (Ca2+) transient in the same extent as seen in young and adult hearts, which is attributed the decreased functionality of the L-type sarcolemmal Ca2+ channel, leading to a minor increase in Ca2+ influx. [25] This apparent alteration was suggested to work as an adaptive mechanism to prevent the increased risk of Ca2+ overload as this is related to proteolysis and cell death. [20, 28]

Another factor that contributes to β-AR dysfunction with aging is the impaired function of the β-AR-G protein adenylyl cyclase complex and alterations in adenylyl cyclase catalytic subunit, which ultimately leads to a reduction in the phosphorylation of proteins that are essential to the contractile response, via decreased activated protein kinase A (PKA) by a reduction in the ability to generate cyclic adenosine monophosphste (cAMP). [26, 29]

Adenylyl cyclase is a key enzyme to produce cAMP as a secondary messenger downstream to β-Adrenergic signaling and adenylyl cyclase type 5 is its major form in the heart. [24] It was observed that adenylyl cyclase type 5 knock-out mice had prolonged lifespan and were protected from cardiac aging, exhibiting a reduced age-dependent cardiac hypertrophy, fibrosis, systolic dysfunction and apoptosis, which are all critical factors for cardiac dysfunction. [30] Moreover, mice disruption of adenylyl cyclase type 5 was shown to prevent hypertrophy, apoptosis and failure in an animal model of chronic catecholamine stimulation or aortic banding. It was also observed a prolonged lifespan, which might be mediated through a decreased activation of cAMP, leading to decreased production of PKA, which in turn is associated with an increased

activation and activity of the anti-apoptotic, anti-oxidative and cell-survival Raf-1/pMEK/pERK pathway. [31, 32] With aging, the adenylyl cyclase type 5 portein levels and mRNA leves decline progressively in rat heart. [33]

1.3.2 Excitation-Contraction coupling and calcium handling proteins in Aging Heart

Intracellular Ca2+ homeostasis and Ca2+ handling proteins are also submitted to the progressive influence of aging in the heart. [34] As the kinetic of Ca2+ handling is a core process in excitation-contraction (E-C) coupling and consequently in the inotropic performance of the heart, an age-associated dysfunction in this process lead to a less effective cardiac behavior and, in part, may contribute for the development of cardiac pathological states. [34] The main cause for the altered Ca2+ handling that senescent heart exhibit [20, 28, 35] are the age-related modifications of expression, regulation and function of Ca2+ handling proteins, leading to a impairment in Ca2+ homeostasis. All together, these modifications contribute to a decrease in peak contraction, prolongation of contraction duration and a predisposition to a reduced threshold for Ca2+ overload observed in myocytes from aged rats. [35, 36]

1.3.2.1 SERCA

The sarcoplasmic reticulum Ca2+-ATPase (SERCA) plays a vital role in Ca2+ handling and in E-C coupling. Its cardiac major isoform, SERCA2a, transports Ca2+ from cytoplasm into the sarcoplasmic reticulum (SR) during diastole, leading to muscle relaxation by lowering cytosolic Ca2+ levels. This is an essential step for the later release of Ca2+ by ryonadine receptors, crutial for contraction of the sarcommere. [28, 37] Alterations in the amount of this protein with ageing seem to be species-dependent since while in the rat heart no apparent changes were detected while SERCA2a levels were reduced in the senescent human and mice heart. [34] [38, 39] [28]. Regarding to its activity, there seems to occur an age-associated decline in the Ca2+-sequestering activity of SERCA2a in rodent hearts, which may underlie the depression of cardiac relaxation. [34, 38, 40, 41] This impaired activity of SERCA2a may be explained by the unbalanced interaction between SERCA2a and its inhibitor phospholamban (PLB), as suggested by the decreased SERCA2a/PLB ratio

that progressively occurs with age. [37, 42] Also, the age-associated impairment in β-AR signaling results in decreased PKA-dependent phosphorylation of PLB. [29] In addiction, Ca2+/calmodulin-dependent protein kinase (CaMK) that also phosphorylate PLB, as been shown to modulate SERCA2a by direct phosphorilation. [43] Importantly, aging causes a 50% reduction in the amount of CaMK (δ-isoform). [41]

1.3.2.2 L-Type Ca2+ channels

L-Type Ca2+ channels (LCC) are voltage sensitive and in cardiomyocytes the Ca2+ current through this channels is the central pathway for the Ca2+ influx from extracellular space into the cytoplasm. [37] Given that the peak L-type Ca2+ current is the primary trigger for SR Ca2+ release, the LCC is fundamental for the regulation of cardiac contractile strength and E-C coupling. [37, 44] The age-associated changes in LCC properties are ambiguous. Regarding the peak L-type Ca2+ current, some studies reveal increased [45], decreased [46], or unchanged Ca2+ transients. [25] Nonetheless, a reduction in LCC Ca2+ currents leads to a compensatory SR Ca2+ release in order to preserve the contractile capacity. This condition results in Calcineurin/nuclear factor of activated T cells (calcineurin/NFAT) signaling activation that promotes the development of hypertrophy and cardiac dysfunction. [47]

Together, the slow inactivation of the peak L-type Ca2+ current in old rat myocyte, ergo prolonged cystolic Ca2+ transient and consequent prolonged contraction, with a reduction in outwardly directed potassium (K+) transient, might in part account for the prolonged action potential (AP) in senescent hearts. [20, 28, 48] Prolonged AP and contraction can be viewed as adaptive rather than dysfunctional adaptation, because reduced velocity of myocardial shortening is energy-efficient, allowing for continued blood ejection during the late systole, advantageous to the age-related increased in central arterial stiffness. [20] Also, the age-associated impairment in the β-AR signaling cascade compromises the functionality of LCC. [25]

1.3.2.3 Ryanodine Ca2+-release channel

The Ryanodine-sensitive Ca2+-release channel (RYR) is located at the junctional portion of the SR and RYR2 is the most expressed isoform in the heart. [49]

The release of Ca2+ from intracellular stores is a crucial part of E-C coupling and the RYR2 plays a fundamental role on that process by mediating the Ca2+ release from SR to cytoplasm. [44, 49, 50] Age-associated alterations in the expression and function of RYR have been reported in animals and humans. [37, 41, 51] The hyperphosphorylation of RYR by PKA has been implicated in both diastolic and systolic dysfunction in the aged failing heart, resulting in abnormal intracellular SR Ca2+ leak. [37, 51] CaMKII-mediated phosphorilation of RYR can either depress or enhance RYR activity and it has been seen that CaMKII-mediated phosphorilation of RYR increases resting SR Ca2+ leak. [52] Nonetheless, it has been reported a significant reduction in the CaMKII protein content and phosphorilation of RYR with aging [41] , and also diminished protein expression of RYR in cardiac tissue of aged Wistar rats [53], but not in Fisher 344 rats. [41]

Zhu and coworkers [39] measured single channel properties of RYR and unitary SR Ca2+ release events (Ca2+ sparks) in senescent rat ventricular myocytes. They observed decreased intracellular Ca2+ transient amplitude and an increased time constant of the intracellular Ca2+ transient decay, which correlated with a reduced SR Ca2+ content. [39] Also, senescent myocytes displayed an increase in Ca2+ spark frequency, along with a reduction in Ca2+ spark duration, width and amplitude, given by the increased RYR open probability and shorter mean open time. [39]

An increase in frequency of spontaneous Ca2+ sparks may account for the reduced SR Ca2+ content by an increase in Ca2+ leak from the SR [35], which may result in posterior cardiac functional alterations, such as diastolic dysfunction by a slower decline of the intracellular Ca2+ transient and increase in diastolic intracellular Ca2+ content, and systolic dysfunction by a reduced SR Ca2+ content available for RYR release and an overall reduced cardiac myocyte threshold for Ca2+ overload. [20, 28]

1.3.2.4 Na+-Ca2+ exchanger

The Na+-Ca2+ exchanger (NCX) is an important membrane cation transporter. Its major myocardial isoform, NCX1, serves as the main transsarcolemmal Ca2+ extrusion mechanism and has an essential role in the maintenance of the intracellular Ca2+ homeostasis and cardiac E-C coupling. [28, 37, 54]

The NCX has the capacity to reverse the direction of Ca2+ flow in response to the cell sosium (Na+) gradient. Working in forward mode, the NCX generates an inward current that carries 3 Na+ into cytoplasm and 1 Ca2+ to extracellular space and in reverse mode produces an outward Ca2+ current, which is important for the late repolarization phase of cardiac myocytes and the later Ca2+ clearance phase of the intracellular Ca2+ transient, essential for the relaxation properties of the myocardium.[28, 54, 55]

It is known that NCX is upregulated at the transcriptional level in hearts submitted to increased cardiac loading [56] or increased protein levels in pathological states, such as heart failure [54], which can be a possible adaptive mechanism for the associated reduced SERCA2a activity. [37] Age-related alterations in the protein levels and activity of NCX have been reported. Regarding NCX protein levels, studies in aged rodent hearts suggest either unchanged [57] or decreased levels. [53] The NCX activity in aged myocardium follows the same course, exhibiting either increased [58], decreased [59] or unchanged activity. [60] However, a recent study reports an age-dependent increase in NCX forward mode activity, leading to increased NCX-mediated Ca2+ clearance during relaxation. [57] This age-dependent increase in NCX forward mode, could generate a depolarizing current and contribute to the prolongation on the late AP duration, which in turn leads to the age-associated prolonged contraction and also could serve in part to compensate the age-related decline in SERCA2a activity. [35, 57]

1.3.2.5 Calsequestrin

Calsequestrin (CSQ) has an important role in Ca2+ storage in SR and CSQ2 is the main expressed isoform in the heart. [37] Studies have shown that cardiac CSQ expression does not alter with aging. [39, 41] Nevertheless, these studies centered only in CSQ protein levels and more recent evidence suggest

that Ca2+ in the SR lumen interacts with CSQ2 which in turn interacts with the integral membrane proteins triadin (TRD) and junctin (JNC), that induce changes in luminal Ca2+ to the RYR2. Consequently, it influences SR Ca2+ release by reducing RYR2 activity during and afterward SR CA2+ release when SR Ca2+ concentrations decrease and also by stimulating RYR2 activity when diastolic SR Ca2+ concentrations are above normal levels. [37, 50]

So, in addition to Ca2+ storage reservoir in the SR, CSQ, via interaction with JCN and TRD, controls the RYR2 opening, serving as a luminal Ca2+ sensor for RYR2. [50] It might be interesting to examine the effects of age in the interaction activity of the CSQ with TRD and JCN and so, the influence of the luminal Ca2+ dynamics on the RYR2 Ca2+ - mediated release from the SR.

1.3.3 Cardiac metabolism and the influence of Sirtuins in the aging heart

The heart, in an organ with a very high-energy demand and needs a constant supply of adenosine-5'-triphophate (ATP) to sustain an adequate contractility behavior, ion homeostasis and basal metabolic processes. [61] Accordingly, the heart has a high metabolic flexibility to sustain a sufficient ATP generation. It uses several different carbon substrates as source of energy (fatty acids, glucose, lactate and ketone bodies), which is an important feature to face alterations of nutrient status and hemodynamic stress, granting certain adaptive properties to the heart. [62]

Under normal physiological state, >95 % of the ATP production arises from mitochondrial oxidative phosphorylation, with the surplus coming from glycolysis. Also, the heart has a preference for oxidation of fatty acids (FA), since 50 % - 70 % of ATP is from FA with the remainder largely by carbohydrate oxidation. [61, 63] Since FA utilization is the main pathway for heart source of energy, here we confine to its major properties and alterations with the effect of the aging process in the heart. Circulating long-chain FA are transferred to the cardiomyocyte by passive diffusion via flip-flop mechanism across the lipid bi-layer and by protein carrier-mediated pathway.[61] The main protein carrier is the fatty acid translocase CD36 (FAT/CD36), which has been shown to mediate 50% - 60% of the FA uptake into cardiomyocytes and so with central importance in FA oxidation within the mitochondria. [64] Another important step for FA oxidation is the passage of cytoplasmatic FA to mitochondria, which is

done by the enzyme carnitine palmitoytransferase (CPT) that catalyzes the conversion of long-chain acyl CoA into long-chain acylcarnitine and is subsequently shuttled into mitochondria. [63] So, alterations in CPT activity influences ATP generation by changes in mitochondrial β-oxidation. There are evidences that disturbances in the heart energy metabolism are related to alterations in the above-mentioned pathways, probably contributing to the progressive decline of cardiac function . [63, 65-67] In fact, studies suggest that besides impairment of FA oxidation [67, 68] , also the whole oxidative metabolism is decreased with aging. Regarding this, decreased ketone and glucose oxidation were observed in the aged heart, leading to a significant reduced acetil CoA-derived ATP production. [65, 69] Some studies suggest an increase in glucose oxidation as a mechanism for compensation the age-associated decrease in FA oxidation. [64]

Another important feature in the modification of heart metabolism with age is the increased accumulation of lipids in non-adipose tissues, as cardiomyocytes. [65, 70] This results in ectopic deposition of reactive lipid species and has the potential to cause organ-specific lipotoxicity, compromising cardiomyocyte normal functionality [70] and therefore a contributor to the age-related cardiac dysfunction. One age-related mechanism that could contribute to the increased cardiomyocyte lipid accumulation is the balance between FA uptake and oxidation. Accordingly, it has been shown a dramatic increase in FAT/CD36 expression in the aged heart [65], conferring to the aged heart a more susceptible state for cardiomyocyte lipid accumulation. Also, it was shown a decreased CPT activity in the aged heart [71] and given its membrane integrity-dependent activity, the deleterious effects of reactive oxygen species (ROS) in the outer mitochondrial membrane that happens with age, may be a possible explanation for the reduced CPT activity. Nevertheless, it suggest an impaired FA transfer to mitochondrial and subsequent decreased ATP generation. In addition, peroxisome proliferator-activated receptor-α (PPAR- α), that acts as a transcriptional regulator of multiple target genes, which controls FA and glucose oxidation, has also been shown to suffer a marked depression on protein expression in aged heart. [69] Consequently, also may be a potential mechanism to the age-related decrease in FA and glucose oxidation.

1.3.3.1 Sirtuins in the aged cardiac metabolism

In recent years, sirtuins have been proposed to have a determinant role for the heart homeostasis at several levels. In mammals seven sirtuins family members exist and they regulate a variety of cellular functions including DNA repair, the cell cycle and more robustly the metabolism. [72] One particular property is the regulation of mitochondrial number, turnover and activity by both mitochondrial and non-mitochondrial sirtuins, acting as metabolic sensors in a nicotinamide adenine dinucleotide (NAD+)-dependent pathway to deacetylate specific protein lysine residues, activating or inhibiting their activity.[68] Sirtuins activation is intrinsically linked to the energetic and redox status of the cell, measured and influenced by the NAD+/NADH ratio and by absolute levels of NAD+ and NADH. [73]

Sirtuin-3 (SIRT-3) is activated by metabolic stress conditions as caloric restriction or fasting. [73] SIRT-3 deficient mice exhibit a remarkable mitochondrial protein hyperacetylation, which was not seen in SIRT-4 and SIRT-5, suggesting that SIRT-3 is the main mitochondrial deacetylase. [74] The cardiac homeostatic pathways of SIRT-3 include generation of reduced equivalents by mitochondrial catabolism of substrates, control of the electron transfer chain (ETC), control of several pathways in redox status and the oxidation of reactive aldehydes. [73] So SIRT-3 activity in the control of enzymes involved in cardiac energy metabolism is consistent with an overall protector role against the age-related cardiac dysfunction and consequent age-related cardiac diseases. [75] In fact, SIRT-3 deficient mice show loss of regulation capacity of energy homeostasis expressed by a significantly reduced heart ATP basal levels, which is an physiological high oxidative tissue. [76]

The influence of SIRT-3 in β-oxidation is mediated by deacetylation and activation of long-chain acyl-CoA dehydrogenase II and the acid citric cycle (ACC) enzymes, glutamate dehydrogenase, which facilitates the oxidative deamination of glutamate to alpha-ketoglutate and the isocitrate dehydrogenase II, that increases glutathione levels [68, 73], in this manner improving the overall FA β-oxidation status.

Also, has been shown that SIRT-3 deacetylate ETC enzymes and consequently leading to an enhanced state of the oxidative phosphorylation. [68, 73, 75, 76]

Specifically, SIRT-3 physically interacts with complex I subunit NDUFA9, leading to increased activity. [76]

Complex II and V are also a substrate for SIRT-3 activity, through deacetylation of the subunit succinate dehydrogenase (SDH) (complex II) and the subunits ATP5A1 / ATP5F1 (complex V). [77] This is an important feature of SIRT-3 influence, for the rationale that the SDH catalyzes the oxidation of succinate to fumarate and electrons generated by this reaction are transferred to the ACC, ergo the complex II has a unique dual role in mitochondrial metabolism, it is part of ETC and ACC.

Another mechanism of SIRT-3 mediated enhancement of the oxidative phosphorylation is through the deacetylation of cyclophilin D, causing an inhibition of its peptidyl-prolyl isomerase activity, leading to the dissociation of hexokinase II from the mitochondria to the cytosol. This redistribution of hexokinase II enhances the stimulation of the oxidative phosphorylation. [78]

So, with this SIRT-3 mediated myriad of mitochondrial benefits and being the major mitochondrial deacetylase, it is reasonable to assume that being the heart an high mitochondrial density organ, SIRT-3 activity his fundamental to the age-associated cardiac metabolism and ETC dysfunction.

1.3.4 Age-related Cardiac mitochondrial alterations and dysfunction

The relevance of mitochondria to function, homeostasis and viability of a cell is not constrained just to ATP production. Mitochondria cover essential roles in a myriad of major cellular processes, including cellular redox homeostasis, regulation of programmed cell death due to release of proteins that promote apoptosis, ion homeostasis, ROS- regulated inflammation. [23, 24, 79, 80] With aging, there is a progressive loss of mitochondrial function. The resultant intracellular ambience can contribute to compromise the cardiomyocyte cell viability, ultimately leading to cell death. [81] Importantly, mitochondrial dysfunction coupled with a defective oxidant scavenging and impaired mitochondrial maintenance is implicated in pathogenesis of several chronic degenerative diseases, including cardiovascular and neural diseases. [80, 82, 83] Nevertheless, the understanding of the age-induced alterations in mitochondrial function is complex, mainly by the fact that the heart contains two different populations of mitochondria that are structurally similar but

biochemically different. The subsarcolemmal type (SSM) is located beneath plasma membrane and the interfibrilhar type (IFM) is positioned between myofibrils. Importantly, IFM has a higher citrate synthase and SDH activities and also has a 1,5 times faster oxidation of substrates than SSM. [84] In addition, it was verified a decreased rate of oxidative phosphorylation with aging only in IFM. [85] Therefore, this shows the importance of specifying the different populations of mitochondria while studying the effects of age. Also, this may explain the inconsistency found by different investigators in the age-related changes in oxidative phosphorylation. [80, 85] One of the main aspects in cardiac mitochondrial aging is related to the alterations in the ETC. The complex I (NADH dehydrogenase) of the respiratory chain complexes appears to be highly susceptible to loss of function with age. [86] Since there is an age-associated increase in mitDNA damage and that mitDNA encodes 7 subunits of complex I, it is reasonably natural that this complex would suffer disadvantage effects with aging. [80, 87] The complex III (cytochrome-c redutase) and complex IV (cytochrome-c oxidase) also contains mitochondrial DNA (mitDNA) encoded polypeptides and there is evidence for a decline in their activity with age. [86, 88] Also, the complex II (succinate dehydrogenase) which does not have mitDNA encoded polypeptides, seems to be unaltered with aging. [86] The activity of the complex V (ATP-synthase) also exhibits significant decreases with age. [89]

Thus, there is an age-related decline in ETC function which favors a decrease in ATP production and an increased formation of mitochondrial derived-ROS, mainly superoxide (O2.-), by an increment in electron leakage. [24] In addition to being the main mechanism of endogenous ROS production, mitochondria is also particularly vulnerable to oxidative damage by ROS. ROS can induce several oxidative alterations including protein oxidation, lipid peroxidation and mitDNA damage. The latest being specially susceptible to damage since it is located near the inner mitochondrial membrane, is deprived of protective histones and has very limited DNA repair activity, which is mostly done by the base excision repair mechanism. [80, 90] Also, being the heart an organ with a limited regenerative capacity the DNA damage is more severe than what occur in mitotic tissues like liver. In fact cardiomyocytes have a very limited capacity to undergo mitosis and with the O2 consumption and low

antioxidant capacity compared with the liver. [80, 91] Accordingly, there is evidence for an age-related increase in mitROS production in the heart [92] and also an increase in mitDNA oxidative damage. [93] Importantly, there is a positive correlation between age-associated increase in mitDNA damage and age-associated in mitDNA deletions and point mutations. [80] Also there is an inverse correlation of deoxyguanosine levels (indicator of DNA oxidative damage) in heart mitDNA and maximum lifespan.[93] The mitDNA mutations can lead to defective ETC and ATP production, which ultimately can result in a noxious cycle of age-increasing mitROS production, mitDNA oxidative damage and mitochondrial dysfunction. [80, 94]

Due to the elevated transient nature of ROS, the indirect measures of oxidative stress in tissues are needed to assess their alterations and consequently changing the cellular environment. Specially, the redox status is considered an important parameter for assessing the pro-oxidant cellular environment. [95] The ratio of glutathione/oxidized glutathione is thought to be the most abundant redox buffer system, and a decreased ratio indicates a shift of the redox status to a more oxidized, suggesting oxidative stress. [95] So, increased oxidative stress in the heart indicates an age-related shift of the redox status to a more oxidative cellular environment. This mechanism could be related to a blunted capacity to buffer heart-derived ROS with age, even when generated at basal conditions, and so may be an important molecular pathway that underlies the cardiac aging process and the consequent age-associated decline in the functional capacity of the heart.

Critical evidence for the significant role of mitROS in cardiac aging comes from mice overexpressing mitochondrial catalase (mitCAT). This mice shows 20% and 10% increase in median and maximal lifespan, respectively. [96] Also, it shows attenuated age-dependent mitochondrial oxidative damage, prevention of cardiac hypertrophy and improvement of cardiac function. [97] Interestingly, mitCAT overexpression also ameliorated the cardiomyopathy in mice with homozygous mutation of mitDNA polymerase gamma, that are prone to age-dependent accumulation of mitDNA mutations mediated by an increase in mitochondrial oxidative stress. [98] Moreover, lipids and proteins are also substrates to oxidative alteration within cardiac mitochondria. In fact, there is evidence for an increase in age-related lipid peroxidation in cardiac

mitochondria. [92] Importantly, lipid peroxidation is the main cause for the age-related decrease in membrane fluidity, permeability and consequently membrane leakage. [95] This important feature of the effects of age in membrane homeostasis and consequently cell viability is mediated through aldehydic products of lipid peroxidation, 4-hydroxynonenal (HNE) and melondialdehyde (MDA). [99] Specially, HNE is highly reactive with proteins and cause inhibition of protein and DNA synthesis, enzyme inactivation and it is thought to have a major role in oxidative stress-induced cellular dysfunction. [92]

Protein carbonyls is the most commonly used method for studying protein oxidation and so the effects of ROS in protein function. Accordingly, there is evidence for an age-related increase in protein carbonyls in cardiac mitochondria. [92] Also, there is an increased accumulation of oxidized proteins with age as a consequence of the age-related increase in ROS production and an age-associated impairment in the proteolytic pathways that remove the damaged proteins. [95]

It is fundamental for cardiomyocyte homeostasis to have a proper functioning mechanism of removal of damaged organelles, such as dysfunctional mitochondria. This process is specifically called mitophagy, which is a selectively targeted macroautophagy to mitochondria. [100] The main trigger for mitophagy is the loss of membrane potential, in which Parkin and Pink1 are thought to have important roles. [82] Another important trigger for mitophagy is intimately related to the loss of membrane potential after an increase in permeability in the inner mitochondrial membrane to solutes with molecular weight up to 1500 Daltons, leading to the opening of the mitochondrial permeability transition pore (mPTP). Ultimately, this opening can lead to cell death by apoptosis after the release of cytochrome C as a consequence of rupture of the outer mitochondrial membrane. [79] Regarding the molecular regulation of mitophagy, the mTOR/AMPK pathway is thought to be a major mechanism. [101] AMPK exerts a negative regulatory effect on the mammalian target of rapamycin and consequently activates the ULK1-Atg13-FIP200 complex required for the induction of macroautophagy. [101] In addition, AMPK triggers the SIRT-1 deacetylation of PGC-1α and FOXO1, resulting in transcriptional modulation of mitochondrial biogenesis. [82, 101] Therefore, the mTOR/AMPK pathway contributes to a functional pool of mitochondria.

Nevertheless, it has been reported that impairments in macroautophagy result in premature aging and decreased lifespan. [82] Importantly, evidence shows that cardiomyocyte macroautophagy becomes prejudiced with aging, leading to an accumulation within cardiomyocytes of enlarged dysfunctional mitochondria that are prone to ROS production and leakage, also less likely to be autophagocytosed in virtue to their size. [82, 102] This important feature of the impact of mitophagy in cardiomyocyte function with age, can lead to a vicious cycle of age-related autophagic failure and age-related accumulation of dysfunctional mitochondria, leading to an increase in oxidative stress and increased oxidative damage, grandly contributing to cardiomyocyte dysfunction and death.

1.3.5 *Vascular alterations and underlying mechanism with age*

There are compelling evidences that the major central blood vessels undergo several alterations with the development of the aging process. [4, 8, 20, 21] In fact, there is a view that emphasizes the vascular aging as a fundamental underlying factor for the overall aging process. It simply relies in the fact that besides being responsible for adequate supply of nutrients and O2 to all cells, alterations in the dynamic physiological nature of vessels and exposition to stressors as shear stress, oxidized proteins and lipids, results in intense changes in blood vessels cellular activities, causing significant functional alterations. [23, 103] The major age-associated vascular alterations include luminal dilation, intimal and media thickening, vascular stiffening, endothelial dysfunction and a chronic low-grade vascular inflammation . [4, 8, 20, 21, 104]

1.3.5.1 *Age-associated endothelial dysfunction*

Alterations of endothelial function that occur with aging are well characterized in the literature and considerable evidence supports the notion that the age-associated increase in ROS production is involved. [20-22, 24] Nitric oxide (N.O) is one of the most important mechanisms to the local regulation of the blood flow. [105] Importantly, endothelium-derived N.O confers other vasoprotective effects, as inhibition of apoptosis, disruption of pro-inflammatory cytokine-induced signaling pathways and inhibition of platetet aggregation and inflammatory cell adhesion to endothelial cells. [22, 106] There

is evidence for a significantly impairment in the N.O bioavailability with age. [106] In fact, the age-associated increase in ROS production leads to an increased breakdown of N.O to peroxynitrite (ONOO-) due to high concentrations of O2-. [105] Also, an age-related decline in endothelial nitric oxide synthase (eNOS) and intracellular L-arginine availability, an increased expression of inducible nitric oxide synthase (iNOS) and NAD(P)H oxidases activity, aggravate the N.O bioavailability, which leads to a significant age-related vasoreactivity dysfunction. [22, 105, 107] The fundamental role of endothelium-derived N.O for cardiovascular function and its importance in aging was shown through eNOS knockout mice, which display a significantly shorter lifespan and exhibit a premature cardiac aging phenotype with cardiac dilation, hypertrophy and dysfunction. [108]

1.3.5.2 *Age-associated vascular stiffness*

Another hallmark of the vascular aging is the progressive vessel stiffness, which confers important hemodynamic alterations. [4, 8, 109] The hemodynamic consequence of the age-associated vascular stiffness is an altered aortic pulse wave velocity causing an increased systolic pressure and decreased diastolic pressure. The last one leads to a decreased coronary blood flow and the enhanced systolic pressure ultimately results in LV remodeling, diastolic dysfunction and a predisposition to atherosclerotic lesions. Taken all together, significantly enhances the cardiovascular mortality with aging. [22]

Accompanying the increased stiffness, the major elastic arteries also dilate, leading to a significant enhancement of the vascular wall tension, contributing to the age-associated vascular remodeling. [22] Another important vessel alteration is the increased Ca2+ deposition with age, which might also contribute to the loss of vessel distensibility with age. [21]

One crucial contributor to the age-associated vessel stiffness is related to the alterations that affect the extracellular matrix (ECM). There is a significant evidence for a progressive age-induced collagen deposition in the vascular wall, which ultimately leads to impairment in vessel compliance in response to hemodynamic alterations. [4, 8, 110] Importantly, it has been shown a positive relation between increased levels of advanced glycation end products (AGEP) and the age-associated vascular stiffness. [21] Inhibition of AGEP was shown to

prevent the progressive vessel stiffness with aging and cardiac hypertrophy through a reduction in the AGEP-induced cross linkings of the ECM proteins. [111]

Another major alteration in vascular homeostasis with aging is the migration and hypertrophy of vascular smooth muscle cells (vSMC). Accordingly, with the development of the aging process there is a significantly increase in the vSMC migration capacity. This has been linked to Angiotensin II signaling cascade (ANGII), including the activation of the matrix metalloproteinase II (MMPII), transforming growth factor beta (TGF-β1), endothelin-1 (ET-1), nuclear factor kappa light-chain enhancer of activated B cells (NF-KB) and monocyte chemoattractant protein-1 (MCP-1), central effectors in age-associated vascular remodeling. [112]

Interestingly, inhibition of angiotensin converting enzyme (ACE) suppressed the ANGII formation and decreased blood pressure by reducing the vessel stiffness with aging. [113]

1.3.5.3 *Age-associated chronic low-grade vascular inflammation*

Vascular aging is also associated with a chronic low-grade inflammation, which creates a microenvironment favorable to an exaggerated vascular response to injury and potentially leading to the development of atherosclerosis. [23, 104] Importantly, it has been recognized that the age-related oxidative stress may cause vascular inflammation and endothelial dysfunction even in the absence of atherosclerosis. [114] Also, it has been suggested that mit-derived ROS has a key role in the age-induced vascular inflammation. [23, 104] ROS enhances the NF-KB activation and so regulates the gene transcription of various growth factors, cytokines and chemokines. Indeed, it has been suggested that the increased O2- production in aged mitochondria, is dismuted to hydrogen peroxide (H_2O_2) by manganese superoxide dismutase (Mn-SOD) and because H_2O_2 can penetrate the mitochondrial membranes, it promotes the NF-KB activation in the cytoplasm, leading to consequent pro-inflammatory status. [115] Also, some NF-KB-induced proteins act as NK-KB activators, causing a loop that perpetuates the production of inflammatory mediators. [23]

Importantly, it has been reported a significant increase in the transcriptional and binding activity of NF-KB with aging. [116] Therefore, with

aging a pro-inflammatory shift in vascular gene expression takes place. This includes an upregulation of iNOS, cytokines, chemokines and adhesion molecules. [23, 104, 105] In fact, the plasma concentrations of some inflammatory markers as vascular cell adhesion molecule (VCAM), tumor necrosis factor alpha (TNF-α), E-selectin, IL-6, IL-18 and MCP-1, are positively correlated with age. [117]

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EXPERIMENTAL STUDY

AGE-INDUCED CARDIAC DYSFUNCTION IS ATTENUATED BY LONG-TERM ENDURANCE EXERCISE TRAINING

Aging is characterized by a progressive impairment in cardiac maximal function, with a more incidence of diastolic dysfunction in contrast to a relatively well-preserved systolic function. Regular exercise can reduce the risk of cardiac dysfunction and it is assumed that some of the beneficial effects are based on mitochondrial adaptations. Our purpose was to determine whether one year of exercise training could modulate the cardiac functional decline observed in sedentary aging. Female Wistar rats (*n* = 16; weight=130±2.4g), were randomly displaced into the following two groups: sedentary (SED; *n*=8; with restricted movement to the cage space for 52 weeks) and exercise (EX; *n*=8; submitted to treadmill exercise training for 52 weeks, 5 days/week, 60 min/day at 20 m/min). Twenty-four hours after ending the training protocol, all the animals were anesthetized, tracheostomized for mechanical ventilation, the right jugular vein was cannulated and the heart exposed by median sternotomy. After, a pressure-volume catheter was inserted in the left ventricle (LV) for hemodynamic evaluation in baseline conditions and under acute occlusion of the ascending aorta (isovolumetric heartbeats). Samples from LV and RV were collected for biochemical analysis and samples from LV, RV and SPT were also collected for histological CSA evaluation. EX animals presented a lower HR (EX:294.3±4.887 vs. SED:313.9±6.669 bpm) and a decreased EDP (EX:6.288±0.4982 vs. SED:9.023±1.219 mmHg),while MaxP (EX:161.2±2.714 vs. SED:145.1±4.050 mmHg) and ESP (EX:158.8±2.653 vs. SED:141.9±3.771 mmHg) were increased in comparison to the SED group (P<0.05). The training program did not promote significant alterations (P>0.05 vs. SED) in the dP/dt max (EX:9968±178.9 vs. SED:9324±538.7) and dP/dt min (EX:-11383±114.5 vs. SED:-10175±591.9). Under ISO, the EX group exhibited an improved response (P<0.05) as shown by the increased dP/dt max (EX:9846±208.8 vs SED:8720±316.4), decreased EDP (EX:6.969±0.354 vs SED:10.41±1.347 mmHg) and faster Tau (EX:13.55±0.472 vs SED:16.32± 1.149). Exercise training also induced significant increase in the LV cardiomyocyte CSA (P<0.05), although the SED animals show significantly increased RV and SPT cardiomyocyte CSA. At the mitochondrial level EX animals show increased expression levels of SIRT-3 and also increased activity of mitochondrial complex V. (P<0.05) Our data suggest that long-term endurance exercise training of moderate intensity provides a cardioprotective phenotype against

biological aging, manifested by improved cardiac function in baseline and against acute afterload elevations. This benefits can possibly be linked to increased mitochondrial oxidative phosphorylation and SIRT-3 expression.

Key words: Regular physical exercise; Cardiac dysfunction; Mitochondrial dysfunction; Cardioprotective phenotype.

Introduction

Aging is a multifactorial process that results in damage to molecules, cells, and tissues, ultimately leading to progressive organ dysfunction. [1-3] Its effects are even more marked in organs with a limited regenerative capacity, such as the heart. [4] The age-associated cardiac and vascular dysfunction is the basis for the development of pathological states that can lead to cardiovascular diseases (CVD), which are in fact considered the leading cause of death, disability and morbidity in the elderly population. In Portugal, they accounting for 31.9% of deaths every year. [5, 6]

A large variety of cardiovascular alterations, including functional, structural and molecular changes, are thought to affect cardiac performance of the elderly, with a higher predominance on diastolic function and ventricular compliance. [7-9] However, it is unclear if the molecular alterations are the underlying factor for the functional changes or if the latest predispose to the development of the molecular alterations. Nevertheless, it is known that several factors are associated with the age-related cardiac dysfunction, including a depression in the β-adrenergic responsiveness, calcium handling abnormalities and a shift in the myosin heavy chain (MHC) isoforms, from the faster α-MHC to the slower β-MHC. [10-12] Also, cardiac aging is associated with mitochondrial abnormalities, mainly associated with increased reactive oxygen species (ROS) production and a deficient oxidant scavenging that leads to impairments in electron transport chain (ETC) and oxidative phosphorylation, including alterations in ATP-synthase (complex V). [13, 14]

Sedentary behavior induces cardiovascular deconditioning and accelerate cardiac aging. [15, 16] On its turn, physical exercise has been shown to improve the overall health and is unequivocally associated with an increase in medial lifespan in different animal species. [17] Also, clinical studies have shown that regular physical exercise, specially endurance exercise, preserves the cardiac function in the older population. [18] This poses the question if the decline in cardiac function observed in older persons is attributable to a sedentary lifestyle and thus reversed by exercise, or is it a physiologic feature of the aging process.

The purpose of present study was to investigate if the cardiac features normally associated with aging are a consequence of the aging process or from the progressive decline in physical activity.

Material and Methods

Animals and experimental design

Animal experiments were conducted under Portuguese law on animal welfare and according to the *Guide for the Care and Use of Laboratory Animals*, published by the United States National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and approved by the Ethical Committee from the University of Porto. Female Wistar rats (*n* = 16; age = 5wk ; weight = 130±2.4g at the initiation of the experiment) were randomly displaced into the following two groups: *1* sedentary (SED; *n*=8; with restricted movement to the cage space) and *2* exercised (EX; *n*=8; submitted to treadmill exercise training). All animals were maintained in a controlled environment at room temperature of 22º, beneath a 12:12-h light-dark inverted cycle, with food and water *at libitum*.

Exercise training program

Animals displaced to the exercise-training group were acclimated to treadmill for 2 consecutive weeks. During this period the running time and intensity were gradually increased till reach 60 min/day at 20 m/min. After 2 days of rest, the exercised animals trained 5 consecutive days (Monday to Friday) for 52 weeks. All the animals from the exercised training group completed the training program.

Experimental preparations for hemodynamic evaluation

Twenty-four hours after the end of the 52 wk exercise training protocol, and after access their body weight, all the animals were anesthetized by inhalation with a mixture of 4% sevoflurane with oxygen and placed over a heating pad, to maintain the body temperature at 37ºC. After, animals were tracheostomized for mechanical ventilation with oxygen-enriched air (60 cpm, tidal volume set at 1 ml/100 g, model 683: Harvard Small Animal Ventilator). The right jugular vein was cannulated under binocular surgical microscopy (Wild M651.MS-D; Leica, Herbugg, Switzerland) for administration of prewarmed 0.9% NaCL solution in order to balance the perioperative fluid losses. The heart was exposed by a median sternotomy and the pericardium was extensively opened. Ascending aorta was dissected to allow abrupt acute occlusion during

the experimental protocol. Lastly, 1 conductance catheter (SPR-324; Millar Instruments) was positioned by apical puncture on left ventricle (LV) to assess cardiac performance. After instrumentation, all animals were stabilized for 15 min before starting the experimental protocol.

Hemodinamyc measurements

Hemodynamic was performed as previously described in detail. [19] All the animals from EX and SED groups were submitted to hemodynamic measurements in baseline steady-state conditions. In order to assess the cardiac behavior to a stressful condition it was performed an abrupt acute occlusion of the ascending aorta (isovolumetric heartbeats) to rapidly augment the LV afterload. Parameters were recorded and on-line converted to a digital data with a sample frequency of 1,000 Hz, in order to accurately capture all the features of the pressure-volume waveforms produced by the fast-beating rat heart. LV hemodynamic parameters included: Peak systolic pressure (Pmax), end-diastolic (EDP) and end-systolic pressure, peak rate of pressure raise (dP/dtmax) and peak rate of pressure decay (dP/dtmin). The tau (time constant of relaxation rate) was estimated by fitting the isovolumetric pressure fall to a monoexponential function. All the animals completed the experimental protocol. Data were stored and analyzed with Millar conductance data acquisition and analysis software (PVAN3.5)

Tissue collection

After completing the acquisition of the hemodynamic data, all animals were sacrificed through exsanguination. The heart was excised and weighted. Under binocular magnification (×3.5) the LV free wall, RV free wall, and septum (SPT) were dissected and weighted independently. Heart weight, LV and RV were normalized to tibia length. The left gastrocnemius was also removed and weighted. Samples of LV, RV and SPT from all animals were collected and fixed in a solution of 4% (vol/vol) buffered paraformaldehyde for latter histological analysis. Also, samples of LV and RV from all animals were collected for mitochondria isolation, being the remained processed for biochemical analysis.

Histological preparation for light microscopic evaluation

Tissue sections of LV, RV and SPT were fixed in a solution of 4% (vol/vol) buffered paraformaldehyde for 24 hours and posteriorly dehydrated with graded ethanol and included in paraffin blocks. Xilene was used in the exchange between dehydration and impregnation. Serial sections (5 μm of thickness) of the paraffin blocks were cut by a microtome and mounted on silane-coated slides. The slides were dewaxed in xylene an hydrated though graded alcohol concentrations, finishing in phosphate buffered saline solution prepared by dissolving Na2HPO4 (1.44 g), KH2PO4 (0.24 g), NaCl (8 g), KCl (0.2 g) in 1 litre of deionised water and adjusting pH to 7.2. Deparaffinised sections from LV were stained for haematoxylin-eosin, conducted by immersing slides in Mayer's haematoxylin solution for 9.5 min followed by immersion in eosin solution for 4.5 min, dehydration with graded alcohols through xylene and mounted with DPX. Cardiomyocytes surface area (CSA) was measured and only round nucleated myocytes were considered for analysis.

Mitochondria Isolation

Cardiac mitochondria isolation was performed using the conventional methods of differential centrifugation, as previously described. [20] All procedures were performed at 0-4°C. Briefly, after excised the hearts were immediately minced in an ice-cold isolation medium containing 250 mM sucrose, 0.5 mM EGTA, 10 mM HEPES-KOH (pH 7.4), and 0.1 % defatted BSA. The minced blood-free tissue was resuspended in isolation medium containing protease subtilopeptidase A type VIII (1 mg/g tissue) and homogenized with tightly fitted Potter-Elvehjen homogenizer and Teflon pestle. The suspension was incubated for 1 minute (4 °C) and rehomogenized. An 0.5 mL aliquot of cardiac muscle homogenate was reserved for biochemical analysis and the remaining homogenate was centrifuged at 14,500 *g* during 10 minutes. The supernatant fluid was decanted, and the pellet, essentially devoid of protease, was gently resuspended in isolation medium. The suspension was centrifuged at 750 *g* for 10 minutes, and the resulting supernatant was centrifuged at 12,000 *g* for 10 minutes. The pellet was resuspended and repelleted at 12,000 *g* for 10 minutes. The final pellet, containing the mitochondrial fraction, was gently resuspended in a washing medium containing

250 mM sucrose, 10 mM HEPES-KOH, pH 7.4. Phosphatases and proteases inhibitors were added and all the procedures were performed at 0-4 ºC.
Mitochondrial and whole heart protein concentration was spectrophotometrically estimated with the colorimetric method “RC DC protein assay” (Bio-Rad) using bovine serum albumin (BSA) as standard. This assay is based on a modification of the Lowry et al. [21] protocol, allowing the quantification of the protein in the presence of reducing agents and detergents.

Determination of ATP-Synthase activity

For spectrophotometric determination of respiratory chain complex V activity, mitochondrial fractions were disrupted by a combination of freeze-thawing cycles in hypotonic media (25 mM potassium phosphate, pH 7.2) and the activity was measured as previously described. [22] The phosphate produced by hydrolysis of ATP reacts with ammonium molybdate in the presence of reducing agents to form a blue-colour complex, the intensity of which is proportional to the concentration of phosphate in solution. Oligomycin was used as an inhibitor of mitochondrial ATPase activity. Each sample was analyzed in duplicate.

Western blotting analysis

Equivalent amounts of total proteins from each group were electrophoresed on a 12.5 % SDS-PAGE as described by Laemmli. [23] In order to normalize variations among gels, one sample from each group was always applied in the same gel. Gels containing total proteins or mitochondrial proteins (separated by 2-D BN-PAGE) were blotted onto a nitrocellulose membrane (Whatman®, Protan®) and nonspecific binding was blocked with 5 % (w/v) dry non-fat milk in TBS-T (100 mM Tris, 1.5 mM NaCl, pH 8.0 and 0.5 % Tween 20). Membranes were then incubated with primary antibody solution (1:1000 dilution; anti-ATP synthase subunit beta; anti-GAPDH; anti-SIRT-3). After 2 hours incubation, the membrane was washed with TBS-T and incubated with anti-mouse or anti-rabbit IgG peroxidase secondary antibody (1:1000 dilution, Amersham Pharmacia Biotech). Immunoreactive bands were detected with enhanced chemiluminescence reagents (ECL, Amersham Pharmacia

Biotech) according to the manufacturer's procedure and images were recorded using X-ray films (Kodak Biomax light Film, Sigma). The films and the gels were scanned in Molecular Imager Gel Doc XR+ System (Bio-Rad) and analyzed with QuantityOne software version 4.6.3 (Bio-Rad, Hercules, CA). Four independent experiments were considered for analysis. Equal loading was confirmed by staining the membrane with Ponceau S.

MHC isoform determination

Left and right ventricular samples were weighed and transferred to a glass homogenizer. A 1:19 ratio of 100 mM phosphate buffer, pH 7.4, containing 0.02% bovine serum albumin was added. Tissue sections were thoroughly homogenized with tightly fitted Potter-Elvehjen homogenizer and Teflon pestle. Total protein concentration was spectrophotometrically assayed with the colorimetric method “RC DC protein assay” (Bio-Rad) using bovine serum albumin (BSA) as standard. Alpha- and beta-isoforms of cardiac myosin heavy chain were separated by gel electrophoresis following the procedure described by Talmadge and Roy. [24] The amount of protein run on the gel was 1 mg per lane. To avoid inter-gel variation, one sample from each of the groups studied was applied in the same gel. The stacking gel consisted of 30% glycerol and 4 % acrylamide:*N*,*N*’-methylene-bis-acrylamide in the ratio of 50:1, 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% sodium dodecyl sulfate (SDS). The separating gels were composed of 30% glycerol, 8% acrylamide-bis (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS. Polymerization was initiated with 0.05% N,N,N’,N’-tetramethylethylenediamine and 0.1% ammonium persulfate. The gels were run in a Mini-Protean system (Bio-Rad) at 4°C. The running conditions were 70V (constant voltage) for 30 hours. The gels were stained with Coomassie Colloidal, scanned in Molecular Imager Gel Doc XR+ System (Bio-Rad, Hercules, CA, USA) and optical density analysis of MHC bands was performed using QuantityOne Imaging software (v4.6.3, Bio-Rad). Five independent experiments assayed in duplicate were considered for analysis.

Statistical analysis

Data are presented as mean±standard deviation (SD). For comparisons between groups, a unpaired *t*-test was performed considering the normal distribution of the variables. Significance statistical level was set at $P < 0.05$. Statistical analyses were carried out using SPSS 15.0 for Windows software.

Results

Effects of exercise training protocol in LV baseline function

The LV hemodynamic profile at baseline conditions reveled a significant lower heart rate (HR) in EX animals (EX:294.3±4.887 vs. SED:313.9±6.669 bpm) ($P<0.05$ vs. SED). At the systolic level, the EX animals exhibited increased systolic pressures, expressed by maximum pressure (MaxP) (EX:161.2±2.714 vs. SED:145.1±4.050 mmHg) and end-systolic pressure (ESP) (EX:158.8±2.653 vs. SED:141.9±3.771 mmHg) ($P<0.05$ vs. SED). Although exercise training did not promote significant alterations ($P>0.05$ vs. SED) in the dP/dt max (EX:9968±178.9 vs. SED:9324±538.7 mmHg/sec), there was a slightly tendency in the EX group to higher maximum rate of LV pressure elevation. At diastolic level, the EX animals exhibited a significant lower end-diastolic pressure (EDP) (EX:6.288±0.4982 vs. SED:9.023±1.219 mmHg) ($P<0.05$ vs. SED), indicating an enhanced overall diastole by working at lower develop pressure. The exercise training did not promote significant alterations ($P>0.05$ vs. SED) in the diastole velocity-related parameters, express by the dP/dt min (EX:-11383±114.5 vs. SED:-10175±591.9 mmHg/sec) and Tau (EX:11.55±0.2550 vs. SED:11.59±0.3704 m/sec), however there was also presented a slightly tendency in the EX group for a higher maximum rate of LV pressure decay.

Effects of exercise training protocol in LV behavior to acute pressure overload

For examining the LV behavior in response to a stressful condition, it was performed an abrupt occlusion of the ascending aorta, in order to suddenly augment the LV afterload. The animals were roughly submitted to the same overload, given by the similarly reached MaxP ($P>0.05$ vs. SED). For similar afterload elevations, at systolic level the animals LV behavior was different in both groups, with EX animals exhibiting a significant improvement of contractility ($P<0.05$ vs. SED), accessed by maximum rate of LV pressure elevation (dP/dt max) (EX:9846±208.8 vs. SED:8720±316.4 mmHg/sec). The diastolic response of the EX animals was also different from SED group, since the exercise training promoted a significant lower EDP (EX:6.969±0.354 vs SED:10.41±1.347 mmHg) and a faster Tau (EX:13.55±0.472 vs SED:16.32±

1.149), showing that exercise training endow the animals with a faster and lower pressure relaxation. Although the absence of significant alterations among groups in the dP/dt min (P>0.05 vs. SED), there was a tendency for a higher maximum rate of LV pressure decay in EX animals.

Effects of exercise training protocol in the morphometric parameters

Exercise training result in significant increase in body (EX:325.1±11.23 vs. SED:291.8±10.62 g) and left gastrocnemius weight (EX:2.226±0.06033 vs. SED:1.959±0.05029 g) (P<0.05 vs. SED), while it did not promote any significant alterations in the heart, LV, RV and SPT weight (P>0.05). The ratio of heart and LV weight to tibia length did not suffer significant alterations (P>0.05 vs. SED), while the ratio of RV weight to tibia length suffer significant alterations (EX:0.04404±0.001906 vs. SED:0.05789±0.005577) (P<0.05).

Effects of exercise training protocol in cardiomyocyte structure

Fifty two weeks of exercise training resulted in a significant increase in LV cardiomyocyte cross sectional area (CSA) in comparison with SED animals (EX:274±23 vs. SED:249±18 μm^2) (P<0.00005 vs. SED), while the RV cardiomyocyte CSA show a significant increase in SED animals (EX:243±21 vs. SED:252±26 μm^2) (P<0.005 vs. SED). The SPT follows the same statistical significance with an increase in cardiomyocytes CSA in SED animals (EX:238±30 vs. SED:254±31 μm^2) (P<0.0005 vs. SED). This RV CSA results in combination with the basal SED higher pressures, indicate an increased RV afterload, which might be consequent of a pulmonary arterial hypertension development.

Effects of exercise training protocol on MHC

Exercise training did not promote any significant alterations in LV and RV beta/alpha-MHC isoform ratio (P>0.05 vs. SED).

Effects of exercise training protocol in cardiac metabolism and mitochondrial oxidative phosphorylation

At mitochondrial level, 52 weeks of exercise training result in a significantly increased complex V activity. ($P<0.05$ vs. SED) So, EX animals showed an enhanced ability to aerobically produce ATP ($P<0.05$ vs. SED). Western blot analysis of LV and RV ATP-synthase subunit beta was performed in order to validate the protein expression profile observed and no differences were verified between groups ($P>0.05$ vs. SED).

In order to investigate the influence of the exercise training protocol in cardiac glycolysis, western blot analysis of LV and RV GAPDH was performed and no statistical significance in protein expression levels was detected either in LV or RV ($P>0.05$ vs. SED). Also, exercise training induced significantly higher SIRT-3 protein expression levels ($P<0.0005$ vs. SED).

Discussion

Our main purpose was to determine whether one year of forced exercise training could modulate the cardiac functional, structural and biochemical alterations that are typically associated to aging. To our knowledge, this is the first study that combines a 52 weeks of forced exercise training program. Our main results suggest that long-term exercise training at a moderate intensity is able to improve the cardiac function, specially the diastolic behavior. This improvement in the overall cardiac functional capacity may be related to the enhancement of the mitochondrial oxidative phosphorylation though SIRT-3 beneficial activities in cardiac mitochondria.

It is widely recognized that aging impairs the cardiovascular function, with a more incidence in diastolic function, in contrast to a relatively well-preserved systolic function, either in animal species or in humans. [12, 18, 25-27] Exercise training is thought to mitigate several age-related cardiac biological changes, preventing the overall decline in functional capacity. [28-30] The present study shows that exercise training, i.e. an active aging, can ameliorate cardiac function in baseline conditions and increase its in response to a sudden enhanced afterload.

The higher heart rate displayed with sedentary aging was reduced to lower levels by exercise training, thereby lowering the cardiac work and metabolic demands at baseline function. This feature is in line with other studies [31, 32] and according to Leosco et al. [32], this could be related to a exercise training-mediated enhancement of the age-dependent impairment of adrenergic signaling. Active aging also rescued the cardiac pressures profile. In this study, the systolic pressures, and either maximum or at end-systole, were significantly augmented at basal conditions with exercise. The end-diastolic pressures were also completely rescued by exercise training, either in basal or under acute afterload elevation conditions. The systolic pressures corroborate the results found by Choi et al. [9]. They report a reduction of the systolic pressures with aging in rats and an augmentation after 12 weeks of treadmill exercise training. They also report an increase in the diastolic pressures with aging, although exercise did not induce any significant alterations. This absence of diastolic improvements may be related to the short and low intensity exercise training

protocol that they used. However, Lechance et al. [33], using a more extensive program, showed that 9 months of exercise training effectively reduced EDP in rats.

In the present study, active aging significantly improved Tau. Thus exercise training was able to enhance the rate of fall of ventricular pressure during isovolumetric relaxation, i.e, improving the active relaxation properties of the ventricle and diastolic function. These results are not in conformity with the work done by Choi et al. [9]. They report no significant Tau alterations with exercise training and proposed that the deleterious aging effect in diastolic function might overcome the possible benefits of exercise training. Nevertheless, the used exercise protocol might not be long enough to induce significant alterations in the ventricular active relaxation properties. This exercise-mediated enhancement of diastolic function is intimately related with calcium (Ca2+) handling in the contractile apparatus of cardiomyocytes, specially the transport of Ca2+ from cytoplasm to sarcoplasmic reticulum, leading to relaxation by lowering cytosolic Ca2+ levels. [34-36] This is confirmed with the work done by Tate et al. [37], which showed that exercise training was able to increase the rates of Ca2+ uptake and restore the relaxation times back to values seen in young adult rats. In the present study, we also found that exercise training resulted in a significantly increased dP/dtmax, which is a common used parameter that is sensitive to changes in contractility. [38] These results corroborate those exhibited by Choi et al. [9], which report that exercise training augmented the maximum rate of pressure elevation in comparison to the sedentary group. Of note, enhanced dP/dtmax is known to be highly related to increased activity of SERCA2a [39] and a more efficient transport of calcium to the sarcoplasmic reticulum [40].

A fundamental feature in cardiac contractility is the oxidative phosphorylation energetic supply of ATP to cardiomyocytes contractile apparatus. [41] Aging is associated with impairments in ETC and oxidative phosphorylation, including reduced activity of ATP-synthase [13, 14], mainly due to increased mitochondrial ROS production and a lower antioxidant activity. [42, 43] In this study, exercise training significantly increased the ATP-synthase activities, i.e. rescued the loss of oxidative phosphorylation seen with sedentary

aging. These results corroborate the findings from Padrão et al. [44], which reveal increased ATP-synthase activity with a lifelong active lifestyle in mice.

Sirtuin-3 has been shown to mediate a myriad of mitochondrial benefits and also is the main mitochondrial deacetylase. [45, 46] Among the benefits in cardiac function, SIRT-3 has been shown to deacetylate ETC enzymes and consequently enhancing the oxidative phosphorylation state. Specially, SIRT-3 interacts with ATP-synthase subunits ATP5A1 and ATP5F1. [47] In this study, exercise training promoted to a significant increase in SIRT-3 protein expression levels. So this may in part explain the increase in ATP-synthase activity seen in exercised animals, suggesting that exercise training-increase in SIRT-3 expression may play an important role in preventing cardiac aging.

Another important feature of the cardiac contractility behavior is the MHC content, which has been shown to be associated with alterations in cardiomyocyte power output properties. [48] With aging, a shift in MHC takes place and is characterized by a decrease in α-MHC and a increase in β-MHC content.[49] However, there are evidences suggesting that an increase in the ratio of β / α-MHC has no deleterious effects in contractile function. [50] Our results indicate that the ratio of β / α-MHC did not suffered significant alterations with exercise training, which are in line with previous studies. [49, 51]

Regarding the RV, and contrarily to what we were expecting, SED animals displayed more hypertrophy (increased RV mass and cardiomyocyte cross sectional area) than EX. We attribute this finding to the possible presence of increased pulmonary vascular resistance as we found an elevated ratio of lung weight/tibia length, and possibly to higher RV peak systolic pressure due to arterial stiffness [52-54].

Conclusion

The present work shows that long-term exercise training improves cardiac performance, counteracting the degenerative heart phenotype associated with advanced chronological age. The underlying mechanisms of the exercise-induced protective effects are complex and remain incompletely understood but they possibly include improvements at the mitochondrial level.

Acknowledgements

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FIGURE LEGENDS

Figure 1: Effects of exercise training on LV (**A**), RV (**B**) and SPT (**C**) cardiomyocyte CSA. Values are presented as Mean±SD (n=8 per group). ** P<0.005 vs SED, *** P<0.0005 vs SED, **** P<0.00005 vs SED. (LV: left ventricle, RV: right ventricle, SPT: septum, CSA: cross sectional area)

Figure 2: Effects of exercise training on LV and RV GAPDH expression. Values are presented as Mean±SD (n=8 per group). SED: sedentary, EX: exercise, LV: left ventricle, RV: right ventricle.

Figure 3: Effects of exercise training on LV and RV ATP-Synthase expression. Values are presented as Mean±SD (n=8 per group). SED: sedentary, EX: exercise, LV: left ventricle, RV: right ventricle.

Figure 4: Effects of exercise training on LV SIRT-3 expression. Values are presented as Mean±SD (n=8 per group). *** P<0.0005 vs SED. SED: sedentary, EX: exercise, LV: left ventricle

Figure 5: Effects of exercise training on LV and RV MHC isoform ratio. Values are presented as Mean±SD (n=8 per group). SED: sedentary, EX: exercise, LV: left ventricle, RV: right ventricle.

Figure 6: Effects of exercise training on LV ATP-synthase activity, assessed by spectrophotometry. Values are presented as Mean±SD (n=8 per group). ** P<0.005 vs SED. SED: sedentary, EX: exercise, LV: left ventricle.

Table 1. General morphometric characteristics

	SED	EX
Morphometry		
BW (g)	291,8 ± 10,62	325,1 ± 11,23*
Gast (g)	1,959 ± 0,05	2,226 ± 0,06**
Lungs (g)	2,655 ± 0,71	2,221 ± 0,22
Lungs / Tibia	0,703 ± 0,20	0,560 ± 0,05
Heart / Tibia	0,270 ± 0,01	0,263 ± 0,01
LV / Tibia	0,148 ± 0,004	0,145 ± 0,006
RV / Tibia	0,057 ± 0,005	0,04 ± 0,001*

LV: left ventricle, RV: right ventricle, BW: body weight, Gast: left gastrocnemius. Values are presented as Mean±SD. *P<0.05 vs SED; **P<0.005 vs SED.

Table 2. Hemodynamic evaluation at basal conditions

	SED	EX
Hemodynamic		
Basal Conditions	LV	LV
HR (bpm)	313,9 ± 6,66	294,3 ± 4,88*
MaxP (mmHg)	145,1 ± 4,05	161,2 ± 2,71**
MinP (mmHg)	3,473 ± 0,88	2,158 ± 0,39
ESP (mmHg)	141,9 ± 3,77	158,8 ± 2,65***
EDP (mmHg)	9,023 ± 1,21	6,288 ± 0,49*
dP/dt_{max} *(mmHg/s)*	9462 ± 418,9	9701 ± 178,9
dP/dt_{min} *(mmHg/s)*	-10452 ± 458,7	-11039 ± 164,1
Tau (ms)	11,59 ± 0,37	11,55 ± 0,25

LV: left ventricle, RV: right ventricle, HR: heart rate, MaxP: maximum pressure, MinP: minimum pressure, ESP: end systolic pressure, EDP: end diastolic pressure, dP/dtmax: maximum rate of pressure elevation, dP/dtmin: maximum rate of pressure decay, Tau: isovolumic relaxation Constant. Values are presented as Mean±SD. *P<0.05 vs SED; **P<0.005 vs SED; ***P<0.0005 vs SED.

Table 3. Hemodynamic evaluation under isovolumetric conditions

	SED	**EX**
Hemodynamic		
Isovolumetric Conditions	LV	LV
HR (bpm)	298,5 ± 8,45	291,2 ± 4,53
MaxP (mmHg)	208,9 ± 6,79	226,3 ± 5,40
MinP (mmHg)	7,076 ± 1,20	3,717 ± 0,302*
ESP (mmHg)	208,4 ± 6,79	225,0 ± 5,80
EDP (mmHg)	10,41 ± 1,34	6,969 ± 0,351*
dP/dt_{max} *(mmHg/s)*	8720 ± 316,4	9846 ± 208,8**
dP/dt_{min} *(mmHg/s)*	-5434 ± 374,1	-5814 ± 369,6
Tau (ms)	16,32 ± 1,14	13,55 ± 0,473*

LV: left ventricle, HR: heart rate, MaxP: maximum pressure, MinP: minimum pressure, ESP: end systolic pressure, EDP: end diastolic pressure, dP/dtmax: maximum rate of pressure elevation, dP/dtmin: maximum rate of pressure decay, Tau: isovolumic relaxation Constant. Values are presented as Mean±SD. *P<0.05 vs SED; **P<0.005 vs SED.

FIGURE 1

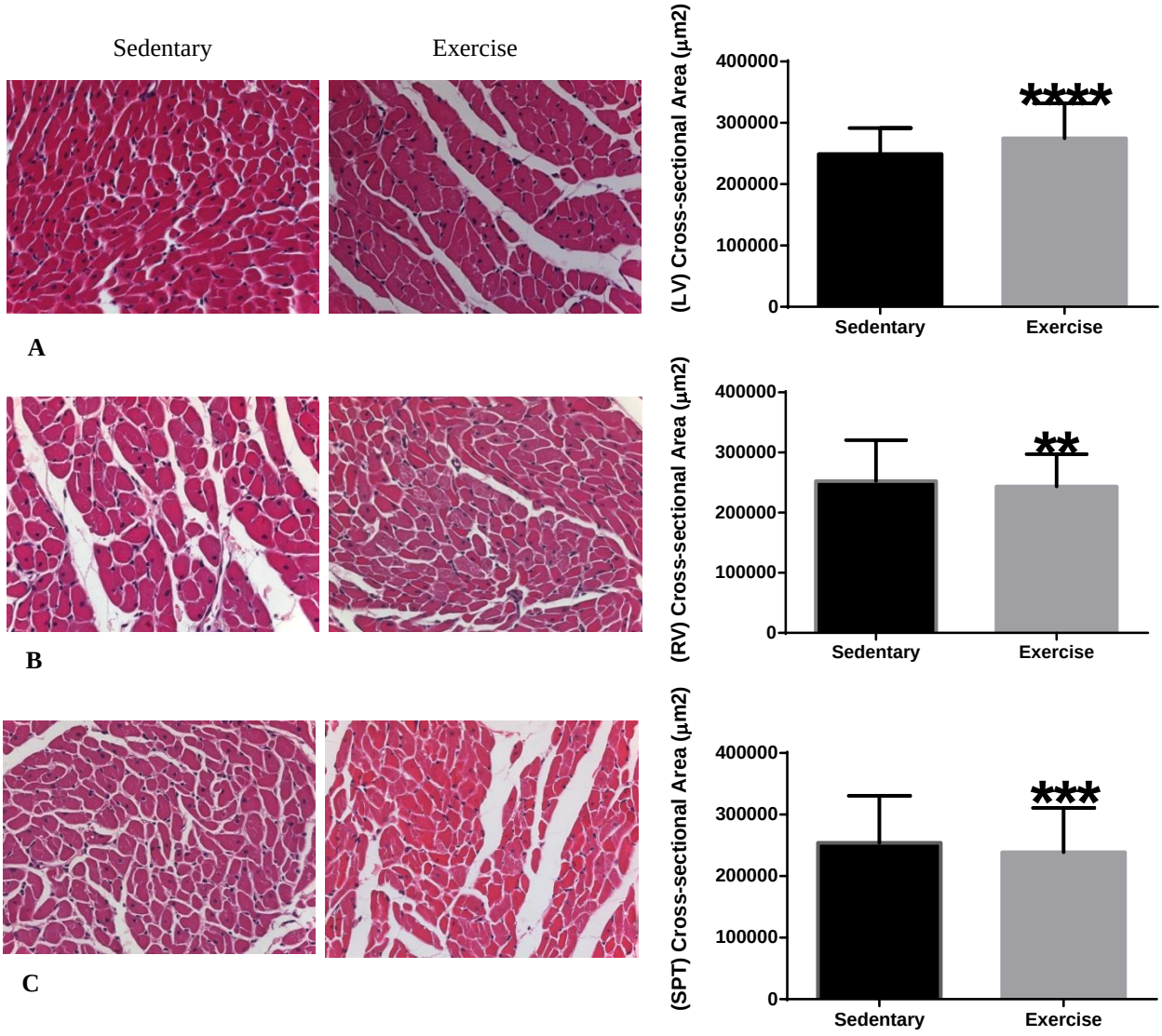
Sedentary
Exercise
A
B
C
(LV) Cross-sectional Area (µm2)
(RV) Cross-sectional Area (µm2)
(SPT) Cross-sectional Area (µm2)
400000
300000
200000
100000
0
Sedentary
Exercise
****
**
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FIGURE 2

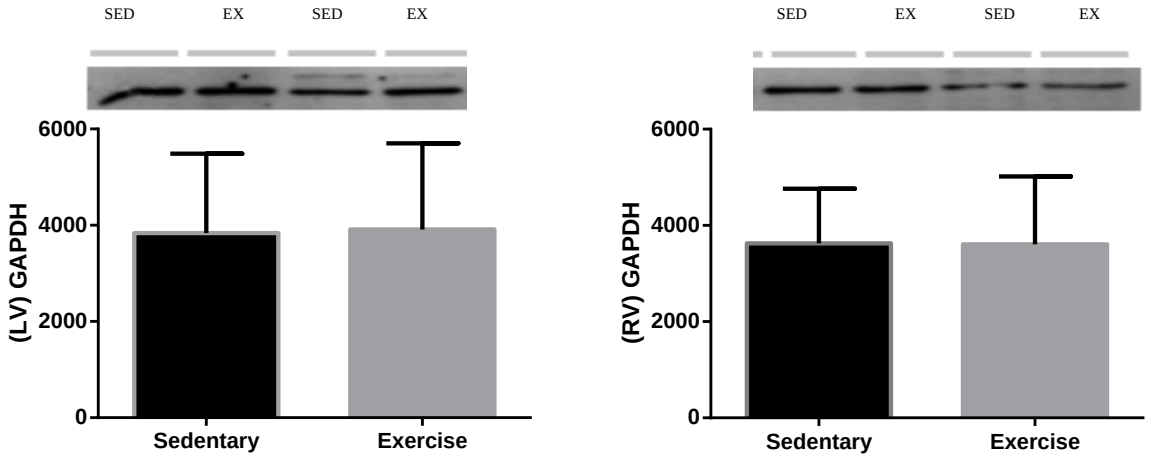


FIGURE 3

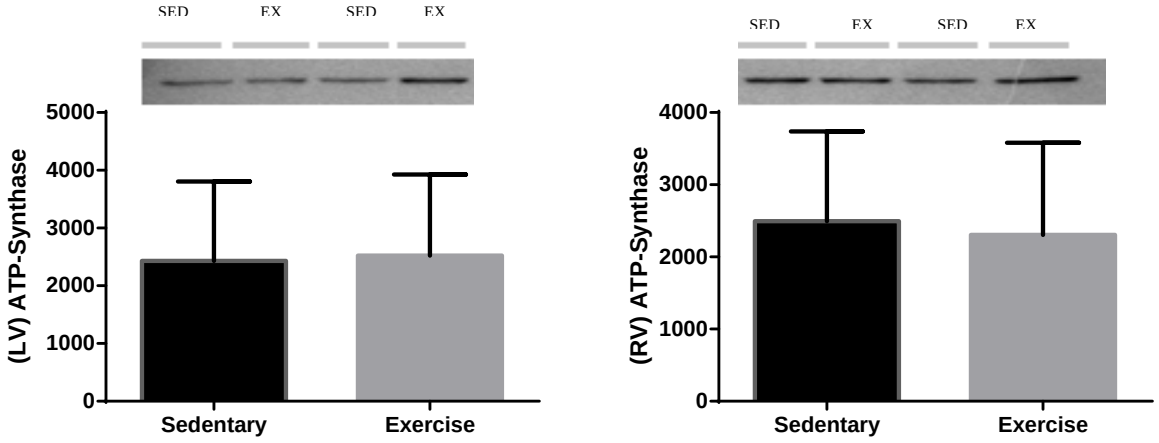


FIGURE 4

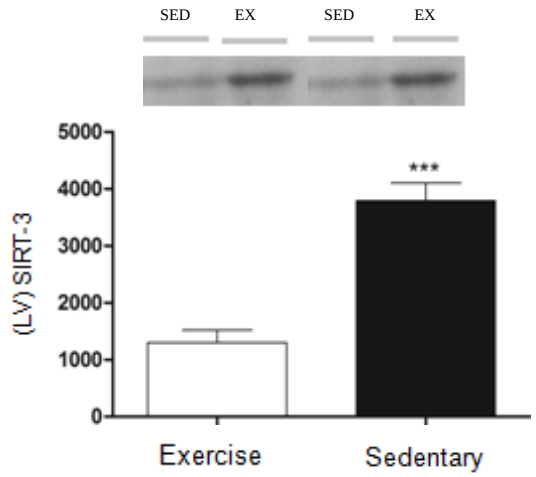


FIGURE 5

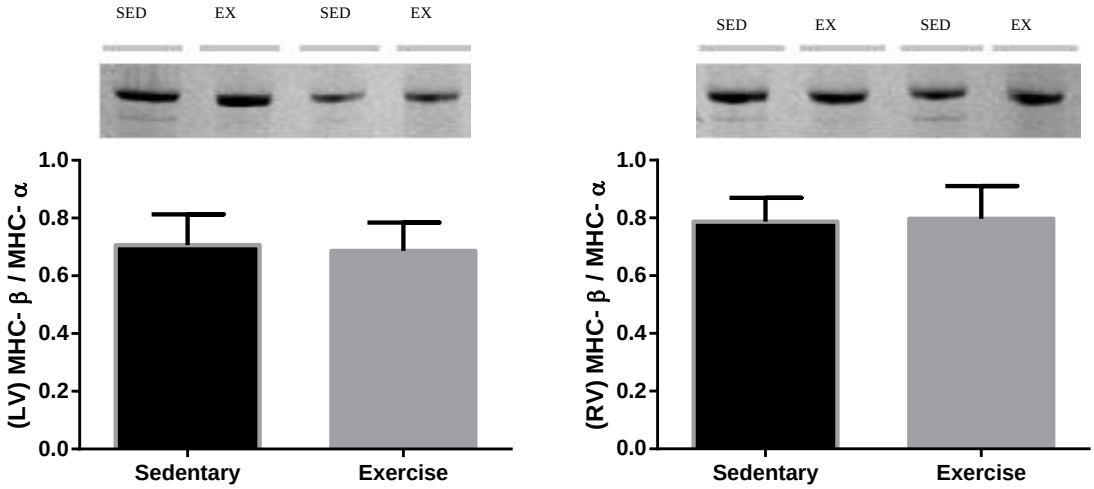
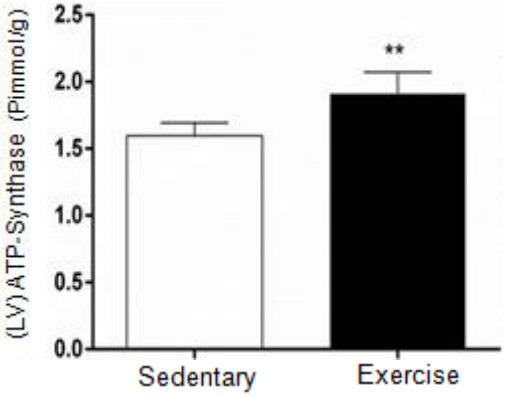


FIGURE 6



3. MAIN CONCLUSION

Considering the overall findings supported by our study, the main conclusions that must be highlighted are:

1. Long-term exercise training of moderate intensity is able to improve the LV cardiac function in baseline conditions and in response to a sudden increase in LV afterload;

2. The loss of cardiac function associated with sedentary behavior is rescued by long-term exercise training;

3. The positive effects of long-term exercise training may be related to the enhancement of the mitochondrial oxidative phosphorylation possibly mediated by SIRT-3.